OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
AG055367

b. Agency Routing Number

2. DATE SUBMITTED	**Application Identifier**	**c. Previous Grants.gov Tracking Number**

5. APPLICANT INFORMATION **Organizational DUNS*:** 0729333930000

Legal Name*: UNIVERSITY OF SOUTHERN CALIFORNIA
Department: Contracts and Grants
Division: 195-1642394A1
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: CA
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701

Person to be contacted on matters involving this application
Prefix: Mr. First Name*: Steven Middle Name: Last Name*: Misuraca Suffix:
Position/Title: Contracts and Grants Officer
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701
Phone Number*: 213-740-8207 Fax Number: 213-740-6070 Email: misuraca@research.usc.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 195-1642394A1

7. TYPE OF APPLICANT* O: Private Institution of Higher Education
Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2018 03/31/2023

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-037

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Caleb Middle Name: E. Last Name*: Finch Suffix: Ph.D

Position/Title: Professor
Organization Name*: University of Southern California
Department: Davis School of Gerontology
Division:
Street1*: 3715 McClintock Avenue
Street2: Department of Contracts & Grants
City*: Los Angeles
County: CA
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0191
Phone Number*: 213-740-1758 Fax Number: 213-740-0853 Email*: cefinch@usc.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$12,616,207.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$12,616,207.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Mr. First Name*: Steven Middle Name: Last Name*: Misuraca Suffix:

Position/Title*: Contracts and Grants Officer
Organization Name*: University of Southern California
Department: Contracts and Grants
Division:
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701
Phone Number*: 213-740-8207 Fax Number: 213-740-6070 Email*: misuraca@research.usc.edu

Signature of Authorized Representative*
Steven Misuraca

Date Signed*
05/25/2017

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:CoverLetter_P01_2017_Final.pdf

424 R&R and PHS-398 Specific
Table Of Contents

Component Summary

Components	Component Project Title	Organization Name	Contact PD/PI Name or Project Lead Name
Overall	Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms	UNIVERSITY OF SOUTHERN CALIFORNIA	Finch, Caleb E.
Admin-Core-001 (724)	Administrative Core	University of Southern California	Finch, Caleb E.
Core-001 (257)	Neuroimaging Core	University of Southern California	THOMPSON, PAUL M
Core-002 (249)	Environment Exposure & Neurotoxicology Core	University of Southern California	Sioutas, Constantinos
Project-001 (987)	Traffic-Related Air Pollutants and Alzheimer's Disease: Risk, Susceptibility and Mechanisms in Women	University of Southern California	Chen, Jiu-Chiuan
Project-002 (634)	Urban Air Pollution and Pathological Brain Aging: A Nationwide Twin Study in Men	The Regents of University of California UC San Diego	FRANZ, CAROL Elaine
Project-003 (425)	Age-sex-ApoE allele interactions in neuronal and white matter vulnerability to air pollution	University of Southern California	FINCH, CALEB E
Project-004 (437)	Urban air pollution and cerebral hypoperfusion: aging and sex influences	University of Southern California	Mack, William J

Project/Performance
Site Location(s) Summary

Applicant Organization	City	State/Province	Country
UNIVERSITY OF SOUTHERN CALIFORNIA	Los Angeles	CA	UNITED STATES

Organization Name	City	State/Province	Country	Component
University of California Riverside	Riverside	CA	UNITED STATES	Project-002 (634)
University of California San Diego	La Jolla	CA	UNITED STATES	Project-002 (634)
University of Southern California	Los Angeles	CA	UNITED STATES	Admin-Core-001 (724)
University of Southern California	Los Angeles	CA	UNITED STATES	Core-001 (257)
University of Southern California	Los Angeles	CA	UNITED STATES	Core-002 (249)
University of Southern California	Los Angeles	CA	UNITED STATES	Overall
University of Southern California	Los Angeles	CA	UNITED STATES	Project-001 (987)
University of Southern California	Los Angeles	CA	UNITED STATES	Project-003 (425)
University of Southern California	Los Angeles	CA	UNITED STATES	Project-004 (437)
University of Washington	Seattle	WA	UNITED STATES	Core-002 (249)
VA Puget Sound Health Care system	Seattle	WA	UNITED STATES	Project-002 (634)
Wake Forest University Health Sciences	Winston-Salem	NC	UNITED STATES	Project-001 (987)

Human Subjects
Clinical Trials
Vertebrate Animals
HESC
Summary

Components	Human Subjects	Clinical Trial	Phase III Clinical Trial	Vertebrate Animals	HESC
Overall	Y	N	N/A	Y	N
Admin-Core-001 (724)	N	N	N/A	N	N
Core-001 (257)	N	N	N/A	Y	N
Core-002 (249)	N	N	N/A	Y	N
Project-001 (987)	Y	N	N/A	N	N
Project-002 (634)	Y	N	N/A	N	N
Project-003 (425)	N	N	N/A	Y	N
Project-004 (437)	N	N	N/A	Y	N

Senior/Key Personnel
Summary

Name	Organization	Role on Project	Components
Finch, Caleb E.	University of Southern California	PD/PI(Contact)	Overall
Chen, Jiu-Chiuan	University of Southern California	PD/PI(MPI)	Overall
Braskie, Meredith Nicole	University of Southern California	Co-Investigator	Core-001 (257)
Cen, Steven Yong	University of Southern California	Other: Biostatistician	Project-001 (987)
Chen, Jiu-Chiuan	University of Southern California	Other: Core Co-Lead	Admin-Core-001 (724)
Chen, Jiu-Chiuan	University of Southern California	Co-Investigator	Core-002 (249)
Chen, Jiu-Chiuan	University of Southern California	Other: Project Lead	Project-001 (987)
Espeland, Mark A.	Wake Forest University Health Sciences	Co-Investigator	Project-001 (987)
FENNEMA-NOTESTINE, CHRISTINE	University of California San Diego	Co-Investigator	Project-002 (634)
FINCH, CALEB E	University of Southern California	Other: Project Lead	Project-003 (425)
Finch, Caleb E.	University of Southern California	Other: Core Lead	Admin-Core-001 (724)
FORMAN, HENRY Jay	University of Southern California	Co-Investigator	Core-002 (249)
FRANZ, CAROL Elaine	University of California San Diego	Other: Project Lead	Project-002 (634)
Gatz, Margaret	University of Southern California	Co-Investigator	Project-001 (987)
HAGLER, DONALD J	University of California San Diego	Co-Investigator	Project-002 (634)
JACOBS, RUSSELL E	University of Southern California	Co-Investigator	Core-001 (257)
Kaufman, Joel Daniel	University of Washington	Co-Investigator	Core-002 (249)
Kisler Elliott, Kassandra J	University of Southern California	Other: Research Associate	Core-001 (257)
KREMEN, WILLIAM S.	University of California San Diego	Other: Project Co-Lead	Project-002 (634)
Liu, Collins Yuchen	University of Southern California	Co-Investigator	Project-001 (987)
MACK, WENDY JEAN	University of Southern California	Co-Investigator	Project-003 (425)
MACK, WENDY JEAN	University of Southern California	Co-Investigator	Project-004 (437)
Mack, William J	University of Southern California	Other: Project Lead	Project-004 (437)
Millstein, Joshua	University of Southern California	Co-Investigator	Project-001 (987)
Montagne, Axel	University of Southern California	Co-Investigator	Core-001 (257)
MORGAN, TODD E	University of Southern California	Co-Investigator	Project-003 (425)

Nation, Daniel A	University of Southern California	Co-Investigator	Project-001 (987)
Nelson, Amy R	University of Southern California	Post Doctoral Scholar	Core-001 (257)
Petkus, Andrew John	University of Southern California	Co-Investigator	Project-001 (987)
PIKE, CHRISTIAN J	University of Southern California	Co-Investigator	Project-003 (425)
Sheppard, Elizabeth A Lianne	UNIVERSITY OF WASHINGTON	Co-Investigator	Core-002 (249)
Sioutas, Constantinos	University of Southern California	Other: Core Lead	Core-002 (249)
SMITH, NICHOLAS L	UNIVERSITY OF WASHINGTON	Co-Investigator	Project-002 (634)
THOMPSON, PAUL M	UNIVERSITY OF SOUTHERN CALIFORNIA	Other: Core Lead	Core-001 (257)
Tu, Xin M.	University of California San Diengo	Co-Investigator	Project-002 (634)
Vanos, Jennifer	University of California San Diego	Co-Investigator	Project-002 (634)
Zhang, Honqiao	University of Southern California	Co-Investigator	Core-002 (249)
Zlokovic, Berislav V	University of Southern California	Other: Core Co-Lead	Core-001 (257)

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Finch, Caleb E

eRA COMMONS USER NAME (credential, e.g., agency login): cefinch

POSITION TITLE: Professor, Biological Sciences and Gerontology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Yale University, New Haven, CT	B.S.	05/61	Biophysics
Rockefeller University, NY, NY	Ph.D.	06/69	Cell Biology
Rockefeller University, NY, NY	Postdoc	08/70	Comparative Pathology

A. Personal Statement

I have long-standing interests in environmental influences on aging, which began with an invited review in Science that showed heritable factors account for <35% individual differences in lifespan (Finch & Tanzi, Science, 1997). The environment clearly has had a major role in the increase of lifespans since 1800 through diminishing inflammation and infection (Finch & Crimmins, Science 2004). As founding PI of the USC Alzheimer Center in 1984, I have a long record of leadership in brain sciences. My work on Alzheimer disease lead me to consider the role of diet and most recently, air pollution factors in brain aging. Discussions with Rob McConnell and other USC epidemiologists on their findings of air pollution particles and atherosclerosis motivated me to consider the impact on brain aging. Since 2010, I have collaborated with Costas Sioutas for rodent exposure studies. Our models show the adverse impact of nano-sized traffic-related air pollution (TRAP) on the brain throughout life. Most recently in collaboration with JC Chen, we showed that the ApoE4 Alzheimer risk factor increased dementia risk increased vulnerability to TRAP in his studies of the WHIMS cohort, and in our studies of EFAD mice. We also continue long-standing neuroendocrine studies on the peri-menopause that show altered glial neurotrophic activity with onset of cycle lengthening and sex-specific effects of APOE alleles on cerebral hemorrhages.

B. Positions and Honors

Positions and Employment

1969 - 1972 Assistant Professor of Anatomy, Cornell University Medical College
1972 - 1975 Assistant Professor of Biological Sciences, University of Southern California
1975 - 1978 Associate Professor Gerontology and Biological Sciences, University of Southern California
1978 - present Professor Gerontology and Biological Sciences, University of Southern California
1984 - present ARCO Professor in Gerontology, University of Southern California.
1985 - 2005 Founding Director, Alzheimer Disease Research Center (NIA); Assoc Dir, 2005-present.

Honors

1985 RW Kleemeier Award of the Gerontological Society of America
1985 Brookdale Award for Contributions to Gerontology
1986 Allied-Signal Incorporated Award for Achievement in Biomedical Research on Aging
1987 Research Award of the American Aging Association
1987 - 1990 Member, National Advisory Council on Aging (NIA)
1988 - 1995 NIH LEAD Award in Alzheimer disease
1994 Sandoz Prize, Premier Award of the International Association of Gerontology

1995 Ipsen Foundation Prize for Research on Longevity (Paris)
1996 American Federation of Aging Research, Irving Wright Award
2013 Vincent Cristofalo Lecturership on Aging, Wistar Institute
2014 Tulane University distinguished Lecturer in Gerontology
2015 UCSF-Gladstone Inst, Grand Rounds speaker, Memory and Aging Clinic.

C. Contributions to Science

Neurobiology of aging Since 1965, I have worked on biological mechanisms in aging using a systems approach to cell and organ interactions. My thesis work was the first analysis of gene expression in relationship to neuroendocrine changes with aging (#1). I pioneered concepts that brain neurotransmitter functions and neuroendocrine functions change across the lifespan and that clinical phases of aging changes are outcomes of life-long aging processes. My initial independent research focused on monoamine neurotransmitters in rodent models, which showed slowed turnover in aging mice (#2). Human brain samples from the first brain bank for normal brain aging (with Bengt Winblad, #3) revealed that healthy middle-aged humans and rodent share progressive slow declines of dopamine receptors (#4). Because aging rodents are free of atherosclerosis, our findings of receptor loss during middle-age showed the first ischemia-independent processes of neuron aging. The same decline of 5% per decade after age 40 was later shown for cortical synapse, grey matter atrophy, and cognitive processing speed.

1. **Error! Hyperlink reference not valid. CE**, Foster JR, Mirsky AE (1969) Aging and the regulation of cell activities during exposure to cold. J Gen Physiol 54:690-712. PMID 4391049
2. **Finch CE** (1973) Catecholamine metabolism in the brains of ageing male mice. Brain Res 52:261-276. PMID: 4700706
3. Severson JA, Marcusson J, Winbald B, **Finch CE** (1982) Age-correlated loss of dopaminergic binding sites in human basal ganglia. J Neurochem 39:1623-1631. PMID: 7142992
4. Morgan DG, May PC, **Finch CE** (1987) Dopamine and serotonin systems in human and rodent brain: Effects of age and degenerative disease. J Am Ger Soc 35:334-345. PMID: 3600950

Female reproductive aging We innovated a systems approach to ovary-brain interactions during middle-age that cause the rodent hypothalamus to become desensitized to estrogen. Hypothalamic interactions with the aging ovary were analyzed by heterochronic ovarian transplantation between different ages and by long-term ovariectomy, which showed that the rodent hypothalamus is vulnerable to cumulative effects of estrogen (#1). Despite complete impairment of the estrogen-induced pre-ovulatory LH surge, there was no change in the number of hypothalamic LHRH neurons (#2). This was the first example of a major functional brain aging change in the absence neuron loss. Moreover, hypothalamic astrogliosis is driven by estrogen exposure. This finding led to recent studies on steroidal regulation of GFAP in relation to neurotrophic activity (#3) in the mouse peri-menopause, when the transition to irregular cycling increased GFAP transcription with decreased neurotrophic activity. Moreover, the onset of irregular cycling impairs hippocampal long-term-potentiation with a shift in gene expression different from regular cyclers of the same age (#4). This systems approach to ovary-brain interactions documented the plasticity of aging processes down to the level of gene expression.

1. Felicio LS, Nelson JF, Gosden RG, **Finch CE** (1983) Restoration of ovulatory cycles by young ovarian grafts in aging mice: Potentiation by long-term ovariectomy decreases with age. Proc Nat Acad Sci 80:6076-6080. PMID 6850036
2. Hoffman GE, **Finch CE** (1986) LHRH neurons in the female C57BL/6J mouse brain during reproductive aging: No loss up to middle-age. Neurobiol Aging 7:45-48. PMID: 3513038
3. Arimoto JM, Wong A, Rozovsky I, Lin SW, Morgan TE, **Finch CE** (2013) Age increase of estrogen receptor alpha (Erα) in cortical astrocytes impairs neurotrophic support in male and female rats. Endocrinology, 154: 2101–2113. PMID: 23515288 PMCID 3740484486.
4. Yin F, Yao J, Sancheti H, Feng T, Melcangi RC, Morgan TE, **Finch CE**, Pike CJ, Mack WJ, Cadenas E, Brinton RD. 2015. The perimenopausal aging transition in the female rat brain: decline in Bioenergetics systems and synaptic plasticity. Neurobiol Aging. 36: 2282-2295 PMID:25921624; PMCID: PMC4416218.

ApoE Genes with altered expression during Alzheimer disease in human and rodent models included apoE (#1) and several complement factors. Soon, ApoE4 was recognized as the first AD risk factor. In 1999, the USC Alzheimer Center in collaboration with U Washington and McGill showed further that women ApoE4

carriers had the highest risk (#2). Sex-apoE4 interactions include cerebral microbleeds, which show a male-bias, opposite to the higher amyloid load in women and mice carrying apoE4 (#3). ApoE continues to surprise: it has cognitive benefits in the Tsimane, an indigenous Amazonian tribe living under poor hygiene (#4). This finding is consistent with its evolutionary history as the ancestral allele, with pleiotropies that are not advantageous under conditions of low infection and excess nutrients.

1. Poirier J, Hess M, May PC, **Finch CE**. (1991) Astrocytic apolipoprotein E mRNA and GFAP mRNA in hippocampus after entorhinal cortex lesioning. Brain Res Mol Brain Res. 11:97-106. PubMed PMID: 1661818.
2. Bretsky PM, Buckwalter JG, Seeman TE, Miller CA, Poirier J, Schellenberg GD, **Finch CE**, Henderson VW. (1999) Evidence for an interaction between apolipoprotein E genotype, gender, and Alzheimer disease. Alzheimer Dis Assoc Disord. 13:216-21. PubMed PMID: 10609670.
3. Cacciottolo M, Christensen A, Pike CJ, Sullivan PM, Morgan TE, **Finch CE**. 2016 The APOE4 allele shows opposite sex bias in microbleeds and Alzheimer's Disease of humans and mice. Neurobiol Aging, 37:47-57. PMCID 4687024.
4. Trumble, BC, Stieglitz J, Blackwell AD, Allayee H, Beheim B, **Finch CE**, Gurven M, Kaplan H. 2016. ApoE4 alleles are associated with improved cognitive function in individuals with a high parasite burden. FASEB J. 31: 1508-11515. PMID:28031319

Environmental factors in brain aging are now a major thrust. An invited review by Science on the genetics of aging (#1) showed the surprising generality, that the heritable variance of life expectancy is less than 35% in humans and in lab animals, pointing to a major influence of environmental factors. Accordingly, I developed collaborations to understand environmental factors that enabled the major increase of human life expectancy since 1800. Studies with demographer E Crimmins shows the role of infection and inflammation in the progressively improving cohort mortality across the lifespan (#2). In 2010, JC Chen and I founded the AirPollBrain multi-disciplinary program on air pollution in brain aging and dementia http://envneurosci.usc.edu/APB/). In collaboration with C Sioutas, we developed rodent models for exposure to particulate material (PM) from urban traffic, collected in suspension for re-aerosolization. Under controlled time and dose of exposure to PM, mice show selective alterations in glutamate receptors (#3). The APOE4 allele enhanced amyloid deposits in the EFAD mouse, consistent with the greater vulnerability of women to dementia in the WHIMS cohort to high levels of air particles PM2.5 (#4). Gene-environment interactions are being sought for other AD-risk factors.

1. **Finch CE**, Tanzi RE 1997 The genetics of aging. Science 278:407-411.
2. **Finch C**, Crimmins E 2004 Inflammatory exposure & historical changes in lifespans. Science 305: 1736-39.
3. Morgan TE, Davis DD, Iwata N, Tanner JM, Snyder D, Ning Z, Kam W, Hsu YT, Winkler JW, Chen JC, Petasis NA, Baudry M, Sioutas C, **Finch CE** (2011) Glutamatergic neurons in rodent models respond to nanoscale particulate urban air pollutants *in vivo* and *in vitro*. Env Health Perspect 119:1003-1009.
4. Cacciottolo M, Wang X, Driscoll I, Woodward N, Saffari A, Reyes J, Serre ML, Vizuete W, Sioutas C, Morgan TE, Gatz M, Chui HC, Shumaker SA, Resnick SM, Espeland MA, **Finch CE**, Chen JC. 2017. Particulate air pollutants, APOE alleles, and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. Transl Psych 7:e1022. doi: 10.1038/tp.2016.280. PMID: 28140404.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/caleb.finch.1/bibliography/47376704/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1. R01 AG051521 Finch (PI) 09/30-2015-09-29/2020. Amyloid and inflammation: modulation by apoE, gender, air pollution, and drugs.
2. P01 ES022845 McConnell (PI) 07/01/13-05/31/18 NIH/NIEHC & EPA Project 3 on rodent exposure to urban traffic derived air pollution particles. Pre-and postnatal exposure of mice urban traffic derived air pollution particles for effects on adult behavior and neuron structure. Role: Co-I
3. P01 AG05142-31 Chui (PI) 04/01/15-3/31/20 Alzheimer Disease Research Center (ADRC) The ADRC focuses on the molecular and cellular analysis of changes during Alzheimer's disease and on clinical methods for early detection and the identification of subtypes. Role: Associate Director

4. R21 AG050201 (Finch PI) 04/01/2016-03/30/2018). Air pollution nano-particulate matter, APP processing, and glutamate receptors.
5. R01 AG054442 (Kaplan H, PI)(04/2017-03/2022). Brain atrophy, cognitive impairment and Alzheimer's in a low CVD-risk population. Role, Finch, Co-I.
6. PD160021P1 (Brundin P, Finch CE, Chen H, coPIs)(10/01/2017-09/30/2021) Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System.
7. PO1 AG026572, Brinton R (PI) 06/01/2016 - 05/31/2021 Perimenopause in Brain Aging and Alzheimer’s Disease. Project 2. Role: Co-I

Pending:
1. P01 AG055367-01A1, re-submitted 05/25/2017: Finch, coPI: Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms.

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Chen, Jiu-Chiuan

eRA COMMONS USER NAME (agency login): jc_chen

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Taipei Medical University, Taipei, Taiwan	MD	06/1992	Medicine
Harvard School of Public Health, Boston, MA	MPH	06/1998	Environmental and Occupational Health
Harvard School of Public Health, Boston, MA	ScD	06/2002	Environmental Health Sciences

A. Personal Statement

The long-term goal of this program is to better define the individual risk, heterogeneity, and biological basis of Alzheimer's disease (AD) associated with exposure to ambient air pollution in aging populations. Dr. Finch and I will be the MPI of this new P01 application. I will also lead the Project-1, which is designed to investigate the contribution of traffic-related air pollution (TRAP) to the risk, susceptibility and mechanisms of AD and related dementias (ADRD) in a nationwide cohort of post-menopausal and older women from the Women's Health Initiative (WHI) Memory Studies (WHIMS). Working with Dr. Sioutas who directs the Environmental Exposure and Neurotoxicology Core (Core C), I will be coordinating the effort to support the study design and conduct of large-scale air pollution/spatial epidemiologic studies on geographically-diverse cohorts; harmonize the spatiotemporal air pollution modeling approaches across two population studies (WHIMS; Vietnam Era Twin Study of Aging [VETSA]); and contribute my knowledge/expertise in studying air pollution-neuroepidemiology of brain aging. The collaborative infrastructure of proposed P01 is the outgrowth of the AirPollBrain (APB) Network (PIs: Finch & Chen), a research development program funded by USC since 2010. The APB allows us to develop collaborative team projects across USC. For this application, we have assembled faculty investigators and scientists with complementary expertise in neurobiology of AD (Finch; Pike; Morgan), population & clinical neuroimaging (Thompson; Braskie; Liu), mouse brain imaging (Zlokovic; Thompson), neuroinformatics (Thompson; Braskie) and high-dimensional data analyses (Thompson; Millstein); brain vascular biology (Zlokovic; Mack), clinical neurology (Liu) and neurosurgery (Mack), cognitive neurosciences and neuropsychology (Gatz; Nation; Petkus), latent structure modeling (Petkus), epidemiology of AD (Gatz; Chen), air pollution/environmental-neuroepidemiology (Chen), inhalation exposure assessment and neurotoxicology (Sioutas; Forman). Over the last 2-3 years, we have been extending this "team science" approach to collaborative research partnership with scientists outside USC. For instance, through the NIEHS inter-center collaborative initiative, I developed the MPI R01ES025888 with Dr. Joel Kaufman who leads the MESA-Air (Multi-Ethnic Study of Atherosclerosis and Air Pollution) with state-of-the-art spatiotemporal TRAP modeling tools not available to APB before. Through the NIH-supported IGEMS consortium, Dr. Gatz (APB executive committee member) and I forged the collaboration of APB and VETSA (Project-2) investigators. My leadership in Project-1 builds on my long-standing involvement in WHI research since 2004-9 when I was on the faculty of Department of Epidemiology of the University of North Carolina at Chapel Hill (UNC-CH). By collaborating with senior WHI investigators at UNC and other institutes, I initiated/completed the effort to promote WHI/WHIMS as a unique resource for population environmental neurosciences to address pressing issues and health concerns (e.g., sleep-brain health) most relevant to post-menopausal and older women. As the PI of WHI's ancillary studies#226 & 252, I led the collaborative effort between UNC Depts of Epidemiology and Environ. Sci. & Eng. to enhance the environmental data resources and modeling tools applicable to nationwide cohorts including the WHIMS Suite of Studies. These efforts led to the previous recognition by the National Sleep Foundation, NHLBI Innovative Research Grant Program, the receiving of UNC Junior Faculty Development Award, and the Rosenblith Award from the Health Effects Institute.

In summary, I have demonstrated a track-record of successful and productive multidisciplinary projects as well as the leadership in the emerging field of environmental neurosciences of brain aging in populations. These

experiences make me fully qualified to be the Co-PI of this P01 program, lead the Project-1 in WHIMS, and provide the Core C1 supporting function.

B. Positions and Honors

Positions and Employment

1996 - 1998	Director & Chief Physician, Chia-Yi Christian Hospital, Department of Community Health, Attending Physician, Department of Internal Medicine Chia-Yi City, Taiwan
1999 - 2002	Post-MD Research Fellow, Harvard School of Public Health, Occupational Health Program, Boston, MA
2001 - 2004	Teaching Fellow, *Occupational and Environmental Medicine*/*Environmental and Occupational Medicine and Epidemiology*, Harvard School of Public Health, Department of Environmental Health, Boston, MA
2002 - 2003	Post-Doctoral Research Fellow , Harvard School of Public Health, Department of Environmental Health, Boston, MA
2003 - 2008	Research Associate, Department of Environmental Health, Harvard School of Public Health, Boston, MA
2004 - 2009	Assistant Professor, Department of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, NC
2004 - 2009	Member, Center for Environmental Health and Susceptibility, University of North Carolina, Chapel Hill, NC
2006 - 2007	Special Volunteer Scientist, Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC
2009 -	Associate Professor, Department of Preventive Medicine, Keck School of Medicine, Los Angeles, CA
2011 -	Investigator, USC Memory and Aging Center/Alzheimer's Disease Research Center, Department of Neurology, Los Angeles, CA
2014 -	Member, USC/Nathan Shock Centers of Excellence in the Basic Biology of Aging , Davis School of Gerontology, Los Angeles, CA

Other Experience and Professional Memberships

1995-	Society of Internal Medicine, Taiwan (Board Certified)
1996-	Environmental and Occupational Medicine Association, Taiwan (Subspecialty Board Certified)
2003-	Member, Society of Epidemiologic Research
2003-	Member, International Society of Environmental Epidemiology
2013-	Members, International Society to Advance Alzheimer's Research and Treatment (*ISTAART*)
2006-2007	Advisory Committee, Environmental Epidemiology Training Program, UNC-Chapel Hill
2008	Panel Epidemiologist, Planning Review for EPA National Ambient Air Quality CO Standards
2009-	*Ad hoc* Review Panel, Alzheimer's Association Research Grant Program
2009	International Review Panel, Italian Ministry of Health, Department of Innovation, Office of Scientific Research and Technology
2010	Faculty Judge, Society for Epidemiologic Research Students Dissertation Workshop, Seattle, WA, June 23-26, 1010
2010	International Review Panel, Research Programme Research Grant, Environmental Exposures & Health Initiatives (EEHI), MRC/NERC/DH/ESRC/Defra, UK
2011-2013	External Advisory Board, NIEHS-UCLA Center for Gene-Environment Studies in Parkinson's Disease, UCLA David Geffen School of Medicine, Los Angeles, CA
2011	NIH Study Section on Neurological, Aging, and Musculoskeletal Epidemiology (NAME), *Temporary Member*, June 2-3, 2011
2011	NIH Center for Scientific Review, *Special Emphasis Panel,* Member Conflict: Epidemiology ZRG1 PSE-K (04), November 17-18, 2011
2012	NIH Center for Scientific Review, *Special Emphasis Panel,* Member Conflict: Epidemiology ZRG1 PSE-M (02), June 25-26, 2012
2012-2013	IDM (Institute of Developing Mind) Director Search Committee, Children's Hospital Los Angeles
2013	NIH Center for Scientific Review, *Special Emphasis Panel,* Member Conflict: Epidemiology ZRG1 PSE-R (04), March 4-5, 2013
2014-	Associate Editor, *Stoch Env Re Risk A (SERRA)*

2014-5 NIH Study Section on Neurological, Aging, and Musculoskeletal Epidemiology (NAME), February 13-14, 2014; October 9-10, 2014; June 11-12, 2015
2016 NIH Neurotoxicology and Alcohol (NAL) Study Section, June 13, 2016
2017 NIH Biomedical Computing and Healthcare Informatics (BCHI) Study Section, June 22-23, 2017

Honors

1998 AT&T Oversea Study Award, Taiwan Branch, AT&T
1998 Taiwan Harvard Alumni Association Scholarship Award, Harvard University Alumni Association
1999-2011 Harvard-Liberty Mutual Program Fellowship, Harvard School of Public Health, Boston, MA
2002 Fang Ching Sun Memorial Award Finalist, Harvard School of Public Health
2007 Developmental Research Award, NHLBI Innovative Research Grant Program
2008 Junior Faculty Development Award, UNC Chapel Hill, NC
2008 Young Investigators Award Finalist, National Sleep Foundation & Sleep Research Society
2008 Walter A. Rosenblith New Investigator Award, Health Effects Institute
2009 Recommendation for Promotion to Tenured Associate Professor, Department of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, NC (*Declined*)
2011 Butler-Williams Scholar, National Institute on Aging

C. Contribution to Science

I am a physician-epidemiologist with formal training in Internal Medicine, Environmental and Occupational Medicine, Environmental Health Sciences (EHS), and Epidemiology (Environmental; Occupational; Clinical). My research endeavors cover several key areas of EHS. One defining features of these studies is the "team science" approach. While often requiring the effective coordination of multidisciplinary team/multi-site studies, this approach enables the formulation of new EHS questions by integrating state-of-the knowledge and research tools in toxicology/disease pathogenesis with novel theories from other disciplines. Employed to studying population neurosciences, the team science approach also fosters the development/validation/ application of sophisticated quantitative methods to address statistical issues involving complex study design and multi-dimensional exposure/outcome data.

Exposure Assessment Methods and Study Design for Large Populations

My initial research training was in occupational epidemiology methods for exposure assessment in studying work-related musculoskeletal disorders. My doctoral study demonstrated an efficient study design approach to assessing occupational exposure by using validation data to develop statistical instruments for exposures that are hardly or not directly measurable in large cohorts. This work was the first introducing the efficient study design to assessing low-frequency whole-body vibration (WBV, a form of oscillating mechanical energy transmitted to human body through supporting surfaces) exposure in professional drivers. This approach has been adapted in subsequent occupational WBV studies conducted in Canada, Japan, Europe, and China to better predict occupational exposures and quantify WBV dosages. My doctoral study was a product of multidisciplinary team science, for which I brought together mechanical engineers and biomechanics ergonomists working with clinicians, health psychologists, musculoskeletal epidemiologists and biostatisticians to launch the largest occupational cohort of professional taxi drivers in Taipei City, Taiwan. While at UNC Dept. of Epidemiology, my interest in occupational/environmental epidemiologic methodology evolved into the development/validation of environmental exposure models applicable to nationwide large population-based cohorts. I worked closely with geostatisticians at the UNC Bayesian Maximum Entropy (BME) Modeling Lab to address two fundamental issues (temporal upscaling in multi-scale estimation; non-stationarity of spatial processes) in air pollution exposure modeling for large-scale cohorts.

a. **Chen JC**, Chang WR, Shih TS, Chen CJ, Chang WP, Dennerlein JT, Ryan LM, Christiani DC. Using exposure prediction rules for exposure assessment: an example on whole-body vibration in taxi drivers. Epidemiology. 2004;15(3):293-9. PMID: 15097009
b. **Chen JC**, Dennerlein JT, Shih TS, Chen CJ, Cheng Y, Chang WP, Ryan LM, Christiani DC. Knee pain and driving duration: a secondary analysis of the Taxi Drivers' Health Study. Am J Public Health. 2004;94(4):575-81. PMCID: PMC1448301
c. Yu HL, **Chen JC**, Christakos G, Jerrett M. BME estimation of residential exposure to ambient PM10 and ozone at multiple time scales. Environ Health Perspect. 2009;117(4):537-44. doi: 10.1289/ehp.0800089. PMCID: PMC2679596
d. Akita Y, **Chen JC**, Serre ML. The moving-window Bayesian maximum entropy framework: estimation of $PM_{2.5}$ yearly average concentration across the contiguous United States. J Expo Sci Environ Epidemiol. 2012;22(5):496-501. doi:10.1038/jes.2012.57. PMCID: PMC3601029

Mechanistic Mediators of and Individual Susceptibility to Cardiovascular Effects of Airborne Particles

My pursuit of air pollution health effects research started during my post-doctoral work with faculty at the Particle Research Core of the Harvard-NIEHS Center of Environmental Health and Harvard-EPA Particulate Matter (PM) Center. The initial focus was on studying the mechanistic mediators/identifying the determinants of individual susceptibility to cardiovascular effects of airborne particles. To this end, I contributed to integrating the knowledge of particle toxicity in animals with the analyses of gene expression data from human panel studies to better understand the mechanistic pathways involved in cardiophysiological and inflammatory responses. My post-doctoral work was the first to demonstrate the utility of the Framingham Cardiovascular Disease (CVD) Risk Score (a widely used clinical tool for CVD risk stratification and clinical management) to better characterize the individual susceptibility to air pollution-related cardiac effects. This useful strategy was recognized in the American Heart Association's Scientific Statement on Air Pollution and CVD. My subsequent work demonstrated the first link between systemic inflammation with *long-term* exposure to particulate air pollutants, and identified obesity and metabolic syndrome as new indicators of susceptibility of PM exposures.

a. **Chen JC**, Stone PH, Verrier RL, Nearing BD, MacCallum G, Kim JY, Herrick RF, You J, Zhou H, Christiani DC. Personal coronary risk profiles modify autonomic nervous system responses to air pollution. J Occup Environ Med. 2006; 48(11):1133-42. PMID: 17099449
b. Wang Z, Neuburg D, Li C, Su L, Kim JY, **Chen JC**, Christiani DC. Global gene expression profiling in whole-blood samples from individuals exposed to metal fumes. Environ Health Perspect. 2005;113(2):233-41. PMCID: PMC1277870
c. **Chen JC**, Cavallari JM, Stone PH, Christiani DC. Obesity is a modifier of autonomic cardiac responses to fine metal particulates. Environ Health Perspect. 2007;115(7):1002-6. PMCID: PMC1913600
d. **Chen JC**, Schwartz J. Metabolic syndrome and inflammatory responses to long-term particulate air pollutants. Environ Health Perspect. 2008;116(5):612-7. doi: 10.1289/ehp.10565. PMCID: PMC2367655

Environmental Health and Aging

While at UNC-CH, I expanded my research to epidemiology of aging, primarily focused on examining whether and how environmental stressors in workplaces and communities affect development and progression of chronic diseases (e.g., CVD, osteoarthritis, sleep disorders) as well as the quality of life as people age. One example was my contribution to a new line of collaborative research with the Thurston Arthritis Research Center to investigate how social factors (e.g., workplace policies; occupational physical activities; lead exposure) determine health disparities in osteoarthritis. After I left UNC, several clinical fellows and junior investigators continued on this important albeit understudied area of environmental health and aging.

a. **Chen JC**, Linnan L, Callahan LF, Yelin EH, Renner JB. Workplace policies and prevalence of knee osteoarthritis: the Johnston County Osteoarthritis Project. Occup Environ Med. 2007;64(12):798-805. PMCID: PMC2095393
b. Allen KD, **Chen JC**, Callahan LF, Golightly YM, Helmick CG, Renner JB, Jordan JM. Associations of occupational tasks with knee and hip osteoarthritis: the Johnston County Osteoarthritis Project. J Rheumatol. 2010;37(4):842-50. doi: 10.3899/jrheum.090302. PMCID: PMC4051278
c. Allen KD, **Chen JC**, Callahan LF, Golightly YM, Helmick CG, Renner JB, Schwartz TA, Jordan JM. Racial differences in knee osteoarthritis pain: potential contribution of occupational and household tasks. J Rheumatol. 2012; 39(2):337-44. doi: 10.3899/jrheum.110040. PMCID: PMC4031236
d. Nelson AE, Shi XA, Schwartz TA, **Chen JC**, Renner JB, Caldwell KL, Helmick CG, Jordan JM. Whole blood lead levels are associated with radiographic and symptomatic knee osteoarthritis: a cross-sectional analysis in the Johnston County Osteoarthritis Project. Arthritis Res Ther. 2011;13(2):R37. doi:10.1186/ar3270. PMCID: PMC3132016

Ambient Air Pollution, Neurodevelopment, and Brain Aging

Since 2009, my primary research effort has been devoted to investigating whether and how exposures to neurotoxic air pollutants affect the neurobehavioral development and cognitive functions across the life span. These studies involve a wide range of unique populations, including children, community-dwelling residents, nationwide survey samples, and large population-based cohorts. This novel line of research in population environmental neurosciences began with the first epidemiologic evidence supporting neurotoxic effect of ambient ozone exposure in adults and then making the first effort to tackle neurotoxic mixture with correlated air pollution exposure contexts for their impact on autism risk. In the last few years, I helped create a vibrant program of research, including mechanistic studies with inhaled particle exposures in animal models, which has grown into several funded projects (R21AG040683; R21AG040753; RF1AG051521). The first NIH-funded R01 (AG033078; PI: Chen) on air pollution and cognitive aging had produced compelling data (including the new discovery of potential white matter loss associated with $PM_{2.5}$) to redefine air pollution neurotoxicology concerning the relative importance of neurovascular damage and structural neurotoxicity in late life.

a. **Chen JC**, Schwartz J. Neurobehavioral effects of ambient air pollution on cognitive performance in US adults. Neurotoxicology. 2009; 30(2):231-9.doi:10.1016/j.neuro.2008.12.011. PMID: 19150462

b. **Chen JC**, Wang X, Wellenius GA, Serre ML, Driscoll I, Casanova R, McArdle JJ, Manson JE, Chui HC, Espeland MA. Ambient air pollution and neurotoxicity on brain structure: Evidence from women's health initiative memory study. Ann Neurol. 2015;78(3):466-76. doi: 10.1002/ana.24460. PMCID: PMC4546504
c. Casanova R, Wang X, Reyes J, Akita Y, Serre ML, Vizuete W, Chui HC, Driscoll I, Resnick SM, Espeland MA, **Chen JC**. A Voxel-Based Morphometry Study Reveals Local Brain Structural Alterations Associated with Ambient Fine Particles in Older Women. *Front Hum Neurosci.* 2016;10:495. PMC:5061768
d. Cacciottolo M, Wang X, Driscoll I, Woodward N, Saffari A, Reyes J, Serre ML, Vizuete W, Sioutas C, Morgan TE, Gatz M, Chui HC, Shumaker SA, Resnick SM, Espeland MA, Finch CE, **Chen JC**. Particulate air pollutants, APOE alleles and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. *Transl Psychiatry.* 2017;7(1):e1022. PMC:5299391

One of the AirPollBrain (http://envneurosci.usc.edu/APB/) Network's important missions is to develop an interdisciplinary educational and training program in Environmental Neurosciences. Over the last few years, the AirPollBrain Network has created a unique intellectual environment, interdisciplinary research infrastructure, educational resources and mentoring capacities that have helped several junior faculty and trainees successfully compete for NIH funding to support their great endeavors to understand "*Neighborhood Context, Social Relationships, and Health: Examining the Pathways*" in elderly (R00AG039528), characterize "*Neurotoxicity of Airborne Particles: Roles of Chronic Cerebral Hypoperfusion*" (R01ES024936; ONES Award 2015), conduct "*Prospective Evaluation of Air Pollution, Cognition, and Autism from Birth onward*" (R01ES023780), examine the link between "*Early-Life Exposure to Urban Air Pollution and Externalizing Behaviors in Youth*" (F31ES025080), and study the associations of *"Air pollution, gestational diabetes, and autism spectrum disorder"* (F31 ES027340).

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/jiu-chiuan.chen.1/bibliography/40329132/public/?sort=date&direction= descending

D. Research Support

Ongoing Research Support

R01ES025888 Chen & Kaufman (MPI) 09/30/2016 – 07/31/2021
"Environmental Determinants of Pathological Brain Aging in the WHI Memory Studies"
Role: Principal Investigator
This project supports the further development of advanced nationwide spatiotemporal air pollution modeling and applies the resulting tools to substantiate the evidence of pathological brain aging associated with exposure to *regional* ambient air pollutants from mid- to late life.

RF1AG054068 Chen (PI) 09/01/2016 – 06/30/2021
"*Alzheimer's Disease & Related Dementias: Geography, Environments & Mechanisms*"
Role: Principal Investigator
The long-term goal of this project is to better understand the geographic disparities in Alzheimer's disease and related dementias (ADRD) by studying the neuropsychological trajectories and clinical progression to increased ADRD risk as related to geographic indicators, identifying the contributing environmental factors and examining their interactions, and elucidating the possible mechanistic mediators.

R21AG051113 Casanova & Chen (MPI) 09/01/2015 – 04/30/2018 (NCE)
"*Regional Neurotoxicity & Early Biomarkers of Air Pollution Effects on Brain Aging*"
Role: Principal Investigator
This study employs high-dimensional voxel-wide analyses to examine the brain region-specific effects of ambient air pollution on brain volumes and identify their spatial patterns to predict AD risk.

Completed Research Support

R01AG033078 Chen (PI) 03/15/2011 – 02/28/2015
"Environmental Determinants of Cognitive Aging in the WHI Memory Study"
This cohort study aims to investigate *regional* ambient air pollutants as novel environmental determinants of cognitive aging to improve our understanding of the neurocognitive effects of environmental factors in later life.

R21ES022369 Chen & Baker (MPI) 08/02/2013 – 07/31/2015
"*Neurodevelopment in Urban Environments: Role of Exposure to Ambient Air Pollution*"
This study aims to better understand how urban environmental factors, especially neurotoxic air pollutants during vulnerable time periods, contribute to neuropsychological development in adolescents/young adults.

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Braskie, Meredith Nicole

eRA COMMONS USER NAME (credential, e.g., agency login): mbraskie

POSITION TITLE: Assistant Professor of Research

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
College of William and Mary, Williamsburg, VA	B.B.A.	05/1994	Accounting
University of California, Los Angeles	Ph.D.	12/2006	Neuroscience
University of California, Berkeley	Postdoc	01/2009	Neuroscience
University of California, Los Angeles	Postdoc	08/2011	Neuroscience

A. Personal Statement

My research focuses mainly on neuroimaging Alzheimer's disease (AD) risk and cognitive aging, with an emphasis on preclinical AD and early mild cognitive impairment. In particular, I evaluate how genetic and environmental risk factors for AD relate to brain structure, function, and connectivity throughout adulthood. I also seek to understand the biological mechanisms and signaling pathways that may contribute to or protect against AD-related changes, using relevant blood and cerebrospinal fluid measures and multimodal imaging. My research projects and publications to date have all have involved various modes of brain imaging, including structural MRI (using both tensor based morphometry and FreeSurfer analyses), fMRI, diffusion tensor imaging, and white matter hyperintensity assessments on FLAIR images. I am experienced in performing positron emission tomography (PET) analyses and have worked on/published studies using PET with various ligands: FDDNP (amyloid plaques and neurofibrillary tangles), FDG (glucose metabolism), AV45 (amyloid), and FMT (dopamine synthesis capacity). My publication record includes 37 co-authored peer-reviewed journal articles (as lead author for 17), as well as 1 submitted manuscript. Four of my first authored papers were published in **The Journal of Neuroscience**, including one about the relationship between the AD risk gene *CLU* and white matter integrity that received worldwide recognition. As a post doc, I proposed and wrote the majority of an R01 examining the genetics of Alzheimer's disease as relates to functional and structural brain connectivity (PI Thompson). I later won a small local grant and directed the analyses that led to a publication. These analyses also provided preliminary data for another R01, on which I am currently directing the work of a Research Assistant, a Post Doctoral Scholar, a Neuroscience PhD Student, and undergraduate volunteers. Now as junior faculty at the University of Southern California, I am the project leader for Project 2 of our Alzheimer's Disease Research Center grant (PI Chui) and am ensuring that the goals of that project are being met. I am committed to Alzheimer's disease research personally and professionally and have extensive experience with a variety of neuroimaging modalities and AD research, as is evidenced by my productive publication history.

B. Positions and Honors

Positions and Employment

1994 - 1998 Certified Public Accountant, Arthur Anderson LLP
1998 - 2001 Certified Public Accountant, Joseph D. Kalicka & Co.
2000 - 2000 Research Assistant, independent study, U. Massachusetts, Amherst
1999 - 2001 Research Assistant and volunteer, U. Massachusetts, Amherst
2002 - 2002 Graduate Student Researcher (lab rotation), U. California, Los Angeles
2002 - 2006 Graduate Student Researcher, U. California, Los Angeles
2006 - 2009 Postdoctoral fellow, U. California, Berkeley

2009 - 2011 Postdoctoral fellow, U. California, Los Angeles
2011 - 2013 Assistant researcher, U California, Los Angeles
2013 - present Assistant Professor of Research, University of Southern California

Honors

2011 Turken Award for Alzheimer's Disease Research
2009 - 2010 Ruth L Kirschstein National Research Service Award (NRSA)
T32 NS048004, PI - Dr. Nelson Freimer, Neurobehavioral Genetics – UCLA
2004 - 2006 Achievement Rewards for College Scientists (ARCS) fellowship (Underwritten in part by John Douglas French Alzheimer's Foundation and the Erteszek Foundation through September 2006)
2003 - 2006 Ruth L Kirschstein National Research Service Award (NRSA), F31 NS45425, PI - Meredith Braskie
2003 Travel award, Organization for Human Brain Mapping annual meeting

C. Contribution to Science

1. Genetic associations with brain measures

Understanding how genetic variants affect brain structure and connectivity is an important first step in designing treatments for the disease that are affected by the systems those genes influence. In my graduate work, I was involved in a study by Burggren et al.,that was the first to show how the Alzheimer's disease (AD) genetic risk variant *APOE*4 related to the thickness of hippocampal area subregions. My role in that highly cited study was to help with the scanning, hippocampal unfolding, and data interpretation, and to edit the manuscript.

In my postdoctoral work, I became a fellow supported by an institutional NRSA grant at UCLA in Neurobehavioral Genetics (PI Nelson Freimer) and continued to study genetic effects on the brain, this time related to structural connectivity. We were the first to publish how the newly confirmed genetic risk allele C of the clusterin (*CLU*) gene variant rs11136000, related to brain measures in living humans. We found that each C allele of the *CLU* variant was associated with lower diffusion tensor imaging fractional anisotropy (DTI FA), a widely accepted measure of white matter integrity, broadly throughout the brain, including in regions having preferential degeneration in AD. Clusterin is implicated in β-amyloid transport. However, our 398 young adult subjects were likely too young to have amyloid deposits in their brains. Our results suggest another way by which the *CLU* C allele may make the brain vulnerable to AD-related cognitive decline, decades before an AD diagnosis would be expected. Our findings were reported by newspapers in at least 15 countries, widely on the internet, and by KCBS radio news in San Francisco. **The Journal of Neuroscience** paper that resulted has been cited nearly 130 times.

In another paper published in **The Journal of Neuroscience**, we found that a genetic variant in the *NTRK1* (also known as *TRKA*) gene was associated with white matter integrity in a large sample of healthy young adults. NTRK1 is a high affinity receptor for nerve growth factor (NGF), which has been implicated in normal brain development, AD, schizophrenia, and other neurological disorders. My role in these two studies was to conceive the research idea, perform statistical analyses, interpret the results, write the initial draft of the paper, and to implement all subsequent revisions.

I was also lead author on two fMRI studies of people from families that carry rare genetic mutations that confer AD in middle age in an autosomal dominant manner. In one of these, published in **Neurobiology of Aging**, fMRI activity during a novelty encoding task increased the closer AD mutation carriers got to the expected age of disease onset, suggesting that the increase in activity related to disease processes rather than to developmental differences related to the gene mutation. In a follow-up paper, published in **Human Brain Mapping**, preclinical mutation carriers showed greater fMRI activation during memory retrieval, and this activation was greatest in those who performed most poorly on the task. This suggests that pathological processes rather than benign compensation are responsible for the increased activity that occurs prior to clinically relevant cognitive decline. In these studies, I planned and executed the analyses, interpreted the results, and wrote and revised the manuscript.

a. Burggren AC, Zeineh MM, Ekstrom AD, **Braskie MN**, Thompson PM, Small GW & Bookheimer SY 2008 Reduced cortical thickness in hippocampal sub-regions among cognitively normal apolipoprotein E e4 carriers. Neuroimage, 41(4):1177-83. PMCID: PMC2601686

b. **Braskie MN**, Jahanshad N, Stein JL, Barysheva M, McMahon KL, de Zubicaray GI, Martin NG, Wright MJ, Ringman JM, Toga AW & Thompson PM 2011 Common Alzheimer's disease risk variant within the CLU gene affects white matter microstructure in young adults.. The Journal of Neuroscience, 31(18):6764-70. PMCID: PMC3176803

c. **Braskie MN**, Medina LD, Rodriguez-Agudelo Y, Geschwind DH, Macias-Islas MA, Cummings JL, Bookheimer SY & Ringman JM 2010 Increased fMRI signal with age in familial Alzheimer's disease mutation carriers. Neurobiology of Aging, 33(2):424.e11-21. PMCID: PMC3097258

d. **Braskie MN**, Jahanshad N, Stein JL, Barysheva M, Johnson K, McMahon KL, de Zubicaray GI, Martin NG, Wright MJ, Ringman JM, Toga AW & Thompson PM 2012 Relationship of a variant in the NTRK1 gene to white matter microstructure in young adults. The Journal of Neuroscience, 32(17):5964-72. PMCID: PMC3393752

2. **Amyloid PET in cognitively intact older adults**

I was the lead author on the first paper published online to demonstrate that amyloid PET signal was associated with cognition in cognitively intact older adults. This paper, published in **Neurobiology of Aging**, has been cited >100 times. We used the (18F)FDDNP-PET ligand, which labels amyloid plaques and, to a lesser extent, neurofibrillary tangles. Using MRI-derived cortical surface models, aligned across subjects with sulcal matching, we showed that in AD patients, those with MCI, and healthy older controls, AD pathology was significantly correlated with cognitive performance in a pattern that mirrored a classic pattern of pathological progression. When only cognitively intact older controls were considered, FDDNP signal in small regions of the frontal and parietal cortex was still significantly higher in those who had worse cognition. My role in this project was to conceive of the idea, perform MRI scanning and analysis, interpret the data, and write and revise the manuscript. A graduate individual NRSA grant on which I was the principal investigator funded this work. In Scott et al., 2016, we found in cognitively intact older adults, that greater amyloid burden at baseline was associated with greater WMH accrual over a two-year period of time. This association was especially pronounced in those with hypertension, but the relationship remained significant even after controlling for age and hypertension. Our results suggest that the contribution of possible amyloid deposition should be considered as an independent factor when evaluating white matter lesion etiology.

a. **Braskie MN**, Klunder AD, Hayashi KM, Protas H, Kepe V, Miller KJ, Huang S-C, Barrio JR, Ercoli LM, Siddarth P, Satyamurthy N, Liu J, Toga AW, Bookheimer SY, Small GW & Thompson PM 2010 Plaque and tangle imaging and cognition in normal aging and Alzheimer's disease. Neurobiology of Aging, 31(10):1669-78. PMCID: PMC2891885

b. Scott JA. **Braskie MN**, Tosun D, Maillard P, Thompson P, Weiner MW, DeCarli C, Carmichael OT. Cerebral Amyloid Is Associated With Greater White Matter Hyperintensity Accrual in Cognitively Normal Elderly 2016. Neurobiology of Aging. 48:48-52. PMCID in process.

3. **Somatic health and brain aging**

One of my major interests is how general health, particularly cardiovascular and metabolic health and the immune system, affects brain aging and AD risk. We previously discovered that even within the normal range of systolic blood pressure and body mass index, higher values were associated with greater fMRI activity in regions vulnerable to AD. Cardiovascular deficits and AD frequently co-occur. Our results suggest that part of the AD-related pattern frequently seen in those with increased risk may relate to cardiovascular risk. Conversely, the pattern of brain changes associated with cardiovascular risk may create a vulnerability to cognitive deficits when AD-related pathology is present. In this study, I conceived the idea for the study, performed all analyses, interpreted the results, and wrote and revised the manuscript.

More recently, we examined the relationship of total brain volume and voxelwise brain volume (assessed using tensor-based morphology) with reported physical activity intensity and blood serum levels of TNFα, a pro-inflammatory cytokine. We found that lower physical activity intensity and higher levels of TNFα were both independently associated with smaller brains, and that higher TNFα was associated with lower voxelwise brain volume in the inferior parietal lobule. TNFα and physical activity intensity were not significantly correlated with one another. Our results suggest that the effect of physical activity on brain volume is largely independent of any effect it might have on inflammation. For this paper, I planned the study, conceived the idea for and wrote the grant that funded the study, performed all imaging analyses, interpreted the results, and wrote and revised the manuscript. In Scott et al., 2015, we found that amyloid deposition and hypertension were independently associated with white matter hyperintensity volume in older adults.

a. **Braskie MN**, Small GW & Bookheimer SY 2010 Vascular health risks and fMRI activation during a memory task in older adults. Neurobiology of Aging, 31(9):1532-42. PMCID: PMC2965069

b. **Braskie MN**, Boyle CP, Rajagopalan P, Gutman BA, Toga AW, Raji CA, Kuller LH, Becker JT, Lopez OL & Thompson PM 2014 Physical activity, TNFα, and volume of the aging brain. Neuroscience, 273:199-209. PMCID: PMC4076831

c. Scott JA, **Braskie MN**, Tosun D, Thompson PM, Weiner M, DeCarli C, Carmichael OT Cerebral Amyloid and hypertension are independently associated with white matter lesions in the elderly. Frontiers in Aging Neuroscience. PMCID: PMC4664630

4. **Dopamine synthesis capacity, early adult development, and cognitive aging**

In our **Journal of Neuroscience** paper, in which I was the lead author, we used the (PET) radiotracer 6-[(18)F]fluoro-l-m-tyrosine (FMT), a marker for activity of the dopamine-synthesizing enzyme, aromatic amino acid decarboxylase (AADC), to measure dopamine synthesis capacity in the caudate in healthy older and younger adults. We evaluated how FMT signal in the caudate related to frontal lobe function and age. Prior studies using FDOPA as a radiotracer to measure striatal dopamine synthesis had inconsistent results. However, FDOPA signal is subject to some aspects of post-release processing, which may themselves change with aging, while FMT signal is a purer measure of AADC function. Consistent with prior work in non-human primates, we found that FMT signal in the dorsal caudate increased with age, suggesting compensation for deficits elsewhere in the dopamine system. In contrast, in younger adults, FMT signal in the dorsal caudate was lower with age, possibly due to ongoing developmental in young adulthood. Younger adults who performed worse on tests of frontal lobe function showed greater FMT signal in the right dorsal caudate, independent of age effects. Together this suggests that higher striatal higher striatal FMT signal is associated with non-optimal dopamine processing.

In a follow-up study, we found older cognitively intact adults showed less fMRI deactivation in the posterior cingulate cortex and precuneus during a delayed recognition working memory task than younger adults. These regions are an integral part of the default mode network, which shows deficits in many disease and disorders, including AD. We also found that in young adult subjects, greater task-induced fMRI deactivations were associated with higher FMT signal in the caudate, and with worse memory performance. This suggests that dopamine synthesis may help modulate default mode network activity, and changes to this system may contribute to working memory deficits in older adults.

For both of these studies, I recruited and performed MRI scanning all the younger subjects in this study as well as coordinating their neuropsychological testing and PET scanning. I analyzed the PET and MRI scans included in the study and trained, supervised, and checked the analyses of the others who assisted me. I also wrote and revised the manuscripts.

a. **Braskie MN**, Wilcox CE, Landau SM, O'Neil JP, Baker S, Madison CM, Kluth JT & Jagust WJ 2008 Relationship of striatal dopamine synthesis capacity to age and cognition. Journal of Neuroscience, 28(52):14320-8. PMCID: PMC2880923

b. **Braskie MN**, Landau SM, Wilcox CE, Taylor SD, O'Neil JP, Baker S, Madison CM & Jagust WJ 2011 Correlations of striatal dopamine synthesis with default network deactivations during working memory in younger adults. Human Brain Mapping, 32(6):947-61. PMCID: PMC3176660

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1H5KN3R_gyckc/bibliography/44271641/public/?sort=date&direction=ascending

D. Research Support

USC CTSI Pilot Grant (PI Yassine) 02/01/17-01/31/18
The delivery of the essential omega-3 fatty acid DHA to the brain
We will evaluate the effect *APOE*4 status has on delivery of DHA to the brain by assessing CSF DHA levels before and after DHA supplementation. We will relate changes in CSF DHA before and after DHA supplementation to MRI measures of brain structure, function and connectivity.
Role: Co-Investigator – My team will perform the analyses of structural MRI, diffusion MRI, and resting state fMRI scans.

R56 AG054073 O'Bryant (PI) 09/01/2016 – 08/31/2017
NIH/NIA
Health Disparities in Alzheimer's Disease among Mexican Americans
We will examine neuroimaging biomarkers and blood-based biomarkers associated with MCI and AD among Mexican Americans and non-Hispanic whites. We will also examine the utility of our blood-based AD screening tool as the first-step in a multi-stage diagnostic process among Mexican Americans.
Role: Co-Investigator – Helped design the imaging portion and aided in writing the grant. I am responsible for structural MRI analyses, white matter lesion segmentations, and structural equation modeling, and drafting or editing the resulting imaging-related publications.

P50 AG005142-31 Chui (PI) 05/02/15-03/31/20
NIH/NIA
Alzheimer's Disease Research Center Project 2 – Metabolic Factors in AD
We will investigate how insulin and insulin-like growth factor, and cholesterol efflux capacity relate to AD pathology and brain structure and function in older cognitively intact adults and those with mild cognitive impairment.
Role: Project leader of Project 2 – Designed the study with input of collaborators and wrote the initial draft of the grant. I am responsible for structural and functional image analyses, statistical analyses, project oversight & administration, and drafting and/or editing all resulting publications.

R01 AG041915 Thompson (PI) 09/15/14-08/31/19
NIH/NIA
Growth factors, neuroinflammation, exercise, and brain integrity
We will investigate how three key processes (inflammation, brain growth factors, and physical activity) relate to brain structure and brain decline over a 2-year period. Physical exercise boosts the ability of growth factors to promote regeneration in the hippocampus, which is important in memory. Inflammation decreases that ability. Our work will help provide a mechanistic understanding of diseases and disorders such as Alzheimer's disease and schizophrenia.
Role: Co-Investigator – Designed the study and wrote the grant. Responsible for project oversight, performing or supervising all image analyses and statistical analyses, and drafting and/or editing all resulting publications.

R01AG040060 Thompson (PI) 09/01/11-05/31/17
NIH/NIA
Alzheimer's disease risk analyzed using population imaging genomics
We will use MRI measurements of this connectivity as an AD risk proxy to better characterize how known AD risk genes affect the brain, helping researchers to improve treatment focus. We will also use this proxy to identify new possible AD risk genes, allowing researchers to assess more homogeneous samples of people.
Role: Co-Investigator – Designed 2/3 of grant aims and wrote associated grant text. Responsible for rs-fMRI image analyses, statistical analyses related to a subset of DTI and fMRI studies, and drafting and editing a subset of publications.

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Steven Yong Cen, PhD

eRA COMMONS USER NAME (credential, e.g., agency login): stevencen

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Shanghai University of Traditional Chinese Medicine and Pharmacology	MBBS	07/1994	Medicine
Cal State University, Northridge	MS	07/1999	Exercise Physiology
University of Southern California	MS	07/2000	Biometry
University of Southern California	PhD	12/2005	Biostatistics

A. Personal Statement

The long-term goal of this program is to better define the individual risk, heterogeneity, and biological basis of Alzheimer's disease (AD) associated with exposure to ambient air pollution in aging populations. I will participate in the Project-1, which is designed to investigate the contribution of traffic-related air pollution (TRAP) to the risk, susceptibility and mechanisms of AD and related dementias (ADRD) in a nationwide cohort of post-menopausal and older women from the Women's Health Initiative (WHI) Memory Studies (WHIMS).

I have been working with Dr. JC Chen (Project-1 PI) on the NIA-funded R01 entitled "Environmental Determinants of Cognitive Aging in the WHI Memory Studies," serving as Data Core Director. I bring to the project team a track record of my prior productivity and success in the design and data analyses for multi-center projects involving complex dataset development and statistical analyses of multidimensional longitudinal research data.

I will commit to this study as the lead biostatistician. I will oversee the statistical analysis, data integration and data quality control. I have extensive research experience in database and statistics with application in imaging study and clinical trial. Currently I serve as the director of data core for Roxanna Todd Hodges Stroke Center, director of research database of Interventional Cardiology, and the in-house lead biostatistician for Dept. of Neurology and Dept. of Radiology at USC Keck School of Medicine. I am currently the biostatistician of two large NIH funded studies*: Novel Myocardial Perfusion Stress Test using Arterial Spin Labeling,* and *Human Connectomes for Low Vision, Blindness, and Sight Restoration*. I am serving as the statistical advisor as part of editorial board for BMJ-Heart. I have served as the co-director of Data Management and Analysis Center (DMAC) for two NIH funded large scale multi-center landmark phase III clinical trials in stroke rehabilitation history— Locomotor Experience Applied Post-Stroke (LEAPS) Trial (2006-2011) and Interdisciplinary Comprehensive Arm Rehabilitation Evaluation Stroke Initiative (I-CARE) Trial (2008-2015). I have served as a biostatistician / data manager and then the biostatistics core co-director for NIH funded project study Pacific Rim Transdisciplinary Tobacco & Alcohol Use Research Center from 08/2000 to 07/2011. In sum, I have the expertise, experience, and motivation necessary to successfully carry out my role in the proposed research project.

a. Dong Y, Dobkin BH, **Cen SY**, Wu AD, Winstein CJ. Motor cortex activation during treatment may predict therapeutic gains in paretic hand function after stroke. *Stroke*. 2006;37:1552-1555
b. Jones JG, **Cen SY**, Lebel RM, Hsieh PC, Law M. Diffusion tensor imaging correlates with the clinical assessment of disease severity in cervical spondylotic myelopathy and predicts outcome following surgery. *AJNR Am J Neuroradiol*. 2013;34:471-478
c. Matcuk GR, Jr., **Cen S**Y, Keyfes V, Patel DB, Gottsegen CJ, White EA. Superolateral hoffa fat-pad edema and patellofemoral maltracking: predictive modeling. AJR Am J Roentgenol. 2014;203(2):W207-12.
d. Huhdanpaa H, Hwang D, **Cen S**, Quinn B, Nayyar M, Zhang X, et al. CT prediction of the Fuhrman grade of clear cell renal cell carcinoma (RCC): towards the development of computer-assisted diagnostic method. Abdom Imaging. 2015;40(8):3168-74.
e. Shiroishi MS, **Cen SY**, Tamrazi B, D'Amore F, Lerner A, King KS, Kim PE, Law M, Hwang DH, Boyko OB, Liu CS., Predicting Meningioma Consistency on Preoperative Neuroimaging Studies., Neurosurg Clin N Am. 2016 Apr;27(2):145-54
f. Chen F, Gulati M, Hwang D, **Cen S,** Yap F, Ugwueze C, Varghese B, Desai M, Aron M, Gill I, Duddalwar V., Voxel-based whole-lesion enhancement parameters: a study of its clinical value in differentiating clear cell renal cell carcinoma from renal oncocytoma. Abdom Radiol (NY). 2016 Sep 6.
g. Saremi F, **Cen S,** Tayari N, Alizadeh H, Emami A, Lin L, Fleischman F. A correlative study of aortic valve rotation angle and thoracic aortic sizes using ECG gated CT angiography. Eur J Radiol. 2017 Apr;89:60-66
h. 62. Yoon AJ, Do HP, **Cen S,** Fong MW, Saremi F, Barr ML, Nayak KS. Assessment of segmental myocardial blood flow and myocardial perfusion reserve by adenosine-stress myocardial arterial spin labeling perfusion imaging. J Magn Reson Imaging. 2017 Feb 2 PMID: 28152238

B. Positions and Honors

Positions and Employment

2001-2006 Biostatistician, Division of Biostatistics, Department of Preventive Medicine, University of Southern California, Los Angeles, CA
2006-2007 Postdoctoral Research Associate, Division of Biostatistics, Department of Preventive Medicine, University of Southern California, Los Angeles, CA
2007-2013 Assistant Professor, Biokinesiology & Physical Therapy Division, School of Dentistry University of Southern California, Los Angeles, CA
2013- Assistant Professor of Research, Department of Neurology, University of Southern California, Los Angeles, CA
2013- Assistant Professor of Research (dual appointment), Department of Radiology, University of Southern California, Los Angeles, CA

Other Experience and Professional Memberships

2000- Member, American Statistics Association
2005- Member, American Public Health Association
2007 Reviewer, Nicotine and Tobacco Research
2011 Reviewer, Biomedical Engineer Online
2013- Editorial Board Statistical Adviser, British Medical Journal of Heart

Honors

2000 Academic Achievement Awards, University of Southern California, Los Angeles, CA
2003 Phi Tau Phi Scholarship, Phi Tau Phi Scholastic Honor Society of America, Pasadena, CA
2011 Dorothy Briggs Memorial Scientific Inquiry Award 2011 (as a major coauthor), American Physical Therapy Association
2013 First prize winner of the Stroke Progress and Innovation Award (as a major coauthor), American Stroke Association

C. Contribution to Science

1. I have extensive experience in study design and statistical analysis in imaging study. As the in house lead statistician of USC radiology department, I have led the study design and data analysis for all clinical researches in radiology department and translational research projects from other departments with imaging as the key outcome. My main contribution in this project is study design and statistical analysis.

    a. Dong Y, Dobkin BH, **Cen SY**, Wu AD, Winstein CJ. Motor cortex activation during treatment may predict therapeutic gains in paretic hand function after stroke. *Stroke*. 2006;37:1552-1555
    b. Xiao L, Bechara A, **Cen S**, Grenard JL, Stacy AW, Gallaher P, et al. Affective decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in 10th-grade chinese adolescent smokers. *Nicotine Tob Res*. 2008;10:1085-1097
    c. Jones JG, **Cen SY**, Lebel RM, Hsieh PC, Law M. Diffusion tensor imaging correlates with the clinical assessment of disease severity in cervical spondylotic myelopathy and predicts outcome following surgery. *AJNR Am J Neuroradiol*. 2013;34:471-478
    d. Matcuk GR, Jr., **Cen SY**, Keyfes V, Patel DB, Gottsegen CJ, White EA. Superolateral hoffa fat-pad edema and patellofemoral maltracking: Predictive modeling. *AJR Am J Roentgenol*. 2014;203:W207-212
    e. Huhdanpaa H, Hwang D, **Cen S**, Quinn B, Nayyar M, Zhang X, et al. CT prediction of the Fuhrman grade of clear cell renal cell carcinoma (RCC): towards the development of computer-assisted diagnostic method. Abdom Imaging. 2015;40(8):3168-74.
    f. Shiroishi MS, **Cen SY**, Tamrazi B, D'Amore F, Lerner A, King KS, Kim PE, Law M, Hwang DH, Boyko OB, Liu CS., Predicting Meningioma Consistency on Preoperative Neuroimaging Studies., Neurosurg Clin N Am. 2016 Apr;27(2):145-54
    g. Chen F, Gulati M, Hwang D, **Cen S,** Yap F, Ugwueze C, Varghese B, Desai M, Aron M, Gill I, Duddalwar V., Voxel-based whole-lesion enhancement parameters: a study of its clinical value in differentiating clear cell renal cell carcinoma from renal oncocytoma. Abdom Radiol (NY). 2016 Sep 6.
    h. Saremi F, **Cen S,** Tayari N, Alizadeh H, Emami A, Lin L, Fleischman F. A correlative study of aortic valve rotation angle and thoracic aortic sizes using ECG gated CT angiography. Eur J Radiol. 2017 Apr;89:60-66
    i. Yoon AJ, Do HP, **Cen S,** Fong MW, Saremi F, Barr ML, Nayak KS. Assessment of segmental myocardial blood flow and myocardial perfusion reserve by adenosine-stress myocardial arterial spin labeling perfusion imaging. J Magn Reson Imaging. 2017 Feb 2 PMID: 28152238

2. My other contribution to science in this proposed study is to oversee the data management and quality control. I have served as the co-director of Data Management and Analysis Center (DMAC) and director of the database for two multi-center landmark phase III clinical trials in stroke rehabilitation history—Locomotor Experience Applied Post-Stroke (LEAPS) Trial and Interdisciplinary Comprehensive Arm Rehabilitation Evaluation Stroke Initiative (I-CARE) Trial. The database/informatics system I have developed successfully implemented the study protocol and delivered the high quality data. As the result, sever papers have published in high impact peer review journals including New England Journal of Medicine, American Journal of Public Health and BMJ. This R01 study will require the collaboration in data collection across different departments. My experience in informatics and data management will support the study in integrating data from different sources and delivery the high quality data.

    a. Unger JB, Chou CP, Palmer PH, Ritt-Olson A, Gallaher P, **Cen S**, et al. Project flavor: 1-year outcomes of a multicultural, school-based smoking prevention curriculum for adolescents. *Am J Public Health*. 2004;94:263-265
    b. Duncan PW, Sullivan KJ, Behrman AL, Azen SP, Wu SS, Nadeau SE, et al. Body-weight-supported treadmill rehabilitation after stroke. *N Engl J Med*. 2011;364:2026-2036
    c. Attenello FJ, Wen T, **Cen SY**, Ng A, Kim-Tenser M, Sanossian N, et al. Incidence of "never events" among weekend admissions versus weekday admissions to us hospitals: National analysis. *Bmj*. 2015;350:h1460
    d. Winstein CJ, Wolf SL, Dromerick AW, Lane CJ, Nelsen MA, Lewthwaite R, **Cen SY**, Azen SP; Interdisciplinary Comprehensive Arm Rehabilitation Evaluation (ICARE) Investigative Team. Effect of a Task-Oriented Rehabilitation Program on Upper Extremity Recovery Following Motor Stroke: The ICARE Randomized Clinical Trial, JAMA. 2016 Feb 9;315(6):571-81. doi: 10.1001/jama.2016.0276.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/myncbi/collections/48228979/?msg=publicURL

D. Research Support

Ongoing Research Support

California State Fund (First5 LA) Mulligan (PI) 3/012013 – 02/28/2018
Children's Health and Maintenance Program - an oral health promotion program for children ages 0 to 5
The goal of this study is to investigate socioeconomic and health insurance status as factors attributable to the oral health condition of at-risk children aged 0 to 5 years and to improve access to care by providing oral health education and services at various community sites.
Role: Director, Center of Informatics, Data Management and Statistics

R01 HL 130494 (Nayak) 7/1/16 – 6/30/20 1 calendar month
NIH / NHLBI $1,802,672 total direct
Novel Myocardial Perfusion Stress Test using Arterial Spin Labeling
This proposal will develop a new ultra-safe method for measuring the rate of blood flow to the heart muscle at rest and during stress. If successful, this could improve heart disease screening in patients with poor kidney function and patients who require frequent checkups.

NIH U01 EY025864 Bosco Tjan (PI) 09/2015 – 06/2019
NIH $3,720,277 (total 5 year direct costs)
Human Connectomes for Low Vision, Blindness, and Sight Restoration
The overall objective of this study is to characterize in unprecedented detail the relationships between retinal pathologies and their downstream impact on the central visual pathway over the natural courses of blinding diseases and their treatments. We will make available to the scientific community data sets that include advanced ophthalmic and neural imaging data collected from the same subjects, and new software tools for analyzing these data.
Role: Co-Investigator and Lead Biostatistician

California Community Foundation Christopher Lee (PI) 4/1/2015 - 3/31/2017
USC Lung Cancer Screening Program
The USC Department of Radiology and the Norris Patient Education and Outreach Center in partnership with USC's Health Sciences Campus Community Partnerships office propose to lead outreach efforts to link high risk individuals to USC's Lung Cancer Screening Program which will provide a seamless system of care of those enrolled. The goal of the program is to save lives by preventing and reducing the devastating effects of lung cancer for residents of the Centinela Valley through education, early detection, diagnosis and treatment, and integrated preventive services, with special emphasis on the underserved and those most affected.
Role: Co-investigator, Informatics

Completed Research Support

1UH2EB019889-01 Susan Love (PI) 08/01/2015 – 07/31/2016
Validation Study of Low-Cost Portable Computer-Aided Diagnosis Ultrasound System for Breast Cancer Triage in Low to Middle-Income Countries (LMIC) Environments
The goal of this study is to validate software to triage palpable breast lumps between those that may be malignant to those they may be benign. This would enable stressed health care delivery systems to focus resources on the women most likely to benefit from their efforts.
Role: Director, Data Core and Lead Biostatistician

R01NS056256 Winstein; Wolf; Dromerick (PIs) 08/01/2008 – 07/31/2015
Interdisciplinary Comprehensive Arm Rehabilitation Evaluation Stroke Initiative (I-CARE) Trial

The goal of the study was to improve outpatient therapy for arm paresis after stroke. This Phase III, single blind, multi-center, randomized control trial will investigate the effectiveness of ASAP (Accelerated Skill Acquisition Program), a focused, intense, evidence-based, upper extremity rehabilitation program.
Role: Co-Director, Data Management/Statistical Analysis Center

H133E080024 Winstein (PI) 10/01/2008 - 09/30/2012

Rehabilitation Engineering Research Center for Technologies for Successful Aging with Disability

The goal of this project was to develop and deliver cutting-edge technologies for identification, evaluation, and rehabilitation of the motor processes that facilitate or impede functional performance, employment, and community participation for the intended beneficiaries.
Role: Lead Biostatistician, Data Management and Analysis Center

R01AG033078 Chen (PI) 03/15/2011- 02/28/2015
Environmental Determinants of Cognitive Aging in the WHI Memory Study
The goal of the study was to investigate ambient air pollutants as novel environmental determinants of cognitive aging to improve our understanding of the neurocognitive effects of environmental factors in later life.
Role: Director, Data Core

R01 NS050506 Duncan (PI) 10/01/2006 - 09/30/2011
Locomotor Experience Applied Post-Stroke (LEAPS) trial
The goal of the study was to determine if there is a difference in the proportion of participants who recover walking ability at one year post-stroke when randomized to a specialized locomotor training program (LTP), conducted at 2- or 6-months post-stroke, or those randomized to a home based non-specific, low intensity exercise intervention (HEP) provided 2 months post-stroke.
Role: Co-Director, Data Management and Analysis Center

P50CA084735 Johnson (PI) 07/01/2004 – 06/30/2011
Pacific Rim Transdisciplinary Tobacco & Alcohol Use Research Center (PR TTAURC)
The goal of the study was to study genetic and environmental factors that influence tobacco and alcohol use among adolescents in China and the United States.
Role: Biostatistician/Co-investigator

P50CA084735 Johnson (PI) 08/01/2000 – 01/31/2005
Transdisciplinary Tobacco Use Research Center (TTURC)
The goal of the study was to develop and evaluate culturally tailored (Latino, Pacific Islander and Chinese) behavior intervention program to prevent tobacco use in adolescent.
Role: Biostatistician / Data Manager

R01 NS 45485-01 Winstein (PI) 09/01/2002 – 06/30/2006
Brain and Behavior Correlates of Arm Rehabilitation
The goal of the study was to examine the motor control and cerebral activity changes associated with constraint-induced (CI) movement therapy for patients with sub-acute stroke who are between 3-9 months post stroke.
Role: Lead Biostatistician/Data Manager

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Mark Andrew Espeland

eRA COMMONS USER NAME (credential, e.g., agency login): MESPELAN

POSITION TITLE: Professor, Public Health Sciences

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Arizona State University, Tempe, AZ	B.S.	05/74	Mathematics
University of Rochester, Rochester, NY	M.A.	05/76	Statistics
University of Rochester, Rochester, NY	Ph.D.	12/81	Statistics

A. Personal Statement

I look forward to continuing my long-term collaboration with Dr. Jiu-Chiuan Chen to study mechanisms linking air pollution to Alzheimer's disease. Dr. Chen is conducting important and impactful research in this area. I am well-prepared for this collaboration. I am founding Chair of the Department of Biostatistical Sciences at the Wake Forest School of Medicine and a Fellow of the American Statistical Association (1996) for my formative work in missing data and misclassification methods and a Fellow of the Society for Clinical Trials (2011) for my work in the design and conduct of multi-center studies. Later into my career, my primary research has shifted from methodology to have greater concentration on identifying approaches to preserving brain health during later life and the interfaces among aging, diabetes, obesity, and cognitive function. This shift in focus began in 1995 when I joined with Dr. Sally Shumaker to develop the Women's Health Initiative Memory Study, which is still ongoing and has spawned several inter-locking programs related to brain health. Since then, I am currently PI of the multi-center Look AHEAD coordinating center and past-PI of the Look AHEAD Brain MRI study. I have growing experience in diabetes and neuroepidemiology: 104 of the 114 published articles I have authored/co-authored since 2010 are focused on brain health and/or lifestyle intervention. The Modan Award I received from the American Diabetes Association in 2012 was based on my work examining trajectories of cognitive decline among women with diabetes. I also have considerable prior experience in cardiovascular disease: the Remington Award I received from the American Heart Association in 1996 was based on my work towards developing and introducing missing data methods for use in clinical trials with atherosclerotic outcomes. My induction in the American Association for the Advancement of Science in 2014 drew from my biomedical and biostatistical work spanning several research fields. I have held leadership roles in dozens of coordinating centers. I am a frequent consultant to the NIH and industry and to the Alzheimer's Association, a past member of the NIA Clinical Trials Advisory Panel, a current member of the National Academy of Sciences Committee on Reducing Risks of Alzheimer's Disease, and statistical editor for the Journal of Gerontology. I have served on or chaired dozens of DSMBs for NIH and industry. I direct the Data Analysis Core for the Wake Forest Alzheimer's Disease Center. I have authored/co-authored 296 papers on statistical methodology and biomedical research (and 68 additional consortium publications). Four of these that support my ability to conduct the research related to cognitive health are:

1. Cacciottolo M, Wang X, Driscoll I, Woodward N, Saffari A, Reyes J, Serre ML, Vizuete W, Sioutas C, Morgan TE, Gatz M, Chui HC, Shumaker SA, Resnick SM, **Espeland MA,** Finch CE, Chen JC. Particulate air pollutants, APOE alleles, and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. Translational Psychiatry 2017;7;e1022;doi:10;1038/tp.2016.289. PMCID: PMC5299391
2. **Espeland MA,** Erickson K, Neiberg RH, et al. Brain and white matter hyperintensity volumes after ten years of random assignment to lifestyle intervention. Diabetes Care 2016;39:764-771. PMCID: PMC4839171

3. **Espeland MA,** Luchsinger JA, Baker LD, Neiberg R, Kahn SE, Arnold SE, Wing RR, Blackburn GL, Bray G, Evans M, Hazuda HP, Jeffery RW, Wilson VM, Clark JM, Coday M, Demos-McDermott K, Foreyt JP, Greenway F, Hill JO, Horton ES, Jakicic JM, Johnson KC, Knowler WC, Lewis CE, Nathan DM, Peters A, Pi-Sunyer X, Pownall H, Wadden TA, Rapp SR for the Look AHEAD Study Group. Effect of a long-term intensive lifestyle intervention on prevalence of cognitive impairment. Neurology 2017 (in press).
4. **Espeland MA,** Rapp SR. Bray GA, Houston DK, Johnson KC, Kitabchi AE, Hergenroeder AL, Williamson J, Jakicic JM, Van Dorsten B, Williamson J, Kritchevsky SB. Long-term impact of behavioral weight loss intervention on cognitive function: The Action for Health in Diabetes Movement and Memory Study. J Gerontol Med Sci 2014;69;1101-8. PMCID: PMC4158413.

B. Positions and Honors

Positions

1979-81	Assistant in Biostatistics, Div. of Biostatistics, Univ. of Rochester Medical Center, Rochester, NY
1981-83	Assistant Professor, School of Public Health, Univ. of Illinois of Chicago, Chicago, Il
1983-86	Coordinator of Biostatistics and Computing, Eastman Dental Center, Rochester, NY
1986-87	Assistant Professor, Center for Prev. Res. and Biometry, Wake Forest Univ, Winston-Salem, NC
1987-94	Associate Professor, Dept. Public Health Sciences, Wake Forest Univ., Winston-Salem, NC
1993-98	Section Head, Section on Biostatistics, Wake Forest Univ. Health Sciences, Winston-Salem, NC
1994-	Professor, Dept./Div. of Public Health Sciences, Wake Forest University, Winston-Salem, NC
2006-12	Chair, Dept. of Biostatistical Sciences, Wake Forest Univ. Health Sciences, Winston-Salem, NC

Honors

1996	Remington Lecturer, American Heart Association
1996	Fellow, American Statistical Association
2011	Fellow, Society for Clinical Trials
2011	Established Investigator Clinical Sciences Research Award, Wake Forest School of Medicine
2012	Michaela Modan Award for Epidemiological Research, American Diabetes Association
2014	Fellow, American Association for the Advancement of Science
2014	Research Team Science Award, Wake Forest School of Medicine

Other Experience (selected)

1987-1995	Co-Principal Director, Coord Center, Postmenopausal Estrogen/Progestin Intervention Study
1987-1994	Co-I, Primary Statistician, Administrative Center, Asymptomatic Carotid Artery Plaque Study
1991-1997	PI, Coordinating Center, Trial of Nonpharmacologic Interventions in the Elderly
1994-1998	Co-Principal Investigator, Postmenopausal Estrogen/Progestin Intervention Continuation Study
1994-1999	Co-I, PEPI Safety Follow-up Study
1995-2004	Co-PI, Women's Health Initiative Memory Study
1996-2000	Co-I, Cardiovascular Health Study Brachial Artery Substudy
1997-2003	PI, Autoimmune Inner Ear Disease Trial
1999-2011	Co-PI, Women's Health Initiative Study of Cognitive Aging
1999-2001	Co-I, Insulin Resistance and Atherosclerosis Study
2000-2007	Co-I, Study of Tamoxifen and Raloxifene Cognition Study
2001-2005	Co-I, Type 1 Diabetes Genetics Consortium
2003-2007	Co-I, Lifestyle Interventions and Independence for Elders (LIFE) pilot study
2004-2007	Co-PI, Women's Health Initiative Memory Study – Magnetic Resonance Imaging
2004-	Co-PI, Women's Health Initiative Memory Study Extension
2006-2009	Co-PI, Translating Research into Prevention of Diabetes Trial
2007-2010	PI, Seniors Health and Activity Research Program pilot
1999-	PI, Action for Heath in Diabetes (Look AHEAD)
2009-2016	PI, Study of Novel Approaches to Prevention (SNAP)
2009-2014	Co-PI, Look AHEAD Physical and Cognitive Function Ancillary Study
2009-	Co-PI, Lifestyle Interventions and Independence for Seniors trial
2011-2014	PI, Look AHEAD Brain MRI Study
2010-	Co-I, Women's Health Initiative Southeast Regional Center
2013-	PI, (Subcontract): Environmental Determinants of Cognitive Aging
2013-	Co-I, Beta-cell function and cognition in the Restoring Insulin Secretion (RISE) Study
2015-2017	Co-I, Enabling Reduction of Low-Grade Inflammation In Seniors Vanguard Study
2016-	mPI, Cocoa Supplement and Multivitamin Outcome Study of the MIND
2016-	Core Director, Wake Forest Alzheimer's Disease Core Center

2016- PI, Study of Novel Approaches to Prevention Extension (SNAP-E)
2016- PI, Subcontract to Alzheimer's Therapeutic Research Institute, EARLY clinical trial

C. Contribution to Science

Influence Of Diabetes On Cognitive Health

Lately in my career, I have become very interested in understanding the impact of diabetes and obesity on the late-life brain health. This has drawn from several papers from the WHI which helped to establish their influence on cognitive trajectories and brain MRI outcomes. One of these led to my recognition from the American Diabetes Association Michaela Modan Award for Epidemiological Research. I have led the first paper from the Look AHEAD study group to examine the impact of its weight loss intervention on cognition. My current paper describing the primary findings of the Look AHEAD brain MRI study is currently in press.

1. **Espeland MA,** Miller ME, Goveas JS, Hogan PA, Coker LH, Williamson JD, Naughton MJ, Resnick SM. Cognitive function and fine motor speed in older women with diabetes mellitus: results from the Women's Health Initiative Study of Cognitive Aging. Journal of Women's Health 2011;20(10):1435-1443. PMCID: PMC3186442
2. **Espeland MA,** Bryan RN, Goveas JS, Robinson J, Siddiqui MS, Liu S, Hogan PE, Casanova R, Coker LH, Yaffe K, Masaki K, Rossom R, Resnick SM. Influence of type 2 diabetes on brain volumes and changes in brain volumes: results from the Women's Health Initiative Magnetic Resonance Imaging Studies. Diabetes Care 2013;36:90-97.PMCID: PMC3526228
3. **Espeland MA,** Brinton RD, Manson JE, Yaffe K, Hugenschmidt C, Vaughan L, Craft S, Edwards BJ, Casanova R, Masaki K, Resnick SR. Postmenopausal hormone therapy, type 2 diabetes mellitus, and brain volumes, Neurology 2015;85:1131-38. PMCID: PMC4603880
4. **Espeland MA,** Brinton RD, Hugenschmidt C, Manson JE, Craft S, Yaffe K, Weitlauf J, Vaughan L, Johnson KC, Padula CB, Jackson R, Resnick SM for the WHIMS Study Group. Impact of type 2 diabetes mellitus and postmenopausal hormone therapy on incidence of cognitive impairment in older women. Diabetes Care 2015;38:2316-24. PMCID: PMC4657616

Impact of Postmenopausal Hormone Therapy on the Brain

I have been an investigator of the Women's Health Initiative (WHI) studies related to brain health since 1995. At that time, postmenopausal hormone therapy was often recommended as a treatment for the preservation of cognitive function in older women based on cohort studies and considerable basic science work. We tested this assumption in the WHI randomized clinical trial of hormone therapy and found that, in women aged 65 years and older, hormone therapy markedly increased risk of dementia and mild cognitive impairment. This has led to a marked drop in the prescription of hormone therapy to older women and has saved lives. My 2009 publication listed below sets the basis for understanding the potential mechanism for this: hormone therapy increases brain atrophy which accounts for the increased risk. My 2015 Neurology paper listed above furthers our understanding of the mechanism for this, which is related to estrogen's "healthy cell bias" role in regulating energy metabolism in the brain. My 2013 paper below supports this mechanism demonstrating that while hormone therapy does not benefit cognition overall in younger women, it does not appear to have harmful effects. While my recent award as a fellow of the AAAS is as a biostatistician, my work in neuro-epidemiology also contributed to this award.

1. **Espeland MA,** Rapp SR, Shumaker SA, Brunner R, Manson JE, Sherwin BB, Hsai J, Margolis KL, Hogan PE, Wallace R, Dailey M, Freeman R, Hays J. Conjugated equine estrogens and global cognitive function in postmenopausal women. JAMA 2004;291:2959-2968.
2. **Espeland MA**, Tindle HA, Bushnell CA, Jaramillo SA, Kuller LH, Margolis KL, Mysiw WJ, Maldjian JA, Melhem ER, Resnick SM. Brain volumes, cognitive impairment, and conjugated equine estrogens. Journal of Gerontology: Medical Sciences 2009;64A:1243-50 PMCID: PMC2773813.
3. **Espeland MA**, Brunner RL, Hogan PA, Rapp SR, Coker LH, Legault C, Granek I, Resnick SM. Long term effects of conjugated equine estrogens therapies on domain-specific cognitive function: Results from the Women's Health Initiative Study of Cognitive Aging (WHISCA) Extension. J Am Geriatr Soc 2010;58:1263-1271. PMCID: PMC2917208.
4. **Espeland MA,** Shumaker SA, Leng I, Manson JE, Brown CM, LeBlanc ES, Vaughan L, Robinson J, Rapp SR, Goveas JS, Lane D, Wactawski-Wende J, Stefanick MS, Li W, Resnick SM. Long term effect on cognitive function of postmenopausal hormone therapy prescribed to women aged 50-55 years. JAMA Internal Medicine 2013;173:1429-36. PMCID: PMC3844547.

Lifestyle, Behvarioral Intervention, and Brain Health

I have been engaged in a number of major multicenter clinical trials involving behavioral interventions, in addition to Look AHEAD. These include ACT, TONE, LIFE-P, LIFE, HELP, and SNAP. I also was PI for a single site pilot study of behavioral approaches to improving cognitive function, the Seniors Health and Activity Research Program (SHARP). Collectively, these studies have had a profound influence on contributing to the effective use of behavioral intervention in health promotion.

1. **Espeland MA,** Newman AB, Sink K, Gill TM, King AC, Miller ME, Guralnik J, Katula J, Church T, Manini T, Reid KF, McDermott MM. Associations between ankle-brachial index and cognitive function: Results from the Lifestyle Interventions and Independence for Elders Trial. J Am Med Dir Assoc 2015;16:682-689. PMCID: PMC4516564
2. Sink K, **Espeland MA,** Castro C, Church T, Cohen R, Dodson JA, Guralnik J, Hendrie HC, Jennings J, Katula J, Lopez OL, McDermott M, Pahor M, Reid KF, Rushing J, Verghese J, Rapp S, Williamson J, for the LIFE Study investigators. Effect of physical activity on cognitive outcomes in sedentary older adults. Results from the LIFE Randomized Clinical Trial. JAMA 2015;314:781-790. PMCID: PMC4154884
3. **Espeland MA,** Lipska K, Miller ME, Rushing J, Cohen RA, Verghese J, McDermott MM, King AC, Strotmeyer ES, Blair SN, Pahor M, Reid K, Demons J, Kritchevsky SB. Effects of physical activity intervention on physical and cognitive function in older, sedentary adults with and without diabetes. J Gerontol A BiolSci Med Sci 2016 EPub PMID: 27590629 PCMID in progress
4. Driscoll I, Gaussoin S, Wassertheil-Smoller S, Limacher M, Casanova R, Yaffe K, Resnick S, **Espeland MA**. Obesity and brain structural integrity in older women: the Women's Health Initiative Magnetic Resonance Imaging Study (WHIMS-MRI). J Gerontol A Biol Sci Med Sci 2016 EPub PMCID: PMC4978361

Methods for Multicenter Studies

I have collaborated on the development of many innovations for the conduct of large research programs. My first experience with study coordination began in 1981 when I became an investigator at the coordinating center for the NHLBI-funded Cooperative Study of Sickle Cell Disease and published a JASA paper on missing data methods I developed for that cohort study. During the subsequent 34 years, I have been continuously involved in study collaboration and leadership and directed groups that have made major advances in this field. These include the development of integrated web-based data management systems and the development of on-line monitoring reports. This also includes advances in study design and analyses. Recognition of this work supported my award of Fellow to the Society for Clinical Trials and to the American Statistical Association.

1. **Espeland M,** Hui S. A general approach to analyzing epidemiologic data that contain misclassification errors. Biometrics 1987;43:1001-1012.
2. **Espeland MA,** Platt OS, Gallagher D for the Cooperative Study of Sickle Cell Disease. Joint estimation of incidence and diagnostic error rates from irregular longitudinal data. J Am Statist Assoc 1989;84:972-979.
3. **Espeland MA,** Craven TE, Miller ME, D'Agostino R. 1996 Remington Lecture: Modeling multivariate longitudinal data that are incomplete. Ann Epidemiol 1999;9:196-205.
4. **Espeland MA,** Gill TM, Guralnik, Miller ME, Fielding R, Newman AB, Pahor M. Designing clinical trials of intervention for mobility disorder: results from the Lifestyle Interventions and Independence for Elders Pilot (LIFE-P) Trial. J Gerontol Med Sci 2007;62A;1237-1243. PMCID: PMC2376827.

Assessment of Atherosclerosis

Early in my career, I was lead statistician for some of the major clinical trials in which carotid ultrasonography framed the primary outcome. These trials (ACAPS, PLAC-2, PREVENT) helped to lay the basis for establishing the efficacy of statin therapy. I wrote a series of papers on how to interpret ultrasonographic data, which led to my recognition from the American Heart Association with its Remington Award for formative statistical work. The 2005 paper listed below is highly cited and provides the basis for the use of carotid ultrasongraphic endpoints as surrogates for major cardiovascular disease events in statin trials.

1. **Espeland MA,** Byington RP, Hire D, Davis VG, Hartwell T, Probstfield J. Analysis strategies for serial multivariate ultrasonographic data that are incomplete. Statist Med 1992;11:1041-1056.
2. **Espeland MA,** Craven TE, Riley WA, Corson J, Romont A, Furberg CD. Reliability of longitudinal ultrasonographic measurements of carotid intimal-medial thicknesses. Stroke 1996;27:480-485.
3. **Espeland MA,** Tang R, Terry JG, Davis SH, Mercuri M, Crouse JR. Associations of risk factors with segment-specific intimal-medial thickness of the extracranial carotid artery. Stroke 1999;30:1047-1955.
4. **Espeland MA,** O'Leary DH, Terry JG, Morgan T, Evans G, Mudra H. Carotid intimal-media thickness as a surrogate for cardiovascular disease events in trials of HMG-CoA reductase inhibitors. Curr Cont Trials Cardiovasc Med 2005;6:3 (http://cvm.controlled-trials.com). PMCID: PMC555546.

A full list of my published work is available: http://www.ncbi.nlm.nih.gov/myncbi/mespelan@wfubmc.edu/cv/.

D. Research Support

R01 DL099164-01 (Craft) 07/01/2013 – 06/30/2017
Beta-cell Function and Cognition in the Restoring Insulin Secretion (RISE) Study
This project examines the effects of induced insulin resistance on APP, and A in older adults with Mild Cognitive Impairment (MCI) and Alzheimer's disease, as well as normal older adults. Role: Co-I

R01 HL127341 (Wing) 09/01/2015 – 05/31/2019
Preventing Weight Gain in Young Adults Extension (SNAP-E) This represents a subcontract with Miriam Hospital. SNAP-E will follow participants of the Study of Novel Approaches to Weigh Gain Prevention (SNAP) for an additional 4 years to determine if the benefits of those interventions can be maintained with only minimal intervention and thus provide a public health approach to the problem of weight gain in young adults.
Role: Co-I

HHSN268201600004C (Shumaker) 10/15/2015 – 10/14/2020
Women's Health Initiative Extension 2015-2020 This study is designed to collect high quality data from WHI participants and retain participants in the study. Facilitate the advancement and dissemination of scientific findings for WHI through the development of competitive ancillary studies and sub-studies, papers and presentations. Role:

5 U01 DK57136-18 (Espeland) 02/01/2016 – 01/31/2021
16/16 Action for Health in Diabetes Extension Study Biostatistics Research Center
The Look AHEAD-Extension will follow approximately 3800 individuals for an additional 4.5 years to determine whether random assignment to an intensive lifestyle intervention focused on weight loss achieved through healthy eating and increased physical activity relative to a control group leads to improved long-term health in later life. This extended follow-up will provide important information about the long-term beneficial effects of a lifestyle intervention in a growing segment of the population-namely those who are older, overweight or obese, and have type 2 diabetes. Role: PI

1R01AG050657-01A1 (Baker) 06/01/2016-02/28/2021
Cocoa Supplement and Multivitamin Outcome Study-Mind (COSMOS-Mind) The proposed study, COSMOS-Mind, will enroll 2,000 women and men from the parent trial (>65 years old), and conduct standardized and validated cognitive assessments via telephone at baseline (before randomization) and annually over 3 years of follow-up to establish whether daily use of cocoa flavanols with or without a multivitamin can protect cognitive function and reduce incidence of cognitive impairment, including Alzheimer's disease. Role: Co-I

1R01AG054069-01 Chen (PI) 09/01/2016 – 05/31/2021
The Macrovascular and Microvascular Contributions to Alzheimer's Disease: MESA VASCAD
This project holds the promise to generate new knowledge about environmental risk factors in late life that are amenable to intervention, and better understanding why places matter in Alzheimer's disease and related dementias (ADRD) risks may contribute new approaches to developing effective prevention modalities, a national goal set in the National Plan to Address Alzheimer's Disease by 2025. Role: Co-I

R01AG054068-01 Chen (PI) 09/01/2016-06/30/2021
Alzheimer's Disease & Related Dementias: Geography, Environments & Mechanisms The long-term goal of this project is to better understand the geographic disparities in Alzheimer's disease and related dementias (ADRD) by studying the neuropsychological trajectories and clinical progression to increased ADRD risk as related to geographic indicators, identifying the contributing environmental factors and examining their interactions, and elucidating the possible neurobiological mediators. Role:

P30 AG049638-01A1 Craft (PI) 07/01/2016-06/30/2021
Wake Forest Alzheimer's Disease Core Center To determine the long-term effects of randomization to weight loss on a performance-based measure of mobility disability (400-m walk time; primary outcome) and physical function (short physical performance battery, leg strength and power; secondary outcomes).
Role: Co-I

R01ES025888 Chen/Kaufman (PI) 09/30/2016-07/31/2021
Environmental Determinants of Pathological Brain Aging in WHI Memory Studies This project will support the development/refinement of state-of-the-art spatiotemporal air pollution modeling tools and apply the resulting comprehensive new exposure data (from 1990s to 2016) to better understand the long-term influences of ambient air pollutants including particulate mixture on increased dementia risk and further investigate novel mediators and pathways leading to pathological brain aging in late life. Role: Co-I

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Christine Fennema-Notestine

eRA COMMONS USER NAME (credential, e.g., agency login): cfennema

POSITION TITLE: Associate Professor of Psychiatry & Radiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Inst. of Technology, Cambridge, MA	B.S.	06/1990	Cognitive Science
University of California, San Diego, La Jolla, CA	M.A.	06/1992	Experimental Psychology
University of California, San Diego, La Jolla, CA	Ph.D.	06/1995	Cognitive Science & Psychology
University of California, San Diego, La Jolla, CA	Post-doc	07/1996	Neuroimaging

A. Personal Statement

The proposed P01 aims to better define the individual risk, heterogeneity, and biological basis of Alzheimer's disease (AD) associated with exposure to ambient air pollution in aging populations. Multimodal human neuroimaging data will be explored with respect to spatiotemporal data on air pollution and integrated with translational mechanistic studies in mice, with an emphasis on effects within the white matter in particular. As a cognitive neuroscientist with extensive expertise in multi-modal neuroimaging and the effects of aging and Alzheimer's disease on the brain, I am well qualified to contribute to the proposed study. My research broadly emphasizes MRI methods development and validation, and the clinical application of these methods to normal aging, neurodegenerative disease, HIV, and psychiatric disorders, including my ongoing collaboration with the Vietnam Era Twin Study of Aging (VETSA; PI W. Kremen), a nationwide cohort to be leveraged in the proposed study. My work in neuroimaging and biomedical informatics includes assessing method performance and validation to improve outcomes and allow pooling of data across multiple sites, which has led to the development of large datasets, as well as scientific advances in our understanding of phenotypes associated with risk and progression of diseases including Alzheimer's disease and HIV. Of particular relevance to the proposed work, I began collaborating with the VETSA study during its first wave, and became a key investigator in the neuroimaging aspects of VETSA Waves 2 and 3, continuing to work closely with Drs. William Kremen and Carol Franz. I lead the image analysis for the white matter hyperintensities, and I play a key role in the structural and diffusion work as well as in quality control. Over time, I have served as the Director of the Biomedical Informatics Research Network Data Repository, the HIV Neurobehavioral Research Center (HNRC) Neuroimaging Core, and the multi-site CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) Neuroimaging Core. Currently, in addition to my role in VETSA, I lead a study on the *Impact of Cannabinoid Use on HIV-related Brain Alterations in Young Adults,* direct the neuroimaging aspects of the recently funded *Age Related Determinants of HAND: A 12 Year Follow-up of CHARTER Participants*; and play a key role in the Translational Methamphetamine AIDS Research Center (TMARC) Neuroimaging Core and several clinical trial studies. Research in these programs involves cross-sectional and longitudinal morphological, diffusion, and MR spectroscopy image analysis, development and validation of methods, and associated studies in relation to cognition, immune recovery, biomarkers of inflammation, metabolic disorders, and substance use. For the proposed work, I will provide consultation and support related to the neuroimaging data from the VETSA cohort, with an emphasis on the structural and white matter measures, and scientific integration of findings.

B. Positions and Honors

Positions and Employment

2003-2007 Associate Project Scientist, Department of Psychiatry, UC San Diego, La Jolla, CA
2006-2009 Director, Biomedical Informatics Research Network (BIRN) Data Repository
2007-2011 Assistant Professor of Psychiatry & Radiology, UC San Diego, La Jolla, CA
2011-Present Associate Professor of Psychiatry & Radiology, UC San Diego, La Jolla, CA

Other Experience and Professional Memberships (selected recent)

2011-Present Center for Functional MRI Strategic Planning Committee, UCSD
2012 Associate Editor, *Journal of Alzheimer's Disease*
2014-Present Grant Review Ad Hoc Panel Member (recent selected): *Creative and Novel Ideas in Research Awards program (CNIHR), USA; National Institute of Neurological Disorders and Stroke, USA; National Research Foundation, South Africa; National Science Foundation, USA; University of California Center for Accelerated Innovation (UC CAI), USA*
2013-Present Ad Hoc Peer Review (recent selected): *Brain and Behavior; Human Brain Mapping; IEEE Transactions in Medical Imaging; Journal of Clinical and Experimental Neuropsychology; J. of MRI; J. of Neuroscience Methods; J. of Psychiatry and Neuroscience; Neurobiology of Aging; NeuroImage; NeuroImage: Clinical; Neuroinformatics; Neurology; Neuropsychology; Psychiatry Research: Neuroimaging; Journal of Infectious Diseases; PLoS ONE; Radiology*

C. Contributions to Science

1. My early work emphasized methodology and biomedical informatics related to image analysis and data sharing, including methods validation (a, b) and improved pooling of data across multiple sites (b). With the national Biomedical Informatics Research Network (BIRN) and related testbeds, I aimed to enable multi-site clinical imaging projects and foster data sharing and integration. As Director of the BIRN Data Repository, I worked with all sites on infrastructure development to enable public data sharing including development of enhanced database infrastructures (c). I continue to work with collaborative informatics and data sharing efforts, most recently with the Cerebral Blood Flow BIRN (CBFBIRN) data repository (d), a successful effort in sharing of data and MRI sequences [https://cbfbirn.ucsd.edu/].
    a. **Fennema-Notestine** C, Ozyurt IB, Clark CP, Morris S, Bischoff-Grethe A, Bondi MW, Jernigan TL, Fischl B, Segonne F, Shattuck DW, Leahy RM, Rex DE, Toga AW, Zou KH, & Brown GG. (2006). Quantitative evaluation of automated skull-stripping methods applied to contemporary and legacy images: Effects of diagnosis, bias correction, and slice location. *Human Brain Mapping, 27*(2), 99-113. Epub date 2005/06/30.
    b. **Fennema-Notestine** C, Gamst AC, Quinn BT, Pacheco J, Jernigan TL, Thal L, Buckner R, Killiany R, Blacker D, Dale AM, Fischl B, Dickerson B, & Gollub RL. (2007). Feasibility of multi-site clinical structural neuroimaging studies of aging using legacy data. *Neuroinformatics, 5*(4), 235-245. Epub date 2007/11/14.
    c. Ozyurt IB, Keator DB, Wei D, **Fennema-Notestine** C, Pease KR, Bockholt J, & Grethe JS. (2010). Federated web-accessible clinical data management within an extensible neuroimaging database. *Neuroinformatics, 8*(4), 231-249. Epub Date 2010/06/23. PMCID: 2974931.
    d. Shin DD, Ozyurt IB, Brown GG, **Fennema-Notestine** C, & Liu TT. (2015). The Cerebral Blood Flow Biomedical Informatics Research Network (CBFBIRN) data repository. *Neuroimage*. Epub Date 2015/05/05. PMCID: 4651708.
2. Catalyzed by this work, I led the development of large, comprehensive datasets to explore the neuroimaging profiles of mild cognitive impairment and Alzheimer's disease (a,b), and to advance our understanding of phenotypes associated with risk and progression of disease (b,c,d). One overarching goal is to improve effectiveness of clinical trials, to bring us closer to treatment strategies that will slow the progression of or prevent neurodegeneration. This original work provides a strong foundation for our understanding of brain alterations associated with mild cognitive impairment and Alzheimer's disease relevant to the proposed study.
    a. **Fennema-Notestine** C, Hagler DJ, Jr., McEvoy LK, Fleisher AS, Wu EH, Karow DS, & Dale AM for the Alzheimer's Disease Neuroimaging Initiative. (2009). Structural MRI biomarkers for preclinical and mild Alzheimer's disease. *Human Brain Mapping, 30*(10), 3238-3253. Epub Date 2009/03/12. PMCID: 2951116.
    b. **Fennema-Notestine** C, McEvoy LK, Hagler DJ, Jr., Jacobson MW, & Dale AM for the Alzheimer's Disease Neuroimaging Initiative. (2009). Structural neuroimaging in the detection and prognosis of pre-clinical and early AD. *Behavioural Neurology, 21*(1), 3-12. Epub Date 2009/10/23. PMCID: 2873895.

c. Jacobson MW, McEvoy LK, Dale A, & **Fennema-Notestine** C. (2009). Cognitive phenotypes, brain morphometry and the detection of cognitive decline in preclinical AD. *Behavioural Neurology, 21*(1), 29-37. Epub Date 2009/10/23. PMCID: 2864725.
d. Walhovd KB, Fjell AM, Dale AM, McEvoy LK, Brewer J, Karow DS, Salmon DP, & **Fennema-Notestine** C for the Alzheimer's Disease Neuroimaging Initiative. (2010). Multi-modal imaging predicts memory performance in normal aging and cognitive decline. *Neurobiology of Aging, 31*(7), 1107-1121. Epub Date 2008/10/08. PMCID: 3947581.

3. My work expanded into the study of normal aging, emphasizing the genetic and environmental influences on the brain, collaborating with the VETSA team (W. Kremen and C. Franz) leading this component of the proposed P01. This large scale project of male twins in midlife includes structural, diffusion, perfusion, and functional connecvity measures. Supporting training of a post-doctoral fellow, I explored whether neuroimaging phenotypes were genetically driven and distinct from each other (a). Subsequently, I led a study defining the genetic and environmental influences of the brain's structural phenotypes in middle-age (b). In this cohort, I also examined the impact of carrying the ApoE ε4 allele, a genetic risk factor for Alzheimer's disease, on brain structure, demonstrating differences in the frontal cortex prior to clinical impairment (c). This work and subsequent VETSA studies demonstrating interactions between ApoE and other modifying factors provides guidance for the proposed study. Recently, our work demonstrated strong associations between hypertension and white matter microstructure, using diffusion tensor imaging, supporting associations between cardiovascular health and brain structure (d), and a study of the heritability of white matter hyperintensities and hypertension is in progress.
    a. Panizzon MS, **Fennema-Notestine** C, Eyler LT, Jernigan TL, Prom-Wormley E, Neale M, Jacobson K, Lyons MJ, Grant MD, Franz CE, Xian H, Tsuang M, Fischl B, Seidman L, Dale A, & Kremen WS. (2009). Distinct genetic influences on cortical surface area and cortical thickness. *Cerebral Cortex, 19*(11), 2728-2735. Epub Date 2009/03/21. PMCID: 2758684.
    b. Kremen WS, Prom-Wormley E, Panizzon MS, Eyler LT, Fischl B, Neale MC, Franz CE, Lyons MJ, Pacheco J, Perry ME, Stevens A, Schmitt JE, Grant MD, Seidman LJ, Thermenos HW, Tsuang MT, Eisen SA, Dale AM, & **Fennema-Notestine** C. (2010). Genetic and environmental influences on the size of specific brain regions in midlife: the VETSA MRI study. *Neuroimage, 49*(2), 1213-1223. Epub Date 2009/09/30. PMCID: 3397915.
    c. **Fennema-Notestine** C, Panizzon MS, Thompson WR, Chen CH, Eyler LT, Fischl B, Franz CE, Grant MD, Jak AJ, Jernigan TL, Lyons MJ, Neale MC, Seidman LJ, Tsuang MT, Xian H, Dale AM, & Kremen WS. (2011). Presence of ApoE epsilon4 allele associated with thinner frontal cortex in middle age. *Journal of Alzheimer's disease : JAD, 26 Suppl 3*, 49-60. Epub Date 2011/10/06. PMCID: 3302177.
    d. McEvoy LK, **Fennema-Notestine** C, Eyler LT, Franz CE, Hagler DJ, Jr., Lyons MJ, Panizzon MS, Rinker DA, Dale AM, & Kremen WS. (2015). Hypertension-related alterations in white matter microstructure detectable in middle age. *Hypertension*. Epub Date 2015/06/10. PMCID: 4499000.
4. Of particular relevance to my work with white matter disease, I brought my neuroimaging expertise to the study of HIV infection, including structural, diffusion, and spectroscopy MRI explorations and training. Building on prior work demonstrating white matter damage in HIV infection, I found that increases in CD4 cell count, evidence of immune recovery, were associated with an increase in white matter abnormalities, suggesting neuroinflammation may be occurring in the CNS during recovery (a). It is possible that these immune recovery-associated changes are transient and may resolve once recovery has plateaued, although immune recovery lags viral suppression and may continue for years. In another study, we found structural associations, including white matter volume, with metabolic factors (e.g., body mass index) in HIV infection (c). With trainees, we found an association between distal neuropathic pain and cortical gray matter (b), and our recent MR spectroscopy study suggested that monocyte activation and chemotaxis continue to contribute to brain abnormalities in anti-retroviral-treated individuals (d). Recently, I started a study of the Impact of Cannabinoid Use on HIV-related Brain Alterations in Young Adults (NIH R21DA037667); the high rate of cannabis use may impact the progression of HIV infection, given cannabinoids link to greater immune suppression and potential anti-inflammatory effects.
    a. **Fennema-Notestine** C, Ellis RJ, Archibald SL, Jernigan TL, Letendre SL, Notestine RJ, Taylor MJ, Theilmann RJ, Julaton MD, Croteau DJ, Wolfson T, Heaton RK, Gamst AC, Franklin DR, Jr., Clifford DB, Collier AC, Gelman BB, Marra C, McArthur JC, McCutchan JA, Morgello S, Simpson DM, & Grant I for the CHARTER Group. (2013). Increases in brain white matter abnormalities and subcortical gray

matter are linked to CD4 recovery in HIV infection. *Journal of NeuroVirology, 19*(4), 393-401. Epub Date 2013/07/11. PMCID: 3776609.

b. Keltner JR, **Fennema-Notestine** C, Vaida F, Wang D, Franklin DR, Dworkin RH, Sanders C, McCutchan JA, Archibald SL, Miller DJ, Kesidis G, Cushman C, Kim SM, Abramson I, Taylor MJ, Theilmann RJ, Julaton MD, Notestine RJ, Corkran S, Cherner M, Duarte NA, Alexander T, Robinson-Papp J, Gelman BB, Simpson DM, Collier AC, Marra CM, Morgello S, Brown G, Grant I, Atkinson JH, Jernigan TL, & Ellis RJ. (2014). HIV-associated distal neuropathic pain is associated with smaller total cerebral cortical gray matter. *Journal of NeuroVirology, 20*(3), 209-218. Epub Date 2014/02/20. PMCID: 4040150.

c. Archibald SL, McCutchan JA, Sanders C, Wolfson T, Jernigan TL, Ellis RJ, Ances BM, Collier AC, McArthur JC, Morgello S, Simpson DM, Marra C, Gelman BB, Clifford DB, Grant I, & **Fennema-Notestine** C. (2014). Brain morphometric correlates of metabolic variables in HIV: the CHARTER study. Journal of NeuroVirology, 20(6), 603-611. 2014/09/18. PMCID: 4268263.

d. Anderson AM, **Fennema-Notestine** C, Umlauf A, Taylor MJ, Clifford DB, Marra CM, Collier AC, Gelman BB, McArthur JC, McCutchan JA, Simpson DM, Morgello S, Grant I, & Letendre SL. (2015). CSF biomarkers of monocyte activation and chemotaxis correlate with magnetic resonance spectroscopy metabolites during chronic HIV disease. *Journal of NeuroVirology*. Epub Date 2015/06/13. NIHMSID: 700013.

5. In addition, my broadly collaborative efforts have yielded several outcomes to advance our understanding of the impact of normal aging and Alzheimer's disease on the brain. Early work supports the need for careful evaluation of brain and cognitive profiles for which "normal" may be defined differentially in very old age (a & c). We explored the interaction between the ApoE ε4 allele status and age on the rate of decline in AD. Young-old individuals with AD had more severe deficits and steeper declines in cognition and brain structure than the very-old, and the young-old AD carrying an ε4 allele were the most significantly impacted. This work underscores the importance of integrating age and genetic susceptibility when examining neuropsychological and neuroimaging changes in aging and early AD. Continuing my work with the ADNI sample, I led a study examining the association between cognitive reserve and cortical thickness in aging and mild cognitive impairment (MCI), and we found that early-life education did not provide a protective buffer, in terms of greater cortical thickness, against cognitive decline (b). Several of my prior works were referenced in a recent update by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (d).

a. Stricker NH, Chang YL, **Fennema-Notestine** C, Delano-Wood L, Salmon DP, Bondi MW, & Dale AM for the Alzheimer's Disease Neuroimaging Initiative. (2011). Distinct profiles of brain and cognitive changes in the very old with Alzheimer disease. *Neurology, 77*(8), 713-721. Epub Date 2011/08/13. PMCID: 3164395

b. Pillai JA, McEvoy LK, Hagler DJ, Jr., Holland D, Dale AM, Salmon DP, Galasko D, & **Fennema-Notestine** C for the ADNI. (2012). Higher education is not associated with greater cortical thickness in brain areas related to literacy or intelligence in normal aging or mild cognitive impairment. *Journal of Clinical and Experimental Neuropsychology*, 34(9), 925-935. Epub Date 2012/08/22. PMCID: 3488147.

c. Chang YL, **Fennema-Notestine** C, Holland D, McEvoy LK, Stricker NH, Salmon DP, Dale AM, & Bondi MW for the ADNI. (2013). APOE interacts with age to modify rate of decline in cognitive and brain changes in Alzheimer's disease. *Alzheimer's and Dementia: the Journal of the Alzheimer's Association, 10*(3), 336-348. Epub Date 2013/07/26. PMCID: 3815680.

d. Jack CR, Jr., Barnes J, Bernstein MA, Borowski BJ, Brewer J, Clegg S, Dale AM, Carmichael O, Ching C, DeCarli C, Desikan RS, **Fennema-Notestine** C, Fjell AM, Fletcher E, Fox NC, Gunter J, Gutman BA, Holland D, Hua X, Insel P, Kantarci K, Killiany RJ, Krueger G, Leung KK, Mackin S, Maillard P, Malone IB, Mattsson N, McEvoy L, Modat M, Mueller S, Nosheny R, Ourselin S, Schuff N, Senjem ML, Simonson A, Thompson PM, Rettmann D, Vemuri P, Walhovd K, Zhao Y, Zuk S, & Weiner M. (2015). Magnetic resonance imaging in Alzheimer's Disease Neuroimaging Initiative 2. *Alzheimer's & Dementia : the Journal of the Alzheimer's Association, 11*(7), 740-756. Epub Date 2015/07/22. OPEN Access: http://www.sciencedirect.com/science/article/pii/S1552526015001685.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/christine.fennemanotestine.1/bibliography/45429187/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

R21 DA037667 (MPI Fennema-Notestine, C & Nichols, S) 05/01/2015-04/30/2017
NIH/NIDA
Impact of Cannabinoid Use on HIV-related Brain Alterations in Young Adults
The project will develop a multimodal approach to elucidate the combined effects of HIV infection and cannabis use on the brain in youth, using multimodal neuroimaging, cognitive assessments, and plasma biomarkers.
Role: Principal Investigator

2 R01 AG022381-12A1 (Kremen, William) 07/01/2015-03/31/2020
NIH/NIA
The VETSA Longitudinal MRI Twin Study of Aging
This project continues to follow a community sample of male twins entering their late 60s/early 70s with multi-modal neuroimaging, neuropsychological, and physical testing to understand age-related changes in the brain.
Role: Co-Investigator

R01MH107345 (Heaton R./Letendre S.) 09/24/2015-06/30/2020
NIH/NIA
Age Related Determinants of HAND: A 12 Year Follow-up of CHARTER Participants
A long term, longitudinal study to determine the presence, nature and mechanisms of premature or accelerated CNS aging in HIV infection. Neuromedical, cognitive, genetic, and neuroimaging outcomes will be explored in HIV infected adults who received initial examinations between 2003 and 2007.
Role: Co-Investigator

P50 DA26306 (Grant, Igor) 06/01/2014-05/31/2018
NIH
Translational Methamphetamine AIDS Research Center (TMARC)
A center that examines the combined effects of methamphetamine exposure and HIV-related neuropathology on cognitive, reward and motivational function.
Role: Co-Investigator

P30 MH62512 (Heaton, Robert K) 04/01/2016-03/31/2021
NIH
HIV Neurobehavioral Research Center (HNRC)
This center integrates and supports research into etiology, pathogenesis, phenomenology, treatment, and prevention of neuroAIDS, including a specific neuroimaging core component.
Role: Co-Investigator

R01 NS080655 (Thompson, Paul) 08/01/2012-07/31/2016
NIH
Predicting Brain Changes in HIV/AIDS
This program will implement novel innovations in MRI and diffusion tensor imaging to chart the dynamics of HIV disease in the brain, revealing factors that predict clinical decline and brain decline.
Role: Co-Investigator

1 R01 DA039775 (Bharti, Ajay) 08/01/2015-05/31/2020
NIH/NIDA
Impact of HCV Therapy on CNS Outcomes
The project aims to explore the impact of early treatment of HCV in substance using populations to prevent or treat its effect on the brain as well as the liver, which may shift the current approach to identifying candidates for therapy. Outcomes include change in medical, cognitive, neuromedical, and neuroimaging measures.
Role: Co-Investigator

1 R01 AG048650-01A1 (Ellis, Ronald Joseph) 09/01/2015-05/31/2020
NIH/NIA
Phase II Trial of Tesamorelin for Cognition in Aging HIV-infected Persons
The multi-site project will explore a novel treatment strategy to improve cognition and brain health in HIV+ individuals by reducing both bad fat in those with abdominal obesity and associated inflammation associated.
Role: Co-Investigator

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Henry Jay Forman

eRA COMMONS USER NAME (credential, e.g., agency login): hforman

POSITION TITLE: Professor of Research Gerontology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Queens College, City Univ. N.Y.	B.A.	1963-67	Chemistry
Columbia University, N.Y. (with Philip Feigelson)	Ph.D.	1967-71	Biochemistry
Duke University, Durham, N.C. (with Irwin Fridovich)	Postdoctoral	1971-73	Biochemistry

A. Personal Statement

My primary research is in the fields of redox signaling and adaptation to oxidative stress. My interest in redox biology began during my graduate studies, beginning with studies on the kinetics of an enzyme that had superoxide as an intermediate, postdoctoral work on the enzymology of the superoxide dismutases, continuing through studies on induction of antioxidant enzymes that continues today, pioneering work on redox signaling that also continues. Our current studies focus on both the oxidative aspects of sickle cell disease and, in this proposal, on the age-induced alterations in redox signaling that result in greater basal expression of antioxidant enzymes and pro-inflammatory cytokines, but a blunted antioxidant response that inhibits protective adaptive changes in the elderly. I have published over 200 peer-reviewed articles and over 40 book chapters. I am Past President of the Society for Free Radical Biology and Medicine and served many years as the Reviews Editor for Free Radical Biology & Medicine. I am now the Editor-in-Chief of Archives of Biochemistry and Biophysics. While my research has been very focused on redox biology, my training and teaching experience has ranged from organic chemistry to physiology. This broad experience has influenced the approaches I take to research. In 2016, I won both the Award Lectureship from the Society for Free Radical Research – Europe and the Lifetime Achievement Award from the Society for Redox Biology and Medicine. For this proposal, I will supervise part of Core C and participate in planning of the experiments that produce the samples to be assayed in the core. In addition to being a Professor of Research Gerontology at USC, I am a Distinguished Professor Emeritus of Biochemistry at UC Merced.

B. Positions and Honors

Univ. Kansas Medical School and VA Hosp., K.C., MO: Research Associate, 1973-74; Investigator, 1974-77; Adjunct Asst. Professor of Biochemistry, 1977
Univ. Pennsylvania: Asst. Professor of Physiology, 1978-82; Associate Professor, 1982-86
Univ. Southern California: Assoc. Professor of Pediatrics, Pathology, and Toxicology, 1986-89; Head, Cell Biology Group, Childrens Hospital, 1989-93; Professor of Molecular Pharmacology & Toxicology, Pediatrics and Pathology, 1989-99
Univ. Alabama, Birmingham: Professor and Chair of Environmental Health Sciences, 1999-2003
Univ. California, Merced: Professor of Chemistry and Biochemistry, 2003-15 (Distinguished Professor, 2010-15), Distinguished Professor Emeritus, 2015-
Univ. Southern California: Research Professor of Gerontology, 2009-

Editorial and Review Boards: Archives of Biochemistry and Biophysics, 1991-, Reviews Editor, 2015, Editor-in-Chief, 2015-
Free Radical Biology & Medicine, 1985-2015, Associate Editor 1996-2015, Reviews Editor 2006-2014 American Journal of Respiratory Cell and Molecular Biology, 1995-2003
Member of Environmental Health Sciences Review Committee, NIEHS, 1996-99
Numerous NIH study sections and special emphasis panels, 1981-
NIEHS Advisory Board of Scientific Counselors, 2009
Member of the Governing Board, San Joaquin Valley Air Pollution Control District, 2008-13

Honors: Queens College Honor Chemistry Society, 1969
Columbia University Fellowship, 1967-71
N.I.H. Postdoctoral Fellowship, 1971-73
John Morgan Society (University of Pennsylvania)
Fellow of the Oxygen Society (now Society for Free Radical Biology & Medicine)
Charles Krown/Pharmacy Alumni Professorship (University of Southern California), 1995-99
Sigma Xi Distinguished Scientist Award, UC Merced Chapter 2008
Distinguished Professor, University of California Merced, 2010
Lifetime Membership in International 4-Hydroxynonenal Club, 2012
Distinguished Service Award, Society for Free Radical Biology and Medicine, 2014
Award Lectureship Society for Free Radical Research – Europe, 2016
Lifetime Achievement Award, Society for Redox Biology and Medicine, 2016

C. Contribution to Science

1. Early work on superoxide biochemistry. My graduate work was on the kinetics, catalytic mechanism and allosteric regulation of tryptophan oxygenase in which I demonstrated that superoxide was an intermediate. My interest in superoxide led me to a postdoctoral fellowship with Irwin Fridovich in which I did some of the early work on superoxide dismutase (SOD) and in using SOD as a tool to investigate redox chemistry. A study on the role of zinc in cytosolic SOD led others year later to investigate the role of zinc binding in an aberrant form of SOD producing an inherited form of Amyotrophic Lateral Sclerosis.

Forman, H.J. and Feigelson, P. Kinetic evidence indicating the absence during catalysis of an unbound ferroprotoporphyrin form of tryptophan oxygenase. Biochemistry 10: 760-763, 1971.

Forman, H.J. and Fridovich, I. Electrolytic univalent reduction of oxygen in aqueous solution demonstrated with superoxide dismutase. Science 175: 339, 1972.

Forman, H.J. and Fridovich, I. On the stability of bovine superoxide dismutase: the effects of metals. J. Biol. Chem. 248: 2645-2649, 1973.

2. Mitochondrial production of superoxide. In the course of investigating the role of dihydroorotate dehydrogenase in mammalian pyrimidine biosynthesis, I found that the enzyme, which transfers electrons to complex III in mitochondria, could also cause production of superoxide. In that first study, I demonstrated that mitochondrial SOD was responsible for pulling the reaction forward by displacing a kinetically and thermodynamically unfavorable reaction, which subsequently was shown to be the autooxidation of ubisemiquinone in complex III. In the 1970s and early 1980s, there was significant skepticism about the physiological significance of mitochondrial superoxide production, but now, it is thought to be responsible for a large variety of physiological and pathological processes.

Forman, H.J. and Kennedy, J.A. Role of superoxide radical in mitochondrial dehydrogenase reactions. Biochem. Biophys. Res. Commun. 60: 1044-1050, 1974

Forman, H.J. and Kennedy, J. Superoxide production and electron transport in mitochondrial oxidation of dihydroorotic acid. J. Biol. Chem. 250: 4322-4326, 1975.

3. Pulmonary oxidative stress, induction of antioxidant enzymes, and early studies on signal transduction in macrophages. I joined Aron Fisher at the University of Pennsylvania in a program project on pulmonary oxygen toxicity. We did a number of studies on oxygen and paraquat toxicity, which I continued for many years and are now relevant to studies of sickle cell disease and other hemoglobinopathies, which have a large oxidative component due to hemolysis. But my focus turned toward two other areas that continue as interests to this day; adaptation to oxidative stress and oxidant-altered signal transduction. We demonstrated that several antioxidant enzymes increased in type II pneumocytes in the lungs of rats that adapted to breathing high O_2 concentrations

that permitted rats to survive in subsequent exposure to 100% O_2. Although many studies were focused on how oxidative stress caused irreversible damage and death, realizing that we get sick many more times than we die, led me to study altered cell function by oxidants, particularly focusing on altered calcium signaling. Along the way, I also realized that to study pathophysiology meant I needed to investigate normal physiology and biochemistry as well. Thus, we showed that in the normal respiratory burst of macrophages, NADPH rather than NADH was the required substrate for the NADPH oxidase.

Forman, H.J., Nelson, J. and Fisher, A.B. Rat alveolar macrophages require NADPH for superoxide production in the respiratory burst. J. Biol. Chem. 255: 9879-9883, 1980.

Forman, H.J. and Fisher, A.B. Antioxidant enzymes in rat granular pneumocytes: constitutive levels and effect of hyperoxia. Lab. Invest. 45: 1-6, 1981.

4. Glutathione biosynthesis. One of the major adaptive responses to oxidative stress is an increase in glutathione (GSH) through induction of both subunits of the first enzyme in its de novo, glutamate cysteine ligase (GCL) and an essential enzyme in the so-called GSH scavenger pathway, γ-glutamyl transpeptidase (GGT). My laboratory demonstrated that quinones, which can both conjugate to protein thiols and redox cycle to generate superoxide and H_2O_2 induced GCL and GGT. My lab has continued to make important advances in understanding how the transcription of these genes are regulated by coordination of Nrf2/EpRE and AP-1/TRE signaling pathways. More recently, we have demonstrated that both GCL genes and other genes regulated by Nrf2 change from being inducible by oxidants in young mice become uninducible in aging mice at least in part through an increase in the Nrf2 inhibitors c-Myc and Bach1 with age. We also did studies on GSH transport in cystic fibrosis, demonstrating its regulation by CFTR.

Shi, M.M., Kugelman, A., Iwamoto, T., Tian, L. and Forman, H.J. Quinone-induced oxidative stress elevates glutathione and induces γ-glutamylcysteine synthetase activity in rat lung epithelial L2 cells. J. Biol. Chem. 269: 26512-26517, 1994.

Levy, S., and Forman, H.J. c-Myc is a Nrf2-interacting protein that negatively regulates phase II genes through their electrophile responsive elements. IUBMB Life 62: 237-246, 2010

Zhang, H., Liu, H., Davies, K.J.A., Sioutas, C., Morgan, T., Finch, C.E., and Forman, H.J. Nrf2-regulated phase II enzymes are induced by chronic ambient nanoparticle exposure in young mice with age-related impairments. Free. Radic. Biol. Med. 52: 2038-2046, 2012.

5. Redox signaling. Studies on redox signaling, which overlaps with the other areas above began with the realization that oxidants at low concentration enhanced rather than inhibited functions in alveolar macrophages. This led us to investigate the role of endogenously generated H_2O_2 as a second messenger. An early publication in this area was our demonstration that H_2O_2 made by stimulated macrophages activated NF-κB signaling (43). At that time, phagocytes were the only cells known to produce H2O2 upon stimulation of an NADPH oxidase rather than as a byproduct of metabolism. Many other studies had previously demonstrated signaling by the addition of exogenous H_2O_2. So, our report was looked upon as a curiosity of phagocytes until the discovery by Lambeth of the multiple and ubiquitous NADPH oxidases. Regardless, how H_2O_2 functioned as a second messenger was not revealed until it became apparent that some of the targets formed protein mixed disulfides or adducts to protein cysteines. We demonstrated the glutathionylation of PTP1B by endogenously generated H_2O_2 and suggested that this and other H_2O_2-mediated signaling was enzymatically driven rather than dependent on non-enzymatic protein cysteine oxidation that is far too slow in most cases to account for the observed activation. The involvement of an enzymatic process was then demonstrated in the peroxiredoxin/thioredoxin oxidation mediated activation of JNK in stimulated macrophages. We have also demonstrated that electrophiles, such as the commonly generated lipid peroxidation product 4-hydroxy-2-nonenal, have specific target thiols, including another protein tyrosine phosphatase, SHP-1. We have shown that TGF-β1 activation of Src kinase involves the oxidative modification of Src cysteines. We have also studied NF-κB dependent signaling stimulated in macrophages by environmentally relevant levels of silica particles and determined that it depends on both the activation of H_2O_2 production and the presence of iron on the surface that causes a lipid raft disruption through very minor lipid peroxidation. Subsequently, the products from the NF-κB pathway can activate the Nrf2 signaling pathway in macrophages.

Kaul, N. and Forman, H.J. Activation of NF-κB by the respiratory burst of macrophages. Free Radic. Biol. Med. 21: 401-405, 1996.

Rinna, A. Torres, M., and Forman, H.J. Stimulation of the alveolar macrophage respiratory burst by ADP causes selective glutathionylation of protein tyrosine phosphatase 1B. Free Radic. Biol. Med. 41: 86-91, 2006.

Premasekharan, G., Nguyen, K., Contreras, J., Ramon, V., Leppert, V.J., and Forman, H.J.

Iron-mediated lipid peroxidation and lipid raft disruption in low-dose silica-induced macrophage cytokine production. Free Radic. Biol. Med. 51: 1184–1194, 2011

Zhang, H., Davies, K.J.A., and Forman, H.J. TGFβ1 rapidly activates Src through a non-canonical redox signaling mechanism. Arch. Biochem. Biophys. 568: 1-7, 2015

Zhang, H., Zhou, L., Yuen, J., Birkner, N., Leppert, V., O'Day, P.A., and Forman, H.J. Delayed Nrf2-regulated antioxidant gene induction in response to silica nanoparticles. Free Radic Biol Med. 108: 311-319, 2017

Peer reviewed articles can be found at http://www.ncbi.nlm.nih.gov/sites/myncbi/henry.forman.1/bibliography/41145654/public/?sort=date&direction=ascending while all my publications including books and book chapters can be found at https://sites.google.com/site/hjforman/home/publications'

D. Additional Information: Research Support and/or Scholastic Performance

R01 ES023864-01 Forman, PI 2/9/15-10/30/20
Human models of the particulate-induced inflammatory/antioxidant axis in aging
This grant focuses on the effect of aging on the balance between pro-inflammatory and anti-oxidant defenses in response to air pollution nanoparticles.

R01 ES 003598 NIH Davies, PI 7/1/13-6/30/18
Oxygen radical toxicity and protein degradation
This focuses on the role of the 20S proteasome, the immunoproteasome, and the Pa28 proteasome regulator in adaptation to oxidative stress.
Role: Investigator

U54 HL117718 NIH Coates, PI 7/1/13-6/30/18
Multimodal biophysical markers of vascular disease in hemoglobinopathies
This focuses on identification of the physiological and biochemical differences within sickle cell populations leading to differences in outcome.
Role: PI on subcontract

Completed in past three years

R56 ES023864 Forman, PI 6/1/14 - 5/31/15
Human models of the particulate-induced inflammatory/antioxidant axis in aging
See R01 ES023864 above

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Carol E. Franz, PhD

eRA COMMONS USER NAME (credential, e.g., agency login):CEFRANZ

POSITION TITLE: ASSOCIATE PROFESSOR

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Gordon College, Wenham, MA	BA	06/80	Psychology
Boston University, Boston, MA	PhD	06/88	Psychology
Harvard University, Cambridge, MA	Post-Doc	06/92	Human Motivation and Adult Development

A. PERSONAL STATEMENT.

I am Associate Professor in the UCSD Department of Psychiatry and associate director of the twin research laboratory at the Center for Behavioral Genomics. I have been a co-investigator and co-director on all of the VETSA studies (VETSA 1 & 2; VETSA MRI 1 & 2; VETSA Cortisol; VETSA MRI 3 and VETSA 3) since their inception in 2002. My expertise is in genetic and environmental influences on cognitive aging as it relates to psychosocial and health factors with a focus on stress, adaptive mechanisms, and aging. I developed the psychosocial/personality batteries for the first two VETSA data collections and was part of the team that developed the health measures. I am trained as a personality psychologist, specializing in adult development; my training emphasized secondary data analysis and longitudinal methods. In my postdoctoral fellowship at Harvard University with Dr. David McClelland examined adult development and human motivation across 40 years of life, thus solidifying my knowledge of mid-life development at a time when few researchers investigated that life period. I later trained with Dr. Jack Block, at UC Berkeley, working on issues related to life course development and longitudinal design. At UC Davis, I was mentored by Richard Kravitz, MD, MPH in health services research with a focus on depression, dementia, health care, and barriers to care. With VETSA and other investigators I have worked on a variety of papers linking life experiences, stress, personality/psychosocial characteristics, health, biological systems (e.g. cortisol) with cognition, and brain. My expertise in Alzheimer's disease (AD) and cognitive aging includes my collaboration with Ladson Hinton MD at UC Davis on studies of caregivers of elderly with dementia and the role of primary care in care for elderly adults with AD. With Dr. Kremen I am co-director of the VETSA projects and the Center for Behavior Genetics of Aging at UCSD. I have worked closely on research studies with Drs. Kremen, Fennema-Notestine, Hagler, and Smith for over a decade. In summary, my demonstrated experience as a researcher on aging, my scientific background in genetic and environmental influences on aging, health, personality and life course development, my technical skills, and effective collaborations position me to be an effective PI for this study. This project moves the VETSA study in a new direction for these investigators—towards the exploration of putative environmental influences in the context of a genetically informed study.

B. POSITIONS AND HONORS

Positions and Employment

1981-1987 Graduate Research Assistant; Boston University
1987-1992 Post-Doctoral Research Fellow: Co-Principal Investigator of Midlife follow-up of Sears,

	Maccoby and Levin Patterns of Childrearing sample, Harvard University
1987-1992	Research Associate, Center for Applied Social Science, Boston University
1991-1992	Lecturer, Department of Psychology, Boston University
1992-1993	Visiting Assistant Professor, Dept. of Psychology, University of Michigan, Ann Arbor
1993-1994	Visiting Assistant Professor, Dept. of Psychology, Williams College, MA
1994-1996	Research Psychologist, Institute of Human Development, University of California, Berkeley
1997-1998	Research Manager, Center for Health Services Research in Primary Care, University of California, Davis, Department of Internal Medicine
1998-2002	Research Manager, University of California, Davis, Department of Psychiatry
2002-2004	Senior Research Analyst, Center for Health Services Research in Primary Care, UC Davis
2004-2009	Associate Project Scientist, Department of Psychiatry, University of California, San Diego
2009-2015	Assistant Professor, Department of Psychiatry, University of California, San Diego
2004-present	Associate Director, Twin Research Laboratory, Department of Psychiatry, University of California, San Diego
2015-	Associate Professor, Department of Psychiatry, University of California, San Diego

Committees and Professional Memberships

1985-	American Psychological Association
1998-2002	Research Advisory Board, Department of Psychiatry, UC Davis, 1998-2002
1998-2002	Faculty-Alumni Research Award Committee, Department of Psychiatry, UC Davis 1998-2002
1998-2002	Mental Health Services Research Group, Department of Psychiatry, UC Davis, 1998-2002
2002-2004	Center for Health Services Research in Primary Care (CHSRPC), Research Leadership Committee, UC Davis, 2002-2004
2003-	Gerontological Society of America
2008-	Behavior Genetics Association
2005-	International Society of Twin Studies
2013-	Association for Psychological Science
2015	Planning committee: UCSD Annual Women's Conference
2015	Planning committee: 2015 Behavior Genetics Annual Meeting, held in San Diego, CA
2015-	Member: UCSD Human Research Protections Review Committee
2016-	Chair's Advisory Committee on Diversity Issues
2016-	Chair's Task Force on Health Sciences Faculty Climate Survey; Leader of "Behavior and Civility" Task Force.

Honors and Awards

1980	Summa cum laude, Gordon College
1984	Clara Mayo Award for Outstanding Research on Women, Boston University
1986-87	Graduate School Dissertation Scholarship Award, Boston University
2006	Society for General Internal Medicine Article of the Year award (JAMA, 2005 co-author)
2006	AcademyHealth Article of the Year award (JAMA 2005: co-author): Kravitz RL, Epstein R, Feldman MD, **Franz CE**, Azari R, Wilkes MS, Hinton LH, Franks P (2005). Influence of patients' requests for direct-to-consumer advertised antidepressants: A randomized controlled trial. JAMA, 1995-2002. PMCID 1924631

C. CONTRIBUTIONS TO SCIENCE.

List of Published Work (Journal Articles, Book, Book Chapter) From NCBI:

http://www.ncbi.nlm.nih.gov/sites/myncbi/carol.franz.1/bibliography/40386178/public/?sort=date&direction=ascending

D.Research Support.

ACTIVE

R01 AG046413 (Franz) 09/1/13—08/31/16
NIH/NIA
Archiving the Vietnam Era Twin Studies of Aging (VETSA): New Uses for Old Data
The goal of this grant is to electronically archive all grants associated with the VETSA projects, making them DDI compliant and to archive the data in the national archives at ICPSR. Role: PI

R01 AG037985 (Pedersen) 09/01/11—08/31/15
NIH/NIA
Gene-Environment Interplay of Social Contexts and Aging-Related Outcomes
The goal of this grant (aka the "iGEMS" consortium) is to identify social and environmental measures common to 8 different twin studies of aging, so as to examine the influence of gene by environment interactions on aging. Role: Co-Investigator

*R01AG022381 (Kremen) 07/01/15 - 03/30/20
NIH/UC San Diego
The VETSA Longitudinal MRI Twin Study of Aging
To study cognitive and psychological function and brain development in a 50-60 year-old sample of veteran twins.

*R01AG050595 (W. Kremen & M Lyons) 9/01/2015–8/31/2019
NIH/NIA \
The VETSA Longitudinal Twin Study of Cognition and Aging
The goal of this study is to collect a third wave of data to determine genetic and environmental influences on cognitive, personality/ psychosocial, and health/medical measures in middle aged twins. The study examines the inter-relationships among these domains and their ability to predict cognitive and adaptive aging. Role: Co-Investigator. **C. CONTRIBUTIONS TO SCIENCE.**

1. Stress and aging: It has long been recognized that stress affects multiple aspects of the aging body and brain, including physical and mental health, as well as cognition; however mechanisms by which stress and aging are linked are poorly understood. With the VETSA studies my colleagues and I have made unique contributions to our understanding of the genetic and environmental influences on the HPA axis, new findings regarding associations between cortisol and brain structures, and on longitudinal influences on the relationships between midlife cortisol and cognition. I was lead author on all of this work.

a. Franz CE, York TP, Eaves LJ, Mendoza SP, Hauger RL, Hellhammer DH, Jacobson KC, Levine S, Lupien SJ, Lyons MJ, Prom-Wormley E, Xian H, Kremen WS (2010) Genetic and environmental influences on cortisol regulation across days and contexts in middle-aged men. Behavior Genetics, 40, 467-479.

b. Kremen WS, O'Brien RC, Panizzon MS, Prom-Wormley E, Eaves LJ, Eisen SA, Eyler LT, Hauger RL, Fennema-Notestine C, Fischl B, Grant MD, Hellhammer DH, Jak AJ, Jacobson KC, Jernigan TL, Lupien SJ, Lyons MJ, Mendoza SP, Neale MC, Seidman LJ, Thermenos HW, Tsuang MT, Dale AM, **Franz CE** (2010) Salivary Cortisol and Prefrontal Cortical Thickness in Middle-Aged Men: A Twin Study. Neuroimage, 53, 1093-1102.

c. **Franz CE**, O'Brien RC, Hauger RL, Mendoza SP, Panizzon MS, Prom-Wormley E, Eaves LJ, Jacobson K, Lyons MJ, Lupien S, Hellhammer D, Xian H, Kremen WS. Cross-sectional and 35-year longitudinal assessment of salivary cortisol and cognitive functioning: The Vietnam Era Twin Study of Aging. *Psychoneuroendocrinology.* Aug 2011;36(7):1040-1052. PMCID 3130089.

d) **Franz, C. E.**, Lyons, M. J., Spoon, K. M., Hauger, R. L., Jacobson, K. C., Lohr, J. B., McKenzie, R., Panizzon, M. S., Thompson, W. K., Tsuang, M. T., Vasilopoulos, T., Vuoksimaa, E., Xian, H., & Kremen, W. S. (2014). Post-traumatic Stress Symptoms and Adult Attachment: A 24-year Longitudinal Study. *Am J Geriatr Psychiatry, 22,* 1602-1612.

2. Mild cognitive impairment/Alzheimer's Disease. The above studies focus more on my contributions to normal or typical adult functioning; yet a key aspect of aging is pathological cognitive aging. This problem affects individuals, families, institutions, and public policy. Solving the important problem of the growing number of elderly with dementia requires both research that addresses identification of and causes of dementia; as well as social and institutional barriers to care. One of my contributions to science includes my approach to research which emphasizes the necessity of using multiple methods to fully understand a problem--shown here as ranging from in-depth qualitative analysis of caregivers of elderly with dementia to examination of the heritability of MCI and its association with brain structure. This is highlighted in the

influential multi-method, multi-cohort work I have done with different groups to better understand cognitive aging, mild cognitive impairment and Alzheimer's Disease.

a) Hinton WL, **Franz CE**, Friend J. (2004). Pathways to dementia diagnosis in a multiethnic sample: A qualitative study of help-seeking patterns and family concerns about the quality of their care. *Alzheimer Disease and Associated Disorders. 18, 134-144.*

b) **Franz CE,** Kravitz RL, Barker JC, Flores Y, Krishnan S, Hinton L. Non-medical influences on the use of cholinesterase inhibitors in dementia care. *Alzheimer Disease and Related Disorders*, 21, 241-248.

c) Kremen, W. S., Jak, A. J., Panizzon, M. S., Spoon, K. M., **Franz, C. E.**, Thompson, W. K., Jacobson, K., Vasilopoulos, T., Vuoksimaa, E., Xian, H., Toomey, R., & Lyons, M. J. (2014). Early identification and heritability of mild cognitive impairment. *Int J Epidemiol, 43*(2), 600-610. PMCID: 3997374

d) Jak, A.J.**,** Panizzon, M.S., Spoon, K.M., Fennema-Notestine, C., **Franz, C.E.**, Thompson, W.K., Jacobson, K.C., Xian, H., Eyler, L.T. Vuoksimaa, E., Toomey, R., Lyons, M.J., Neale, M. C., Fischl, B., Tsuang, M.T., Dale, A.M., & Kremen, W.S. (2015). Hippocampal Atrophy Varies by Neuropsychologically-Defined Subtype of MCI among Men in Their 50s. *American Journal of Geriatric Psychiatry*, 23: 456-465.

3) Physical Health and Aging. Two key aspects of aging are the contributions of physical and psychological health to cognitive aging. Through my work with VETSA and other studies, I have made important and unique contributions to both areas. Here I highlight work focusing on physical health factors that play key roles in aging; behavior genetic studies of aging starting in middle-age have been rare and importantly contribute to early identification of cognitive and brain aging and associated risks.

a) **Franz CE**, Grant MD, Jacobson KC, Kremen WS, Eisen SA, Xian H, Romeis J, Thompson-Brenner H, Lyons MJ. (2007) Genetics of body mass stability and risk for chronic disease: A 28-year longitudinal study. Twin Research and Human Genetics, 10, 537-545.

b) Xian, H., Vasilopoulos, T., Liu, W., Hauger, R. L., Jacobson, K. C., Lyons, M. J., . .**Franz, C. E.** (2017). Steeper change in body mass across four decades predicts poorer cardiometabolic outcomes at midlife. *Obesity (Silver Spring), 25*(4), 773-780. doi:10.1002/oby.21791

c) Vasilopoulos, T., Kremen, WS , Grant, MD, Panizzon, MS, Xian, H. Toomey, RT, Lyons, MJ, , Jacobson KC., **Franz, CE** (2015) Individual Differences in Cognitive Ability at Age 20 Predict Pulmonary Function 35 Years Later, Journal of Epidemiology & Community Health, 69, 261-265.

d) Panizzon, M.S., Hauger, R.L., Sailors, M, Lyons, MJ, Jacobson, K.C., McKenzie, R.M., Rana, B, Vasilopoulos, T. Vuoksimaa, E. Xian, H., Kremen, W.S., and **Franz, C.E.*,** 2016, A new look at the genetic and environmental coherence of metabolic syndrome components, *Obesity.**** Senior and Corresponding Author**

4) Psychological Health, Personality and Aging. Psychological health and personality play important roles in cognitive and brain aging yet there is very little study of these factors starting in middle age, especially with regard to genetic and environmental influences. Through my work with VETSA and other studies, I have made important and unique contributions to both areas.

a) **Franz CE**, Lyons MJ, O'Brien RC, Panizzon MS, Kim K, Bhat R, Grant MD, Toomey R, Eisen S, Xian H, Kremen WS (2011) A 35-Year Longitudinal Assessment of Cognition and Midlife Depression Symptoms: The Vietnam Era Twin Study of Aging. American Journal of Geriatric Psychiatry, 19, 559-570.

b) Finkel, D.*, **Franz, C.E**.*, Horwitz, B., Christensen, K., Gatz, M., Johnson, W., Kaprio, J., Korhonen, T., Neiderhiser, J., Petersen, I., Rose, R.J., Silventoinen, K., & IGEMS Consortium. (in press). Marital status moderates gender differences in genetic and environmental influences on subjective health. Psychology and Aging. *Joint senior authors.

c) McCaffery, J. M., **Franz, C. E**., Jacobson, K., Leahey, T. M., Xian, H., Wing, R. R., Lyons, M. J., & Kremen, W. S. (2011). Effects of social contact and zygosity on 21-y weight change in male twins. *Am J Clin Nutr, 94*(2), 404-409. PMCID: 3142719

d) Lewis G, Panizzon MS, Eyler L, Fennema-Notestine C, Chen CH, Neale MC, Jernigan T, Lyons MJ, Dale AM, Kremen WS, & **Franz CE (2014)**, Heritable Influences on Amygdala and Orbitofrontal Cortex Contribute to Genetic Variation in Core Dimensions of Personality, Neuroimage, 103C, 309-315.

5) Early influences on Aging. Since the beginning of my career I have been interested in associations between childhood/young adult experiences and aging. Here my most important contributions, and highly cited papers, focused on three types of risk/protective factors: childhood SES and familial experiences, early cognitive ability; and combat exposure. Although attachments (close relationships) as a core feature of adult

development and adjustment is now widely accepted; this was not the case in 1985 when I wrote my first theoretical paper on the overlooked role of attachments in life course development. Since the original paper I have published a number of important papers examining the origins and outcomes of key relationships from a variety of studies and types of relationships. This includes an influential study published in JAMA for which I was senior project manager and co-investigator examining the role of physician patient relationships in the diagnosis and treatment of depression (Kravitz et al 2005, JAMA).

The range of my research expertise is also one of my unique contributions to science in that I have led high quality complex projects to completion across my career ranging from qualitative projects, to experimental studies, to large-scale longitudinal epidemiological studies.

a) Koestner R, **Franz CE**, & Weinberger J. (1990) The family origins of empathic concern: A 26 year longitudinal study. *Journal of Personality and Social Psychology*, 58, 709-717.

b) **Franz CE**, McClelland DC, & Weinberger J. (1991) Childhood antecedents of conventional social accomplishment in midlife adults. *Journal of Personality and Social Psychology*, 60, 586-595.

c) Franz CE, York TP, Eaves LJ, Prom-Wormley E, Jacobson KC, Lyons MJ, Grant MD, Xian H, Panizzon MS, Jimenez E, Kremen WS. Adult romantic attachment, negative emotionality, and depressive symptoms in middle aged men: a multivariate genetic analysis. *Behav Genet.* Jul 2011;41(4):488-498. PMCID 3121938.

d) **Franz, C. E**., Spoon, K., Thompson, W., Hauger, R. L., Hellhammer, D. H., Jacobson, K. C., Lupien, S., Lyons, M. J., McCaffery, J., McKenzie, R., Mendoza, S. P., Panizzon, M. S., Ramundo, A., Shahroudi, A., & Kremen, W. S. (2013). Adult cognitive ability and socioeconomic status as mediators of the effects of childhood disadvantage on salivary cortisol in aging adults. *Psychoneuroendocrinology, 38*(10), 2127-2139. PMCID:

List of Published Work (Journal Articles, Book, Book Chapter) From NCBI:

http://www.ncbi.nlm.nih.gov/sites/myncbi/carol.franz.1/bibliography/40386178/public/?sort=date&direction=ascending

D. Research Support.

ACTIVE

R56 AG037985 (Pedersen) 9/1/16-8/31/17
NIH/NIA
Gene-Environment Interplay of Social Contexts and Aging-Related Outcomes
The goal of this grant (aka the "iGEMS" consortium) is to identify social and environmental measures common to 8 different twin studies of aging, so as to examine the influence of gene by environment interactions on aging. Role: PI of subaward

R01AG022381 (Kremen) 07/01/15 - 03/30/20
NIH/UC San Diego
The VETSA Longitudinal MRI Twin Study of Aging
The goal of this study is to collect a third wave of data to determine genetic and environmental influences on associations between brain structure and cognitive, personality/ psychosocial, and health/medical outcomes in middle aged twins. The study examines the inter-relationships among these domains and their ability to predict cognitive and adaptive aging.
Role: Co- Investigator; Co-director of the VETSA project.

R01AG050595 (W. Kremen & M Lyons) 9/01/2015–8/31/2019
NIH/NIA
The VETSA Longitudinal Twin Study of Cognition and Aging
The goal of this study is to collect a third wave of data to determine genetic and environmental influences on cognitive, personality/ psychosocial, and health/medical measures in middle aged twins. The study examines the inter-relationships among these domains and their ability to predict cognitive and adaptive aging.
Role: Co- Investigator; Co-director of the VETSA project.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH
DO NOT EXCEED FIVE PAGES.

NAME: Margaret Gatz

eRA COMMONS USER NAME: margat

POSITION TITLE: Professor of Psychology and Gerontology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Rhodes College, Memphis, TN	B.A.	06/1966	Psychology
Duke University, Durham, NC	Ph.D.	09/1972	Clinical Psychology
Center for the Study of Aging and Human Development, Duke University Medical Center	Postdoctoral Research Fellow	08/1973	Gerontology

A. Personal Statement

I have a background in psychology, with a focus on clinical psychology and aging. My primary scholarly focus is the use of longitudinal and twin studies to identify potentially modifiable risk and protective factors across the lifespan, influences that may work in interaction with genes to influence emotional, cognitive, and physical well-being in old age. These interests led me to initiate the Study of Dementia in Swedish Twins and by extension to identifying cases of dementia throughout the Swedish longitudinal twin studies. With these data, we have demonstrated the contribution of cardiovascular disorders, diabetes, and obesity to the risk for Alzheimer's disease and dementia; the role of depression, anxiety, and other psychosocial variables in dementia and cognitive change trajectories; the protective role of education, occupation, and leisure activities; and most recently geographic variation in occurrence of dementia in Sweden. We have also considered sex differences in the relative influence of genetic and environmental factors for liability to Alzheimer's disease and dementia, and gender differences in the importance of various lifestyle and environmental risks for dementia. Presently, as part of the Interplay of Genes and Environment across Multiple Studies (IGEMS) consortium, I am participating in harmonizing data and analyses across multiple twin studies from around the world—including the VETSA study that is in Project-2 of the current application. These data are being harnessed in particular to address health disparities related to socioeconomic class and sex. Additionally, I am working with the Health and Medicine Division of the National Academies of Sciences, Engineering, and Medicine to develop the National Academy of Sciences-National Research Council Twin Registry (WW II Twin Registry) as a scientific resource. Since 2010, I have been a part of the USC AirPollBrain group with Drs. Chen and Finch, serving on the executive committee. I will participate in Project-1 of the current application, which is designed to investigate the contribution of traffic-related air pollution (TRAP) to the risk, susceptibility and mechanisms of AD and related dementias (ADRD) in a nationwide cohort of post-menopausal and older women from the Women's Health Initiative (WHI) Memory Studies (WHIMS). I will facilitate the integration of Project-1 and -2 findings about cognitive functions and cross-comparison of the analytic results.

B. Positions and Honors

Positions and Employment

1973-78 University of Maryland, Dept. of Psychology: Asst. Prof. (1973-78), Assoc. Prof. (1978)
1979-- University of Southern California, Dept. of Psychology: Assoc. Prof. (1979-85), Full Prof. (1985--), Chair of Department (2007-2013)
2000-- Foreign Adjunct Professor, Karolinska Institutet, Dept. of Medical Epidemiology & Biostatistics

Other Experience and Professional Memberships

2003-04 Committee on a Research Agenda for the Social Psychology of Aging, National Academy of Sciences
2003-16 Committee on Twins Studies, Institute of Medicine (Chair, 2013-2016)

Honors

1997 Distinguished Mentorship Award, BSS Section, Gerontological Society of America
1999 Master Mentor Award, American Psychological Assoc., Div. 20/Retirement Research Foundation
2003-04 Zenith Award, Alzheimer's Association
2005 Distinguished Research Achievement Award, American Psychological Association, Div. 20
2006 Donald F. Kent Award, Gerontological Society of America
2008 M. Powell Lawton Award for Distinguished Contributions to Clinical Geropsychology, American Psychological Association, Div. 12 Section II
2009 Developmental Health Award, American Psychological Association, Div. 38
2011 Medicine Hedersdoktor (MDhc), Karolinska Institutet
2012 Fellow, American Association for the Advancement of Science

C. Contributions to Science

1. Several of my early publications challenged prevailing views about depression in older adults, which on the one hand exaggerated its prevalence, and on the other hand normalized depression as an expected consequence of growing older. I have also been concerned with how depression is (or is not) detected by health practitioners as well as how depression is measured in surveys of older adults. More recently, with colleagues and students, I have argued that depression with its first onset late in life may represent a prodromal symptom of dementia, and we have identified shared genetic variation between late onset depression and vascular factors.
    a. Gatz, M., & Hurwicz, M-L. (1990). Are old people more depressed? Cross-sectional data on CES-D factors. *Psychology and Aging, 5*, 284-290.
    b. Brommelhoff, J.A., Gatz, M., Johansson, B., McArdle, J.J., Fratiglioni, L., & Pedersen, N.L. (2009). Depression as a risk factor or prodomal feature for dementia? Findings in a population-based sample of Swedish twins. *Psychology and Aging, 24,* 373-384. PMCID: PMC2713179
    c. Kendler, K.S., Fiske, A., Gardner, C.O., & Gatz, M. (2009). Delineation of two genetic pathways to major depression. *Biological Psychiatry, 65*, 808-811. PMCID: PMC2744314
    d. Karlsson, I.K., Bennet, A.M., Ploner, A., Andersson, T., Reynolds, C.A., Gatz, M., & Pedersen, N.L. (2015). Apolipoprotein E ε4 genotype and the temporal relationship between depression and dementia. *Neurobiology of Aging, 36,* 1751-1756. PMCID: PMC4380668

2. Working with a team of collaborators, I have documented the substantial heritability of Alzheimer's disease, indicating the importance of genetic risk for predicting who will develop Alzheimer's disease. Our observations have provided a point of reference for those seeking to identify genes underlying Alzheimer's disease. At the same time, we also pointed to variability in intra-pair differences in age of onset between monozygotic twins concordant for the disorder, demonstrating the complementary and interacting role of the environment.
    a. Gatz, M., Pedersen, N.L., Berg, S., Johansson, B., Johansson, K., Mortimer, J.A., Posner, S.F., Viitanen, M., Winblad, B., & Ahlbom, A. (1997). Heritability for Alzheimer's disease: The Study of Dementia in Swedish Twins. *Journals of Gerontology: Medical Sciences, 52A*, M117-125.
    b. Pedersen, N.L., Gatz, M., Berg, S., & Johansson, B. (2004). How heritable is Alzheimer's disease late in life? Findings from Swedish Twins. *Annals of Neurology, 55,* 180-185.
    c. Gatz, M., Fratiglioni, L., Johansson, B., Berg, S., Mortimer, J.A., Reynolds, C.A., Fiske, A., & Pedersen, N.L. (2005). Complete ascertainment of dementia in the Swedish Twin Registry: the HARMONY study. *Neurobiology of Aging, 26*, 439-447.
    d. Gatz, M., Reynolds, C.A., Fratiglioni, L., Johansson, B., Mortimer, J.A., Berg, S., Fiske, A., & Pedersen, N.L. (2006). The role of genes and environments for explaining Alzheimer's disease. *Archives of General Psychiatry, 63*, 168-174.

3. I have in particular used the twin model in helping to provide evidence for factors that may increase or decrease an individual's risk for dementia, and at the same time worked to disseminate accurate information based on scientific evidence on dementia risk reduction to the larger community.
    a. Gatz, M., Mortimer, J.A., Fratiglioni, L., Johansson, B., Berg, S., Reynolds, C.A., & Pedersen, N.L. (2006). Potentially modifiable risk factors for dementia in identical twins. *Alzheimer's & Dementia, 2*, 110-117.
    b. Gatz, M., Prescott, C.A., & Pedersen, N.L. (2006). Lifestyle risk and delaying factors. *Alzheimer's Disease & Associated Disorders, 20* (Suppl. 2), S84-S88.
    c. Xu W, Atti AR, Gatz M, Pedersen NL, Johansson B, Fratiglioni L (2011). Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. *Neurology, 76*, 1568-1564. PMCID: PMC3100125
    d. Russ, T.C., Gatz, M., Pedersen, N.L., Hannah, J., Wyper, G., Batty, G.D., Deary, I.J., & Starr, J.M. (2015). Geographical variation in dementia: Examining the role of environmental factors in Sweden and Scotland. *Epidemiology, 26,* 263-270. PMCID: PMC4467562

4. I have focused on measurement issues and harmonization of data across studies of older adults, including use of registry data and effect of context of responding to questionnaires.
    a. Jin, Y-P., Gatz, M., Johansson, B., & Pedersen, N.L. (2004). Sensitivity and specificity of dementia coding in two Swedish disease registries. *Neurology, 63*, 739-741.
    b. Sharp, E.S., Suthers, K.M., Crimmins, E., & Gatz, M. (2009). Does "No" mean "Sometimes"? How older adults respond to the same depression symptoms with different response formats. *Clinical Gerontologist, 32,* 1-8. PMCID: PMC2885734
    c. Gatz, M., Reynolds, C.R., Finkel, D., Hahn, C.J., Zhou, Y., & Zavala, C. (2015). Data harmonization in aging research: Not so fast. *Journal of Experimental Aging Research, 41*, 475–495. PMID 26524232

5. I am committed to making databases available to other users, mainly working through the National Archive of Computerized Data on Aging (NACDA), and to fostering collaborative use of datasets.
    a. Gatz, M. & Pedersen, N.L. (2013). Study of Dementia in Swedish Twins. *Twin Research and Human Genetics, 16,* 313-316. PMCID: PMC3860317
    b. Pedersen, N.L., Christensen, K., Dahl, A., Finkel, D., Franz, C.E., Gatz, M., Horwitz, B.N., Johansson, B., Johnson, W., Kremen, W.S., Lyons, M.J., Malmberg, B., McGue, M., Neiderhiser, J.M., Petersen, I., & Reynolds, C.A. (2013). IGEMS: The Consortium on Interplay of Genes and Environment across Multiple Studies. *Twin Research and Human Genetics. 16,* 481-489. PMCID: PMC3699700
    c. Gatz, M., Harris, J.R., Kaprio, J., McGue, M., Smith, N.L., Snieder, H., Spiro III, A., & Butler, D.A. (2014) Cohort profile: The National Academy of Sciences-National Research Council Twin Registry (NAS-NRC Twin Registry). *International Journal of Epidemiology, 44,* 819-25. PMCID: PMC4521123

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/margaret.gatz.1/bibliography/41151679/public/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 AG054442 Kaplan/Gurven/Finch/Thomas (PIs) 04/15/17-03/31/22
Brain atrophy, cognitive impairment, and Alzheimer's in a low CVD-risk population

The purpose of this project is to investigate incidence, prevalence, and predictors of Alzheimer's disease (AD) in Tsimane Amerindians in Bolivia, an indigenous population living a traditional pre-industrial lifestyle. The central hypothesis being tested is that, compared to Westerners, the low rate of atherosclerosis among Tsimane will be paralleled by a slower rate of cerebral atrophy, and reduced age-related cognitive impairment and dementia.
Role: Co-Investigator

P30 AG017265-17S1 Gatz (Project Director), Crimmins (PI on parent grant) 09/15/16-06/30/17
The NAS-NRC WWII Twin Registry as a Scientific Resource

The purpose of this supplement is to expand the data archive comprised of the National Academy of Sciences-National Research Council Twin Registry of twin pairs born 1917-1927 in which both were in the military. We are digitalizing additional information, updating the NACDA archive, and updating dates of death.
Role: Project Director

R56 AG037985 Pedersen/Gatz (PIs) 09/15/16-08/31/17
Gene-Environment Interplay of Social Contexts and Aging-Related Outcomes

The Interplay of Genes and Environment across Multiple Studies (IGEMS) consortium seeks to characterize gene-environment interplay in aging-related outcomes toward understanding mechanisms for socioeconomic status inequalities in cognitive and physical health outcomes. IGEMS includes 15 twin studies from 5 countries. The project includes developing commensurable measures of occupations and education across participating IGEMS studies, then evaluating a model whereby social class differences in health arise in part through gene-environment processes such that individuals with greater education and occupational status are able to manage genetic risk for disease and cognitive impairment through lifestyle choices made possible by social and economic resources that accompany attainment of high SES.
Role: MPI

R01 AG056163 Prescott (PI) 09/15/16 – 04/30/21
Risk for Alzheimer's Disease and Cognitive Decline in Project TALENT

Project Talent is a longitudinal study that began in 1960 with 377,000 U.S. high school students from 1,200 schools, including 2,300 sets of twins and 80,000 other siblings. This project will follow up 7,500 of the original participants (now aged 70-75) to answer questions about the mechanisms by which higher educational attainment is associated with preserved cognitive performance and reduced risk for Alzheimer's Disease (AD) and other dementias. Participants will be assessed for dementia status and cognitive performance using a contemporary battery of cognitive measures harmonized with several of the original 1960 PT measures, allowing direct measurement of change across 1960 to 2017 on multiple cognitive domains. Other measures include educational and occupational outcomes, and a broad range of health and well-being measures, enabling comparisons with other studies of aging. This unique merger of within-family, between-family, within-school and between-school designs controls for genetic and environmental factors that are confounded in other cohort studies and provides an unprecedented opportunity to address causal hypotheses about the mechanisms underlying individual differences in risk for AD, other dementias and cognitive decline.
Role: Co-Investigator

R01 ES025888 Chen/Kaufman (PIs) 09/30/16-07/31/21
Environmental Determinants of Pathological Brain Aging in the WHI Memory Studies

This project will support the development/refinement of state-of-the-art spatiotemporal air pollution modeling tools and apply the resulting comprehensive new exposure data (from 1990s to 2016) to better understand the long-term influences of ambient air pollutants including particulate mixture on increased dementia risk and further investigate novel mediators and pathways leading to pathological brain aging in late life.
Role: Co-Investigator (PI on satellite account)

R01 AG054068-01 Chen (PI) 09/01/16 – 08/31/21
Alzheimer's Disease & Related Dementias: Geography, Environments & Mechanisms

The long-term goal of this project is to better understand the geographic disparities in Alzheimer's disease and related dementias (ADRD) by studying the neuropsychological trajectories and clinical progression to increased ADRD risk as related to geographic indicators, identifying the contributing environmental factors and examining their interactions, and elucidating the possible neurobiological mediators.
Role: Co-Investigator (PI on satellite account)

Completed Research Support

R01 AG037985 Pedersen (PI) 09/15/10 – 08/31/16
Gene-Environment Interplay of Social Contexts and Aging-Related Outcomes

The purpose is to establish a collaboration among seven longitudinal twin and family studies to lay the foundation for studies of gene-environment interplay through harmonization of the data sets and to use the database to test hypotheses about the impact of early life experiences and adversity and mid and late-life social context on individual differences in late-life functioning.
Role: Co-Investigator

R01 AG043656 Prescott (PI) 09/13/13-06/30/16
A 55-Year Follow-up Study of Project TALENT Twins and Siblings
The goal of this project is to create a U.S. twin registry and archived dataset on twins, their siblings, and non-twin sibling sets who attended the same schools. This project aims to locate and reassess original Project TALENT twins and siblings, assign zygosity to twin pairs, and link genetic and environmental data to analyze moderators of individual differences in cognitive abilities and occupational attainment.
Role: Co-Investigator

R21 AG039572 Gatz (PI) 04/01/11 - 03/31/16
The Greatest Generation: The NAS-NRC WWII Twin Registry as a Scientific Resource
The purpose is to establish a data archive comprised of the National Academy of Sciences-National Research Council Twin Registry of twin pairs born 1917-1927 in which both were in the military and to use the database to test hypotheses about relationships between experiences during the year before and in the military in relation to late life outcomes and survival.
Role: PI

P50 AG05142 Chui (PI) 04/01/10–03/31/15
Alzheimer Disease Research Center
The USC Alzheimer Disease Research Center focuses on basic mechanisms and the cognitive/behavioral impact of Alzheimer disease and cerebrovascular disease among ethnically diverse populations.
Role: Director of Education and Information Core

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Donald J. Hagler, Jr., Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): cogscidh

POSITION TITLE: Assistant Professor of Radiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Northwestern University, Evanston, IL	BA	06/96	Integrated Science Program, Biology
University of California, San Diego, La Jolla, CA	PhD	03/02	Biology
University of California, San Diego, La Jolla, CA	Postdoc	08/07	Cognitive Science

A. Personal Statement

I have the expertise and training necessary to successfully carry out my role in the proposed research project. I have a quantitative neuroscience background, with expertise in the development and use of innovative multimodal brain imaging analysis methods. As a postdoctoral fellow with Martin Sereno at UCSD, I gained experience in cognitive neuroscience, functional magnetic resonance imaging (fMRI), and cortical surface-based analysis. After joining the Multimodal Imaging Laboratory (MMIL) at UCSD, co-directed by Drs. Anders Dale and Eric Halgren, I received training in the use and development of computational methods for multimodal integration and broadened my areas of expertise to include magnetoencephalography (MEG) and diffusion MRI (dMRI). As principal investigator on an NIH K01 career development award, I developed expertise in multimodal integration, developing an fMRI retinotopy-constrained source estimation method for MEG/EEG visual evoked responses. As a participant in a variety of collaborations, I have become experienced with large-scale processing and analysis of multimodal neuroimaging data. In addition, I have developed leadership and instructional skills by training and supervising staff research associates, graduate students, post-doctoral fellows, and medical residents. I have authored several peer-reviewed publications and been a co-author on many more, demonstrating a record of productive research. The current proposal builds upon my previous work developing and applying innovative methods for the processing and analysis of multimodal imaging data. As Co-Investigator on project 2, I will continue my ongoing collaboration with PIs Kremen and Franz to implement our analytic strategy and contribute to the communication of results. My role will also be to communicate with the USC neuroimaging core about the MRI data in VETSA that is being used in Project 2 and work on ways to harmonize this data with other data. I have a demonstrated record of productive research projects, and my expertise and experience have prepared me to be Co-Investigator for the proposed project.

a. Chen CH, Fiecas M, Gutierrez ED, Panizzon MS, Eyler LT, Vuoksimaa E, Thompson WK, Fennema-Notestine C, Hagler DJ, Jr., Jernigan TL, Neale MC, Franz CE, Lyons MJ, Fischl B, Tsuang MT, Dale AM, Kremen WS. 2013. Genetic topography of brain morphology. Proc Natl Acad Sci U S A
b. Rimol LM, Panizzon MS, Fennema-Notestine C, Eyler LT, Fischl B, Franz CE, Hagler DJ, Lyons MJ, Neale MC, Pacheco J, Perry ME, Schmitt JE, Grant MD, Seidman LJ, Thermenos HW, Tsuang MT, Eisen SA, Kremen WS, Dale AM. 2010. Cortical thickness is influenced by regionally specific genetic factors. Biol Psychiatry 67(5):493-9. PMCID: 3184643

c. Hagler DJ, Jr., Ahmadi ME, Kuperman J, Holland D, McDonald CR, Halgren E, Dale AM. 2009. Automated white-matter tractography using a probabilistic diffusion tensor atlas: Application to temporal lobe epilepsy. Hum Brain Mapp 30(5):1535-47. PMCID: 2754725
d. Perry ME, McDonald CR, Hagler DJ, Jr., Gharapetian L, Kuperman JM, Koyama AK, Dale AM, McEvoy LK. 2009. White matter tracts associated with set-shifting in healthy aging. Neuropsychologia 47(13):2835-42. PMCID: 2749901

B. Positions and Honors

Positions and Employment

2007-2009 Assistant Project Scientist, Radiology, Univ. California, San Diego
2009- Assistant Professor, Radiology, Univ. California, San Diego

Other Experience and Professional Memberships

2004- Member, Society for Neuroscience
2007- Ad hoc reviewer, *NeuroImage, Journal of Neuroscience, Journal of Neurophysiology, Cerebral Cortex, Surgical and Radiologic Anatomy, Journal of Neuroscience Methods, Clinical Anatomy, Psychiatry Research, Human Brain Mapping, Brain Structure and Function, Psychological Medicine*
2010 Ad hoc reviewer, National Science Foundation
2012 Ad hoc reviewer, Canadian Institutes of Health Research
2015 Ad hoc reviewer, NIH Cognition and Perception Study Section

Honors

1995 Awarded Barry M. Goldwater Scholarship
1996 NSF Fellowship Honorable Mention
1996 Elected to Phi Beta Kappa, Northwestern University
1996 Graduated from Northwestern University with Distinction

C. Contributions to Science

1. Maps of visual space in the brain allow humans and other species to process visual information about the world around them. My work was the first to demonstrate the existence of topographic maps of visual space in frontal and prefrontal cortex. I also extended previous work to better characterize the multiple spatial maps in posterior parietal cortex. These maps in higher level areas appear to be involved in the application of top-down attentional control and tracking task-relevant objects in coordination with the numerous maps that have been identified in occipital cortex. My role in these studies related to the acquisition and analysis of functional magnetic resonance imaging (fMRI) data and the implementation of cortical surface-based analysis methods.

a. Hagler DJ, Jr., Sereno MI. 2006. Spatial maps in frontal and prefrontal cortex. Neuroimage 29(2):567-77.
b. Hagler DJ, Jr., Saygin AP, Sereno MI. 2006. Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. Neuroimage 33(4):1093-103. PMCID: 1785301
c. Hagler DJ, Jr., Riecke L, Sereno MI. 2007. Parietal and superior frontal visuospatial maps activated by pointing and saccades. Neuroimage 35(4):1562-77. PMCID: 2752728
d. Filimon F, Nelson JD, Hagler DJ, Sereno MI. 2007. Human cortical representations for reaching: mirror neurons for execution, observation, and imagery. Neuroimage 37(4):1315-28. PMCID: 2045689

2. The relatively poor temporal resolution of fMRI and the difficulties of source localization for electroencephalography (EEG) and MEG have presented significant challenges for the noninvasive study of visual processing in humans. I developed a novel method for estimating the time courses of visual responses in individual visual cortical areas using MEG/EEG, fMRI retinotopic mapping, and cortical surface reconstructions derived from structural MRI (sMRI). After further improvements to this method, I have begun to study the timing of evoked responses in early visual areas, something not previously possible. My role in this

work has been as primary investigator and has included data acquisition and analysis and multimodal integration and source estimation methods.

a. Hagler DJ, Jr., Halgren E, Martinez A, Huang M, Hillyard SA, Dale AM. 2009. Source estimates for MEG/EEG visual evoked responses constrained by multiple, retinotopically-mapped stimulus locations. Hum Brain Mapp 30(4):1290-309. PMCID: 2754810
b. Hagler DJ, Jr., Dale AM. 2013. Improved method for retinotopy constrained source estimation of visual-evoked responses. Hum Brain Mapp 34(3):665-83. PMCID: 3299883
c. Hagler DJ, Jr. 2014. Optimization of retinotopy constrained source estimation constrained by prior. Hum Brain Mapp. 35(5):1815-33.
d. Hagler DJ, Jr. 2014. Visual field asymmetries in visual evoked responses. J. Vis. 14(14):13.

3. Early methods for identifying white matter tracts involved manual selection of streamlines created using diffusion tensor imaging (DTI). I developed an automated method for white matter tract segmentation that uses atlas-based information about tract locations and local orientations. Because it is completely automated, this method is a much more efficient way – and much more practical for large-scale studies – to create regions of interest (ROIs) for extracting imaging-derived measures for white matter tracts. Another advantage is that the method is applied in an unbiased, uniform manner across participants. An advantage over popular, probabilistic tractography methods is that only pre-determined, validated white matter tracts are included, excluding artifactual connections. My role in these studies has been the implementation and development of diffusion magnetic resonance imaging (dMRI) data processing methods, development of the tract segmentation method, and data analysis.

a. Hagler DJ, Jr., Ahmadi ME, Kuperman J, Holland D, McDonald CR, Halgren E, Dale AM. 2009. Automated white-matter tractography using a probabilistic diffusion tensor atlas: Application to temporal lobe epilepsy. Hum Brain Mapp 30(5):1535-47. PMCID: 2754725
b. Ahmadi ME, Hagler DJ, Jr., McDonald CR, Tecoma ES, Iragui VJ, Dale AM, Halgren E. 2009. Side matters: diffusion tensor imaging tractography in left and right temporal lobe epilepsy. AJNR Am J Neuroradiol 30(9):1740-7. PMCID: 2759860
c. Perry ME, McDonald CR, Hagler DJ, Jr., Gharapetian L, Kuperman JM, Koyama AK, Dale AM, McEvoy LK. 2009. White matter tracts associated with set-shifting in healthy aging. Neuropsychologia 47(13):2835-42. PMCID: 2749901
d. McDonald CR, Hagler DJ, Jr., Girard HM, Pung C, Ahmadi ME, Holland D, Patel RH, Barba D, Tecoma ES, Iragui VJ, Halgren E, Dale AM. 2010. Changes in fiber tract integrity and visual fields after anterior temporal lobectomy. Neurology 75(18):1631-8. PMCID: 3385464

4. Research projects with multimodal neuroimaging for large numbers of participants present a number of technical and practical challenges. I have been the primary developer of a high-throughput processing and analysis stream for neuroimaging data, including sMRI, dMRI, fMRI, PET, and MEG. Stemming from this work and my expertise in the analysis of multimodal neuroimaging data, I have participated in a number of collaborations, investigating topics such as epilepsy, schizophrenia, Alzheimer’s disease, brain tumor, autism, genetics, aging, and development.

a. Fennema-Notestine C, Hagler DJ, Jr., McEvoy LK, Fleisher AS, Wu EH, Karow DS, Dale AM. 2009. Structural MRI biomarkers for preclinical and mild Alzheimer's disease. Hum Brain Mapp 30(10):3238-53. PMCID: 2951116
b. Stein JL, Medland SE, Vasquez AA, Hibar DP, Senstad RE, Winkler AM, Toro R, Appel K, Bartecek R, Bergmann O, Bernard M, Brown AA, Cannon DM, Chakravarty MM, Christoforou A, Domin M, Grimm O, Hollinshead M, Holmes AJ, Homuth G, Hottenga JJ, Langan C, Lopez LM, Hansell NK, Hwang KS, Kim S, Laje G, Lee PH, Liu X, Loth E, Lourdusamy A, Mattingsdal M, Mohnke S, Maniega SM, Nho K, Nugent AC, O'Brien C, Papmeyer M, Putz B, Ramasamy A, Rasmussen J, Rijpkema M, Risacher SL, Roddey JC, Rose EJ, Ryten M, Shen L, Sprooten E, Strengman E, Teumer A, Trabzuni D, Turner J, van Eijk K, van Erp TG, van Tol MJ, Wittfeld K, Wolf C, Woudstra S, Aleman A, Alhusaini S, Almasy L, Binder EB, Brohawn DG, Cantor RM, Carless MA, Corvin A, Czisch M, Curran JE, Davies G, de Almeida MA, Delanty N, Depondt C, Duggirala R, Dyer TD, Erk S, Fagerness J, Fox PT, Freimer NB, Gill M, Goring HH, Hagler DJ, Hoehn D, Holsboer F, Hoogman M, Hosten N, Jahanshad N, Johnson MP, Kasperaviciute D, Kent JW, Jr., Kochunov P, Lancaster JL, Lawrie SM, Liewald DC, Mandl R,

Matarin M, Mattheisen M, Meisenzahl E, Melle I, Moses EK, Muhleisen TW, Nauck M, Nothen MM, Olvera RL, Pandolfo M, Pike GB, Puls R, Reinvang I, Renteria ME, Rietschel M, Roffman JL, Royle NA, Rujescu D, Savitz J, Schnack HG, Schnell K, Seiferth N, Smith C, Steen VM, Valdes Hernandez MC, Van den Heuvel M, van der Wee NJ, Van Haren NE, Veltman JA, Volzke H, Walker R, Westlye LT, Whelan CD, Agartz I, Boomsma DI, Cavalleri GL, Dale AM, Djurovic S, Drevets WC, Hagoort P, Hall J, Heinz A, Jack CR, Jr., Foroud TM, Le Hellard S, Macciardi F, Montgomery GW, Poline JB, Porteous DJ, Sisodiya SM, Starr JM, Sussmann J, Toga AW, Veltman DJ, Walter H, Weiner MW, Bis JC, Ikram MA, Smith AV, Gudnason V, Tzourio C, Vernooij MW, Launer LJ, DeCarli C, Seshadri S, Andreassen OA, Apostolova LG, Bastin ME, Blangero J, Brunner HG, Buckner RL, Cichon S, Coppola G, de Zubicaray GI, Deary IJ, Donohoe G, de Geus EJ, Espeseth T, Fernandez G, Glahn DC, Grabe HJ, Hardy J, Hulshoff Pol HE, Jenkinson M, Kahn RS, McDonald C, McIntosh AM, McMahon FJ, McMahon KL, Meyer-Lindenberg A, Morris DW, Muller-Myhsok B, Nichols TE, Ophoff RA, Paus T, Pausova Z, Penninx BW, Potkin SG, Samann PG, Saykin AJ, Schumann G, Smoller JW, Wardlaw JM, Weale ME, Martin NG, Franke B, Wright MJ, Thompson PM. 2012. Identification of common variants associated with human hippocampal and intracranial volumes. Nat Genet 44(5):552-61. PMCID: 3635491

c. Tamnes CK, Walhovd KB, Dale AM, Ostby Y, Grydeland H, Richardson G, Westlye LT, Roddey JC, Hagler DJ, Jr., Due-Tonnessen P, Holland D, Fjell AM. 2013. Brain development and aging: overlapping and unique patterns of change. Neuroimage 68:63-74

d. Docherty AR, Hagler DJ, Jr., Panizzon MS, Neale MC, Eyler LT, Fennema-Notestine C, Franz CE, Jak A, Lyons MJ, Rinker DA, Thompson WK, Tsuang MT, Dale AM, Kremen WS. 2015. Does degree of gyrification underlie the phenotypic and genetic associations between cortical surface area and cognitive ability? Neuroimage 106:154-60. PMCID: 4313767

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1vkei-XT-mykd/bibliography/47782276/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

1R01DA038958-01A1 Akshoomoff, Brown (PI) 07/01/2014-05/31/2017
Examination of Neurobehavioral Development Using the PING Data Resource
The Major goals of this project are to study the developmental relationships between brain structure and function and behavior, specifically related to executive function.
Role: Co-Investigator

1R01AG050595-01 Kremen (PI) 07/01/2015 – 06/30/2020
The VETSA Longitudinal Twin Study OF Cognition and Aging (VETSA 3)
Major goals: The major goal of this project is to determine genetic and environmental influences on brain structure and brain aging via MRI. The study will include morphometric analysis of 3-D images, diffusion sensor imaging, and T2*-weighted mapping of hippocampus, and will examine these measures in relation to other measures in the related study listed after this project
Role: Co-Investigator

5R01MH099645-03 Halgren (PI) 08/30/2012 – 05/31/2017
Multiresolution Modeling of Human Thalamocortical Upstates and Downstates
Major goals: The major goal of this project is to create multiresolution models of thalmocortical upstates and downstates that occur during sleep.
Role: Other significant contributor

5R01EB009282-06 Sejnowski (PI) 05/01/2013 – 04/30/2017
Integrated Empirical and Multi-scale Modeling of Human Sleep Spindles
Major goals: The major goal of this project is to create realistic, large-scale models of sleep spindles that integrate neural network models with cortical surface modeling and MEG/EEG forward modeling.
Role: Other significant contributor

1R01 DC012797-01 Mayberry (PI) 12/01/2012 -11/30/2017
Age of acquisition effects on sign language development and brain processing
Major Goals: This project investigates the effects of age of first-language acquisition on sign language comprehension and production and the neural correlates of these effects.
Role: Other significant contributor

ONR N00014-13-1-0672 Bazhenov (PI) 07/01/2013 – 06/30/2018
Memory Consolidation During Sleep in Humans, Rodents and Computational Models
Major goals: to test and refine models of the interplay of sleep rhythms in orchestrating and modulating memory consolidation processes in humans.
Role: Other significant contributor

Completed Research Support

K01MH079146 Hagler (PI) 08/21/2008 - 07/31/2012
Spatiotemporal Mapping of Visual Evoked Cortical Activity and Spatial Attention
The major goals of this grant are to develop methods for estimating time courses of activation in early visual areas and applying those methods to the study of modulation of visual processing by stimulus contrast and spatial attention. Dr. Hagler was responsible for overseeing all aspects of this project.
Role: Principal Investigator

5R01AG022381-11 Kremen (PI) 09/30/2009 – 08/31/2014
The VETSA Longitudinal MRI Twin Study of Aging
Major goals: The major goal of this project is to determine genetic and environmental influences on brain structure and brain aging via MRI. The study will include morphometric analysis of 3-D images, diffusion sensor imaging, and T2*-weighted mapping of hippocampus, and will examine these measures in relation to other measures in the related study listed after this project
Role: Other significant contributor

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Russell E. Jacobs

eRA COMMONS USER NAME (credential, e.g., agency login): jacobsre

POSITION TITLE: Research Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Tulane University, New Orleans, LA	B.S.	1972	Philosophy & Chemistry
Stanford University, Stanford, CA	Ph.D.	1977	Physical Chemistry
University of Illinois, Urbana, IL	Postdoc	1977-1979	

A. Personal Statement

I have more than 30 years experience in the theory, hardware/software development and application of high resolution preclinical MRI. Extensive history of supervising successful Post Doctoral fellows, graduate students, undergraduates, and technicians. Animal models understudy have included: embryonic development, multiple sclerosis, Alzheimer's Disease, cancer, and substance abuse. I am also heavily involved in several multimodal imaging efforts including contrast agent development, implementation of a simultaneous dual μPET/μMRI scanner, and quantitative analysis of MR images using an array of computational warping and statistical parametric analyses. Simultaneous PET/MRI is a truly synergistic merging of technologies. In particular, our work with agents visible in both modalities allows unequivocal identification of the multimodal agent within its anatomical context (MRI) at picomolar sensitivity (PET). "Unequivocal" because it must manifest in *both* modalities at the *same place and time*. For the past 7 years I have been collaborating with Drs. Colcher at City of Hope using our in vivo imaging technologies to visualize potential immunotherapies in animal models. Recent encouraging preliminary results using focused ultrasound melded with *in vivo* longitudinal imaging prompt the current proposal. Our current preclinical efforts with simultaneous μPET/μMRI will be ideally suited to cell tracking studies, elucidation of effects of focused ultrasound and rodent brain imaging (functional and structural). Moreover, with advent of clinical PET/MRI systems, agents and methodologies developed in this work will be directly translatable to patients.

B. Positions and Honors

1972	Graduated Cum Laude; elected to Phi Beta Kappa
1977	Illinois Heart Association Postdoctoral Fellow
1979-80	Visiting Associate, Chemistry Department, Washington State University
1980-86	Assistant Research Biophysicist, Department of Physiology and Biophysics, UC Irvine
1986-88	Associate Research Biophysicist, Department of Physiology and Biophysics, UC Irvine
1988-90	Assistant Professor in Residence, Department of Physiology and Biophysics, UC Irvine
1990-91	Associate Professor in Residence, Department of Physiology and Biophysics, UC Irvine
1991-2016	Member of the Beckman Institute, Division of Biology, California Institute of Technology
2016-present	Professor of Research Physiology and Biophysics, USC Keck School of Medicine
1987-1992	Established Investigator of the American Heart Association
1989	Sylvia Sorkin Greenfield Award - Awarded annually by the American Association of Physicists in Medicine for the best paper published in Medical Physics
1994–1999	Editorial Board NeuroImage
1996–2002	Member of OECD Megascience Forum - Biological Informatics Working Group-Neuroinformatics Subgroup
2001–2006	Editorial Board Journal of Anatomy
2003-2009	Division Committee, Group Ampere, Division of Spatially Resolved Magnetic Resonance

C. Contribution to Science

Lipid-bilayer / membrane protein interactions: My early work used solid-state MRI of deuterated lipids, calorimetry, and some simple computer modeling to gain insights into how steroids, hydrophobic peptides and membrane proteins associate with biomembranes. At that time the only known membrane protein "structure" was that of bacterial rhodopsin (determined via cryo-electron microscopy) and even now fewer that ~30 such structures are determined yearly - an extremely small number consider that ~30% of the mammalian proteome is comprised of membrane proteins. Thus, it was and is important to characterize lipid – peptide/protein interactions with a variety of experimentally methods as well as computational models. I used solid-state NMR, calorimetry, and binding constant determinations to describe how well defined hydrophobic peptides interacted with lipid bilayer membranes. With Prof. White, we developed an "interfacial hydrophobicity scale" that allows one to examine the consequences of different assumptions about the average hydrogen bond status of polar side chains. I did or supervised all the experimental work, including building a solid-state NMR spectrometer, designed experiments, wrote computer programs to analyze and model data, and wrote manuscripts and NIH proposals to support these efforts.

a. Jacobs R, Oldfield E *Deuterium nuclear magnetic resonance investigation of dimyristoyllecithin--dipalmitoyllecithin and dimyristoyllecithin--cholesterol mixtures.* Biochemistry. 1979 Jul 24;**18**(15):3280-5.
b. Jacobs, R.E. and S.H. White, *The Nature of the Hydrophobic Binding of Small Peptides at the Bilayer Interface - Implications for the Insertion of Transbilayer Helices.* Biochemistry, 1989. **28**(8): 3421-3437.
c. White, S.H. and R.E. Jacobs, *Statistical Distribution of Hydrophobic Residues Along the Length of Protein Chains - Implications for Protein Folding and Evolution.* Biophysical Journal, 1990. **57**(4): 911-921.
d. White, S.H. and R.E. Jacobs, *Observations Concerning Topology and Locations of Helix Ends of Membrane-Proteins of Known Structure.* Journal of Membrane Biology, 1990. **115**(2): 145-158.

Development & Applications of high resolution *in vivo* μMRI : In the late 1980's, MRI microscopy was in its infancy (Blackband published the first "single cell" MR image in 1986). In collaboration with Dr. Cho's group at UC Irvine we modified our 7T widebore NMR spectrometer to an MRI scanner. I personally designed and built several gradient and RF coils, as well as writing image collection and processing software. Applications is developmental biology demonstrated for the first time that μMRI could be used in cell fate mapping in the early embryo, revealing information not obtainable by optical methods due to sample opacity. In collaboration with Meade, Fraser, & Louie; we developed the first "smart" MRI contrast agent – an agent activated *in vivo* by cleavage with β-galactosidase. I contributed to the design of the agent and experiments to test it *in vivo*, as well as supervising all aspects of the MR imaging. Further, the use of timelapse *in vivo* μMRI in embryology provided insight into the 3 dimensional character of morphological development, while *ex vivo* μMRI provided data for 3D atlases of the mouse, quail, and lemur (http://atlasserv.caltech.edu). In collaboration with BIRN my μMRI mouse brain images and notions of using atlases as entrées to other types of information found implementation (http://www.birncommunity.org/data-catalog/mouse-3d-mr-minimum-deformation-atlas/)

a. Cho, Z.H., C.B. Ahn, S.C. Juh, H.K. Lee, R.E. Jacobs, S. Lee, J.H. Yi and J.M. Jo, *NMR microscopy with 4-um resolution theoretical study and experimental results.* Med Phys, 1988. **15**(6): 815-824. [Sylvia Sorkin Greenfield Award - Awarded annually by the American Association of Physicists in Medicine for the best paper to be published in Medical Physics]
b. Jacobs, R.E. and S.E. Fraser, *Magnetic Resonance Microscopy of Embryonic Cell Lineages and Movement.* Science, 1994. **263**: 681-684.
c. Papan, C., B. Boulat, S.S. Velan, S.E. Fraser and R.E. Jacobs, *Formation of the dorsal marginal zone in Xenopus laevis analyzed by time-lapse microscopic magnetic resonance imaging.* Developmental Biology, 2007. **305**(1): 161-171.
d. Louie, A.Y., M.M. Huber, E.T. Ahrens, U. Rothbacher, R. Moats, R.E. Jacobs, S.E. Fraser and T.J. Meade, *In vivo visualization of gene expression using magnetic resonance imaging.* Nat. Biotechnol., 2000. **18**(3): 321-325.

μMRI in mouse models of neuro-diseases: The overarching goal of this work is to gain insight into the etiology of neurological diseases (e.g. Alzheimer's Disease, multiple sclerosis, and drug abuse) using mouse models so that this increased understanding can be applied in the human condition. The work with Bloom & later with Voskuhl established that μMRI provides quantitative detailed information about changes in brain volume & structure through comparison of μMRI with gold standard stereology and histology. Manganese enhanced MRI provides a methodology to evaluate changes in neuronal circuitry in mouse models. My major contributions in this area are to import and refine two technologies from other fields: stereotaxic injection of

neuronal tracer (used routinely in histology context) and statistical parametric mapping (used routinely in human fMRI analysis). Together these provide an unbiased quantitative way to compare neuronal circuits across time/stimulus/genotype.

a. Redwine, J.M., B. Kosofsky, R.E. Jacobs, D. Games, J.F. Reilly, J.H. Morrison, W.G. Young and F.E. Bloom, *Dentate gyrus volume is reduced before onset of plaque formation in PDAPP mice: A magnetic resonance microscopy and stereologic analysis.* Proc Natl Acad Sci U S A, 2003. **100**(3): 1381-1386.
b. MacKenzie-Graham, A., S.K. Tiwari-Woodruff, G. Sharma, C. Aguilar, K.T. Vo, L.V. Strickland, L. Morales, B. Fubara, M. Martin, R.E. Jacobs, G.A. Johnson, A.W. Toga, and R.R. Voskuhl, *Purkinje cell loss in experimental autoimmune encephalomyelitis.* Neuroimage, 2009. **48**(4): 637-651.
c. Gallagher JJ, Zhang X, Hall FS, Uhl GR, Bearer EL, Jacobs RE. Altered Reward Circuitry in the Norepinephrine Transporter Knockout Mouse. PLoS One. 2013;8(3):e57597. doi: 10.1371/journal.pone.0057597.
d. Malkova, N. V., J. J. Gallagher, C. Z. Yu, R. E. Jacobs and P. H. Patterson (2014). *Manganese-enhanced magnetic resonance imaging reveals increased DOI-induced brain activity in a mouse model of schizophrenia.* Proc Natl Acad Sci U S A **111**(24): E2492-2500.

Simultaneous μPET & μMR Multimodal imaging – development and applications: Collaborations are about projects, but collaborations are between. In Profs. Cherry & Louie I have been blessed with insightful, productive, and collegial associates. My role in the collaboration is threefold: 1) management – as PI with overall responsibility; 2) MRI engineering aspects and overall integration of the PET component into our MR scanner; and 3) scientific applications including cancer immunotherapies and agents for detection of vulnerable vascular plaque. Along with the Pichler group in Germany, in 2008 we published a demonstration of a relatively easy to use μPET system 'insert' that fits in a preclinical MR scanner allowing synchronized μPET/μMR image acquisition *in vivo* in small animals. Our applications of this technology emphasize imaging of phenomena that require examination over both time and space (*e.g.* tracking of immune system molecules and cells, as well as how they modulate the tumor milieu). An important underlying goal of my lab is to employ synchronized μPET/μMR to develop & evaluate PET and MR biomarkers in tandem, so that in the future MRI biomarkers can be employed clinically to minimize the need for PET scans.

a. Catana, C., D. Procissi, Y.B. Wu, M.S. Judenhofer, J.Y. Qi, B.J. Pichler, R.E. Jacobs and S.R. Cherry, *Simultaneous in vivo positron emission tomography and magnetic resonance imaging.* Proc Natl Acad Sci U S A, 2008. **105**(10): 3705-3710.
b. Ng, T.S.C., D. Procissi, Y.B. Wu and R.E. Jacobs, *A robust coregistration method for in vivo studies using a first generation simultaneous PET/MR scanner.* Medical Physics, 2010. **37**(5): 1995-2003.
c. Ng, T.S.C., J.R. Bading, R. Park, H. Sohi, D. Procissi, D. Colcher, P.S. Conti, S.R. Cherry, A.A. Raubitschek and R.E. Jacobs, *Quantitative, Simultaneous PET/MRI for Intratumoral Imaging with an MRI-Compatible PET Scanner.* Journal of Nuclear Medicine, 2012. **53**(7): 1102-1109.
d. Tu, C., T. S. Ng, R. E. Jacobs and A. Y. Louie (2014). *Multimodality PET/MRI agents targeted to activated macrophages.* J Biol Inorg Chem **19**(2): 247-258.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1pMPjK7bdOn55/bibliography/40530948/public/?sort=date&direction=ascending

D. Research Support

ACTIVE

1P01AG052350-01 (Zlokovic/Toga) 09/30/16-05/31/21 0.6 calendar
NIH/NIA $1,792,396
Vascular contributions to dementia and genetic risk factors for Alzheimer's disease
Program project to study imaging and molecular biomarkers of neurovascular dysfunction in individuals at genetic risk for AD both familial and sporadic.

5R01NS090904-18 (Zlokovic) 09/30/14-07/31/19 1.0 calendar
NIH/NINDS $461,504
Activated Protein C system in stroke models
The goal is to develop late post-ischemic brain repair therapy for stroke with activated protein C analogs.

5R01AG023084-12 (Zlokovic) 06/15/15-03/31/20 1.2 calendar

NIH/NIA (Renewal) $395,012
Cerebrovascular β-Amyloidosis: Aβ CNS Transport Pathways
The role of PICALM in Aβ blood-brain barrier clearance and Alzheimer's neurodegeneration.

1R01NS100459-01 (Zlokovic) 09/30/16-08/31/21 1.2 calendar
NIH/NIA $500,000
The role of pericytes in white matter disease
To study the role of pericytes in white matter degeneration using novel *in vivo* animal model systems.

1R01EB022744-01 (Shi) 09/22/16-06/30/17 0.24 calendar
NIH/NIBIB $4,500 (Jacobs's share only)
Computational tools for modeling human and mouse connectome with multi-shell diffusion imaging
To fully unleash the potential of multi-shell diffusion MRI, in this project we will develop a suite of novel computational tools that jointly estimate fiber orientation distributions and compartmental parameters.
PENDING

2R01 NS076794-06A1 (Town) 09/30/17-08/31/22 0.24 calendar
NIH/NINDS (Renewal) $403,287
Peripheral TGF-beta Pathway Inhibitor Therapy in Alzheimer's Rats
The key goals of this project are to evaluate the role of TGF-beta signaling on hematogenous macrophages as a potential therapeutic approach for Alzheimer's disease using small molecule-based TGF-beta-Smad 2/3 inhibitors and a pre-clinical rodent model of the disease. A critical understanding of this important area of neuroimmunology will likely lead to new therapeutic targets.

NSF NeuroNex (Dong/Toga) 07/01/17-06/30-22 1.2 calendar
NSF $29,304 (Jacobs's share only)
Comparative Connectomics: Bridging Neural Circuit Structure Across Species
In this project we develop and apply sophisticated multidirection multishell diffusion weighted MRI techniques to map out neuronal connectivity in the live mouse brain and merge this information with histological based neural tract tracing and genetic tracing techniques.

Zumberge Interdisciplinary Research Award (Jacobs) 07/01/17-06/30/18 0.0 calendar
Zumberge Research and Innovation Fund at USC $85,000
Targeted Tumor Treatment with Systemic Immunotherapy
Combined modality regimens in immunotherapies for tumors are an exciting emerging development that hold great promise. We propose to combine low power focused ultrasound (LOFU) plus tumor specific immunocyokine (ICK) treatment: LOFU to alter the local tumor immune environment with the ICK acting as a synergistic immune enhancer.

R01 (Wang) 09/01/17-08/31/21 0.12 calendar
NIH $24,655 (Jacobs's share only)
Foundations of Resting State FMRI - Multiscale Imaging of Brain Dynamics
In this project, we will develop and employ functional MRI to investigate the neuronal source of network dynamics by applying a chemogenetic tool (DREADDs) to selectively activate or silence excitatory and inhibitory neurons in mouse brain. We test the hypothesis that selectively activating or silencing excitatory neurons in the motor cortex increases/decreases the complexity of high frequency local field potentials and low frequency fMRI signal fluctuations, as well as the complexity of temporal dynamics of functional connectivity within the motor network.

Pilot Innovator Grant (Kelland) 07/01/18-06/30/19 0.12 calendar
Conrad N. Hilton Foundation $9,739 (Jacobs's share only)
Angiotensin 1-7: a treatment to promote remyelination in models of MS
The goal of this project is to determine whether angiotensin 1-7 is an ideal treatment for patients with either relapsing-remitting or progressive MS in order to alleviate progression and promote repair.

OVERLAP None

BIOGRAPHICAL SKETCH

NAME: Kaufman, Joel

eRA COMMONS USER NAME (agency login): JOELKAUFMAN

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Michigan, Ann Arbor, MI	BA	08/1982	Biomedical Sciences
University of Michigan, Ann Arbor, MI	MD	06/1986	Medicine
University of Washington, Seattle, WA	MPH	06/1990	
Boston City Hospital, Boston, MA	Resident	06/1988	Internal Medicine
University of Washington, Seattle, WA	Fellow	06/1990	Occupational and Environmental Medicine

A. PERSONAL STATEMENT

Dr. Kaufman is a physician-scientist and epidemiologist, with research focused on human health effects of environmental agents, with research spanning epidemiology, toxicology, clinical medicine, and exposure sciences. He has led several investigations regarding the effects of environmental agents on the incidence and progression of chronic diseases including cardiovascular diseases, using new methods to estimate individual-level pollutant exposures for epidemiology. Dr. Kaufman has led the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air) project, building on the NHLBI's MESA cohort, which represents an unprecedented effort to characterize cohort members' exposures to air pollutants of ambient origin. He has also worked with several other cohorts to develop specialized exposure assessment systems for hundreds of thousands of study participants and locations, including in the Women's Health Initiative and the NIEHS Sister Study. Through these efforts, the research group has led state-of-the-art cohort-specific monitoring campaigns, and developed advanced spatiotemporal modeling approaches incorporating numerous geographic covariates, which can accurately estimate air pollutant concentrations at point locations. This experience, and the datasets and products developed by his research group, provides the basis for his contributions to this project.

1. Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson G, **Kaufman JD**. Long-term exposure to air pollution and incidence of cardiovascular events in women. N Engl J Med 2007;356:447-58. PubMed PMID: 17267905.
2. **Kaufman JD**, Adar SD, Barr RB, Budoff M, Burke GL, Curl CL, Daviglus ML, Diez Roux AV, Gassett AJ, Jacobs DR, Kronmal R, Larson TV, Navas-Acien A, Olives C, Sampson PD, Sheppard L, Siscovick DS, Stein JH, Szpiro AA, Watson KE. Air Pollution and Coronary Artery Calcification within Six Metropolitan Areas in the USA. The Multi-Ethnic Study of Atherosclerosis and Air Pollution. The Lancet 2016; 388:696-704. PMID: 27233746. PMCID: PMC5019949 [Available on 2017-08-13]
3. Chi GC, Hajat A, Bird CE, Cullen MR, Griffin BA, Miller KA, Shih RA, Stefanick ML, Vedal S, Whitsel EA, **Kaufman JD**. Individual and Neighborhood Socioeconomic Status and the Association Between Air Pollution and Cardiovascular Disease. Environ Health Perspect.124:1840-1847. PMID: 27138533. PMCID: PMC5132637
4. Weuve J, **Kaufman JD**, Szpiro AA, Curl C, Puett RC, Beck T, Evans DA, Mendes de Leon C: Exposure to Traffic-Related Air Pollution in Relation to Progression in Physical Disability among Older Adults. *Environ Health Perspect* 2016, 124: 1000-1008. PMCID: PMC4937863

B. POSITIONS AND HONORS

Positions and Employment

1986 - 1988	Medical Intern and Resident, Boston City Hospital, Boston, MA
1987 - 1988	Teaching Fellow in Medicine, Boston University School of Medicine, Boston, MA
1988 - 2000	Senior Fellow in Occupational and Environmental Medicine, Departments of Medicine and Environmental Health, University of Washington, Seattle, WA
1990 - 1997	Associate Medical Director for Safety and Health Assessment and Research for Prevention (SHARP) Program, Washington Department of Labor & Industries, Olympia, WA
1990 - 2021	Diplomate in Internal Medicine, American Board of Internal Medicine (recertified 2011)

1991 -	Diplomate in Occupational Medicine, American Board of Preventive Medicine
1991 - 1997	Clinical Assistant Professor, University of Washington, Departments of Medicine and Environmental Health, Seattle, WA
1997 - 2006	Associate Professor, University of Washington, Departments of Environmental & Occupational Health Sciences and Medicine, Seattle, WA
2002 -	Director, Occ and Env Medicine Program, University of WA, Seattle, WA
2003 - 2005	Member, Institute of Medicine Committee on the Gulf War and Health: Selected Environmental Agents, Pollutants, and Synthetic Chemical Compounds
2005 -	Fellow, American College of Physicians
2005 -	Fellow, American College of Occupational and Environmental Medicine
2006 -	Professor, Depts. of Environmental & Occupational Health Sciences, School of Public Health, Seattle, WA
2006 -	Professor, Department of Medicine, School of Medicine, Seattle, WA
2006 -	Professor, Department of Epidemiology, School of Public Health, Seattle, WA
2009 - 2010	Member, USEPA Clean Air Scientific Advisory Committee (CASAC), CO Panel
2013 - 2017	Member, USEPA Clean Air Scientific Advisory Committee (CASAC), NO Panel
2015 - 2018	Member, USEPA Clean Air Scientific Advisory Committee (CASAC), Particulate Matter Review Panel
2015 - 2019	Member, NIH Cancer, Heart, and Sleep Epi Study Section B (CHSB, previously CASE)

Other Experience and Professional Memberships

Member, Intl Society for Environmental Epidemiology; Chair 2014 International Conference
Member, American Public Health Association
Associate Editor, Environmental Health Perspectives
Member, American Thoracic Society
Editorial Board Member, American Journal of Respiratory and Critical Care Medicine

C. Contribution to Science

As a physician-epidemiologist studying environmental and occupational health factors in disease and disability, my work has focused on addressing problems at the nexus of epidemiology, environmental health sciences, toxicology, and clinical medicine. Leveraging advances in each of these disciplines, and conducting multi-investigator collaborations which include state-of-the-art quantitative methods, is common to each area.

1. Exposure to ambient air pollutants is associated with chronic diseases. Our epidemiological work has focused on well-conducted studies with good control of potential confounding factors and excellent outcome assessment. We approach our work with equipoise and have observed and published both null and positive findings. Our findings are most compelling and show more consistent effects for studies of long-term exposure than for short-term exposures.
   a. Miller KA, Siscovick DS, Sheppard K, Sullivan JH, Anderson G, **Kaufman JD**. Long-term exposure to fine particulate matter air pollution and cardiovascular events in women. New England J Med 2007; 356:447-58.
   b. Sullivan JH, Schreuder A, Sheppard L, Siscovick D, **Kaufman JD**. Short-term fine particulate matter exposure and onset of myocardial infarction in a community-based myocardial infarction treatment trial. Epidemiology 2005;16: 41-48.
   c. Young MD, Sandler DP, DeRoo LA, Vedal S, **Kaufman JD**, London SJ. Ambient air pollution exposure and incident adult asthma in a nationwide cohort of US women. Am J Respir Crit Care Med 2014. PMCID: PMC4299575
   d. Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC Jr, Whitsel L, **Kaufman JD**. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 2010;12:2331-2378. PMID: 20458016
2. Improved exposure assessment greatly improves the quality of research investigating the health effects of air pollutants. Sophisticated exposure monitoring campaigns combined with advanced statistical models can be built that accurately estimate exposures where people live.
   a. Keller JP, Olives C, Kim S-Y, Sheppard L, Sampson PD, Szpiro AA, Oron AP, Lindström J, Vedal S, **Kaufman JD**: A Unified Spatiotemporal Modeling Approach for Predicting Concentrations of

Multiple Air Pollutants in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. *Environmental health perspectives* 2014, 123(4):301-309. PMCID: PMC4384200

b. Wang M, Sampson PD, Hu J, Kleeman MJ, Keller JP, Olives C, Szpiro AA, Vedal S, **Kaufman JD**. Combining Land-Use Regression and Chemical Transport Modeling in a Spatio-temporal Geostatistical Model for Ozone and PM2.5. Environ Sci Technol. 2016; 50: 5111-8. PMID: 27074524 PMCID: PMC5096654 [Available on 2017-05-17]

c. Cohen MA, Adar SD, Allen RW, Avol E, Curl CL, Gould T, Hardie D, Ho A, Kinney P, Larson TV, Sampson P, Sheppard L, Stukovsky KD, Swan SS, Liu LJS, **Kaufman JD**. Approach to estimating participant pollutant exposures in the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). Environmental Science & Technology 2009;43:4687-93. PMCID: PMC2727607

d. Kioumourtzoglou MA, Spiegelman D, Szpiro AA, Sheppard L, **Kaufman JD**, Yanosky JD, Williams R, Laden F, Hong B, Suh HH. Exposure measurement error in PM2.5 health effects studies: A pooled analysis of eight personal exposure validation studies. Environ Health 2014;13:2. PMCID: PMC3922798

3. Experimental findings, including in human studies, that use realistic exposure systems, provide key corroborative evidence that environmental factors can affect the cardiovascular system. We have built and use a customized laboratory facility for generating exposures for translation of inhalation toxicology to enhance the clinical and mechanistic framework.

   a. Peretz A, Sullivan JH, Leotta DF, Trenga CA, Sands FN, Allen J, Carlsten C, Wilkinson CW, Gill EA, **Kaufman JD**. Diesel exhaust inhalation elicits acute vasoconstriction *in vivo.* Environmental Health Perspectives 2008;116(7):937-42. PMCID: PMC2453163

   b. Cosselman KE, Krishnan RM, Oron AP, Jansen K, Peretz A, Sullivan JH, Larson TV, **Kaufman JD**. Blood pressure response to controlled diesel exhaust exposure in human subjects. Hypertension 2012;59:943-8. PMID: 22431582 PMCID: PMC3654814

   c. Allen J, Trenga CA, Peretz A, Sullivan JH, Carlsten CC, **Kaufman JD**. Effect of diesel exhaust inhalation on antioxidant and oxidative stress responses in adults with metabolic syndrome. Inhalation Toxicology 2009;21:1061-1067. PMCID: PMC3075948

   d. Gould T, Stewart J, Slater D, McEwen N, **Kaufman JD**, Larson T. A controlled inhalation diesel exhaust exposure facility with dynamic feedback control of PM concentration. Inhalation Toxicology 2008;20:49-52 PMID: 18236222

4. Association of environmental factors with subclinical and intermediate disease markers can provide key insights into mechanisms of effects.

   a. Hajat A, Allison M, Diez-Roux AV, Jenny NS, Jorgensen N, Szpiro AA, Van Hee VC, Vedal S, **Kaufman JD**. Long-term exposure to air pollution and markers of inflammation, coagulation and endothelial activation: A repeat measures analysis in the Multi-Ethnic Study of Atherosclerosis (MESA). Epidemiology. 2015 May;26(3):310-20. PMCID: PMC4455899

   b. Adar SD, **Kaufman JD**, Diez-Roux AV, Hoffmann EA, D'Souza J, Stukovsky KDH, Rich SS, Rotter JI, Guo X, Raffel LJ, Sampson PD, Oron AP, Raghunathan T, Barr RG. Air pollution and percent emphysema identified by computed tomography in the Multi-Ethnic Study of Atherosclerosis. Environ Health Perspect. 2015 Feb;123(2):144-51. PMCID: PMC4314244

   c. Leary PJ, **Kaufman JD**, Barr RG, Bluemke DA, Curl CL, Hough CL, Lima JA, Szpiro AA, Van Hee VC, Kawut SM. Traffic related air pollution and the right ventricle: The Multi-Ethnic Study of Atherosclerosis. Am J Respir Crit Care Med. 2014 May 1;189(9):1093-100. PMCID: PMC4098110

   d. Adar SD, Klein R, Klein BE, Szpiro AA, Cotch MF, Wong TY, O'Neill MS, Shrager S, Barr RG, Siscovick DS, Daviglus ML, Sampson PD, **Kaufman JD**. Air Pollution and the Microvasculature: A cross-sectional assessment of in vivo retinal images in the population-based Multi-Ethnic Study of Atherosclerosis (MESA). PLoS Med. 2010 Nov 30;7(11):e1000372. PMCID: PMC2994677

5. Epidemiological research is enhanced by incorporating cutting-edge methodologies that span disciplines, including new statistical methods and potential confounding and complicating features.

   a. Szpiro AA, Sheppard L, Adar SD, **Kaufman JD**. Estimating acute air pollution health effects from cohort study data. Biometrics 2014 70:164-174. PMCID: PMC4080094

   b. Hajat A, Diez-Roux AV, Adar AD, Auchincloss AH, Lovasi GS, O'Neill MS, Sheppard L, **Kaufman JD**. Air Pollution and Individual and Neighborhood Socioeconomic Status: Evidence from the Multi-Ethnic Study of Atherosclerosis (MESA). Environmental Health Perspectives 2013;121:1325-1333. PMCID: PMC3855503

c. Hicken M, O'Neill MS, Auchincloss AH, Magzamen SL, **Kaufman JD**, Diez Roux AV. Do psychosocial stress and social disadvantage modify the association between air pollution and blood pressure? The Multi-Ethnic Study of Atherosclerosis. Am J Epidemiol 2013;178:1550-1562. PMCID: PMC3888274

d. Jones MR, Diez-Roux AV, Hajat A, Kershaw KN, O'Neill MS, Guallar E, Post W, **Kaufman JD**, Navas-Acien A. Race/ethnicity, residential segregation and exposure to ambient air pollution: the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Public Health 2014. PMCID: PMC4202969

Complete List of Published Work in My Bibliography (of >175 original articles):
www.ncbi.nlm.nih.gov/myncbi/joel.kaufman.1/bibliography/40707694/public/?sort=date&direction=descending

D. RESEARCH SUPPORT

Ongoing Research Support

R01 ES025888 (Kaufman, Chen, MPI) 7/1/2016-6/30/2021
Environmental Determinants of Pathological Brain Aging in WHI Memory Studies
This project will address the impact of long-term exposures on dementia/AD incidence, latent trajectories of internally validated neuropsychological biomarkers, evaluate the hypothesized mediation of these outcomes, and explore the associations between PM exposure sources/compositions and brain aging.

R01 ES023500 (Kaufman, Hansel, MPI) 02/24/14-10/31/18
SPIROMICS - Air Pollution Study
This multi-site, prospective cohort study is evaluating impact of air pollutants on progression and exacerbations of Chronic Obstructive Pulmonary Disease This project adds air pollution exposure monitoring and modeling to an NHLBI cohort study--the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS). Hansel and Kaufman are MPIs.

R56ES026528 (Kaufman, PI) 2/1/2017 – 1/30/2018 (estimate)
Air Pollution, Heart Failure and Atrial Fibrillation in MESA
This award funds air pollutant exposure assessment activities in the MESA cohort during the sixth clinical examination. This project is funded under the High Priority, Short-Term Project Award (R56) mechanism. NIH uses this one- or two-year mechanism to "underwrite highly meritorious applications which have fallen just beyond an established payline".

R01ES026187 (Li, Sheppard, MPI) 9/30/2016-7/31/2021
Title: Air Pollution, The Aging Brain and Alzheimer's Disease
The project examines the effects of air pollution on cognitive decline, all-cause dementia and AD incidence, and brain neuropathologies in the Adult Changes in Thought (ACT) study. We will collect new air pollution measurements to develop for ACT participants state-of-the-art long-term average air pollution predictions for ambient PM2.5, O3, NOx, and NO2. Our overarching goal is to identify air pollution risk factors and quantify their effects in order to promote healthy aging.
Role: Co-Investigator

1UG3OD023271-01 (Karr, Bush, LeWinn, Sathyanarayana, Tylavsky, MPIs) 09/21/2016 – 08/31/2018
Prenatal and Early Childhood Pathways To Health: An Integrated Model of Chemical and Social Exposures, Biological Mechanisms, and Sex-Specific Effects on Neurodevelopment and Respiratory Outcomes
This multi-PI cohort study involves over 3000 mother-child dyads as part of a new national program to understand the role of early life exposures in the development of core pediatric health outcomes. The Pathways study will evaluate exposures to air pollution, phthalates, and stress during pregnancy and impacts on early and later childhood development of asthma, atopy, and neurodevelopmental health.
Role: Co-Investigator

R01 HD078501, NIH/NICHD (Crowder, PI) 08/22/14-04/30/18
Title: Demographic Vulnerability, Neighborhood Pollution, and Racial Health Disparities
The project focuses on the ways in which social and economic conditions at the individual-, family-, and neighborhood-levels interact with neighborhood concentrations of environmental pollution to affect racial disparities in health and mortality. We assess neighborhood pollution, along with information on other neighborhood characteristics, to longitudinal data for a diverse group of individuals in the national Panel Study of Income Dynamics, to study long-term effects of pollution on mortality, self-rated health, physical limitations, and cancer, cardiovascular disease, and asthma.
Role: Co-Investigator

T42 OH008433, Centers for Disease Control and Prevention (Kalman, PI) 7/1/13-6/30/20

Northwest Center for Occupational Health and Safety
This is a multidisciplinary NIOSH Education and Research Center providing training for occupational health and safety professionals.
Role: Director of the occupational medicine component.

5P30ES007033, NIH/NIEHS (Kavanagh, PI) 4/1/16-3/31/21
PI: Kavanagh
Interdisciplinary Center for Exposures, Diseases, Genomics, and Environment (EDGE Center)
This is a renewal of a longstanding NIEHS Core Center. Dr. Kaufman is Deputy Center Director and the Director of the Integrated Environmental Health Sciences Facility Core
Role: Deputy Director, Core Director

RD-83479601, USEPA (Vedal, PI) 12/01/10-11/30/17
PI: Vedal
UW Center for Clean Air Research
The aim of the five project UW CCAR is to disentangle features of this complex mixture to provide insight into those that are especially toxic to the cardiovascular system. The ultimate aim is to identify the specific near-roadway emission sources and interactions that produce the greatest toxicity.
Role: Project lead for two projects

15PRE25680066, AHA (Cosselman, PI) 7/1/15-6/30/17
Mechanisms and Markers of the Cardiovascular Response to Diesel Exhaust in Humans
Doctoral award. This fellowship proposal integrates basic science with clinical measures to examine aspects of the vascular response in humans exposed to diesel exhaust, a model traffic-related pollutant.
Role: Mentor (unfunded).

Completed Research Support

R01ES020871 (Wellenius, PI) 7/15/12-3/31/17
Ambient Air Pollution and Incidental Stroke
The association between exposure to ambient particulate matter (PM) and PM chemical components and the risk of cerebrovascular events within the Women's Health Initiative (WHI) cohort is being investigated. Long-term concentrations of PM and PM components will be estimated using a national spatio-temporal model that makes use of national pollution monitoring data, geographic data and geostatistical estimation methods.
Role: Co-Investigator

R831697 (Kaufman, PI) 8/1/04-7/31/14
US Environmental Protection Agency
Prospective Study of Atherosclerosis, Clinical Cardiovascular Disease, and Long-Term Exposure to Ambient Particulate Matter and Other Air Pollutants in a Multi-Ethnic Cohort
This major, multi-site, prospective cohort study ("MESA Air") was conducted in the NIH/NHLBI Multi-Ethnic Study of Atherosclerosis (MESA) and examined the relationship between air pollutants, the progression of subclinical atherosclerosis, and incidence of cardiovascular events in several US communities.

P50 ES015915 (Kaufman, PI) 6/1/08 – 5/31/15
DISCOVER Center: Cardiovascular Disease and Traffic-Related Air Pollution
This specialized center examined the mechanisms by which traffic-related air pollution causes myocardial infarction and other cardiovascular diseases, by integrating a diverse set of research approaches from basic science to clinical research to population-based studies. There were five projects within this Center.
Role: Center Director, PI Project 1, Director of Administrative Core

R01 ES009411 (Spiegelman, PI; Kaufman, PI on UW sub) 05/01/09-02/28/15
Measurement Errors in Environmental Epidemiology
Primary aim of UW activity was to conduct a validation study relating personal exposure of individuals in epidemiological studies to routine air pollution exposure assessment methods, and advance methods to integrate measurement error correction into epidemiological analyses.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Kisler Elliott, Kassandra

eRA COMMONS USER NAME (credential, e.g., agency login): silverbow

POSITION TITLE: Research Associate

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
San Jose City College, San Jose, CA	AA	05/96	Physics (Honors)
University of California at Davis, Davis, CA	BAS	12/99	Applied Physics (Honors), Chemistry (Honors; dual major)
Cornell University, Ithaca, NY	MS PhD	05/06 08/09	Applied Physics Applied Physics
Cornell University, Ithaca, NY	Postdoctoral	07-12/09	Applied Physics/ Chemical and Biomolecular Engineering/ Microbiology and Immunology
Keck School of Medicine of the University of Southern California, Los Angeles, CA	Postdoctoral	03/16	Physiology and Biophysics/ Neuroscience
Keck School of Medicine of the University of Southern California, Los Angeles, CA	Research Associate	4/16-present	Physiology and Biophysics/ Neuroscience

A. Personal Statement

My formal training is in Applied Physics, but my emphasis in graduate school and as a post-doctoral researcher has been in biophysics, and the development and application of advanced imaging techniques to biological problems. As a graduate student in Dr. Manfred Lindau's laboratory, I studied the mechanism of exocytosis, a critical cellular function that has been implicated in various diseases, at the single cell level using the electrochemical technique of amperometry and various fluorescence imaging techniques, including total internal reflection fluorescence (TIRF) and confocal microscopy. I developed microelectrode arrays for amperometric detection of exocytotic release events from single cells, and combined them with then cutting-edge fluorescence microscopy techniques to tease out details of the SNARE proteins involved in the underlying molecular mechanism, resulting in two publications in *PNAS*. Note that I typically publish under my maiden name, *Kassandra Kisler*. At the end of my PhD, I spent a brief but fruitful time in collaboration between Drs. Lois Pollack and Susan Daniel establishing a TIRF microscopy system and developing protocols to investigate viral fusion into supported lipid bilayers using two-color TIRF microscopy and caged (photoactivatable) compounds.

I furthered my training in advanced imaging techniques and electrophysiology in the laboratory of Dr. Robert H. Chow while studying complexin, a protein involved in exocytosis. I used single cell patch clamp electrophysiology to detect differences in exocytotic function in chromaffin cells expressing mutant forms of complexin. Here, I had the opportunity to attend the Advanced Imaging Methods (AIM) Workshop at UC Berkeley, where lecturers have often included Nobel Prize winners. In experiments with our collaborators, I gained experience in multiphoton imaging of mouse retina slices. Additionally, I used "super-resolution" imaging techniques to further my studies of the complexin protein, which included design and construction of a super-resolution microscope for the Chow lab. Because of my broad background and expertise in imaging techniques, I was invited to attend the prestigious European Molecular Biology Organization (EMBO) Advanced Optical Microscopy Course in Plymouth, England as a teaching assistant.

My work in the Zlokovic lab builds upon my experience with imaging techniques and electrophysiology to tackle important questions related to blood-brain barrier (BBB) integrity, vascular regulation and function, its relation to brain electrical activity, and the mechanisms underlying neurodegenerative diseases such as Alzheimer's using transgenic model animals. My work has included the study of transcytosis of amyloid across the BBB using various *in vivo* and *in vitro* techniques, and establishment of *in vitro* electrophysiology techniques for the lab. Furthermore, I have introduced new protocols for *in vivo* intrinsic optical signal imaging, and visualization of cortical electrical activity using high-speed voltage sensitive dye imaging. My current projects, including a recently published study in *Nature Neuroscience*, focus on the role of pericytes in regulating cortical circulation *in vivo* using exogenous tracers with multiphoton microscopy. Here, I have established multiphoton techniques to study vascular dynamics and blood flow changes on the single capillary level in living mice. Furthermore, I have established techniques in the lab for quantitative multiphoton optical measurement of vascular leakage and permeability-surface (PS) constants *in vivo*. Leveraging these techniques has resulted in a method manuscript currently in preparation and two additional studies in preparation for, and under review in top scientific journals. **Overall, my expertise in these techniques provides me with the background necessary to successfully conduct the experiments proposed**.

Relevant publications and abstracts:

Hafez, I., **K. Kisler**, K. Berberian, G. Dernick, V. Valero, M.G. Yong, H.G. Craighead, M. Lindau. "Electrochemical imaging of fusion pore openings by electrochemical detector arrays." *PNAS* 102: 13879-13884 (2005).

Ngatchou, A., **K. Kisler**, Q. Fang, A. M. Walter, Y. Zhao, D. Bruns , J. B. Sørensen, and M. Lindau. "Movement of synaptobrevin C-terminus induces fusion pore formation." *PNAS,* 107, 18463-18468 (2010).

Zhao Z*, A. P. Sagare*, Q. Ma*, M. R. Halliday, P. Kong, **K. Kisler**, E. A. Winkler, A. Ramanathan, T. Kanekiyo, G. Bu, N. C. Owens, S. V. Rege, G. Si, A. Ahuja, D. Zhu, C. A. Miller, J. A. Schneider, M. Maeda, T. Maeda, T. Sugawara, J. K. Ichida, B. V. Zlokovic. "Central role for PICALM in amyloid-β blood-brain barrier transcytosis and clearance." *Nature Neuroscience*. 2015 Jul;18(7):978-87. *Contribute equally.

K. Kisler*, A.R. Nelson*, S. V. Rege*, A. Ramanathan, Y. Wang , A. Ahuja, D. Lazic, P. S. Tsai, Z. Zhao, Y. Zhou, D. A. Boas, S. Sakadžić, B. V. Zlokovic."Pericyte degeneration leads to neurovascular uncoupling and limits oxygen supply to brain." *Nature Neuroscience*. 2017; 20(3):406-416. *Contribute equally.

B. Positions and Honors

Positions and Employment

2000-2001	Manufacturing Test Engineer, Coherent, Inc., Santa Clara, CA
2002-2009	Research Assistant, Laboratory of Manfred Lindau, PhD, Cornell University, Ithaca, NY
2009	Postdoctoral Research Associate, Laboratories of Lois Pollack, PhD and Susan Daniel, PhD, Cornell University, Ithaca, NY
2010-2012	Postdoctoral Research Associate, Laboratory of Robert H. Chow, MD, PhD, University of Southern California Keck School of Medicine, Los Angeles, CA
2012-2016	Postdoctoral Research Associate, Laboratory of Berislav V. Zlokovic, MD, PhD, University of Southern California Keck School of Medicine, Los Angeles, CA

2016- Research Associate, Laboratory of Berislav V. Zlokovic, MD, PhD, University of Southern California Keck School of Medicine, Los Angeles, CA

Teaching Experience

2001-2005 Teaching Assistant/Grader, School of Applied and Engineering Physics, Cornell University, Ithaca, NY
2002- Supervisor/Mentor of multiple students at the high school, undergraduate, and graduate level in the laboratories of Manfred Lindau, Robert H. Chow, and Berislav V. Zlokovic.
2005-2009 Teaching Assistant, Biophysical Methods Advanced Laboratory, School of Applied and Engineering Physics, Cornell University, Ithaca, NY
2005-2015 Guest Lecturer, Biophysical Methods, Physical Methods in Biochemistry, Molecular Biology of Cellular Communication in the Nervous System courses, Cornell University, Ithaca, NY, University of Southern California, Los Angeles, CA
2012 Teaching Assistant, European Molecular Biology Organization (EMBO) Advanced Optical Microscopy Course, Plymouth, England
2015-2017 Los Angeles/Irvine Annual Brain Bee Volunteer Scientist

Professional Memberships

Member, Society for Neuroscience
Member, Biophysical Society
Member, Golden Key International Honor Society
Member, Sigma Pi Sigma Physics Honor Society

Honors

1994, 1995 San Jose City College Outstanding Academic Achiever in Math and Science
1996 San Jose City College Outstanding Graduate in Math and Science
1996, 1998 UC Davis James & Lela Fulmor Scholarship
1998, 1999 UC Davis Departmental Citation for Outstanding Accomplishment in Physics
1999 UC Davis Saxon-Patten Prize in Physics
2006 13th International Symposium on Chromaffin Cell Biology Travel Scholarship
2007 Chosen to attend the Leadership Alliance National Symposium
2007 Featured in the Leadership Alliance 2007-2008 Emerging PhDs Yearbook
2008 Successfully competed to attend the NIH Graduate Research Festival
2008 Cornell Graduate School Travel Grant
2011 Edmund Optics Higher Education Grant Finalist
2012 William Hansen Sandberg Memorial Foundation Postdoctoral Travel Scholarship

C. Contribution to Science

TIRF microscopy and electrophysiology: The exocytotic mechanism, cell surface trafficking, and neuronal function. Much of my graduate and early postdoctoral work focused on the development and use of electrophysiological detectors, and their use in combination with fluorescence and TIRF microscopy to gain insight into the mechanism of exocytosis. Exocytosis is a highly regulated cell process by which cells, most importantly neurons, release signaling molecules or neurotransmitters. Dysregulation of this process is implicated in multiple diseases. My work involved design and development of microfabricated planar electrode arrays sized to fit single cells on glass coverslips. These arrays enabled fluorescent observation of labeled proteins or compartments within the cells of interest simultaneously with amperometric electrophysiology measurement. Using this method, I and my colleagues were able to measure accurate transmitter release and diffusion from single cells, and changes in exocytosis with manipulation of synaptobrevin, a SNARE protein essential for exocytosis.

I also applied the TIRF imaging technique to the study of estrogen receptor-alpha (ERα) at the cell surface in live cells in real time, publishing one of the first papers detailing this technique. To this point, evidence of estrogen receptor presence at the cell membrane and live visualization of its trafficking was virtually unexplored. Our experiments showed that ERα was present at the membrane in live cells, and that binding of ligands to ERα at the cell surface induced endocytosis of the receptor.

More recently, I have mastered patch clamp electrophysiology and multielectrode array measurement techniques. In collaboration with the Ichida Lab, I applied these techniques to study motor neuron degeneration mechanisms in ALS using patient-derived motor neurons, helping to elucidate the mechanism behind the C9orf72 risk gene. This work is currently in revision for a high-impact journal.

1. **Kisler, K**, R. H. Chow, & R. Dominguez. "Fluorescently-labeled estradiol internalization and membrane trafficking in live N-38 neuronal cells visualized with total internal reflection fluorescence microscopy." *Journal of Steroids & Hormonal Science,* 2013, S12:002

2. **Kisler, K.**, B. N. Kim, X. Liu, K. Berberian, Q. Fang, C. J. Mathai, S. Gangopadhyay, K. D. Gillis, and M. Lindau. “Transparent Electrode Materials for Simultaneous Amperometric Detection of Exocytosis and Fluorescence Microscopy.” *Journal of Biomaterials and Nanobiotechnology*, 2012 3(2): 243-253. Featured on cover.

3. Ngatchou, A., **K. Kisler**, Q. Fang, A. M. Walter, Y. Zhao, D. Bruns , J. B. Sørensen, and M. Lindau. “Movement of synaptobrevin C-terminus induces fusion pore formation.” *PNAS,* 107, 18463-18468 (2010).

4. Hafez, I., **K. Kisler**, K. Berberian, G. Dernick, V. Valero, M.G. Yong, H.G. Craighead, M. Lindau. "Electrochemical imaging of fusion pore openings by electrochemical detector arrays." *PNAS* 102: 13879-13884 (2005).

5. Son EY, Ichida JK, Wainger BJ, Toma JS, Rafuse VF, Woolf CJ, Eggan K. "Conversion of mouse and human fibroblasts into functional spinal motor neurons." *Cell Stem Cell*. 2011 Sep 2;9(3):205-18.

In vivo Imaging: Vascular contributions to neurodegenerative diseases

In my work studying the vascular contributions to neurodegenerative diseases such as Alzheimer's, I have focused strongly on the application of *in vivo* imaging techniques to study vascular dynamics and the neurovascular unit in real time. The neurovascular unit is composed of several cell types working in concert to maintain the blood-brain barrier (BBB) and regulate the blood and nutrient supply to the brain. The pericyte, one of the cell types that compose the neurovascular unit, has been shown to play a role in maintenance of the BBB, but its role in vascular dynamics is unclear. Using loss-of-function pericyte-deficient mice and multiple *in vivo* multiphoton and other imaging techniques, we demonstrated that pericyte degeneration leads to diminished CBF responses, neurovascular uncoupling, reduced oxygen supply to brain and metabolic stress, which over time lead to impaired neuronal excitability and neurodegenerative changes. These important findings have led to a publication detailing these observations and follow up studies (in progress). My use of *In vivo* multiphoton imaging has also lead to new information regarding permeability of brain vasculature in pericyte deficient and Alzheimer's disease ApoE4 model animals.

1. **K. Kisler***, A.R. Nelson*, S. V. Rege*, A. Ramanathan, Y. Wang , A. Ahuja, D. Lazic, P. S. Tsai, Z. Zhao, Y. Zhou, D. A. Boas, S. Sakadžić, B. V. Zlokovic."Pericyte degeneration leads to neurovascular uncoupling and limits oxygen supply to brain." *Nature Neuroscience*. 2017; 20(3):406-416. *Contribute equally.

2. "Pericyte ablation leads to disruption of the neurovascular unit." A. M. Nikolakopoulou, **K. Kisler**, P. Kong, D. Lazic, A. P. Sagare, M. D. Sweeney, E. J. Lawson, Y. Yang, A. Go, B. V. Zlokovic, Z. Zhao. Society for Neuroscience Annual Meeting, November 2016.

3. "Apolipoprotein E4 allele differentially modulates cerebral blood flow and blood-brain barrier permeability in Alzheimer’s disease" A. Montagne, J. Pa, D. A. Nation, S. R. Barnes, M. P. Ellis, **K. Kisler**, A. R. Nelson, S. V. Rege, M. D. Sweeney, A. P. Sagare, J. Makshanoff, Y. Shi, R. E. Jacobs, A. W. Toga, M. G. Harrington7, C. Y. Liu, M. Law, H. C. Chui, B. V. Zlokovic. Society for Neuroscience Annual Meeting, October 2015.

4. **K. Kisler***, A.R. Nelson*, A. Montagne*, B. V. Zlokovic. "Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease." *Nat Rev Neurosci*. 2017 May 18. *Contribute equally.

Regulation of PICALM: An Alzheimer's risk gene.
Another focus of my work has been to help uncover the role of the Alzheimer's Disease (AD) risk gene phosphatidylinositol binding clathrin assembly (PICALM) protein in disease progression. It is known that there is less PICALM present in the brains of AD patients then healthy individuals, but how that affects the disease still a topic of debate. Recently, we identified a critical role for PICALM in brain endothelium, where the protein acts as an endocytotic adaptor protein and chaperone to direct clearance of amyloid out of the brain. Furthermore, we found that a reduction of PICALM expression in amyloid overproducing mice retained more amyloid in their brains then littermates with normal PICALM expression. Currently, I am in the process of screening for drugs that affect PICALM regulation, with the goal to develop a new therapy for AD.

1. Zhao Z*, A. P. Sagare*, Q. Ma*, M. R. Halliday, P. Kong, **K. Kisler**, E. A. Winkler, A. Ramanathan, T. Kanekiyo, G. Bu, N. C. Owens, S. V. Rege, G. Si, A. Ahuja, D. Zhu, C. A. Miller, J. A. Schneider, M. Maeda, T. Maeda, T. Sugawara, J. K. Ichida, B. V. Zlokovic. "Central role for PICALM in amyloid-β blood-brain barrier transcytosis and clearance." *Nature Neuroscience*. 2015 Jul;18(7):978-87. *Contribute equally.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/kassandra.kisler elliott.1/bibliography/50249596/public/?sort=date&direction=ascending

D. Research Support

Cure Alzheimer's Fund PI: Berislav V. Zlokovic 10/2015-9/2017
PICALM gene therapy and drug screening for Abeta clearance
This project is aimed at developing drugs and therapies to increase the expression of PICALM in brain vascular endothelium to aid n the clearance of amyloid proteins from the brain.
Role: Postdoc researcher

Completed Research Support

Cure Alzheimer's Fund PI: Berislav V. Zlokovic 9/2012-9/2013
The role of PICALM in vascular clearance of amyloid-β
This pilot project is aimed at studying the role of the PICALM protein in the clearance of amyloid proteins in health and disease.
Role: Postdoc researcher

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: William S. Kremen

eRA COMMONS USER NAME (credential, e.g., agency login): wkremen

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Arizona; Tucson, AZ	B.A.	1974	Psychology
New York University; New York, NY	M.A.	1982	Psychology
Boston University; Boston, MA	Ph.D.	1990	Clinical Psychology

A. Personal Statement

I am a clinical psychologist and research neuropsychologist. I am also Professor in the Department of Psychiatry at the University of California, San Diego, Co-Director of the Center for Behavior Genetics of Aging and Director of the Twin Research Laboratory in the Department's Center for Behavioral Genomics. I have been PI of the Vietnam Era Twin Study of Aging (VETSA) projects since 2001: primary study; VETSA MRI; VETSA Cortisol. Since the start of VETSA, I continue to work very closely with the contact PI, Dr. Franz, who is Co-Director of VETSA and of the Center for Behavior Genetics of Aging. I have also worked closely with Drs. Fennema-Notestine (VETSA MRI 1 & 2) and Hagler (VETSA MRI 2), and with Dr. Reynolds on VETSA 3 and the international twin consortium on Interplay of Genes and Environment (IGEMS). VETSA is a set of longitudinal behavioral genetic studies that focuses on genetic and environmental influences on aging and outcomes of approximately 1500 middle-aged twins living throughout the U.S. VETSA includes extensive cognitive, psychosocial, psychiatric symptom, health/medical, sleep quality, neuroendocrine, and multi-modal neuroimaging assessments. We also have genome-wide genotyping data and plasma stored from wave 1 for assessment of biomarkers. At wave 1, all subjects were 51-60 years old, so we have detailed characterization beginning in midlife. We began data collection for wave 3 in March 2016. Data are also available from prior assessments of VETSA twins at average ages 20, 37, and 42, thus allowing for the study of very long-term trajectories and outcomes, including the role of genetic factors and associations with brain structure. This makes VETSA very strong with respect to cognitive and brain aging, and early identification of risk for mild cognitive impairment (MCI) and Alzheimer's disease. I, thus, have substantial experience in coordinating a large twin study project, in neurocognitive assessment and analysis, and analysis of twin/genetic data. I will ensure that appropriate variables from these studies are incorporated into the database along with the newly collected geocoding/pollutant data. We will be able to extend previous work on the effects of air pollutants on brain and cognition because VETSA has much more extensive data in both of these domains. My responsibilities will be primarily on overseeing the cognitive and imaging data. I will work with the other investigators to refine variables and to maximize comparability of variables for analysis across different studies. I will work closely with the other investigators to provide all relevant VETSA data, and ensure the proper analysis and interpretation of all of the data. I will also play a key role in editing any presentations or manuscripts.

1. Chen, C-H, Gutierrez, E, Thompson, W, Panizzon, M, Jernigan, T, Eyler, L, Fennema-Notestine, C, Jak, A, Neale, M, Franz, C, Lyons, M, Grant, M, Fischl, B, Seidman, L, Tsuang, M, **Kremen, W***, Dale, A*. (*joint senior authors) 2012. Hierarchical genetic organization of human cortical surface area. *Science*, 335: 1634-1636. PMCID: 3690329
2. **Kremen, W**, Jak, A, Panizzon, M, Spoon, K, Franz, C, Thompson, W, Jacobson, K, Vasilopoulos, T, Vuoksimaa, E, Xian, H, Toomey, R, Lyons, M. 2014. Early identification and heritability of mild cognitive impairment. *International Journal of Epidemiology*, 43: 600-610. PMCID: PMC3997374

3. Panizzon, M, Fennema-Notestine, C, Eyler, L, Jernigan, T, Prom-Wormley, E, Neale, M, Jacobson, K, Lyons, M, Grant, M, Franz, C, Tsuang, M, Fischl, B, Seidman, L, Dale, A, **Kremen, W.** 2009. Distinct genetic influences on cortical surface area and thickness. *Cerebral Cortex*, 19: 2728-2735. PMCID: PMC2758684
4. Granholm, E.L., Panizzon, M.S., Elman, J.A., Jak, A.J., Hauger, R.L., Bondi, M.W., Lyons, M.J., Franz, C.E.*, **Kremen, W.S.*** (*joint senior authors) 2017. Pupillary responses as a biomarker of early risk for Alzheimer's disease. *Journal of Alzheimer's Disease*, 56: 1419-1428. PMCID: N/A

B. Positions and Honors

Positions and Employment

1985-1986	Psychology Fellow (Intern), APA-approved Clinical Psychology Internship, Massachusetts Mental Health Center, Harvard Medical School
1987 - 1989	Research Associate; Department of Psychiatry; Harvard Medical School
1988 - 1995	Health Statistician; Brockton/West Roxbury VA Medical Center
1989 - 1995	Associate Director of Neuropsychological Studies; Section of Psychiatric Epidemiology and Genetics; Harvard Medical School/Brockton VA Medical Center
1990 - 1995	Instructor in Psychology; Department of Psychiatry; Harvard Medical School
1995	Staff Psychologist; Biol. Psychiatry Treatment & Research Ctr; Napa State Hospital; Napa, CA
1995 - 1996	Assistant Professor; Department of Psychiatry; University of California, Davis
1996 - 2003	Associate Professor; Department of Psychiatry; University of California; Davis
2003	Professor; Department of Psychiatry; University of California; Davis
2003-present	Professor, Department of Psychiatry; University of California, San Diego
2004-present	Faculty Member, Stein Institute for Research on Aging, University of California, San Diego
2005-present	Editorial Board, Neuropsychology Review
2005-present	Member, Scientific Merit Review Board, VA Health Services R&D Study Section
2007-present	Director, Lifespan Unit, Center of Excellence for Stress and Mental Health (CESAMH), VA San Diego Healthcare System
2010-present	Director, Twin Research Laboratory, Ctr. for Behavioral Genomics, Dept of Psychiatry, UCSD
2014-present	Member, Neuroscience Unit, Center of Excellence for Stress and Mental Health (CESAMH), VA San Diego Healthcare System

Honors

1974	Senior Scholarship Award; University of Arizona
1980 - 1981	Graduate School Scholarship; New York University
1982 - 1985	Presidential University Fellowship, Boston University
1992	Young Investigator Award; National Alliance for Research on Schizophrenia & Depression
1993	Young Investigator Award; International Congress on Schizophrenia Research
1999 - 2001	Faculty/Alumni Research and Development Fund Award; University of California, Davis
2001	Professional Development Leave Award; Academic Federation, University of California, Davis
2012	Faculty of 1000 (F1000.com) ranked VETSA article, Genetic influences on cortical regionalization in the human brain, *Neuron*, 2011, in "the top 2% of published articles in biology and medicine."
2014	University of California, San Diego Equal Opportunity/Affirmative Action & Diversity Award for Department of Psychiatry Advisory Committee on Diversity Issues

C. Contribution to Science

1. Publications in the early stages of my career were focused on neuropsychological function and brain structure in people with schizophrenia and their nonpsychotic biological relatives. The predominant view in the field had been that cognitive deficits were downstream consequences of preoccupation with delusions and hallucinations. It was also unclear whether brain abnormalities in schizophrenia were pre-existing or consequences of illness, and if normal neuropsychological function was possible in people with schizophrenia. These publications showed there were cognitive deficits and hippocampal volume reductions in relatives who were genetically related to people with schizophrenia, but who did not themselves have the illness. After matching for educational attainment, people with schizophrenia had poorer cognitive function than controls but equal or higher estimated premorbid ability. If you matched for IQ, people with schizophrenia had poorer neuropsychological function than controls. This work was among the first to show that some brain abnormalities are likely pre-existing, and that cognitive deficits are core features of schizophrenia. It contributed key findings clarifying the question of risk factors vs. consequences of illness, and it resulted in a 180-degree shift of the predominant view of cognition in

schizophrenia. It also made important contributions to understanding of the trajectory of schizophrenic illness, strongly suggesting that all people with schizophrenia have impaired cognitive function relative to their premorbid ability. I was a co-investigator on all of these studies, and led the science in several of them.

a. **Kremen, W**, Seidman, L, Faraone, S, Pepple, J, Lyons, M, Tsuang, M. 1996. The "3 R's" and neuropsychological function in schizophrenia: An empirical test of the matching fallacy. *Neuropsychology* 10:22-31 PMCID: N/A.
b. **Kremen, W**, Seidman, L, Faraone, S, Toomey, R, Tsuang, M. 2000. The paradox of normal neuropsychological function in schizophrenia. *J Abnormal Psychology* 109: 743-752 PMCID: N/A.
c. **Kremen, W**, Seidman, L, Faraone, S, Tsuang, M. 2001. Intelligence Quotient and neuropsychological profiles in patients with schizophrenia and normal volunteers. *Biological Psychiatry* 50: 453-462 PMCID: N/A.
d. Seidman, L, Faraone, S, Goldstein, J, **Kremen, W**, Horton, N, Makris, N, Toomey, R, Kennedy, D, Caviness, V, Tsuang, M. 2002. Left hippocampal volume as a vulnerability indicator in schizophrenia: An MRI study of nonpsychotic first-degree relatives. *Arch Gen Psychiatry* 58: 839-849 PMCID: N/A.

2. The power of family/genetic studies to change (even reverse) thinking in the field led to greater interest in truly genetically-informative studies, i.e. twin studies. That ultimately led to my NIA-funded VETSA projects, a set of large-multidisciplinary projects with approximately 25 investigators and consultants that I lead. One key VETSA contribution is basic science work needed to better understand the genetic underpinnings of brain structure so it can then be applied to studying the genetics of cognition and psychiatric disorders. Most MRI studies of cortex, including psychiatric disorders, examined cortical volumes. We showed that global cortical surface area and thickness are genetically distinct. Volume (area x thickness) thus conflates two distinct sets of genes. Thus, it is best to examine surface area and thickness separately, particularly for genetic studies. This work has substantial impact as most studies now do this. We then showed that general cognitive ability is driven by genetic associations with surface area rather than thickness. All prior brain atlases were based on structure or function. We created the first brain atlas derived solely on genetically-based information. We were the first to show in living human brains the anterior-posterior gradient of genetic effects, i.e., the same genes that cause anterior cortical area expansion cause posterior contraction. I am PI on all of these studies.

a. Panizzon, M, Fennema-Notestine, C, Eyler, L, Jernigan, T, Prom-Wormley, E, Neale, M, Jacobson, K, Lyons, M, Grant, M, Franz, C, Tsuang, M, Fischl, B, Seidman, L, Dale, A, **Kremen, W.** 2009. Distinct genetic influences on cortical surface area and thickness. *Cerebral Cortex*, 19: 2728-2735. PMCID: PMC2758684
b. Chen, C-H, Panizzon, M, Eyler, L, Jernigan, T, Thompson, W, Fennema-Notestine, C, Jak, A, Neale, M, Franz, C, Hamza, S, Lyons, M, Grant, M, Fischl, B, Seidman, L, Tsuang, M, **Kremen, W***, Dale, A*. (*joint senior authors) 2011. Genetic influences on cortical regionalization in the human brain. *Neuron*, 72: 537-544. PMCID: PMC3222857
c. Chen, C-H, Gutierrez, E, Thompson, W, Panizzon, M, Jernigan, T, Eyler, L, Fennema-Notestine, C, Jak, A, Neale, M, Franz, C, Lyons, M, Grant, M, Fischl, B, Seidman, L, Tsuang, M, **Kremen, W***, Dale, A*. (*joint senior authors) 2012. Hierarchical genetic organization of human cortical surface area. *Science*, 335: 1634-1636. PMCID: 3690329
d. Vuoksimaa, E, Panizzon, M, Chen C-H, Fieacas, M, Eyler, L, Fennema-Notestine, C, Hagler, D Jr, Fischl, B, Franz, C, Jak, A, Lyons, M, Neale, M, Rinker, D, Thompson, W, Tsuang, M, Dale, A, **Kremen, W.** 2015. The genetic association between neocortical volume and general cognitive ability is driven by global surface area rather than thickness. *Cerebral Cortex*, 25: 2127-2137b. PMCID: 4494025

3. The pathological process in AD begins decades before the onset of clinical dementia, and genetic factors are important in Alzheimer's disease (AD). Widespread agreement on these two truths about AD has led to emphasis on the paramount importance of early identification, and of elucidating the genetic underpinnings of cognitive function, cognitive decline, and dementia. A focus of VETSA was to use an extensive neurocognitive battery in order to avoid celling effects in middle-aged adults. With that battery, we were able to identify mild cognitive impairment (MCI) in our subjects when they were only in their 50s. Almost all other studies of MCI focus on adults 70+. Being able to identify MCI in midlife holds great promise for early identification. We provided validation for our diagnoses, showing that there was greater hippocampal atrophy in those with amnestic MCI. To our knowledge this study was also the first to show heritability of MCI. We have also been using multivariate biometrical modeling to elucidate the genetic

architecture of cognitive abilities. For example, we showed that there are different genetic influences underlying different episodic memory tests, and that they each change differently over time. We also showed that task-evoked pupil responses could serve as a biomarker of early risk for MCI or AD even before cognitive deficits are present. These findings highlight the importance of extensive neuropsychological assessment when assessing cognitive change, as well as the value that twin analyses can have for informing genome-wide association studies. I am PI on all of these studies.

a. **Kremen, W**, Jak, A, Panizzon, M, Spoon, K, Franz, C, Thompson, W, Jacobson, K, Vasilopoulos, T, Vuoksimaa, E, Xian, H, Toomey, R, Lyons, M. 2014. Early identification and heritability of mild cognitive impairment. *International Journal of Epidemiology*, 43: 600-610. PMCID: PMC3997374
b. Jak, A.J., Panizzon, M.S., Spoon, K.M., Fennema-Notestine, C., Franz, C.E., Thompson, W.K., Jacobson, K.C., Xian, H., Eyler, L.T., Vuoksimaa, E., Toomey, R., Lyons, M.J., Neale, M.C., Tsuang, M.T., Dale, A.M., and **Kremen, W.S.** 2015. Hippocampal atrophy varies by neuropsychologically-defined MCI Among Men in Their 50s. *American Journal of Geriatric Psychiatry*, 23: 456-465. PMCID: 435132
c. Panizzon, M.S., Neale, M.C., Docherty, A.R., Franz, C.E., Jacobson, K.C., Toomey, R., Xian, H., Vasilopoulos, T., Rana, B.K., McKenzie, R.M., Lyons, M.J., and **Kremen, W.S.** 2015. Genetic and environmental architecture of changes in episodic memory from middle to late middle age. *Psychology and Aging*, 30: 286-300. PMCID: N/A
d. Granholm, E.L., Panizzon, M.S., Elman, J.A., Jak, A.J., Hauger, R.L., Bondi, M.W., Lyons, M.J., Franz, C.E.*, **Kremen, W.S.*** (*joint senior authors) 2017. Pupillary responses as a biomarker of early risk for Alzheimer's disease. *Journal of Alzheimer's Disease*, 56: 1419-1428. PMCID: N/A

4. With our long-term longitudinal data, assessed when subjects' average age was 38 years, we have now begun to examine the very long-term outcomes associated with stress and PTS symptoms. This shows the importance of stress, not just PTSD. There are few longitudinal data on these very long-term outcomes. Importantly, they demonstrate the importance of stress on aging outcomes as even subthreshold PTS symptoms (when individuals meeting criteria for a diagnosis of PTSD were excluded) still have deleterious effects on functioning in middle and late middle age. They also address the question of whether factors are risk factors or consequences of illness. For example, our twin study showed that pre-trauma cognitive ability was a risk factor for PTSD, and that the relationship was due to shared genetic influences. We also examined genetic associations with cortisol (a "stress" hormone), showing negative effects of childhood adversity on cortisol and negative effects of cortisol on brain structure in frontal cortex, but not hippocampus in middle aged VETSA twins. I am PI on all of these studies.

a. **Kremen, W**, Koenen, K, Boake, C, Purcell, S, Eisen, S, Franz, C, Tsuang, M, Lyons, M. 2007. Pretrauma cognitive ability predicts risk for post-traumatic stress disorder: A twin study. *Archives of General Psychiatry*, 64: 361-368. PMCID: N/A
b. **Kremen, W**, O'Brien, R, Panizzon, M, Prom-Wormley, E, Eaves, L, Eisen, S, Eyler, L, Hauger, R, Fennema-Notestine, C, Fischl, B, Grant, M, Hellhammer, D, Jak, A, Jacobson, K, Jernigan, T, Lupien, S, Lyons, M, Mendoza, S, Neale, M, Seidman, L, Thermenos, H, Tsuang, M, Dale, A, Franz, C. 2010. Salivary cortisol and prefrontal cortical thickness in middle-aged men: A twin study. *Neuroimage*, 53: 1093-1102.
c. Franz, C, O'Brien, R, Hauger, R, Mendoza, S, Prom-Wormley, E, Eaves, L, Jacobson, K, Lyons, M, Lupien, S, Hellhammer, D, Xian, H, **Kremen, W.** 2011. Cross-sectional and 35-year longitudinal assessment of salivary cortisol and cognitive functioning: The Vietnam Era Twin Study of Aging. *Psychoneuroendocrinology*, 36: 1040-1052. PMCID: 3130089
d. Franz, C, Spoon, K, Thompson, W, Hauger, R, Hellhammer, D, Jacobson, K, Lupien, S, Lyons, M, McCaffery, J, McKenzie, R, Mendoza, S, Panizzon, M, Shahroudi, A, **Kremen, W.** 2013. Adult cognitive ability and socioeconomic status as mediators of the effects of childhood disadvantage on salivary cortisol in aging adults. *Psychoneuroendocrinology*, 38: 2127-2139. PMCID: 4755320

D. Research Support

R56 AG037985 (N. Pedersen) 09/01/16—08/31/2017
NIH/NIA
Gene-Environment Interplay of Social Contexts and Aging-Related Outcomes
The goal of this grant (aka the "iGEMS" consortium) is to identify social and environmental measures common to 8 different twin studies of aging, so as to examine the influence of gene by environment interactions on aging. Role: Co-Investigator

R01AG050595 (W. Kremen & M. Lyons) 9/01/2015–8/31/2019
NIH/NIA
The VETSA Longitudinal Twin Study of Cognition and Aging
The goal of this study is to collect a third wave of data to determine genetic and environmental influences on cognitive, personality/ psychosocial, and health/medical measures in middle aged twins. The study examines the inter-relationships among these domains and their ability to predict cognitive and adaptive aging.
Role: Principal Investigator

R01AG022381 (W. Kremen) 07/01/15 - 03/30/2020
NIH/UC San Diego
The VETSA Longitudinal MRI Twin Study of Aging
The goal of this study is to determine genetic and environmental influences on brain structure and brain aging via 3D structural MRI and diffusion tensor imaging in VETSA subjects. This is wave 3 of the study with future planned 5-year follow-ups.
Role: Principal Investigator

R01AG054002 (M. Tsuang & S. Glatt) 07/01/2016 - 06/30/2020
Gene Expression Biomarkers for Early Identification of Impairment: A Twin Study
The goals of the study are to collect additional blood samples from VETSA participants for the analysis of RNA transcripts. Using the twin method we will determine the heritability of expression levels of RNA, transcripts, determine the peripheral blood transcriptomic signature of mild cognitive impairment (MCI), and integrate these results with other putative biomarkers of MCI.
Role: Co-Investigator

R01AG054509 (E. Granholm & M. Bondi) 5/31/2017 – 8/31/2020
Pupillary Responses as a Risk and Staging Biomarker of Preclinical Alzheimer's Disease
The goal of this study is to demonstrate the utility of task-evoked pupillary responses as a biomarker of risk for the very early stages of Alzheimer's disease, and to validate it as a biomarker by examining its association with locus coeruleus contrast (an MRI-based indicator of locus coeruleus function) and CSF indices of beta-amyloid and tau.
Role: Co-Investigator

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Liu, Collins Yuchen

eRA COMMONS USER NAME (credential, e.g., agency login): NA

POSITION TITLE: Assistant Professor of Clinical Neurology and Radiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Berkeley, CA	BA	05/1997	Molecular and Cell Bio. Philosophy
New York Medical College, Valhalla, NY	MD	05/2004	Medicine
University of Pittsburgh Medical Center, PA	Internship	06/2005	Internal Medicine
University of Pittsburgh Medical Center, PA	Residency	06/2008	Neurology
Washington University, St. Louis, MO	Residency	06/2010	Nuclear Medicine
UCLA Brain Mapping Center, and VA Greater LA Healthcare System, CA	Fellowship	06/2012	Neuroimaging Behavioral Neurology

A. Personal Statement

The overarching goal of my career is to improve the diagnosis and treatment of cognitive disorders, especially Alzheimer's disease, using advanced neuroimaging techniques. In addition to fellowship training in behavioral neurology, I have special qualification in nuclear imaging as an authorized user of radiotracers and a clinical reader (including PET and SPECT), as well as research training in MRI methods (including VBM, BOLD fMRI, DTI, and ASL), for diagnosing AD and other dementias.

For this P01 project, I will provide evaluation of early biomarkers (sMRI, FDG PET, and amyloid-PET), to correlation with measures of traffic-related air pollution (TRAP) exposure. Specifically I will participate in ADNI and WHIMS-MRI data interpretation and analyses. In addition, I will assist in the cross-comparison of cerebrovascular injury between human and mouse data.

B. Positions and Honors

Positions

2012- Assistant Clinical Professor
Department of Neurology and Radiology, University of Southern California

Honors

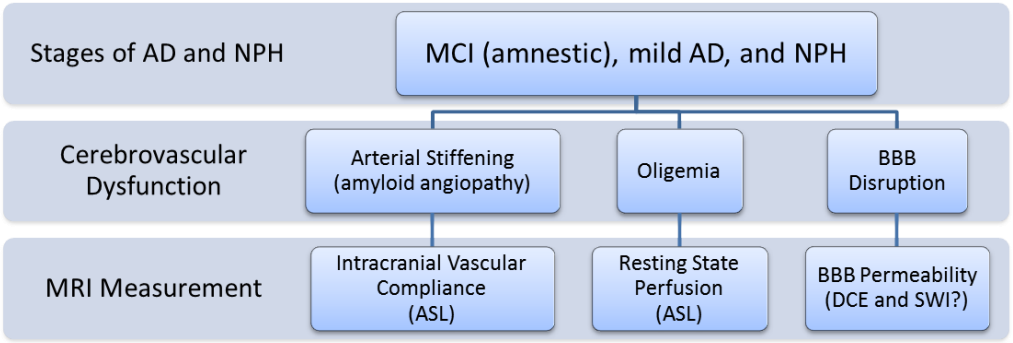
1990 California Alumni Association Scholarship, UC Berkeley
1991 California Honors Society, UC Berkeley
1992-1993 DeWitt Wallace Fellowship, NYU Medical Center, Departmentof Neurosurgery
1994 Departmental Honor, Molecular and Cell Biology, UC Berkeley
1997 Departmental Honor, Philosophy, UC Berkeley
1998-1999 Albert Schweitzer Fellowship
1999-2000 Student Scholar in Stroke Research, American Heart Association
2009-2010 Nuclear Medicine Chief Resident, Washington University
2012 Clinical Scholar, International Society of Magnetic Resonance in Medicine

C. Contributions to Science

1. Characterization of Cerebral Metabolic Activity and Functional Connectivity in Cognitively Normal Elderly with Genetic Risk for Alzheimer's Disease (AD), Early-onset AD (EOAD), and Posterior Cortical Atrophy (PCA). Cortical metabolism and blood oxygen fluctuation (functional connectivity) are important measures of brain activity. AD and other dementias have characteristic hypometabolic patterns, useful for making clinical diagnosis. We have examined the correlation between hypometabolism and cognitive deficits in early-onset AD and posterior cortical atrophy (PCA), a related dementia. These hypometabolic patterns can be understood as networks, with structural and functional connectivity demonstrable by MRI analyses (voxel-based morphometry and blood-oxygen level dependent signal). We have applied these analyses to elderly cognitively normal subjects with genetic risk for AD (Apoe-E4 allele) and early AD patients. A novel measure of BOLD signal complexity (approximate entropy) has also been introduced. These findings help characterize pathological brain activity in Apoe-E4, EOAD, and PCA.

- **APOE4 Allele Disrupts Resting State BOLD Connectivity in the Absence of Amyloid Plaque Burden**. Sheline Y, Morris JC, Snyder A, Price J, Yan Z, **Liu C**, Dixit S, Benzinger T, D'Angelo G, Goate A, Mintun MA. *Journal of Neuroscience*. 2010; 30(50): 17035-17040
- **Posterior cortical atrophy: evidence for discrete syndromes of early-onset Alzheimer's disease.** Tsai P, Teng E, **Liu C**, Mendez MF. *American Journal of Alzheimer's Disease & Other Dementias*. 2011; 26(5): 413-418.
- **Neuropsychological and Neuroimaging Markers in Early-onset vs. Late-onset AD.** Kaiser N, Melrose R, **Liu C**, Sultzer D, Jimenez E, Su M, Monseratt L, Mendez MF. *American Journal of Alzheimers Disease & Other Dementias*. 2012; 27(7): 520-529
- **Complexity and synchronicity of resting state fMRI in normal aging and cognitive decline. Liu C**, Krishnan A, Yan L, Kilroy E, Alger J, Wang JJ. *Journal of MRI*. 2013; 38(1): 36-45

2. Development of Imaging Markers for Cerebrovascular Pathologies in MCI and Alzheimer's Disease. Recently, a vascular hypothesis of AD has emerged, which posits that cerebrovascular dysfunctions, such as arterial stiffening, loss of autoregulation, oligemia, and breakdown of blood-brain barrier (BBB), likely precede and may even cause vascular and parenchymal amyloid deposition. The development of imaging markers for cerebrovascular pathologies might provides a new window of therapy. Using advanced MRI sequences, we have successfully examined cerebral perfusion, BBB permeability, and vascular compliance in in MCI and early AD patients (see Figure).



- **Reliability of pCASL GRASE perfusion in cognitively normal and MCI subjects.** Kilroy E, Apostolova L, **Liu C**, Wang JJ. *Journal of MRI*. 2014; 39(4): 931-939
- **Blood-Brain Barrier Breakdown in the Aging Human Hippocampus.** Montagne A, Sweeney MD, Halladay MR, Sagare AP, Zhao Z, Toga AW, Jacobs RE, **Liu CY**, Amezacua L, Harrington M, Chui H, Meng L, Zlokovic BV. *Neuron*. 2015; 85(2): 296-302
- **Assessing Intracranial Vascular Compliance Using Dynamic Arterial Spin Labeling**. Yan L, **Liu C**, Smith RX, Jog M, Krasileva K, Langhem M, Ringman JM, Wang DJ. *NeuroImage*. 2015; 124(Pt A):433-441.

PubMed: http://www-ncbi-nlm-nih-gov.libproxy1.usc.edu/pubmed/?term=collin+liu

D. Research Support

Ongoing Research Support

ADRC Pilot (PI: Collins Liu, USC) 4/1/2013 – 3/31/2016
Title: Intracranial vascular compliance measured by dynamic arterial spin labeling MRI
USC IRB: HS-13-00222

NIH-NIA ADRC Institutional Grant (PI: Helena Chui and Berislav Zlokovic, USC) 7/1/2015 – 5/31/2020
Title: Vascular Cohort Study
USC IRB: HS-15-00126

NIH-NIA UO1 (PI: John Ringman, USC) 7/1/2015 – 6/30/2020
Title: Neuroimaging, Behavioral and Neurochemical Characterization of Autosomal Dominant Alzheimer's disease and other Neurological Disorders
USC IRB: APP-15-04613

NIH-NIA P01 (PI: John Ringman, USC, and John Morris, Washington University) 1/1/2009 – 6/30/2019
Title: Dominantly Inherited Alzheimer Network
USC IRB: HS-15-00492

Completed Research Support

NIH R21 (PI: Mario Mendez; UCLA) 7/1/2011 – 6/30/2012
Title: Clinical, neuropsychological, and neuroimaging comparisons of early- and late-onset AD

CTSI Pilot (PI: Meng Law, USC) 7/1/2013 – 6/20/2014
Title: Hippocampal BBB breakdown measured by high-resolution DCE (dynamic-contrast enhanced) MRI

ZNI Departmental Fund (PI: Liu and Zlokovic, USC) 7/1/2013 – 6/30/2015
Title: Correlation of novel dynamic MRI markers for cerebrovascular dysfunction with molecular markers for amyloidopathy and neurovascular unit dysfunction in various stages of Alzheimer's disease

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Wendy Mack

eRA COMMONS USER NAME (credential, e.g., agency login): wjmackpi

POSITION TITLE: Professor of Preventive Medicine and Gerontology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Los Angeles	BS	06/1977	Kinesiology
University of Southern California	MS	09/1987	Biometry
University of Southern California	PhD	12/1989	Biometry

A. Personal Statement

As Professor within the Division of Biostatistics in the Department of Preventive Medicine at USC, I have over 25 years of experience in directing biostatistical and data coordination activities for multiple single-centered and multi-centered clinical trials and observational studies. I have directed the biostatistical and data coordination activities of 14 randomized clinical trials (10 of these NIH- or PCORI-funded), and two NIA-funded program projects, and have a wealth of experience and expertise in analysis of longitudinal clinical trial outcomes. Four of these trials have focused on hormonal and soy interventions related to atherosclerosis and cognitive outcomes in postmenopausal women. Importantly, I direct the biostatistical functions including support for design and conduct of clinical trials and clinical studies, of the USC CTSI. In all of this research, I hav e been responsible for all statistical and data-related functions from inception and study design through data collection and oversight, interim reporting, data analyses and final reporting. Therefore, I have extensive expertise in the design, conduct and analysis of longitudinal observational studies and clinical trials. Related to the dissemination of this research, I have contributed directly to the statistical analyses, interpretation of results and preparation of abstracts and manuscripts. For the randomized clinical trials specifically, this has resulted in more than 80 publications over 25 years. I am furthermore deeply committed to training the next generation of clinical investigators and biostatisticians. In my CTSI position and as director of the M.S. programs in Biostatistics and Epidemiology in the Department of Preventive Medicine, I have served as primary (over 100) and secondary (40) mentor to K-awardees, junior faculty, and graduate students (MS and PhD); over 40 of my peer-reviewed publications, co-authored with mentees, have resulted from these mentoring activities.

B. Positions and Honors

Positions and Employment

1988-1989 Research Associate, Department of Preventive Medicine, University of Southern California
1989-1996 Assistant Professor, Department of Preventive Medicine, University of Southern California
1996-2008 Associate Professor, Department of Preventive Medicine, University of Southern California
2008- Professor, Department of Preventive Medicine, University of Southern California
2012- Professor, Davis School of Gerontology, University of Southern California

Other Experience and Professional Memberships

1988- Member, American Statistical Association
1990- Member, Society for Clinical Trials
2004- Member, American Heart Association
2003 Member, NHLBI ZHL1 CCT-L C1 1, CARDIA Trials Review
2003 Member, NCI Special Emphasis Panel, NCI-D GRB W1P, 1 PO1 CA100318-01A1
2004-07 Member, NCCAM Special Emphasis Panel, Clinical Science Review (R21 review)
2007 Member, NIDDK Special Emphasis Panel, ZDK1 GRB-W 02 Cardiovascular Studies in Type 2

Diabetes Ancillary Studies
2008 Member, NIDDK Special Emphasis Panel, ZDK1 GRB-W J1S
2009 Member, NIA Special Emphasis Panel, ZAG1 ZIJ-9 Program Project Review
2007-11 Member, NHLBI Clinical Trials Review Committee Study Section
2012 Member, NIA Special Emphasis Panel, ZAG1 ZIJ-9, Clinical Trials Support Center
2012 Member, NIA Special Emphasis Panel, ZAG1 ZIJ-5, Post-Menopausal Symptoms and Causes
2012 Member, NHLBI Special Emphasis Panel, ZHL1 CSR-D Summer Institute Training in Biostatistics II (T15)
2013 Member, NCCAM Special Emphasis Panel, ZAT1 HS-13 Clinical Studies
2014 Member, NIA Special Emphasis Panel, ZAG1 ZIJ-9, Program Project Review
2014 Chair, NHBLI Special Emphasis Panel, ZHL1 CSR-P S2 1, Pulmonary Hypertension Coordinating Center
2014 Member, NIA Special Emphasis Panel, ZAG1 ZIJ-3(J3), MsFLASH: Living a Healthy Menopause
2015 Member, Special Emphasis Panel, ZRG1 IMST-R (50) R, Pilot Centers for Precision Disease Modeling
2015 Member, NIA Special Emphasis Panel, ZAG1 ZIJ-7 (A1), Mobility-Disability and Muscle Mass
2015 Member, NHLBI Summer Training R25 Review, ZHL1 CSH-H (F2)
2016 Member, NIA Special Emphasis Panel, ZAG1 ZIJ-G(M2), Phase III Clinical Trials for AD
2016 Grant review, California Department of Public Health, Alzheimer's Disease Program
2016-pres Member, NIA Clinical Aging Review Committee
2016 Member, Special Emphasis Panel, ZNS1 SRB-T 19, Small Vessel Contributions to Cognitive Impairment and Dementia (VCID) Biomarkers

Honors
2007 Distinguished Faculty Service Award, University of Southern California

C. **Contribution to Science**

1. **Non-invasive measures of atherosclerosis progression: statistical contributions.** Early in my biostatistics career at the University of Southern California, I was fortunate to include Dr. David Blankenhorn as a faculty mentor. At that time, Dr. Blankenhorn was actively seeking to develop and validate non-invasive measures of subclinical atherosclerosis. Working with Robert Selzer of the CalTech Jet Propulsion Laboratory and Dr. Howard Hodis, we designed and conducted multiple studies to develop carotid ultrasongraphic measures of intima-media thickness (CIMT) and arterial stiffness as valid and highly reproducible atherosclerosis measures. We importantly demonstrated associations of longitudinal measures of CIMT progression with measures of atherosclerosis change measured by invasive coronary angiography as well as CVD event risk. This body of work provided crucial rationale and validation for these non-invasive measures that are now used routinely in both longitudinal observational and clinical trials to assess vascular risk factors as well as efficacy of interventions.

a. Mack WJ, Selzer RH, Hodis HN, Erickson JK, Liu CR, Liu CH, Crawford DW, Blankenhorn DH. One-year reduction and longitudinal analysis of carotid intima-media thickness associated with colestipol/niacin therapy. Stroke 24:1779-1783, 1993
b. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu C-R, Liu C-H, Azen SP. The role of carotid artery intima-media thickness in predicting clinical coronary events. Ann Intern Med 128:262-269, 1998.
c. Mack WJ, LaBree L, Liu C, Selzer RH, Hodis HN. Correlations between measures of atherosclerosis change using carotid ultrasonography and coronary angiography. Atherosclerosis 150:371-379, 2000.
d. Selzer RH, Mack WJ, Lee PL, Kwong-Fu H, Hodis HN. Improved common carotid elasticity and intima-media thickness measurements from computer analysis of sequential ultrasound frames. Atherosclerosis 154:185-193, 2001.

B. **Hormones, cardiovascular disease, and the timing hypothesis: trial designs and reporting.** While observational studies overwhelmingly support the hypothesis that postmenopausal hormone therapy reduces cardiovascular disease risk, randomized clinical trials testing this hypothesis have in general failed to support the hypothesis. Notable differences in the populations evaluated in observational studies compared to clinical trials, as well as animal and human data, led to the proposition of the timing hypothesis that hormone therapy effects on disease risk varies by timing of initiation and the underlying status of the vasculature. In collaboration with Howard Hodis, I have designed, conducted and analyzed three major randomized clinical trials that used the non-invasive CIMT measure as well as coronary

angiographic measures of atherosclerosis as primary outcomes. Major contributions of these trials have shown that: (1) hormone therapy postmenopause reduces the progression of atherosclerosis under primary prevention (reference a), but not under secondary prevention (reference b) conditions, and (2) major mechanisms of benefit are cholesterol modification (HDL increase, LDL decrease). The seminal clinical trial specifically designed to test the timing hypothesis was recently published (reference d).

a. Hodis HN, **Mack WJ**, Lobo RA, Shoupe D, Sevanian A, Mahrer PR, Selzer RH, Azen SP. Estrogen in the Prevention of Atherosclerosis: A randomized, double-blind, placebo-controlled trial. Ann Intern Med 2001;135:939-953.
b. Hodis HN, **Mack WJ,** Lobo RA, Shoupe D, Mahrer PR, Faxon DP, Cashin-Hemphill L, Sanmarco ME, French WJ, Shook TL, Gaarder TD, Mehra AO, Rabbani R, Sevanian A, Shil AB, Torres M, Vogelbach K-H, Selzer RH, Azen SP. Hormone therapy and progression of coronary artery atherosclerosis in postmenopausal women. N Engl J Med 2003;349:535-545.
c. Karim R, Stanczyk FZ, Lobo RA, Hodis HN, **Mack WJ**. Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. J Clin Endocrinol Metab 2008;93:131-138. PMCID: PMC2190735
d. Hodis HN, **Mack WJ**. Henderson VW, Shoupe D, Budoff MJ, Levine-Hwang J, Li Y, Dustin L, Kono N, Stanczyk FZ, Selzer RH, Azen SP. Vascular effects of early versus late postmenopausal treatment with estradiol. N Engl J Med 2016;374:1221-1231.

C. **Vascular contributions to cognitive decline and dementia.** Understanding the contribution of vascular disease and risk factors to the onset and progression of cognitive impairment has potential high impact on both the prevention and treatment of cognitive-impairing disease, most notably Alzheimer disease. For more than 15 years, I collaborated as the primary biostatistician on a program project (Helena Chui, PI) to specifically evaluate the role of vascular disease and risk factors on cognitive impairment. As the biostatistician, I was intimately involved in the study designs and data collection and analysis for these observational studies; critical aspects of study design included definitions of study populations and study groups, sampling methods, and planning of longitudinal assessments. Using longitudinal neuroimaging and cognitive evaluations, our group has reported associations of vascular disease and risk with Alzheimer disease and vascular brain pathology, as well as both MRI- and PET-measures of brain structure and pathology.

a. Marchant NL, Reed BR, Sanossian N, Madison CM, Kriger S, Dhada R, **Mack WJ**, DeCarli C, Weiner MW, Mungas D, Chui HC, Jagust WJ. The aging brain and cognition: contribution of vascular injury and Aβ to mild cognitive dysfunction. JAMA Neurol 2013;70:488-495. PMCID: PMC3771392.
b. Zheng L, Vinters HV, **Mack WJ**, Zarow C, Ellis WG, Chui HC. Cerebral atherosclerosis is associated with cystic infarcts and microinfarcts, but not Alzheimer pathologic changes. Stroke 2013;44:2835-2841. PMCID: PMC4049465
c. Reed BR, Villeneuve S, **Mack W**, DeCarli C, Chui H, Jagust W. Associations between serum cholesterol level and cerebral amyloidosis. JAMA Neurol 2014;71:195-200. PMCID: PMC4083819
d. Villenueuve S, Reed BR, Madison CM, Wirth M, Kriger S, Marchant NL, **Mack WJ**, Sanossian N, DeCarli C, Chui HC, Weiner MW, Jagust WJ. Vascular risk and cerebral beta-amyloid interact to reduce cortical thickness in AD vulnerable brain regions. Neurol 2014;83:40-47. PMCID: PMC4114172

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/45238865/?sort=date&direction=ascending

D. **Research Support**

Ongoing Research Support

1 UL1TR001855 (Buchananl) 07/01/16-05/31/21
NIH/NCATS
Southern California Clinical and Translational Science Institute
Supports development and operation of an institute to develop, promote and support clinical and translational research and training.
Role: Director of Biostatistics, Co-investigator

UF1AG046148 (Brinton, PI) 09/20/13-09/19/18
NIH/NIA
Allopregnanolone Regenerative Therapeutic for MCI/AD: Dose Finding Phase 1
Phase 1 multiple ascending dose trial of allopregnanolone in participants diagnosed with MCI due to AD and early AD.
Role: Co-I, Director of Data Coordinating Center

SC14-1403-13904 (Aranda, PI) 01/01/15-12/31/17
PCORI
Programa Esperanza (Project Hope)
Randomized clinical trial to test the comparative effectiveness of a culturally-modified psychosocial intervention for Spanish-speaking Latino patients with depression compared to enhanced usual care.
Role: Co-I

Helen Diller Family Foundation (Hodis) 01/01/14-12/31/18
Nattokinase Atherothrombotic Prevention Study (NAPS)
Randomized clinical trial to determine whether nattokinase supplementation reduces the progression of atherosclerosis and improves neurocognition in healthy women and men >55 years old
Role: Co-I, Director, Data Coordinating Center

R01ES024936 (Mack, William; PI) 01/15/15-11/30/19
NIH/NIA
Neurotoxicity of Airborne Particles: Role of Chronic Cerebral Hypoperfusion
To determine the impact of particulate matter (PM) exposure on white matter injury and neurocognitive decline, using an experimental murine model.
Role: Co-I

R01OH010665 (Roll) 09/30/2015-09/29/2019
NIOSH
Sonographic Tissue Morphology in Early Stage Work-Related Median Nerve Pathology|
Evaluate the effect of occupational tasks on the development of carpal tunnel syndrome.
Role: Co-I

R01AG046928 (Pa) 09/15/2015-04/30/2020
NIH/NIA
Effects of Physical Activity on Brain Function and Network Connectivity in MCI
To assess the effect of physical activity on remediating early impairments in brain function in sedentary older adults at risk for Alzheimer's disease (AD).
Role: Co-I

R01MD010358 (Goran & Allayee - Multi-P.I.) 04/01/2016-03/31/2020
NIH/NIMHD
Nutrigenetic Intervention to Reduce Liver Fat in Hispanics
Nutrigenetic clinical trial to determine whether dietary sugar reduction will reduce liver fat content in Hispanic pediatric patients with non-alcoholic fatty liver disease (NAFLD) as a function of genotype.
Role: Co-I

R01AI083115 (Lee) 07/01/2009-06/30/2018
NIH/NIAID
Designing T-Cell Based HIV Vaccines Using Peptide-MHC Surface Morphology
To expand our research toward designing prophylactic HIV vaccines.
Role: Co-I

R01AI095066 (Lee) 08/10/2011-02/28/2021
NIH/NIAI
A Single Genomic Assay for HIV Incidence and Transmitted Drug Resistance Mutation Screening

To evaluate genomic signatures within the intrahost HIV sequence population as markers to distinguish incident from chronic infections.
Role: Co-I

R01EY026479 (Fini) 09/01/2016-06/30/2021
NIH/NEI
Clusterin at the Ocular Surface
Investigate previously unrecognized, endogenous protective mechanisms of clusterin on ocular surface.
Role: Co-I

MJFF (Hamm-Alvarez) 01/01/2017-10/31/2018
Michael J. Fox Foundation
Identification of tear biomarkers for Parkinson’s disease patients
To identify protein biomarkers characteristic of the tears of Parkinson's disease (PD) patients.
Role: Co-I

R01HL133169 (Allayee) 02/01/2017-01/31/2021
NIH/NHLBI
Role of Glycine Metabolism in Cardiovascular Disease
Clinical, genetic and bioinformatics to test the hypothesis that glycine metabolism represents a novel sex-specific, protective, and causal pathway for CVD.
Role: Co-I

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: William Jacob Mack, MD, MS

eRA COMMONS USER NAME (credential, e.g., agency login): WJMACK

POSITION TITLE: Associate Professor of Neurosurgery (Clinical Scholar), Neuroscience Graduate Program

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Cornell University, Ithaca, NY	B.A.	6/92	Neurobiology/ Behavior
Columbia University, New York, NY	M.D.	6/01	Medicine
Columbia University, New York, NY	Internship	6/02	Surgery
Columbia University, New York, NY	Residency	6/08	Neurosurgery
University California, Los Angeles, CA	Fellowship	6/10	Neuroendovascular
University Southern California, Los Angeles, CA	MS	12/13	Clinical Investigation

A. Personal Statement

I have a long-standing interest in the role of inflammation in cerebrovascular and neurodegenerative disease processes. A unique clinical background and research training have prepared me well to pursue this scientific focus. I am a cerebrovascular neurosurgeon with a translational science laboratory. My post-doctoral training focused on the vascular biology of cerebral ischemia/ reperfusion injury and the design and implementation of experimental models of cerebrovascular disease. Through interactions with mentors and collaborators at the University of Southern California's *Air Pollution and the Brain Network* and *Alzheimer Disease Research Center (ADRC)*, I have become especially interested in the effects of air pollution in the setting of stroke and cerebral hypoperfusion. I have assumed these understudied areas as my principal research focus. As an Associate Scientific Advisor for *Science Translational Medicine*, I authored editor's choice articles on air pollution: *Clearing the air about stroke* (Mack, 2013); and *Breathe easy, but be concerned* (Mack, 2013) and Alzheimer's disease*: Looking into the crystal ball for Alzheimer's disease* (Mack, 2013). My team has recently demonstrated the detrimental impact of particulate matter exposure on the progression of experimental stroke (Liu, 2016) and has a review paper accepted on the effects of air pollution in the central nervous system. Through support of an ADRC pilot grant, our laboratory established a role for the complement cascade (C5) in white matter injury secondary to chronic cerebral hypoperfusion (Liu, 2013). Leveraging the concepts and models from these studies, we are now examining the joint influence of nanoparticulate matter (nPM) exposure and chronic cerebral hypoperfusion (CCH) on white matter injury, cognitive impairment, and Alzheimer's disease risk. I was granted an NIH/NIEHS ONES award to study the joint effects of nPM exposure and CCH in young male mice (Mack; R01ES024936). I have collaborated closely with Drs. Finch, Chen, Zlokovic, Mack and Sioutas (all investigators on this P01) on these studies. The current project proposal is a logical extension of my scientific interest and prior work.

B. Positions and Honors

Positions and Employment

2014- Associate Professor of Neurological Surgery (Clinical Scholar)
2010- 14 Assistant Professor of Neurological Surgery, University Southern California, Los Angeles, CA
2010- 13 Adjunct Assistant Professor of Radiology, UCLA, Los Angeles, CA
2013- Neuroscience Graduate Program, University of Southern California, Los Angeles, CA

Other Experience and Professional Memberships

2010- Member, American Heart Association/ American Stroke Council

2010-	Member, Congress of Neurological Surgeons
2010-	Member, American Association of Neurological Surgeons
2012-	Reviewer: American Heart Association's BRAIN CLINICAL Grant Peer Review study section
2013-	Reviewer: SC CTSI KL2 Mentored Research Career Development/ TL1 Pre-doctoral grants
2013-16	International Stroke Conference (AHA/ASA): Program Committee
2013-14	Associate Scientific Advisor: Science Translational Medicine
2013-14	Board of Directors: Society of Neurointerventional Surgeons
2013-	Associate Editor/ Editorial Board: Journal of Neurointerventional Surgery
2013-	Editorial Board: World Neurosurgery
2015-2017	Board of Directors: Society of Neurointerventional Surgeons: Education Chair

Honors

1996	Quill and Dagger Senior Honors Society- Cornell University
1996	Graduated with Distinction in all Subjects- Cornell
1996	Golden Key National Honors Society, Cornell University
1996	Phi Kappa Phi National Honors Society, Cornell University
1996	Order of Omega National Honor Society, Cornell University
1996	Award for Outstanding Member- Cornell University Inter-fraternity Council,
1998	Walle J. Nauta Neural Sciences Award: Outstanding Student in the Medical Neural Sciences
1998	Recognition for Outstanding Performance in the Medical Basic Sciences
2001	Alpha Omega Alpha- Columbia University College of Physicians and Surgeons
2012	Fellow of the American Heart Association (FAHA)
2013	Fellow of the American Association of Neurological Surgeons
2014	Hopkins School Alumni Fellow, New Haven, CT
2014	Outstanding Research Scholarly Project Mentor award, University of Southern California
2014-2019	Outstanding New Environmental Scientist (ONES) Program (NIEHS)

C. Contribution to Science

Publication link (155 references)
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47757606/?sort=date&direction=ascending

Cognitive decline in the setting of cerebral hypoperfusion and carotid disease
Vascular mechanisms contribute to both cognitive impairment and Alzheimer's disease. Our group has employed both murine and clinical models of cerebral hypoperfusion to examine the impact of inflammatory mediators on ischemic injury and cognitive outcomes. In a clinical carotid endarterectomy model of cerebral hypoperfusion, our research has demonstrated a roughly 25% incidence of subtle cognitive decline in the absence of overt neurologic change or radiographic evidence of stroke. Our studies have demonstrated that neurocognitve change is associated with elevations in biochemical markers of cerebral injury (S100B,) and upregulation of leukocytes and inflammatory mediators (Mack 2008, Mocco 2006) These putative risk factors parallel those of AD/ vascular dementia and suggest an inflammatory mechanism. We demonstrated the intravenous Magnesium infusion is protective against neurocognitive deficits in a clinical carotid endarterectomy model of cerebral hypoperfusion (Mack, 2009). Our murine chronic cerebral hypoperfusion (CCH) studies have established an association between white matter injury, astrocyte/ microglial reactivity, and working memory deficits. We have demonstrated that the C5 complement component plays a critical role in white matter injury following CCH. Further, we have demonstrated that C5 deficient mice are protected from white matter ischemic injury secondary to CCH (Liu, 2013).

1. Liu Q, He S, Groysman L, Shaked D, Russin JJ, Cen Y, **Mack WJ**: White Matter Injury Due to Experimental Chronic Cerebral Hypoperfusion is Associated with C5 Deposition. *PLoS One.* 2013 Dec 30;8(12):e84802
2. **Mack WJ**, Kellner CP, Sahlein DH, Ducruet AF, Kim GH, Mocco J, Zurica J, Komotar RJ, Haque R, Sciacca RR, Quest DO, Solomon RA, Connolly ES, Heyer EJ: Intraoperative magnesium infusion during carotid endarterectomy: a double-blind placebo-controlled trial. *Journal of Neurosurgery* 2009, 110(5): 961-7.
3. **Mack WJ**, Ducruet AF, Hickman ZL, Zurica J, Starke RM, Garrett MC, Komotar RJ, Quest DO, Solomon RA, Heyer EJ, Connolly ES: Elevation of Monocyte Chemoattractant Protein-1 in atients experiencing neurocognitive decline following carotid endarterectomy. *Acta Neurochir (Wien)* 2008, 150(8):779-84.

4. Mocco J, Wilson DA, Ducruet AF, Komotar RJ, **Mack WJ**, Zurica J, SciaccaRR, Heyer EJ, Connolly ES. Elevations in Preoperative Monocyte Count Predispose to Acute Neurocognitive Decline After Carotid Endarterectomy for Asymptomatic Carotid Artery Stenosis. *Stroke 2006*; 37(1): 240-2.

Air Pollution in Stroke and Cerebrovascular Diseaae

I have contributed an invited editorial to *Science Translational Medicine* on the association between air pollution exposure and stroke incidence/ mortality (Mack 2013). Our group was the first to study the influence of nanoparticulate matter in the setting of experimental stroke. Air pollution is a prevalent environmental source of both inflammation and oxidative stress. These processes are both known to contribute to the progression of stroke. My laboratory has demonstrated that nanoparticulate matter exposure increases stroke damage in an experimental murine model (Liu 2016). Following cerebral ischemia/ reperfusion, mice exposed to nPM exhibit significantly larger infarct volumes and less favorable neurological deficit scores. These mice also demonstrate increases in markers of inflammation and oxidative stress in the region of the ischemic core. We plan on extending these findings to studies in other cerebrovascular pathologies and neurodegenerative disease.

1. Liu Q, Babadjouni R, Radwanski R, Cheng H, Patel A, Hodis DM, He S, Baumbacher P, Russin JJ, Morgan TE, Sioutas C, Finch CE, **Mack WJ**: Stroke Damage is Exacerbated by Nano-Size Particulate Matter in a Mouse Model. *PLoS One*. 2016; 11(4)
2. **Mack, W.J.**: Clearing the Air About Stroke. *Sci. Transl. Med*. 2016 5, 189ec96.
3. **Mack WJ:** Breathe Easy, But Be Concerned. *Sci. Transl. Med*. 2014 6, 219ec10

Inflammation/ oxidative stress and micrvascular failure in the setting of stroke

The effects of microvascular failure on stroke progression are well documented. Imaging studies demonstrate that marginally viable cortical tissue is recruited into a central ischemic core within forty-eight hours of cerebral large vessel occlusion. Moreover, susceptible territories closely match early perfusion deficits, implying that regional flow failure is a prominent feature of tissue ultimately destined for infarction. Our group has examined the temporal characteristics of complement upregulation following experimental stroke (Mack 2006). Additionally, we have investigated the effects of genetic deletion and pharmacologic inhibition of the major components. Our studies have demonstrated ischemic protection in C3 knockout mice and through pharmacologic inhibition of the C3a and C5a receptors (Mocco 2006). C3a receptor modulation of granulocyte infiltration appears to be reperfusion dependent. Together, these results suggest an anaphylotoxin mediated inflammatory mechanism of complement related injury. We have also demonstrated the cerebroprotective effects of citrate/sorbitol-stabilized Dehydroascorbic acid through suppression of excessive oxidative metabolism (Mack 2006) and the feasibility of intracorporeal hypothermia in the setting of acute stroke (Mack, 2003).

1. Mocco J, **Mack WJ**, Ducruet AF, Sughrue ME, Sosunov SA, Nair MN, Laufer I, Komotar RJ, Botto M, Holland MC, Lambris JD, Pinsky DJ, Connolly, Jr. ES: Complement component C3 mediates inflammatory injury following focal cerebral ischemia. *Circulation Research* 2006; 99(2):209-17
2. **Mack WJ**, Mocco J, Ducruet AF, Laufer I, King RG, Zhang, Y, Guo W, Pinsky DJ, Connolly Jr ES. A cerebroprotective dose of intravenous citrate/sorbitol-stabilized dehydroascorbic acid is correlated with increased cerebral ascorbic acid and inhibited lipid peroxidation after murine reperfused stroke. *Neurosurgery* 2006; 59(2): 383-8.
3. **Mack WJ**, Sughrue ME, Ducruet AF, Mocco J, Sosunov SA, Hassid B, Silverberg J, Ten VS, Pinsky DJ, Connolly, Jr. ES: The Temporal Pattern of C1q Deposition following Transient Focal Cerebral Ischemia. *Journal of Neuroscience Research* 2006; Apr; 83(5): 883-9
4. **Mack WJ**, Huang J, Winfree CJ, Kim G, Oppermann M, Dobak J, Inderbitzen B, Yon S, Popilskis SJ, Lasheras J, Sciacca RR, Pinsky DJ, Connolly, Jr. ES. Ultra-Rapid, Convection-Enhanced Intracorporeal Hypothermia: A Feasibility Trial in Non-Human Primate *Stroke* 2003; 34(8): 1994-9.

Inflammation and Blood Brain Barrier Breakdown Following Brain Aneurysm Rupture

Cerebral vasospasm following rupture of a brain aneurysm results in stroke in approximately thirty percent of patients. Evolution in management strategies has been slowed by deficiencies in understanding of the

mechanisms that underlie this process. Coupled with increased permeability of the blood brain barrier, inflammation may incite delayed stroke. My work has focused on elucidating the role of inflammation in the setting of vasospasm following aneurysmal subarachnoid hemorrhage (aSAH). Leveraging serum biomarkers, imaging and clinical data from aSAH patients, our group has demonstrated a role for Intercellular Adhesion Molecule-1 and the C3a complement component in the pathogenesis of cerebral vasospasm (Mack 2002, Mack 2007). We are currently studying the role of Matrix Metalloproteinase 9 (MMP 9). MMP9 is an enzyme involved in breakdown of the blood brain barrier. Prior studies have demonstrated a correlation between elevated MMP 9 levels in the blood and stroke following rupture of brain aneurysms. A clear mechanism is yet to be determined. Our investigation utilize data obtained from advanced MRI perfusion imaging sequences to demonstrate an association between MMP 9 upregulation and breakdown of the blood brain barrier following aneurysm rupture (Mills 2013). The study examines the relationship between compromised blood brain barrier integrity and the occurrence of stroke. A positive correlation would suggest a potential role for MMP 9 as an early diagnostic biomarker and blood brain barrier permeability as a therapeutic target in the management of stroke following rupture of brain aneurysms.

1. Mills J, Mehta V, Russin, JJ, Amar AP, Rajamohan A, **Mack WJ**: Advanced Imaging Modalities in the Detection of Cerebral Vasospasm. *Neurol Res Int.* 2013
2. **Mack WJ**, Ducruet AF, Angevine PD, Komotar RJ, Shrebnick DB, Edwards NM, Smith CR, Heyer EJ, Monyero L, Connolly ES Jr, Solomon RA. Deep hypothermic circulatory arrest for complex cerebral aneurysms: lessons learned. *Neurosurgery*. 2008 Jun; 62(6 Suppl 3): 1311-23.
3. **Mack WJ**, Ducruet AF, Hickman ZL, Garrett MC, Albert EJ, Kellner CP, Mocco J, Connolly, ES: Early plasma complement C3a levels correlate with functional outcome after aneurysmal subarachnoid hemorrhage. *Neurosurgery* 2007; 61(2): 255-60.. PMID: 17762737
4. **Mack WJ**, Mocco J, Hoh D, Huang J, Choudhri TF, Kreiter KA, Lozier AP, Oppermann M, Poisik A, Yorgason J, Solomon RA, Mayer SA, Connolly, Jr. ES: Elevated Serum Intercellular Adhesion Molecule-1 Levels Predict Poor Outcome Following Aneurysmal Subarachnoid Hemorrhage. *J Neurosurg* 2002; 96(1): 71-

Outcomes and health care disparities in patients with cerebrovascular disease and large database
Outcome assessments, cost effectiveness and quality control have become increasingly relevant metrics as our medical delivery paradigm shifts. Using the largest national health outcomes database, our studies have examined critical health care outcomes and disparities in the setting of cerebrovascular disease. We have focused on the incidence of "never events," hospital acquired complications that are deemed preventable by the Center for Medicare Services. We have documented clinical and socioeconomic factors associated with the occurrence of such events (Wen 2014, Wen 2014). Further, we have characterized variances in procedural volumes and admission characteristics that can affect patient outcomes on a large scale (Adamczyk 2102, Attenello 2013).

1. Wen T, He S, Attenello F, Cen S, Kim-Tenser M, Adamczyk P, Amar AP, Sanossian N, **Mack WJ:** The Impact of Patient Age and Comorbidities on the Occurrence of "Never Events" in Cerebrovascular Surgery: An Analysis of the Nationwide Inpatient Sample. *Journal of Neurosurgery*. 2014 June 27:1-7
2. Wen T, Attenello F, He S, Cen Y, Kim-Tenser MA, Sanossian N, Amar AP, **Mack WJ**: Racial and Socioeconomic Disparities in Incidence of Hospital Acquired Complications Following Cerebrovascular Procedures. *Neurosurgery*. 2014 Mar 21.
3. Attenello F, Adamczyk P, Wen G, He S, Zhang K, Russin JJ, Sanossian N, Amar AP, **Mack WJ**: Racial and Socioeconomic Disparities in Access to Mechanical Revascularization Procedures for Acute Ischemic Stroke. *J Stroke Cerebrovasc Dis.* 2013 May 13. doi:pii: S1052-3057(13)00139-0.
4. Adamczyk P, Attenello F, Wen G, He S, Russin J, Sanossian N, Amar AP, **Mack WJ**: Mechanical Thrombectomy in Acute Stroke: Utilization Variances and Impact of Procedural Volume on Inpatient Mortality. *J Stroke Cerebrovasc Dis.* 2012 Sep 25. pii: S1052-3057(12)00270-4.

D. Research Support

Ongoing Research Support

1R01 ES024936 Mack (PI) 12/1/2014-11/30/2019
Neurotoxicity of Airborne Particles: Role of Chronic Cerebral Hypoperfusion.

Examine the joint effects of particulate matter exposure and chronic cerebral hypoperfusion on white matter injury and neurocognitive deficits. Role: PI

U01 MH098937-01 Knowles, Chow (Co-PIs) 09/21/12-05/31/17
Evaluation of Cellular Heterogeneity Using Patchclamp and RNA-Seq of Single Cells
Examine transcriptome variability among ostensibly identical and non-indentical cells in order to validate newest generation RNA-Seq platforms Role: Co-Investigator

R25 NS099008-01 Mack (PI) 07/01/2017-6/31/23
USC Neurosurgery Research Education Training Grant
To train neurosurgery residents for careers as clinician-scientitsts and to obtain career development awards as junior faculty members.

Completed Research Support (past 5 years)

Brain Aneurysm Foundation Mack (PI) 09/01/11-08/31/15
MRI Perfusion Permeability and Matrix Metalloproteinase 9 Levels: Association with Cerebral Vasospasm
Examine blood brain barrier breakdown during cerebral vasospasm following Subarachnoid Hemorrhage. Role: PI

5P30ESO7048-18 NIEHS SCEHS Pilot grant Mack (PI) 04/01/13-03/31/14
Airborne Particulate Matter from Vehicular Exhaust in the Setting of Acute Stroke.
Evaluate the impact of nanoparticulate matter from vehicular exhaust on the progression of murine experimental stroke. Role: PI

12BGIA8700001 AHA Beginning Grant in Aid Mack (PI) 01/01/12-12/31/13
Intra-arterial Magnesium Therapy in Acute Stroke: A Phase II Safety and Feasibility Study.
Assess a novel platform for endovascular delivery for Magnesium as a neuroprotectant agent for acute stroke Role: PI

KL2 RR 31991-1 Mack (PI) 10/01/10-09/31/13
Complement Mediated Injury in a Translation Model of Chronic Cerebral Hypoperfusion
To examine the role of the complement cascade in the setting of experimental chronic cerebral hypoperfusion. Role: PI, Mentors: Caleb E. Finch, PhD and Helena Chui, MD.

P50 AG005142-26A1 ADRC Pilot grant Mack (PI) 1/1/10-12/31/12
The Role of the C5 Complement protein in Chronic Cerebral Hypoperfusion.
Examine the role of the C5 complement protein in the setting of chronic cerebral hypoperfusion. Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Millstein, Joshua	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) JOSHUAMILLSTEIN	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of California, Santa Cruz		05/89	Undergrad Physics
University of Alaska Southeast	B.S.	05/97	Biology
University of Southern California	M.S.	08/02	Biometry
University of Southern California	Ph.D.	08/05	Biostatistics

A. Personal Statement

Throughout my career, my research interests and efforts have focused on the problems of high dimensional data, particularly, biological-based measures in population studies (e.g., population-based genomic and transcriptomic data) in the context of better understanding the pathogenesis of complex diseases. I have developed statistical methods for the analysis of integrative genomic data in the context of animal model, epidemiological, and clinical studies. There are three major areas of statistical methods development that have dominated much of my professional career, i) powerful and computationally efficient approaches to identify epistasis and statistical interactions in general, ii) mediation analysis hypothesis testing, and iii) false discovery rates. The common theme of these approaches is their key importance in extracting knowledge from high-dimensional and biologically-based measures, such as genomic data. These approaches all address the critical need for sound statistical tools applicable to integrate and analyze large-scale complex biological data. For example, there is broad agreement regarding the widespread existence of epistasis, gene-environment interactions, etc., but statistical methods that can handle genomic data remain fairly limited. Mediation analysis is essential in the ability to differentiate between the deluge of dependencies that are not in a disease pathway versus those that are. I have developed the permutation-based FDR approach that does not depend on parametric assumptions that may be difficult to satisfy. My background in neuroscience and statistical methods designed for molecular data in clinical and epidemiological settings compliments the team and provides necessary support for developing strong experimental design and analysis. My experience in developing and applying methodology with few parametric assumptions and advanced quantification of uncertainty in the context of causal inference will be a strong asset for Project 1, Aim 3 in particular. I will also contribute to the dissemination of scientific findings from the Program Project.

B. Positions and Honors

Positions and Employment

2002-2005 Statistician, University of Southern California
2005-2006 Mathematical Statistician, National Oceanic and Atmospheric Administration, National Marine Fisheries Service
2006-2009 Sr. Statistical Geneticist, Rosetta Inpharmatics, Merck & Co., Inc.
2009-2011 Sr. Statistical Geneticist, Sage Bionetworks

2010- Affiliate Investigator, Fred Hutchinson Cancer Research Center, Division of Public Health Sciences, Computational Biology Program
2011-present Assistant Professor, Division of Biostatistics, Dept. of Preventive Medicine, Keck School of Medicine, University of Southern California

Other Experience and Professional Memberships

2004- International Genetic and Epidemiology Society
2006- American Society of Human Genetics
2007- Society for Neuroscience
2011- Editor: BMC Open Network Biology

Manuscript Review

2016-2017 Bioinformatics, Environmental Health Perspecitves, Plos Genetics, Plot One
2015 American Journal of Epidemiology, Bioinformatics
2015 Journal of Exposure Science and Environmental Epidemiology
2014 Statistical Applications in Genetics and Molecular Biology
Earlier Plos Computational Biology, PNAS, and others

Teaching

2013- PM 510: Principles of Biostatistics (onground and online courses)

Honors

2008 Highest ranking within M6 level employees in Merck's Molecular Profiling Division
1997 Highest Achievement in Science, UAS
2010 US Patent: US 8220655, 2012. Hanging Tray for Single Open Beverage [*The SpillNot invention (US8220655) is gaining traction (over 10,000 sold) as an assist to daily living for individuals living with conditions such as ataxia, essential tremor, stroke, MS, muscular dystrophy, and brain cancer, that make it difficult to carry an open beverage without spilling. The SpillNot has raised the quality of life of many people by increasing their independence in performing this daily life activity. It is also used as a tool for science education in many physics classrooms to demonstrate Newton's laws.*]

C. Contributions to Science (link to full list of publications: http://www.ncbi.nlm.nih.gov/sites/myncbi/1T1UacyCvssQm/bibliography/48012715/public/?sort=date&direction=ascending)

Two-step methods to discover gene-gene or gene-environment interactions. The paper below (1), the first publication of this type of statistical approach, has been cited 164 times as of today (5/15), a substantial number for a statistical methods paper, which typically has a relatively small readership. I introduced the concept of using dependencies between features such as SNPs (or SNPs and environmental exposures) as a screening statistic in a two-step analysis designed to detect interactions between those features. Another novel concept presented in this paper (1) was the extreme power advantage of multi-degree-of-freedom tests of main effects and interactions over formal interaction tests. This paper has spawned a number of other methodology papers with approaches designed to detect gene-gene and gene-environment interactions.

1. **Millstein J**, Conti DV, Gilliland FD, Gauderman WJ. (2006) A testing framework for identifying susceptibility genes in the presence of epistasis. Am J Hum Genet 78:15-27. [*A first-in-class paper proposing a novel and powerful 2-stage statistical method for detecting susceptibility genes in the presence of epistasis*.] PMCID: PMC1380213
2. **Millstein J**. (2013) Screening-testing approaches for gene-gene and gene-environment interactions using independent statistics. Frontiers in Genetics | Statistical Genetics and Methodology 4:306.

doi:10.3389/fgene.2013.00306. [*Unique and important insight into screening-testing approaches to epistasis and GxE interactions. First screening testing approach for epistasis or GxE interactions in case study with continuous traits*.] PMCID: PMC3874470

3. **Millstein J**, Siegmund KD, Conti DV, Gauderman WJ. (2005) Identifying susceptibility genes by using joint tests of association and linkage and accounting for epistasis. *BMC Genetics* 6(Suppl 1):S147. [*First screeing-testing approach for epistasis in family-based studies.*]

Causal inference test (CIT). The papers below describe a novel statistical hypothesis test for causal mediation of a measured feature that potentially mediates a known causal relationship between two other measured features. This test is robust and computationally efficient and thereby useful for genomic applications. It has been applied to genomic challenges such as identifying genes whose expression affects sleep phenotypes, identifying DNA methylation loci predictive of epithelial ovarian cancer risk, and identifying cis eQTL genes that mediate trans eQTL effects. Unlike many other mediation approaches commonly used, such as the Sobel test, the publications below demonstrate that is well suited to distinguish reverse causality from causality, minimizing cause-effect confusion.

4. Axelsson A, Mahdi T, Nenonen H, Singh T, Hänzelmann S, Wendt A, Bagge A, Reinbothe T, **Millstein J**, Yang X, Zhang B, Gusmao E, Shu L, Szabat M, Tang Y, Wang J, Salö S, Eliasson L, Artner I, Fex M, Johnson J, Wollheim C, Derry J, Mecham B, Spégel P, Hindrik Mulder, Costa I, Zhang E, and Rosengren A. (2017) *Sox5* regulates beta-cell phenotype and is reduced in type 2 diabetes. Nature Communications (In Press) [*In this work, I applied the CIT to demonstrate that expression of the gene SOX5 mediates the effect of the HDAC inhibitor valproic acid (VPA) on improved insulin secretion*.]
5. **Millstein J**, Chen GK, Breton CV. (2016) cit: hypothesis testing software for mediation analysis in genomic applications. *Bioinformatics*. btw135. PMID: 27153715. PMCID: PMC4965632 [*Years of my personal research went into the development of novel statistical methods and implementation of software that was requested by researchers who used the basic method I developed in 2009 in work published in such journals as Science Translational Medicine, Nature Communication, Nature Biotechnology, Cell Metabolism, Journal of Neurogenetics, Human Molecular Genetics, and PLoS Biology. This software is currently (2016) being used by Leland Taylor in Francis Collins Laboratory of NIH/NHGRI.*]
6. **Millstein J**, Bin Zhang, Jun Zhu, Eric E. Schadt. (2009) Disentangling molecular relationships with a causal inference test. BMC Genetics 10:23. [*First published hypothesis test of causal mediation for a molecular mediator of a QTL*.] PMCID: PMC3224661
7. Breton CV, Yao J, **Millstein J**, Siegmund K, Mack WJ, Whitfield-Maxwell, L, Lurmann F, Hodis HN, Avol E, Gilliland FD. (2016) The effect of prenatal air pollutants and DNA methyl transferase genotypes on newborn LINE1 retrotransposon methylation and childhood cardiovascular phenotypes. Environmental Health Perspecitives. PMID: 27219456 [*Application of causal inference to line1 methylation.*]
8. **Millstein J**, Winrow CJ, Kasarskis A, Owens JR, Zhou L, Summa KC, Fitzpatrick K, Zhang B, Vitaterna MH, Schadt EE, Renger JJ, Turek FW (2011) Identification of causal genes, networks, and transcriptional regulators of REM sleep and wake. SLEEP 34:1469-1477. [*One of the first attempts to apply an integrative genomics approach to identify molecular regulators of sleep*. *Commentary on the importance of this article was published in same issue by Watson NF. Searching for the missing heritability. 1453-1454. PMID: 22043112, PMCID: PMC3198197*] PMCID: PMC3198032

Permutation-based false discovery rate methods (FDR). The papers below are the first publications of a novel, computationally efficient approach for computing confidence intervals for FDR estimates. This work has the potential to have a high impact across scientific disciplines because it can fundamentally change how we approach ‘statistical significance’ in high dimensional testing settings. FDR, recently gaining popularity for addressing the multiple testing problem, can be thought of as a signal-to-noise ratio approach, which is already a substantial departure from the traditional Bonferroni method. FDR confidence intervals allow for another sea change in how we approach multiple testing by freeing the investigator from an a priori significance threshold, which may not yield any discoveries even when there is ample evidence that multiple null hypotheses are false.

9. **Millstein J**, Volfson D. (2013) Computationally efficient permutation-based confidence interval estimation for tail-area FDR. Frontiers in Genetics | Statistical Genetics and Methodology 4(179):1-11. PMCID: PMC3775454
10. **Millstein J**, Volfson D, Lamb JR, Friend S, Dai H, Schadt EE, Bergh J (2010) Permutation-based yet computationally parsimonious FDR point and confidence interval estimators. In JSM Proceedings, ENAR. Alexandria, VA: American Statistical Association 4184-4197.

Joint tests of association and linkage. The papers below describe a novel approach that allows affected-sib-parent studies to be used in a statistically versatile and powerful analysis, where association and linkage effects can be simultaneously estimated in a joint model. Joint estimation allows for increased power using joint tests of association and linkage, but it also allows for conditional tests of linkage given association. The conditional test of linkage will provide signal that will go to zero at the disease locus, providing a novel way of fine mapping a linkage effect without collecting additional data.

11. **Millstein J**, Siegmund KD, Conti DV, Gauderman WJ. (2005) Testing association and linkage using affected-sib-parent study designs. *Genet Epi* 29:225-233.
12. **Millstein J**, Siegmund KD, Conti DV, Gauderman WJ. (2005) Identifying susceptibility genes by using joint tests of association and linkage and accounting for epistasis. BMC Genetics 6(Suppl 1):S147. PMCID: PMC1866788

D. Research Support

Ongoing Research Support

NIH Admin Supplement to 5R01ES02801-04 (Gilliland) 7/01/15–06/30/2016 1.20 CM
NIEHS $256,632
Integrative Genetic Approaches to Gene-Air Pollution Interactions in Asthma

The main purpose is to determine how air pollution exposure affects gene expression in sputum macrophages and whether individual differences in gene expression resulting from DNA variation (eQTL) are modified by air pollution exposure.

3P30CA014089-39S2 (Groshen) 12/01/13–11/30/2015 1.20 CM
NIH $427,487
Biostatistics Core - Comprehensive Cancer Center Core

To meet statistical needs of the USC Norris Comprehensive Cancer Center in areas of study design, study monitoring, and data analysis.

5R01ES022216-02 (Breton) 09/01/2013–06/30/2017 2.33 CM
NIEHS $327,374
Prenatal Tobacco Smoke, Genetic and Epigenetic Changes, and Respirator

The study will leverage an existing comprehensive resource that includes genome-wide association data, linked birth records, and extensive respiratory assessments.

5R01CA172404-02 (Ramus) 08/15/2013–06/30/2018 0.70 CM
NCI $442,287
Identifying Prognostic Markers and Therapeutic Targets for Serous Ovary

The goal is to identify candidate Prognostic genes from a meta-analysis of expression data including subgroups of HGSOC and from an analysis of our survival GWAS.

1R03CA173842-01A1 (Cozen) 01/01/2014-12/31/2015 1.2 CM
NCI $50,000

Comparison of the Fecal Microbiome in Identical Twins Discordant for Colon Polyps

This study will help to elucidate the relationship between gut microbiota and colon polyps, and perhaps provide a path for the development of non-invasive intervention to prevent polyps and subsequent colon cancer, such as probiotics or fecal transplants.

1R01HL118455-01 (Raby) 07/01/2014-06/30/2018 0.72 CM
NIH/NHLBI $696,912
The Integrative Genomics of Acute Asthma Control

Using the Asthma BioRepository for Integrative Genomic Exploration (Asthma BRIDGE), we will perform a series of systems-level genomic analyses that integrate clinical, environmental and various forms of “omic” data (genetics, genomics, epigenetics, and lipidomics) to better understand how molecular processes interact with critical environmental factors to impair asthma control.

Completed Research Support

R03 1R03CA173531-01 Gayther SA (PI) 12/12-11/14
LncRNA Pathways As Novel Biomarkers Derived from the Stroma of Epithelial Ovarian Cancers

AIMS: To use a combination of whole exome sequencing in ovarian cancer families and targeted sequencing of candidates in ovarian cancer populations to identify new susceptibility genes for ovarian cancer
Role: co-investigator

DARPA DARPA-BAA-10-55 **Millstein J** (PI) 09/12-8/18
Reconstruction of a multiscale network of molecular biological timekeepers.
The goal of this study was to identify and describe a network of molecular regulators that through gene expression determines characteristics of biological time.
Role: PI

U54 RFA-CA-09-011 Friend SJ (PI) 06/09-05/11
The Integrative Cancer Biology Program (ICBP): Centers for Cancer Systems Biology (CCSB).
We established a cancer systems biology program to leverage integrative genomics statistical and computational methodology to further cancer research and train junior investigators in cancer systems biology.
Role: Statistical Genetics Lead

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Axel Montagne

eRA COMMONS USER NAME (credential, e.g., agency login): AMONTAGNE

POSITION TITLE: Assistant Professor of Research Physiology and Biophysics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Le Verrier High School, Saint-Lô, FRANCE		Ju 2004	Biology, Physics
University of Caen lower Normandy, Caen, FRANCE	B.S.	Jun 2007	Biology
University of Caen lower Normandy, Caen, FRANCE	M.S.	Jun 2009	Neuroscience, Neuroimaging
University of Caen lower Normandy, Caen, FRANCE	Ph.D.	Oct 2012	Neuroscience, Stroke, magnetic resonance imaging (MRI)
University of Southern California, Los Angeles, USA		Feb 2013 – present	Neuroscience, Alzheimer, Stroke, MRI

A. Personal Statement

I received my PhD in Neuroscience in October 2012, supervised by Pr. Denis Vivien from the University of Caen Lower Normandy. During my PhD, I investigated the impact of a well-known serine protease, called tissue-type plasminogen activator (tPA, the only approved treatment in the acute phase of stroke), on the neurovascular unit and specifically its role on the positive modulation of the glutamatergic neurotransmission involving N-Methyl-D-Aspartate (NMDA) receptors. In parallel, I developed and optimized molecular magnetic resonance imaging (MRI) of endothelial activation with a contrast agent targeting molecules associated with inflammation, specifically vascular cell adhesion molecule-1 (VCAM-1). By examining endothelial cells, diagnosis and non-invasive evaluation of central nervous system (CNS) disorders appear to be feasible even before symptom appearance including cognitive decline. My works clearly showed that it is possible to evaluate inflammation of the CNS with non-invasive and semi-quantitative manners, using very high sensitivity. This approach would allow selecting patients that are the most likely to benefit from anti-inflammatory treatment in numerous neuropathologies including Alzheimer's disease (AD).

I started my postdoctoral training in February 2013 in the outstanding team supervised by Dr. Berislav Zlokovic. I brought my expertise in molecular MRI and other types of MRI analysis, *in vivo* optical imaging, animal surgeries, among other molecular and cellular biology skills. Over the past 4 years, I generated data on the blood-brain barrier (BBB) permeability K_{trans} measurements working closely with Dr. Zlokovic, Dr. Law, Director of the ADRC Imaging Core along with Dr. Toga, and Dr. Chui and other members of the USC imaging team. I also collaborated with Drs. Jacobs and Barnes from the California Institute of Technology (Caltech) to develop and test different paradigms to quantify BBB permeability in the entire brain both in humans and mice using a modified dynamic contrast-enhanced (DCE)-MRI technique. I used several approaches including Patlak's mathematical modeling and Tofts-based pharmacokinetic analysis of DCE-MRI and our newly developed software (*Rocketship*) to compute the blood-to-brain constant K_{trans} reflecting the vascular integrity. I recently developed a new dynamic susceptibility-contrast (DSC) imaging method in order to get quantitative and precise

measurements of cerebral blood flow and volume. I also set up a new high-resolution 3D-T2*-weighted imaging sequence to detect small microhemorrhages, hemosiderin plaques, brain calcification, and amyloid deposits as small as 10-microns diameter in rodents. Finally, in collaboration with Drs. Thompson and Jacobs, I have actively participated in the improvement of a new multi-shell hybrid diffusion imaging (HYDI) method for white matter fibers reconstruction (tractography) from DTI (diffusion tensor imaging)-MRI data acquired both in humans and rodents. The wide range of MRI techniques that I developed and optimized over the past 8 years allow simultaneous study of pathophysiological features in AD and ischemic stroke in both humans and animals. Our new set of high-resolution MRI sequences will improve knowledge of neurovascular dysfunctions that occur in AD and other neurological disorders.

B. Positions and Honors

Positions and Employment

2008-2012 *Graduate student*, Laboratory of Denis Vivien, Ph.D., University of Caen Lower-Normandy, Caen (France).
2013-2016 *Postdoctoral Scholar*, Laboratory of Berislav Zlokovic, M.D., Ph.D., Zilkha Neurogenetic Institute, University of Southern California, Los Angeles (USA).
2016-present *Assistant Professor of Research* Physiology and Biophysics, Laboratory of Berislav Zlokovic, M.D., Ph.D., Zilkha Neurogenetic Institute, University of Southern California, Los Angeles (USA).

Teaching Experience

2011-2012 Teaching Assistant in the Master degree at University of Caen Lower-Normandy (MRI and Contrast Agents).

Honors

2007 B.S. degree Biology specialty with honors, University of Caen Lower-Normandy, Caen (France).
2009 M.S. degree in Neuroscience and Neuroimaging with honors, University of Caen Lower-Normandy, Caen (France).
2012 First place Poster Award (Molecular MRI targeting inflammation - VCAM-1/P-selectin), World Molecular Imaging Congress (WMIC), Dublin, Ireland.
2012 Ph.D. in Neuroscience and Neuroimaging with honors and board's congratulations, University of Caen Lower-Normandy, Caen (France).

Other Experience and Professional Memberships

2009-current Member of the "Societé Française des Neurosciences".
2010-current Member of the European Society for Molecular Imaging (ESMI).
2013-current Member of the Society for Neuroscience.
2014-current NIH eRA commons member.

C. Contribution to Science

Most relevant to the current application

New MRI-based techniques are useful for identifying underlying AD pathology. Using new high-resolution MRI techniques in rodents and homemade pre- and post-processing softwares, we obtained new findings showing that the vascular plasticity plays an important role during normal aging and dementia. We developed molecular MRI targeting neuroinflammation, high-resolution dynamic contrast-enhanced (DCE)-MRI to study the blood-brain barrier integrity, arterial spin labeling (ASL)- and dynamic susceptibility-contrast (DSC)-MRI to get precise blood flow and volume measurements, a new multi-shell diffusion tensor imaging (DTI)-MRI to investigate structural and connectivity changes, and high-resolution 3D-T2*-weighted-based images and susceptibility mapping (SWI) to detect microbleeding events, as well as vascular hemosiderin deposits.

1. **Montagne A**, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, Toga AW, Jacobs RE, Liu CY, Amezcua L, Harrington MG, Chui HC, Law M, Zlokovic BV. Blood-brain barrier breakdown in the aging human hippocampus. Neuron 2015;85(2):296-302. PMID:25611508.
2. **Montagne A**, Toga AW, Zlokovic BV. Blood-brain Barrier Permeability and Gadolinium: Benefits and Potential Pitfalls in Research. JAMA Neurology 2015. doi: 10.1001/jamaneurol.2015.2960. PMID:26524294.
3. **Montagne A,** Pa J, Zlokovic BV. Vascular plasticity and cognition during normal aging and dementia. JAMA Neurology 2015. doi: 10.1001/jamaneurol.2014.4636. PMID:25751405.
4. Barnes SR, Ng TSC, Santa-Maria N, **Montagne A**, Zlokovic BV, Jacobs RE. ROCKETSHIP: a flexible and modular software tool for the planning, processing and analysis of dynamic MRI studies. BMC Medical Imaging 2015;15:19. PMID:26076957.
5. Barnes SR, Ng TSC, **Montagne A**, Law M, Zlokovic BV, Jacobs RE. Optimal acquisition and modeling parameters for accurate assessment of low K_{trans} blood-brain barrier permeability using dynamic contrast-enhanced MRI. Magn Reson Med. 2016 May;75(5):1967-77. PMID:26077645.
6. **Montagne A**, Nation DA, Pa J, Sweeney MD, Toga AW, Zlokovic BV. Brain Imaging of Neurovascular Dysfunction in Alzheimer's Disease. Acta Neuropathol. 2016 May;131(5):687-707. PMID:27038189.
7. Daianu M, Jahanshad N, Villalon-Reina JE, Prasad G, Jacobs RE, Barnes S, Zlokovic BV, **Montagne A**, Thompson PM. 7T multi-shell hybrid diffusion imaging (HYDI) for mapping brain connectivity in mice. Proc SPIE Int Soc Opt Eng. 2015 Mar 20;9413. pii: 941309. PMID:25859293.
8. **Montagne A**, Gauberti M, Macrez R, Jullienne A, Briens A, Raynaud J-S, Louin G, Buisson A, Haelewyn B, Defer G, Docagne F, Vivien D, Maubert E. Ultra-sensitive molecular MRI of cerebrovascular cell activation enables early detection of chronic central nervous system disorders. Neuroimage 2012;63(2):760-70. PMID:22813950.
9. Gauberti M, **Montagne A**, Quenault A, Vivien D. Molecular magnetic resonance imaging of brain-immune interactions. Front Cell Neurosci. 2014 Nov 27;8:389. PMID:25505871.
10. Gauberti M*, **Montagne A***, Marcos-Contreras O, Le Béhot A, Maubert E, Vivien D. Ultra-sensitive molecular MRI of VCAM-1 reveals a dynamic inflammatory penumbra following stroke. Stroke. 2013 Jul;44(7):1988-96. PMID:23743972. * Equal contribution.
11. Gaberel T, Gakuba C, Hébert M, **Montagne A**, Agin V, Rubio M, Emery E, Vivien D, Gauberti M. Intracerebral hematomas disappear on T2*-weighted images during normobaric oxygen therapy. Stroke. 2013 Dec;44(12):3482-9. PMID:24105700.

D. Research support

Ongoing Research Support

ACTIVE

1P01AG052350-01 (Zlokovic/Toga) 09/30/16-05/31/21
NIH/NIA $1,792,396
Vascular contributions to dementia and genetic risk factors for Alzheimer's disease
Program project to study imaging and molecular biomarkers of neurovascular dysfunction in individuals at genetic risk for AD both familial and sporadic.

5P50AG005142-32 (Chui) 04/01/15-03/31/20
Project 1: *Neurovascular Factors in AD* (Zlokovic, Leader) $93,384
Alzheimer Disease Research Center (ADRC)
To evaluate neurovascular function by imaging and molecular biomarkers in participants with no or mild cognitive impairment in three cluster-derived vascular risk profiles from a community-based sample - low vascular risk (lo-VRF), high-hypertension (Hi-Htn) risk and high-metabolic (Hi-Met) risk.

1R01NS100459-01 (Zlokovic) 09/30/16-08/31/21
NIH/NIA $500,000
The role of pericytes in white matter disease
To study the role of pericytes in white matter degeneration using novel *in vivo* animal model systems.

Alzheimer's Association (Zlokovic/Toga) 10/01/16-09/30/21
Vascular contributions to dementia in APOE4 carriers $600,000
To investigate preclinical changes in cerebrospinal fluid and imaging biomarkers of vascular/Blood-brain barrier injury in cognitively normal *APOE4* carriers relative to non-carriers.

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Morgan, Todd Eugene

eRA COMMONS USER NAME (credential, e.g., agency login): temorgan

POSITION TITLE: Research Professor of Gerontology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Dana College, Blair, NE	B.S.	06/1984	Chemistry
Colorado State University, Ft. Collins, CO	Ph.D.	08/1990	Biochemistry
Univ. of Southern California, Los Angeles, CA	Post-Doc	12/1993	Neurobiology

A. Personal Statement

I have a long-standing interest in elucidating the molecular mechanisms underlying normal brain aging and age-related neuropathologies with a particular focus on environmental, dietary and inflammatory contributions. My initial training in Biochemistry was expanded to molecular and cellular biology in living organisms while working with rodents models of aging and age-related diseases. My strengths include the ability to design, orchestrate, analyze and interpret complex rodents studies. This P01 builds on our initial air pollution particulate matter (PM) study (Morgan et al 2011), which has greatly expanded with more recent observations that nPM (a nano-sized sub-fraction of PM2.5) accelerates Abeta production/deposition in ADtg mice (Cacciotollo et al 2017), causes hippocampal CA1 myelin and neurite loss (Woodward et al 2017a), which may involve the TLR4 and TNFa pathways (Woodward et al 2017b). My training and experience provide me with the necessary skill set to successfully contribute to this comprehensive proposal to define new markers and mechanisms for the impact of urban airborne pollution on brain functions.

~~~Morgan TE, Davis DA, Iwata N, Tanner JA, Snyder D, Ning Z, Kam W, Hsu YT, Winkler JW, Chen JC, Petasis NA, Baudry M, Sioutas C, Finch CE. Glutamatergic neurons in rodent models respond to nanoscale particulate urban air pollutants in vivo and in vitro. Environ Health Perspect. 2011 Jul;119(7):1003-9. PubMed PMID: 21724521; PubMed Central PMCID: PMC3222976.

~~~Cacciottolo M, Wang X, Driscoll I, Woodward N, Saffari A, Reyes J, Serre ML, Vizuete W, Sioutas C, **Morgan TE**, Gatz M, Chui HC, Shumaker SA, Resnick SM, Espeland MA, Finch CE, Chen JC. Particulate air pollutants, APOE alleles and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. Transl Psychiatry. 2017 Jan 31;7(1):e1022. doi: 10.1038/tp.2016.280. PubMed PMID: 28140404; PubMed Central PMCID: PMC5299391.

~~~Woodward NC, Pakbin P, Saffari A, Shirmohammadi F, Haghani A, Sioutas C, Cacciottolo M, **Morgan TE**, Finch CE. Traffic-related air pollution impact on mouse brain accelerates myelin and neuritic aging changes with specificity for CA1 neurons. Neurobiol Aging. 2017a May;53:48-58. PubMed PMID: 28212893; PubMed Central PMCID: PMC5388507.

~~~Woodward NC, Levine MC, Haghani A, Shirmohammadi F, Saffari A, Sioutas C, **Morgan TE**, Finch CE. Toll-like receptor 4 in glial inflammatory responses to air pollution in vitro and in vivo. J Neuroinflammation. 2017b Apr 14;14(1):84. doi: 10.1186/s12974-017-0858-x. PubMed PMID: 28410596; PubMed Central PMCID: PMC5391610
~~~

B. Positions and Honors

Positions and Employment

1990 - 1993 Post-doctoral Fellow, School of Gerontology, USC
1994 - 2006 Research Assistant Professor, School of Gerontology, USC
2006 - 2010 Research Associate Professor, School of Gerontology, USC
2009 - Chief Scientific Officer, L-Nutra, Inc
2010 - Research Professor, School of Gerontology, USC

Committee Memberships

2007 - USC Institutional Animal Care & Use Committee (Vice Chair beginning 2010)
2008 - 2016 USC School of Gerontology Faculty Council
2009 - 2011 USC Academic Senate Representative
2009 Special Emphasis Panel, Drug Discovery for Neurodegenerative Diseases, ZRG1 MDCN-B(91)S

C. Contributions to Science

1. Air pollution: Working closely with Drs. Finch and Sioutas, I designed and orchestrated the first nPM mouse exposure study and analyzed all results (Morgan et al 2011). This area of investigation has expanded to examine effects of air pollution prenatally (Davis et al 2013) and in various neurodegenerative models, including stroke (Liu et al 2016). We have investigated how air pollution particles damage the olfactory neuroepithelium, the olfactory bulb, and ultimately the cerebral cortex (Cheng et al 2016). A related P01 ES022845-01 explores that role of diet and air pollution on development and metabolic dysfunction with a tie-in to human studies. To date, we have published 11 peer-reviewed research papers, with 5 more being written.

- **Morgan TE**, Davis DA, Iwata N, Tanner JA, Snyder D, Ning Z, Kam W, Hsu YT, Winkler JW, Chen JC, Petasis NA, Baudry M, Sioutas C, Finch CE. Glutamatergic neurons in rodent models respond to nanoscale particulate urban air pollutants in vivo and in vitro. Environ Health Perspect. 2011 Jul;119(7):1003-9. PubMed PMID: 21724521; PubMed Central PMCID: PMC3222976.
- Davis DA, Bortolato M, Godar SC, Sander TK, Iwata N, Pakbin P, Shih JC, Berhane K, McConnell R, Sioutas C, Finch CE, **Morgan TE**. Prenatal exposure to urban air nanoparticles in mice causes altered neuronal differentiation and depression-like responses. PLoS One. 2013;8(5):e64128. PubMed PMID: 23734187; PubMed Central PMCID: PMC3667185.
- Liu Q, Babadjouni R, Radwanski R, Cheng H, Patel A, Hodis DM, He S, Baumbacher P, Russin JJ, **Morgan TE**, Sioutas C, Finch CE, Mack WJ. Stroke Damage Is Exacerbated by Nano-Size Particulate Matter in a Mouse Model. PLoS One. 2016 Apr 12;11(4):e0153376. PubMed PMID: 27071057; PubMed Central PMCID: PMC4829199.
- Cheng H, Saffari A, Sioutas C, Forman HJ, **Morgan TE**, Finch CE. Nanoscale Particulate Matter from Urban Traffic Rapidly Induces Oxidative Stress and Inflammation in Olfactory Epithelium with Concomitant Effects on Brain. Environ Health Perspect. 2016 Oct;124(10):1537-1546. Epub 2016 May 17. PubMed PMID: 27187980; PubMed Central PMCID: PMC5047762.

2. Soluble Abeta: I was part of the team that initially identified oligomeric Abeta structures (Oda et al 1995; Lambert et al 1998; 2001) which has become a prominent area of investigation in Alzheimers by many labs. I continue to examine oligomeric Abeta in the EFAD mouse model (Cacciottolo et al 2016).

- Oda T, Wals P, Osterburg HH, Johnson SA, Pasinetti GM, **Morgan TE**, Rozovsky I, Stine WB, Snyder SW, Holzman TF. Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A beta 1-42) and forms slowly sedimenting A beta complexes that cause oxidative stress. Exp Neurol. 1995 Nov;136(1):22-31. PubMed PMID: 7589331.
- Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, **Morgan TE**, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL. Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci U S A. 1998 May 26;95(11):6448-53. PubMed PMID: 9600986; PubMed Central PMCID: PMC27787.
- Lambert MP, Viola KL, Chromy BA, Chang L, **Morgan TE**, Yu J, Venton DL, Krafft GA, Finch CE, Klein WL. Vaccination with soluble Abeta oligomers generates toxicity-neutralizing antibodies. J Neurochem. 2001 Nov;79(3):595-605. PubMed PMID: 11701763.

- Cacciottolo M, Christensen A, Moser A, Liu J, Pike CJ, Smith C, LaDu MJ, Sullivan PM, **Morgan TE**, Dolzhenko E, Charidimou A, Wahlund LO, Wiberg MK, Shams S, Chiang GC; Alzheimer's Disease Neuroimaging Initiative, Finch CE. The APOE4 allele shows opposite sex bias in microbleeds and Alzheimer's disease of humans and mice. *Neurobiol Aging*. 2016 Jan;37:47-57. doi: 10.1016/j.neurobiolaging.2015.10.010. Epub 2015 Oct 19. PubMed PMID: 26686669; PubMed Central PMCID: PMC4687024

3. Microglial activation: I have long been working on the role of microglia in brain function. My first studies examined TGF-ß1 when this cytokine was not believed to be produced by brain cells (Morgan et 1993; 1995). I then developed an in vitro protocol for origination and study of microglia and astrocytes from advanced age rodents, where I found that the in vivo phenotypes of glia persist in culture (Rozovsky et al 1998).

- **Morgan TE**, Nichols NR, Pasinetti GM, Finch CE. TGF-beta 1 mRNA increases in macrophage/microglial cells of the hippocampus in response to deafferentation and kainic acid-induced neurodegeneration. Exp Neurol. 1993 Apr;120(2):291-301. PubMed PMID: 8491285.
- **Morgan TE**, Laping NJ, Rozovsky I, Oda T, Hogan TH, Finch CE, Pasinetti GM. Clusterin expression by astrocytes is influenced by transforming growth factor beta 1 and heterotypic cell interactions. J Neuroimmunol. 1995 Apr;58(1):101-10. PubMed PMID: 7730444.
- **Morgan TE**, Rozovsky I, Sarkar DK, Young-Chan CS, Nichols NR, Laping NJ, Finch CE. Transforming growth factor-beta1 induces transforming growth factor-beta1 and transforming growth factor-beta receptor messenger RNAs and reduces complement C1qB messenger RNA in rat brain microglia. Neuroscience. 2000;101(2):313-21. PubMed PMID: 11074155.
- Rozovsky I, Finch CE, **Morgan TE**. Age-related activation of microglia and astrocytes: in vitro studies show persistent phenotypes of aging, increased proliferation, and resistance to down-regulation. Neurobiol Aging. 1998 Jan-Feb;19(1):97-103. PubMed PMID: 9562510.

4. Dietary restriction: My work has expanded our understanding of caloric restriction. I was one of the first to report that caloric restriction attenuated Abeta deposition (Patel et al 2005, shared senior authors). These studies grew out of earlier studies (Morgan et al 1997; 99). I continue to pursue dietary interventions (Brandhorst et al 2015).

- Patel NV, Gordon MN, Connor KE, Good RA, Engelman RW, Mason J, Morgan DG, **Morgan TE**, Finch CE. Caloric restriction attenuates Abeta-deposition in Alzheimer transgenic models. Neurobiol Aging. 2005 Jul;26(7):995-1000. PubMed PMID: 15748777.
- **Morgan TE**, Rozovsky I, Goldsmith SK, Stone DJ, Yoshida T, Finch CE. Increased transcription of the astrocyte gene GFAP during middle-age is attenuated by food restriction: implications for the role of oxidative stress. Free Radic Biol Med. 1997;23(3):524-8. PubMed PMID: 9214592.
- **Morgan TE**, Xie Z, Goldsmith S, Yoshida T, Lanzrein AS, Stone D, Rozovsky I, Perry G, Smith MA, Finch CE. The mosaic of brain glial hyperactivity during normal ageing and its attenuation by food restriction. Neuroscience. 1999 Mar;89(3):687-99. PubMed PMID: 10199605.
- Brandhorst S, Choi IY, Wei M, Cheng CW, Sedrakyan S, Navarrete G, Dubeau L, Yap LP, Park R, Vinciguerra M, Di Biase S, Mirzaei H, Mirisola MG, Childress P, Ji L, Groshen S, Penna F, Odetti P, Perin L, Conti PS, Ikeno Y, Kennedy BK, Cohen P, **Morgan TE**, Dorff TB, Longo VD. A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan. Cell metabolism. 2015; 22(1):86-99. NIHMSID: NIHMS705076 PubMed [journal] PMID: 26094889, PMCID: PMC4509734.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/todd.morgan.1/bibliography/44540372/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1. R01 AG051521 Finch (PI) 09/30-2015-09-29/2020. Amyloid and inflammation: modulation by apoE, gender, air pollution, and drugs. Role: Co-I
2. R21 AG050201 (Finch PI) 04/01/2016-03/30/2018). Air pollution nano-particulate matter, APP processing, and glutamate receptors. Role: Co-I

3. R01 ES023780, Volk H (PI) 08/01/2015 – 04/30/2018 Prospective Evaluation of Air Pollution, Cognition, and Autism from Birth Onward. Subcontract, Role: Co-I
4. PD160021P1 (Brundin P, Finch CE, Chen H, coPIs)(10/01/2017-09/30/2021) Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System. Role: Co-I
5. P01 ES022845 McConnell (PI) 07/01/13-05/31/18 NIH/NIEHC & EPA Project 3 on rodent exposure to urban traffic derived air pollution particles. Pre-and postnatal exposure of mice urban traffic derived air pollution particles for effects on adult behavior and neuron structure. Role: Co-I
6. PO1 AG026572, Brinton R (PI) 06/01/2016 - 05/31/2021 Perimenopause in Brain Aging and Alzheimer's Disease. Core B & Project 2. Role: Co-I

Completed:
1. R21 AG040683, Finch CE (PI) 07/01/2011 - 06/30/2013 Air Pollution and Vulnerability to Alzheimer-like Neurodegeneration in Transgenic Models. Role: Co-I
2. R21 AG040753, Finch CE (PI) 09/01/2011 – 06/30/2013 Aging and sensitivity to traffic-generated air pollutants in male and female mice. Role: Co-I

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Daniel Addison Nation

eRA COMMONS USER NAME (credential, e.g., agency login): DANIEL_NATION108

POSITION TITLE: Assistant Professor of Psychology (USC)

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Santa Cruz, CA	B.A.	2001	Philosophy; Psychology
University of Miami, Miami, FL	M.S.	2006	Psychology
University of Miami, Miami, FL	Ph.D.	2009	Clinical Psychology
University of California, San Diego, CA	Postdoctoral	2009-11	Biological Psychiatry & Neuroscience

A. Personal Statement

My research interests originated in studies of cerebral control of circulation and cardiovascular disease. My early work focused on animal models of atherosclerosis, including apoE knockout mice, and cellular models of vascular inflammation and oxidative stress. More recently, I have applied this background to study vascular contributions to neurodegenerative disease (Nation et al. 2014; Nation et al., 2015), occult cerebrovascular disease (Nation et al 2012) and neuropsychological decline. This research has involved novel approaches to MRI-based assessment of cerebrovascular dysfunction (Nation et al., 2013). Since coming to USC in the fall of 2013, I have continued to focus on mechanisms linking systemic vascular disease to neurodegenerative pathophysiology, and development of clinical and research tools to better assess underlying vascular contributors during early stage disease. My most recent unpublished work has focused on the role of vascular protective factors, including cells expressing hematopoietic and vascular progenitor markers. Using *in vitro* models from patient blood samples, I have found that these progenitor cells are reduced in patients with cognitive impairment, where they correlate with memory performance and cortical thickness. Further research in this area will contribute to efforts aimed at predicting and preventing dementia, as well as identification of new treatment targets.

B. Positions and Honors

2009 - 2011 T32-NIH Postdoctoral Fellow, Department of Psychiatry, UCSD School of Medicine
2011 - 2013 Postdoctoral Fellow—Cognitive Rehabilitation, Veterans Affairs San Diego Healthcare System
2013 - present Assistant Professor, Department of Psychology, University of Southern California

Honors and Awards

2004-8 Outstanding Graduate Research Award
2009 Postdoctoral Research Training Award (T32), National Institute of Mental Health
2010 Program Committee Member, American Psychosomatic Society Annual Meeting
2011 Leon Thal Memorial Travel Award, Veterans Medical Research Foundation
2013 Program Committee Member, International Neuropsychological Society Annual Meeting
2015 Consulting Editor, *Journal of Alzheimer's disease*
2015 Editorial Board, *Neuropsychology*

2016 Keynote Speaker, Inaugural Wake Forest Alzheimer's Disease Center Conference
2016 Affiliate, Zilkha Neurogenetic Institute, University of Southern California, Keck School of Medicine

C. Contributions to Science (selected from over 36 publications)
URL: http://www.ncbi.nlm.nih.gov/pubmed/?term=Nation+DA%5BAuthor%5D

1. *Vascular stiffness contributes to Alzheimer's disease.*
I have contributed to our understanding of how systemic vascular disease relates to AD. Prior work had suggested that age-related arterial stiffening was associated with cognitive decline and that this may be due to cerebrovascular injury secondary to hemodynamic alterations (increased pulse wave velocity and decreased buffering of pressure oscillations). It has been hypothesized that these hemodynamic changes may also impact AD pathophysiology by interfering with amyloid clearance. To investigate these possibilities, I led a series of clinical studies investigating relationships between markers of arterial stiffness and a variety of AD markers (cognitive decline, CSF biomarkers, autopsy). In collaboration with co-authors, I discovered that brachial artery pulse pressure, a marker of arterial stiffening, is associated with cognitive decline and CSF biomarkers of AD in cognitively normal older adults (Nation et al. 2013). Specifically, this research revealed that pulse pressure is associated with both decreased CSF amyloid and increased phosphorylated tau, a novel finding that we have replicated in two separate samples of older adults (Nation et al. 2013, 2015). Finally, I developed an MRI-based marker of cerebrovascular resistance by investigating relationships between blood pressure and cerebral blood flow using arterial spin labeling MRI in older adults and AD patients. Results indicated cortical and subcortical cerebrovascular resistance are elevated across the aging-MCI-AD spectrum (Nation et al. 2013). These findings were extended to a larger cohort where cerebrovascular resistance was found to be increased in patients who were amyloid positive and to correlate with with future regional amyloid deposition. Finally, increased cerebrovascular resistance in conjunction with cerebral amyloidosis was highly predictive of neuropsychological decline, brain atrophy and progression to dementia over several years of follow-up (Yew and Nation, in press). Findings support the hypothesis that arterial stiffening may contribute to cognitive decline and AD pathogenesis through its effects on the neurovascular unit, amyloid clearance, and tau-mediated neurodegeneration.
   1a. Yew, B., Nation, D.A.* (in press). Cerebrovascular resistance and cerebral amyloidosis: Effects on cognitive decline, cortical atrophy and progression to dementia. *Brain*.
   1b. Nation, D.A., Edland, S.D., Bondi, M.W., Salmon DP, Delano-Wood L, Peskind ER, Quinn JF, Galasko DR (2013). Pulse pressure is associated with Alzheimer's biomarkers in cognitively normal older adults. *Neurology*, *81*, 2024-7. PMCID: PMC3854831
   1c. Nation, D.A., Clark, L., Wierenga, C.E., Delano-Wood, L., Bangen, K.J., Jak, A.J., Delis, D.C., Salmon, D.P., Liu, T.T., Bondi, M.W. (2013). Cortical and subcortical cerebrovascular resistance index in Alzheimer's disease and MCI. *Journal of Alzheimer's Disease*. 36(4):689-98.
   1d. Nation, D.A., Edmonds, E.C., Bangen, K.J., Delano-Wood, L., Scanlon, B.K., Han, S.D., Edland, S.D., Salmon, D.P., Galasko, D.R., Bondi, M.W., for the Alzheimer's Disease Neuroimaging Initiative (2015). Pulse pressure in relation to tau-mediated neurodegeneration, cerebral amyloidosis, and progression to dementia in the very-old. *JAMA Neurology*. 72(5):546-553.

2. *Vascular stiffness contributes to cognitive and brain aging.*
I identified brachial artery pulse pressure as a risk factor associated with worse cognitive performance in older adults (Nation et al., 2010). I also led another study in which we showed that ante-mortem pulse pressure elevation greatly increases the risk of occult cerebrovascular disease in AD patient brains at autopsy (Nation et al. 2012). Additional studies indicated that pulse pressure predicted decline in executive function over 5-7 year follow-up, and exhibited greater associations with decline among those at genetic risk for AD due to APOE4 carrier status (Nation et al., 2016). These data suggest that pulse pressure may be a modifiable risk factor for cognitive decline among those at high risk for AD. In a collaborative study investigating an MRI-based marker of cerebrovascular resistance, we found that increased pulse pressure was associated with cognitive decline in those with reduced cerebral blood flow (Clark, Nation, et al., 2015). These studies suggest that pulse pressure may influence cognitive decline by impairing cerebral blood flow.

2a. Nation, D.A., Jak, A.J., Wierenga, C.E., Delano-Wood, L., Delis, D.C., Salmon, D., Bondi, M.W. (2010). Elevated pulse pressure is associated with age-related decline in language functioning. *Journal of the International Neuropsychological Society*. 16(5):933-8.
2b. Nation, D.A., Preis, S.R., Beiser, A., Bangen, K.J., Delano-Wood, L., Lamar, M., Libon, D.J., Seshadri, S., Wolf, P.A., Au, R. (2016). Pulse pressure predicts early brain atrophy and cognitive decline: modifying effects of APOE4. *Alzheimer Disease & Associated Disorders*. 30(3):210-215.
2c. Clark, L., Nation, D.A., Delano-Wood, L., Bangen, K.J., Salmon, D.P., Bondi, M.W. (2015). Elevated cerebrovascular resistance index is associated with cognitive dysfunction in the very-old. *Alzheimer's Research & Therapy*. 7(3):1-9.
2d. Nation D.A., Delano-Wood L., Bangen, K.J., Wierenga, C.E., Jak, A.J., Hansen, L.A., Galasko, D.R., Salmon, D.P., Bondi, M.W. (2012). Antemortem pulse pressure elevation predicts cerebrovascular disease in autopsy-confirmed Alzheimer's disease. *J Alzheimers Dis, 30,* 595-603.

3. *Use of AT1-receptor blockers (ARBs) is linked to neurocognitive benefits in older adults*
AT1-receptor blockers (ARBs) represent one class of antihypertensive medications that have been highlighted for their salutary effects on cognition. ARBs are also thought to specifically attenuate the pulsatile component of blood flow through their vascular "de-stiffening" effects. In a recent study, I found that older adults taking ARBs showed improved AD biomarker profiles in CSF samples, and attenuated progression to dementia over longitudinal follow-up (Nation et al., 2015). In a more recent follow-up study we found that patients taking ARBs that are capable of crossing the blood-brain barrier seem to show particularly salient benefits. Specifically, these individuals exhibit less memory impairment and less white matter lesion burden, potentially suggesting salutary effects on cerebrovascular disease and vascular cognitive impairment (Ho and Nation, in press).
3a. Nation, D.A., Ho, J., Yew, B. (2015). Older adults taking AT1-receptor blockers exhibit reduced cerebral amyloid retention. *Journal of Alzheimer's Disease*. 50(3):779-89.3.
3b. Ho, J., Nation, D.A. (in press). Memory is preserved in older adults taking AT1-receptor blockers. *Alzheimer's Research & Therapy*.

4. *Vascular reserve in cognitive decline and Alzheimer's disease.*
Ongoing work from my own laboratory, and the focus of the present application, involves unpublished studies of circulating angiogenic and vascular-repair factors (e.g., endothelial progenitor cells and circulating angiogenic cells) in age-related cerebrovascular dysfunction, Alzheimer's disease and cognitive decline. I have hypothesized that these factors represent a "vascular reserve" that may become senescent and attenuate in the context of vascular risk and aging, leading to problems maintaining and repairing the cerebrovasculature and neurovascular unit. These studies focused on novel protective factors will substantially advance our understanding of resilience mechanisms in the aging brain and will lead to new insights and targets for therapeutic and risk assessment science. Initial findings indicate that progenitor cells are attenuated in mild cognitive impairment, where they correlate with memory performance and cortical thickness (Nation et al., 2017).
4a. Nation, D.A., Tan, A., McIntosh, E., Yew, B., Ho, J., Dutt, S., Blanken, A., Jang, J., Rodgers, K., Gaubert, A. (2017). Circulating angiogenic cell levels show protective associations with memory function: introducing the vascular reserve hypothesis. Abstract to be presented at the Alzheimer's Association International Conference in London.

5. *Behavioral and neurohypophyseal contributions to cardiovascular disease.*
I have contributed to our understanding of how behavioral and endocrine factors influence the progression of atherosclerotic lesions. Prior work had demonstrated that sedentary lifestyle in humans and physical inactivity in animals can lead to accelerated atherosclerotic disease, but the mechanisms involved remained unclear. Using animal models of atherosclerosis (apoE knockout and WHHL rabbit), I found that physical inactivity leads to increased vascular NAD(P)H oxidase activity (Nation et al. 2008), which may represent a major mechanism by which sedentary lifestyle leads to cardiovascular disease. I also investigated a potential role for the neurohypophyseal peptide, oxytocin, in the amelioration of atherosclerotic disease. Findings indicated that administration of exogenous oxytocin attenuates atherosclerotic lesion progression, vascular inflammation, and adipose tissue inflammation in apoE knockout mice (Nation et al. 2010). In collaboration with colleagues, we found that oxytocin prevents vascular inflammation and oxidant stress *in vitro*, as it reduced NAD(P)H oxidase activity and

proinflammatory cytokine production from cultured endothelial cells, vascular smooth muscle cells, and macrophage-like cells (Szeto et al. 2008). Finally, I aided in the development of improved assay methodologies for the measurement of plasma oxytocin in humans (Szeto et al. 2010).

3a. Nation, D.A., Gonzales, J. A., Mendez, A. J., Zaias, J., Szeto, A., Paredes, J., Brooks, L., D'Angola, A., Schneiderman, N., & McCabe, P.M. (2008). The effect of social environment on markers of vascular oxidant stress and inflammation in the Watanabe Heritable Hyperlipidemic rabbit. *Psychosomatic Medicine*, 70(3):269-75.

3b. Nation, D.A., Szeto, A., Mendez, A.J., Brooks, L., Noller, C.M., Gonzales, J., Zaias, J., Schneiderman, N., McCabe, P.M. (2010). Oxytocin attenuates adipose tissue inflammation and atherosclerosis in apoE-/- mice. *Psychosomatic Medicine*. 72:376-382

3c. Szeto, A., Nation, D.A., Mendez, A.J., Dominguez, R., Brooks, L., Schneiderman, N., McCabe, P.M. (2008). Oxytocin attenuates NADPH-dependent superoxide activity and IL-6 secretion in macrophages and vascular cells. *American Journal of Physiology. Endocrinology and Metabolism*. 295(6):E1495-1501

3d. Szeto, A., McCabe, P.M., Nation, D.A., Tabak, B.A., Rossetti, M.A., McCullough, M.E., Schneiderman, N., Mendez, A.J. (2011). Evaluation of enzyme immunoassay and radioimmunoassay methods for the measurement of plasma oxytocin. *Psychosomatic Medicine*. 73(5):393-400.

D. Research Support

Ongoing Research Support

NIH/NIA 5 P50 AG05142-30 (Chui) 5/01/2015-4/30/2020

USC ADRC Project 1: Vascular Risk Factors and the Neurovascular Unity

This is a competitive renewal of the USC ADRC. Project 1 focuses on the role of blood brain barrier integrity and cerebral blood flow in cognitive and brain structural changes in those with hypertension and diabetes. As Co-Leader of Project 1, my role is to evaluate the relationship between novel CSF- and MRI-based markers of neurovascular unit function and both cerebral blood flow and decline in neuropsychological function in older adults with either low vascular risk, hypertension, or diabetes. I also play a major role in statistical analysis, interpretation and preparation of manuscripts with regard to major study outcomes.

Role: Co-Leader, Project 1

NIH/NIA 1 P01 AG052350-01 (Zlokovic) 9/30/16 – 5/31/21

Vascular Contributions to Dementia and Genetic Risk Factors for Alzheimer's Disease

This is a program project involving four different sites (Mayo Clinic, Banner Alzheimer's Institute Arizona, Washington University ADRC, and USC ADRC). The aims of the study entail investigation of biofluid and MRI-based markers of neurovascular unit function in aging and MCI, presenilin-1 mutation carriers, and animal models of Alzheimer's disease. As Co-leader of Project 1, I lead investigation into how blood-brain barrier breakdown relates to changes in cerebral blood flow and white matter lesion development in carriers of the APOE-ε4 allele and PSEN1 mutations.

Role: Co-Leader, Project 1

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. ***DO NOT EXCEED FIVE PAGES.***

NAME: Amy R. Nelson

eRA COMMONS USER NAME (credential, e.g., agency login): arnelson

POSITION TITLE: Postdoctoral Scholar

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Alabama at Birmingham	B.S.	05/2005	Biology
University of Alabama at Birmingham	M.S.	12/2006	Biology
University of Alabama at Birmingham	Ph.D.	12/2013	Neuroscience
University of Southern California	Postdoc		Neuroscience

A. Personal Statement

Alzheimer's disease (AD) is a debilitating disease first described more than 100 years ago yet the underlying mechanisms causing sporadic AD remain elusive. Interestingly, many neurodegenerative diseases including ALS, Parkinson's disease, Huntington's disease and AD share several underlying similarities including protein misfolding and programmed cell death. Strikingly, disruption of the blood-brain barrier also occurs in these diseases which allow toxins and other molecules to enter the brain. One key player in BBB maintenance is the pericyte. Notably, pericyte number is reduced in AD post-mortem brain tissue. Unfortunately, little is known about how pericyte reduction alters overall brain function. I hypothesize that pericyte maintenance of the BBB is crucial for proper neuronal function. Specifically, I am interested in how pericyte reduction affects the hippocampus, a brain region critical for learning and memory. I have extensive hands-on experience with immunohistochemistry and electrophysiology, and have utilized EFAD and other Alzheimer's related mouse models. Also, I have studied the beneficial effects of estrogen on hippocampal function. Therefore, this P01 is an extension of my experience and interests to understand how air pollution impacts the BBB which could lead to Alzheimer's disease. For this Sub-Core, I will perform tissue immunofluorescent and fluorescent lectin staining and confocal imaging of the NVU and quantification. I am excited to work with Drs. Mack and Finch for Projects 3 and 4 planning and discussion, and will work closely with Drs. Zlokovic, Montagne and Kisler on a daily basis for experimental design, execution and data interpretation. I will spend 20%/2.4 calendar months per year working on this project.

B. Positions and Honors

Positions and Employment

2005 - 2008 Teaching Assistant, Department of Biology, University of Alabama at Birmingham, Birmingham, AL
2007 - 2007 Research Assistant, Microbiology Department, University of Alabama at Birmingham, Birmingham, AL
2007 - 2008 Program Director, Community Outreach Development, Science Education, University of Alabama at Birmingham, Birmingham, AL
2007 - 2013 Graduate Student Assistant, Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL
2014 - present Postdoctoral Scholar, Department of Physiology and Biophysics, Center for Neurodegeneration and Regeneration, Zilkha Neurogenetic Institute, Keck School of Medicine, University of Southern California, Los Angeles, CA
2014 - present USC Leader/Liason, Los Angeles/Irvine Brain Bee
2015 - present Executive Board Member (Treasurer), Young Professionals Committee (YPC), Alzheimer's of Greater Los Angeles

Honors

1997	USA President's List
2004 & 2005	UAB Dean's List
2004	Phi Sigma (Biology Honor Society)
2005 - 2006	Graduate Student Fellowship (for academic merit)
2007	Sigma Xi (nominated for scientific achievement)
2007	Golden Key International Honor Society
2007	Teaching Assistantship (selected as 1 of 10 out of 50 applicants)
2008 - 2013	Graduate Student Fellowship (for academic merit)
2008, 2009 & 2012	Graduate Student Association Travel Fellowship
2010	Alzheimer's Association ICAD Travel Fellowship (Honolulu, HI)
2010	Alzheimer's of Central Alabama Research Grant ($10,000)
2011	International Conference of Alzheimer's Disease Volunteer Fellowship (Paris, France)
2011	Alzheimer's Association Scholarship for Advocacy Forum (Washington D.C.)
2013	Society for Neuroscience Award for SfN Capitol Hill Day (Washington D.C.)
2013 - present	Alzheimer's of Greater Los Angeles (formerly Alzheimer's Association) member
2014	Civitan Travel Award for 2014 Glial Symposium (Birmingham, AL)

C. Contribution to Science

BBB dysfunction in Alzheimer's disease: Vascular insults kick start cellular and molecular events leading to neurodegeneration, cognitive impairment, and dementia. AD genetic risk factors, impaired clearance of Aβ, as well as other vascular risk factors such as environment and lifestyle (e.g. air pollution), lead to cerebral blood flow dysregulation and disruption of the neurovascular unit and the blood-brain barrier, contributing to the onset and progression of dementia and AD.

1. **Nelson, AR**, Sweeney, MD, Sagare, AP, Zlokovic, BV. 2016. Neurovascular dysfunction and neurodegeneration in dementia and Alzheimer's disease. *Biochimica et Biophysica Acta-Molecular Basis of Disease*. 1862(5):887-900. PMID: 26705676
2. Zhao, ZZ, **Nelson, AR**, Betsholtz, C, Zlokovic, BV. 2015. Establishment and dysfunction of the blood-brain barrier. *Cell*. 163(5):1064–1078. PMCID: PMC4655822.
3. Ramanathan A, **Nelson AR**, Sagare AP and Zlokovic BV. 2015. Impaired vascular-mediated clearance of brain amyloid beta in Alzheimer's disease: The role, regulation and restoration of LRP1. *Front. Aging Neurosci.* **7**:136. PMCID: PMC4502358.
4. Winkler EA, Nishida Y, Sagare AP, Rege SV, Bell RD, Perlmutter D, Sengillo JD, Hillman S, Kong P, **Nelson AR**, Sullivan JS, Zhao Z, Meiselman HJ, Wenby RB, Soto J, Abel ED, Makshanoff J, Zuniga E, De Vivo DC, Zlokovic BV. 2015. Glut1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. *Nature Neuroscience*. 18(4):521-530. PMID: 25730668

Pericyte physiology: Pericytes are mural cells along capillaries. There has been a 100 year plus debate regarding whether or not pericytes are contractile cells. We investigated the functional role of pericytes in transgenic mice with reduced number of pericytes and found that they regulate blood flow, tissue oxygenation and metabolism.

1. Kisler, K*, **Nelson, AR***, Rege, S*, Ramanathan, A, Boas, DA, Sakadzic, S, Zlokovic, BV. 2017. Pericyte degeneration leads to neurovascular uncoupling and limits oxygen supply to brain. Nature Neuroscience. 20(3):406-416. PMCID:PMC5323291
2. Kisler, K*, **Nelson, AR***, Montagne, A*, Zlokovic, BV. 2017. Glial and mural cell regulation of cerebral blood flow. *Nature Reviews Neuroscience.* In Press

Beneficial effects of estrogen on hippocampal function: The benefits of estrogen replacement therapy (ERT) on cognition is controversial. However, previous studies have varied the time after menopause at which ERT is initiated. In rats, we found that estrogen replacement restored hippocampal plasticity up to 15 months post-ovariectomy. This beneficial effect was no longer observed 19 months post-ovariectomy. Our studies suggested that there is a critical period during which ERT is beneficial for cognition.

1. Smith CC, Vedder LC, **Nelson AR**, Bredemann TM, and McMahon LL. 2010. Duration of estrogen deprivation, not chronological age, prevents estrogen's ability to enhance hippocampal synaptic physiology. PNAS. 107(45):19543-8. PMCID:PMC2984203

Cholinergic denervation and sympathetic sprouting in Alzheimer's disease: Cholinergic degeneration occurs in AD, and can be mimicked in rats by lesioning cholinergic neurons in medial septum. Hippocampal cholinergic denervation disrupts retrograde transport of nerve growth factor (NGF), leading to its accumulation, which subsequently triggers sprouting of noradrenergic sympathetic fibers from the superior cervical ganglia into hippocampus. Coincident with this sprouting, there is an increase in cholinergic innervation that correlates with a recovery of M1 muscarinic receptor dependent plasticity at CA3-CA1 synapses and visual cortex. M1 mAChRs have been a recent focus as a therapeutic target for AD given their role in cognition and non-amyloidogenic processing of amyloid beta-protein precursor (AβPP). Therefore, we tested the hypothesis that noradrenergic sympathetic sprouting and the associated increase in cholinergic innervation maintains non-amyloidogenic AβPP processing that is dependent upon M1 mAChRs. We found that NGF stimulates sprouting and that sprouting maintains non-amyloidogenic AβPP processing. Furthermore, we showed that $A\beta_{42}$ is not only toxic to central cholinergic fibers innervating hippocampus, but it prevents and reverses noradrenergic sympathetic sprouting and the accompanying cholinergic reinnervation.

1. **Nelson AR**, Kolasa K, McMahon LL. 2014. Noradrenergic sympathetic sprouting and cholinergic reinnervation maintains non-amyloidogenic processing of AβPP. Journal of Alzheimer's Disease. 38(4):867-79. PMCID:PMC4047988

Cancer gene therapy: My earliest work utilized conditionally replicative adenovirus as a cancer gene therapy modality for breast and pancreatic cancer. We developed adenovirus with EGFP core labeling for virus visualization and tracking for *in vivo* imaging. Also, we performed *in vitro* and *in vivo* experiments and found a synergistic effect between an adenovirus developed by the lab that was in early phase clinical trials and commonly used chemotherapeutic agents.

1. **Nelson AR**, Davydova J, Curiel DT, Yamamoto M. 2009. Combination of conditionally replicative adenovirus and standard chemotherapies shows synergistic antitumor effect in pancreatic cancer. Cancer Science. 100(11):2181-7. PMID:19689475

2. Le LP, Le HN, **Nelson AR**, Matthews DA, Yamamoto M, Curiel DT. 2006. Core Labeling of Adenovirus with EGFP. Virology. 351(2):291-302. PMCID:PMC1781517

Complete List of Published Work in MyBibliography:
http://goo.gl/46b1Pb

D. Research Support

Active Research Support

006002-00001 Zlokovic (PI) 10/01/15-09/30/16
Cure Alzheimer's Fund
PICALM gene therapy and drug screening for Abeta Clearance
To explore therapeutic strategies to increase PICALM endothelial expression and measure Aβ clearance and brain accumulation utilizing gene therapy and FDA approved drugs.
Role: Postdoctoral Scholar

2R01AG023084 Zlokovic (PI) 06/15/15-03/31/20
NIH/NIA (Renewal)
Cerebrovascular β-Amyloidosis: Aβ CNS Transport Pathways
The role of PICALM in Aβ blood-brain barrier clearance and Alzheimer's neurodegeneration.
Role: Postdoctoral Scholar

1P01AG052350-01 (Zlokovic/Toga) 09/30/16-05/31/21
NIH/NIA
Vascular contributions to dementia and genetic risk factors for Alzheimer's disease
Program project to study imaging and molecular biomarkers of neurovascular dysfunction in individuals at genetic risk for AD both familial and sporadic.
Role: Postdoctoral Scholar

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME:Andrew John Petkus, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): ANDREWPETKUS24

POSITION TITLE: Assistant Professor of Clinical Neurology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Wisconsin-Oshkosh (Oshkosh, WI)	B.S.	01/2006	Psychology
University of South Florida (Tampa, FL)	M.A.	05/2008	Gerontology
San Diego State University/University of California, San Diego Joint Doctoral Program in Clinical Psychology (San Diego, CA)	Ph.D.	08/2014	Clinical Psychology
University of Southern California (Los Angeles, CA)	Postdoctoral	03/2017	Psychology

A. Personal Statement

The Project-1 Dr. Chen (Project PI) proposed is part of the P01 Program which has a long-term goal to better characterize the individual risk, heterogeneity, and biological basis of Alzheimer's disease (AD) associated with exposure to ambient air pollution in aging populations. Specifically, this project is a longitudinal study designed to investigate the contribution of traffic-related air pollution (TRAP) to the risk, susceptibility and mechanisms of AD and related dementias (ADRD) in a nationwide cohort of post-menopausal and older women from the Women's Health Initiative (WHI) Memory Studies (WHIMS). In this application, we will examine the neurotoxic effects on postmenopausal women enrolled in the WHIMS of Younger Women (WHIMS-Y), focusing our collective effort to investigate the long-term TRAP exposure effects on the new measures of structural brain MRI harmonized by USC investigators using the Neuroimaging Core using Alzheimer's Disease Neuroimaging Initiative (ADNI) biomarkers data.

I am an Assistant Professor in the Department of Neurology at USC. My research interests include utilizing longitudinal data analysis to examine risk factors for cognitive decline and emotional distress in older adulthood. My work has utilized complex data analytic methods including: bivariate dual change score modeling, multilevel latent growth models, longitudinal biometric simplex modeling, gene by environment bivariate biometric twin modeling, survival analysis, and factor analysis. In addition to my work in longitudinal data analysis I have expertise in cognitive assessment and interpreting neuropsychological test data. I will work closely with Dr. Chen and the Project Data Core Director to develop the data analysis plan as related to: (1) applying the latent structural modeling to characterize the longitudinal profiles of cognitive domains pertinent to episodic memory and executive functions; (2) determining how these domain profiles are affected by TRAP exposures or associated with ADNI-derived structural brain MRI biomarkers; (3) constructing the structural equation models to evaluate the proposed mechanistic mediations for their contribution to the TRAP neurotoxic effects. I will also contribute to the publications and presentations over the course of the project.

Relevant work related to this project include:

1. **Petkus, A. J.**, Reynolds, C. A., Wetherell, J. L., Kremen, W. S., Pedersen, N., & Gatz, M. (2016). Anxiety is associated with increased risk of dementia in older Swedish Twins. *Alzheimer's & Dementia, 12*, 399-406, doi:10.1016/j.jalz.2015.09.008.
2. **Petkus, A. J.,** Reynolds, C. A., Wetherell, J. L., Kremen, W. S., & Gatz, M. (2017). Temporal dynamics of anxiety and cognitive performance across older adulthood. *Psychology and Aging,* Advanced online publication, doi: 10.1037/pag0000164.
3. **Petkus, A. J.**, Beam, C., Johnson, W., Kaprio, J., Korhonen, T., McGue, M., Neiderhiser, J.M., Pedersen, N., Reynolds, C., & Gatz, M. for the IGEMS Consortium. (2017). Gene-Environment interplay in depressive symptoms: Moderation by age, sex, and physical illness. *Psychological Medicine,* Advanced online publication, doi:10.1017/S0033291717000290.
4. **Petkus, A. J.,** Reynolds, C. A., Wetherell, J. L., Kremen, W.S., & Gatz, M. (2015). Stability and change in the genetic and environmental contributions to anxiety symptoms in older adulthood. *Behavior Genetics, 46*, 492-505, doi: 10.1007/s10519-015-9772-0.
5. **Petkus, A. J.**, Wetherell, J. L., Stein, M. B., Liu, L., & Barrett-Connor, E. (2012). History of sexual assault is associated with earlier declines in executive functioning in older adults with APOE-4. *Journal of Gerontology Series B: Psychological Sciences, 67,* 653-659, doi: 10.1093/geronb/gbr163.

B. Positions and Honors

Positions and Employment

2013-2014 Psychology Intern, APA-approved Clinical Psychology Internship, VA Long Beach Healthcare System

2014-2017 Postdoctoral Research Associate; Departments of Psychology and Neurology; University of Southern California

Honors:

2008 Masters in Gerontology Comprehensive Examination- Pass with Distinction, University of South Florida

2010 Pre-Dissertation Research Award, Gerontological Society of America Behavioral and Social Sciences Section

2017 Research Career Institute in the Mental Health of Aging, Fellowship, National Institute of Mental Health, Weill Cornell Medicine

C. Contributions to Science

1. My postdoctoral research and predoctoral dissertation have focused on elucidating the complex association between anxiety, depression and cognitive decline. Anxiety is associated with worse cognitive performance in older adulthood; however, research had not examined the temporal dynamics between anxiety and cognitive performance. Before my work it was unknown if higher anxiety was contributing to worse cognitive performance or if worse cognitive performance was associated with subsequently higher anxiety. The extent to which genetic factors shared between anxiety and cognitive performance was contributing to this association was unclear. My work in this area found that cognitive performance was a leading indicator of change in anxiety while anxiety was not associated with changes in cognitive performance. I also found that higher anxiety was associated with increased risk of dementia over the 28-year follow-up period. Co-twin control analyses found that genetic factors common to anxiety were partially mediating this association.

a) **Petkus, A. J.**, Reynolds, C. A., Wetherell, J. L., Kremen, W. S., Pedersen, N., & Gatz, M. (2016). Anxiety is associated with increased risk of dementia in older Swedish Twins. *Alzheimer's & Dementia, 12*, 399-406, doi:10.1016/j.jalz.2015.09.008.

b) **Petkus, A. J.,** Reynolds, C. A., Wetherell, J. L., Kremen, W. S., & Gatz, M. (2017). Temporal dynamics of anxiety and cognitive performance across older adulthood. *Psychology and Aging,* Advance online publication, doi: 10.1037/pag0000164.

c) **Petkus, A. J.**, Gum, A. M., & Wetherell, J. L. (2013). Anxiety is associated with cognitive impairment in homebound older adults. *International Journal of Geriatric Psychiatry, 28,* 989-990.

2. I have served as a project coordinator or therapist on two NIH funded clinical trials examining psychosocial and pharmacological interventions for late life Generalized Anxiety Disorder. My duties on these projects included project recruitment, preparing IRB submissions, clinical assessment, managing data and providing health education. Many clinical trials for anxiety disorders have excluded participants over the age of 60. Because of this, the effects of common psychosocial treatments for anxiety were unclear. This work with Dr. Wetherell found that augmenting pharmacological treatment with Cognitive-Behavioral Psychotherapy prevented relapse of GAD over the subsequent year of follow-up. I was part of another collaboration with Dr. Wetherell documenting that interventions, both psychosocial and pharmacological, are less effective for older adults compared to younger adults. This led to the development of a review paper describing the rationale for testing new intervention methods for later life anxiety such as using mindfulness based Acceptance and Commitment (ACT) therapy.

a) **Petkus, A. J.**, & Wetherell, J. L. (2013). Acceptance and Commitment Therapy with older adults: Rationale and considerations. *Cognitive and Behavioral Practice*, *20*, 47-56.

b) Wetherell, J. L., **Petkus, A. J.**, Stein, M. B., Craske, M. G., Chavira, D., Liu, L., & Roy-Byrne, P. (2013). Age differences in treatment response to a collaborative care intervention for anxiety disorders in primary care. *British Journal of Psychiatry, 203*, 65-72.

c) Wetherell, J. L., **Petkus, A. J.**, White, K. S., Nguyen, H., Kornblith, S., Andreescu, C., Zisook, S., & Lenze, E. J. (2013). Antidepressant medication augmented with cognitive behavioral therapy for Generalized Anxiety Disorder in older adults. *American Journal of Psychiatry, 170,* 782-790.

d) Wetherell, J. L., Afari, N., Ayers, C. R., Stoddard, J. A., Ruberg, J., Sorrell, J. T., Liu, L., **Petkus, A. J.**, Thorp, S. R., Kraft, A., & Patterson, T. L. (2011). Acceptance and Commitment Therapy for Generalized Anxiety Disorder in older adults: A preliminary report. *Behavior Therapy, 42*, 127-134.

3. My contributions to science also include elucidating the long-term impact of early life trauma exposure on later life health, emotional, and cognitive functioning. The long-term impact of trauma in chronically ill, physically impaired homebound older adults was unknown. The long-term impact of trauma early in life on cognitive functioning was also unknown, especially how genetic factors may moderate this association. My work found that homebound older adults who reported a history of trauma exposure were in worse physical and mental health than homebound older adults without this history. In the second examination we found that experiencing multiple sexual assaults was associated with greater declines in executive functioning over time, and the APOE e4 allele moderated this association. My work as a postdoctoral fellow, currently in press in the *Journal of Clinical Psychiatry*, has examined the extent to which childhood trauma is associated with cognitive performance in older adults with anxiety and depressive disorders. We found that childhood trauma was associated with slower processing speed and worse attention and executive functioning in two independent samples of anxious and depressed older adults.

a) **Petkus, A. J.**, Gum, A. M., King-Kallimanis, B., & Wetherell, J. L. (2009). Prior trauma history is associated with psychological distress and somatic symptoms in homebound older adults. *American Journal of Geriatric Psychiatry, 17,* 810-818.

b) **Petkus, A. J.**, Wetherell, J. L., Stein, M. B., Liu, L., & Barrett-Connor, E. (2012). History of sexual assault is associated with earlier declines in executive functioning in older adults with APOE-4. *Journal of Gerontology Series B: Psychological Sciences, 67,* 653-659.

c) **Petkus, A. J.**, Lenze, E., Butters, M., Twamley, & Wetherell, J.L. (in press). Childhood trauma is associated with poorer cognitive performance in older adults. *Journal of Clinical Psychiatry.*

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/1naBkvPy5gpkh/bibliography/42131699/public/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

RF1AG054068 Chen (PI) 09/01/2016 – 06/30/2021
"*Alzheimer's Disease & Related Dementias: Geography, Environments & Mechanisms*"
Role: Co-Investigator
The long-term goal of this project is to better understand the geographic disparities in Alzheimer's disease and related dementias (ADRD) by studying the neuropsychological trajectories and clinical progression to increased ADRD risk as related to geographic indicators, identifying the contributing environmental factors and examining their interactions, and elucidating the possible mechanistic mediators.

National Parkinson's Foundation Research Grant
Petzinger (PI) 08/01/2014-07/31/2018
"Exercise Targeting Cognitive Impairment in Parkinson's Disease
The goal of this study is to examine the association between two types of physical fitness: aerobic and skilled motor fitness, and cognitive performance in Parkinson's patients. The project consists of an intervention study where participants are randomized to receive aerobic exercise, fine motor exercise, or social contact for 12 weeks. The second study is a cross-sectional study to examine the associations between fitness and cognitive performance. In both studies structural and functional neuroimaging is done to examine physiological mediators of this association.

Completed Research Support

F31 AG042218 (Petkus, PI) 04/01/13-07/31/14
Anxiety and Cognitive Performance in Swedish Twins: Genetic and Environmental Influences

The goal of this study is to elucidate the longitudinal association between symptoms of anxiety and cognitive performance across older adulthood. Longitudinal data from the Swedish Adoption Twin Study of Aging was analyzed to examine the extent to which genetic and environmental factors contributed to the stability of anxiety symptoms across older adulthood, the temporal dynamics of the association between anxiety and cognitive performance, and the extent to which anxiety symptoms in midlife were associated with risk of dementia over a 28-year follow-up period.
Role: Principal Investigator

R01 AG037985 (Pedersen, PI) 08/01/14 - 05/31/16

Gene-Environment Interplay of Social Contexts and Aging-Related Outcomes

The purpose is to establish a collaboration among seven longitudinal twin and family studies to lay the foundation for studies of gene-environment interplay through harmonization of the data sets and to use the database to test hypotheses about the impact of early life experiences and adversity and mid and late-life social context on individual differences in late-life functioning.
Role: Postdoctoral Research Associate

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Christian J. Pike

eRA COMMONS USER NAME: cjpike

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Southern California	B.S.	1985	Biological Sciences
University of California, Irvine	Ph.D.	1994	Biological Sciences
University of California, Irvine	Postdoctoral	1995	Neuroscience

A. Personal Statement

I will serve as a Co-Investigator on this P01 application that will evaluate the relationships between *APOE* gentotype, sex and vulnerability to neural injury caused by air pollution. My training and experience in the neurobiology and neuroendocrinology of aging and age-related neurodegenerative diseases are well suited to the proposed application. As outlined in Section C below, my doctoral studies focused on structure-activity relationships of the AD-related protein β-amyloid with an emphasis on its neurodegenerative mechanisms. In my postdoctoral work, I extended my AD interests to include study of factors that regulate disease risk, focusing initially on the sex steroid hormone estrogen and its actions in reducing β-amyloid toxicity and AD-related neurodegeneration. Subsequent research directions broadened this general theme of sex steroids, aging, and AD with the inclusion of ongoing projects with two other sex steroid hormones, progesterone and testosterone. Emerging areas of emphasis in my lab include (i) elucidation of the interactions between obesity, apoE, and AD and, (ii) identification of AD-related therapeutics in preclinical models. I have collaborated with Dr. Caleb Finch for several years. Recently, our laboratories have been jointly pursuing studies on the neural effects of apoE4. Our shared interests in sex differences and apoE are highly relevant to the proposed studies.

Recent publications from my lab that are especially relevant to this grant application include the following:

1. Barron AM, Rosario ER, Eltereifi R, and **Pike CJ** (2013) Sex-specific effects of high-fat diet on indices of metabolic syndrome in 3xTg-AD mice: Implications for Alzheimer's disease. *PLoS ONE* **8** (10): e78554. PMCID: PMC3810257
2. Moser VA and **Pike CJ** (2016) Obesity and sex interact in the regulation of Alzheimer's disease. *Neuroscience & Biobehavioral Reviews.* 67:102-118. PMCID: PMC4912955
3. **Pike CJ** (2017) Sex and the development of Alzheimer's disease. *Journal of Neuroscience Research*. 95:671-680. PMCID: PMC5120614
4. Christensen A and **Pike CJ** (2017) Age-dependent regulation of obesity and Alzheimer-related outcomes by hormone therapy in female 3xTg-AD mice. *PLoS One*. In press.
5. Moser VA and **Pike CJ** (2017) Obesity accelerates Alzheimer-related pathology in *APOE4* but not *APOE3* mice. *eNeuro*. In press.

B. Positions and Honors

Positions

1995-1999 Asst. Research Professor, Institute of Brain Aging & Dementia, University of California, Irvine

1999-2005 Assistant Professor, Davis School of Gerontology, University of Southern California
2005-2012 Associate Professor, Davis School of Gerontology, University of Southern California
2012-present Professor, Davis School of Gerontology, University of Southern California

Professional Experience and Honors

1991-1993 NIMH Predoctoral Training Grant
1996-1998 NIH Peer Review, Neurological Sciences 1 (ad hoc member)
1997 NIH Peer Review, Neurological Sciences 3 (ad hoc member)
1998-2000 Fellow, John Douglas French Alzheimer's Foundation
1998-present Peer Review, Alzheimer's Association
1998-2009 Editorial Board, *Journal of Neurochemistry*
1999 NIH Peer Review, Molecular, Cellular, and Developmental Neuroscience-2 (ad hoc member)
2000-2001 NIH Peer Review, Molecular, Cellular, and Developmental Neuroscience-1 (ad hoc member)
2000 Turken Award, Alzheimer's Association
2000-2005 Hanson Family Assistant Professor of Gerontology
2009-current Editorial Board, *Frontiers in Aging Neuroscience*
2010-current National Scientific Advisory Council, American Federation for Aging Research
2012-current Editorial Board, *Frontiers in Cellular Neuroscience*
2015 NIH/CSR Peer Review, Aging Systems and Geriatrics (AGS) (ad hoc member)
2015-16 NIH/CSR Peer Review, Brain Disorders and Clinical Neuroscience (BDCN) (ad hoc member)

C. Contributions to Science

1. β-Amyloid aggregation and toxicity. A critical step that jumpstarted modern AD research was the mid 1980's identification of a novel protein called β-amyloid as the primary component of senile plaques. When I began my graduate work at UC Irvine in 1989 with mentor Carl Cotman and collaborator Charlie Glabe, very little was known about β-amyloid and its role in AD. In studying the effects of β-amyloid on neurons, I made a seminal discovery: the biological activities of β-amyloid depend upon its physical conformation. My studies demonstrated that the inherent ability of β-amyloid to change from a soluble, monomeric peptide into β-sheet aggregates conferred the peptide with a pathological property: neurotoxicity. Additional work described the process of apoptosis as a key component in the mechanism of β-amyloid toxicity. These findings helped to explain how β-amyloid, a normal protein that is benign in its soluble state, can drive neurodegenerative changes when it accumulates in aggregated forms in the aging brain.

a. **Pike CJ**, Walencewicz AJ, Glabe CG and Cotman CW (1991) In vitro aging of β-amyloid protein causes peptide aggregation and neurotoxicity. *Brain Research*, **563**: 311-314.

b. **Pike CJ**, Burdick D, Walencewicz AJ, Glabe CG and Cotman CW (1993) Neurodegeneration induced by β-amyloid peptides *in vitro*: the role of peptide assembly state. *Journal of Neuroscience*, **13**: 1676-1687.

c. **Pike CJ**, Walencewicz-Wasserman AJ, Kosmoski J, Cribbs DH, Glabe CG and Cotman CW (1995) Structure-activity analyses of β-amyloid peptides: contributions of the β25-35 region to aggregation and neurotoxicity. *Journal of Neurochemistry*, **64**: 253-265.

d. Yao M, Nguyen T-V, and **Pike CJ** (2005) β-Amyloid-induced neuronal apoptosis involves c-Jun N-terminal kinase-dependent downregulation of Bcl-w. *Journal of Neuroscience* **25**: 1149-1158.

2. Testosterone and Alzheimer's disease. Although the most significant risk factor for developing AD is advancing age, there remains a large void in our understanding of which components of normal aging promote AD pathogenesis. One significant consequence of normal aging in men is a gradual depletion of testosterone, which is linked with increased risk of several diseases including sarcopenia and osteoporosis. My research program defined key aspects in the role of age-related testosterone loss in AD, an area largely unexplored when I began my investigations. We demonstrated for the first time that the male human brain is particularly vulnerable to loss of androgens with increasing age and helped establish that testosterone loss is an AD risk factor in men. Further, my lab identified and mechanistically defined three distinct neural actions of testosterone linked to its protective role against AD: (1) reduction of β-amyloid accumulation by a mechanism that involves upregulation of the β-amyloid degrading enzyme neprilysin, (2) neuroprotection mediated by androgen receptor activation of MAPK signaling, and (3) inhibition of tau phosphorylation by inactivating the kinase GSK3β via a signaling pathway dependent upon androgen receptor activation of Akt and PI3 kinase.

a. Rosario ER, Chang L. Stanczyk FZ, and **Pike CJ** (2004) Age-related testosterone depletion and the development of Alzheimer's disease. *Journal of the American Medical Association (JAMA)* **292**: 1431-1432.

b. Nguyen TV, Yao M, and **Pike CJ** (2005) Androgens activate mitogen activated protein kinase signaling: role in neuroprotection. *Journal of Neurochemistry* **94**:1639-1651.

c. Rosario ER, Carroll JC, Oddo S, LaFerla FM, and **Pike CJ** (2006) Androgens regulate development of neuropathology in a triple transgenic mouse model of Alzheimer's disease. *Journal of Neuroscience* **26**: 13384-13389. ['Recommended' by *Faculty of 1000 Biology*]

d. Rosario ER, Chang L, Head EH, Stanczyk FZ, and **Pike CJ** (2011) Brain levels of sex steroid hormones in men and women during normal aging and in Alzheimer's disease. *Neurobiology of Aging* **32** (4): 604-613. PMCID: PMC2930132

3. Estrogen, Progesterone and Alzheimer's disease. Estrogen loss at menopause was established as a risk factor for AD in women prior to my work in the field, but many questions were unanswered. Research by my lab determined that women with AD have low brain levels of estrogen. Notably, we found that depletion of estrogen accelerates pathology in animal models of AD, an effect prevented by estrogen treatment. Because hormone therapy in women usually includes both estrogens and progestins, we investigated the interactions between these hormones in regulation of AD-related pathology. A significant discovery by my group is that that the beneficial effects of estrogen are strongly regulated by progesterone. Protective estrogen actions are attenuated when progesterone is co-delivered in a continuous manner, but increased when progesterone is co-delivered in a more natural, cyclic pattern. One important action of estrogen associated with protection against AD and other age-related neurodegenerative diseases is protection from neuronal death. My research group has made important advances in the field's understanding of estrogen neuroprotection by defining key mechanisms.

a. **Pike CJ** (1999) Estrogen modulates neuronal Bcl-x_L expression and β-amyloid-induced apoptosis: relevance to Alzheimer's disease. *Journal of Neurochemistry*, **72**: 1552-1563.

b. Carroll JC, Rosario ER, Chan L, Stanczyk FZ, Oddo S, LaFerla FM, and **Pike CJ** (2007) Progesterone and estrogen regulate Alzheimer-like neuropathology in female 3xTg-AD mice. *Journal of Neuroscience* **27**:13357-13365.

c. Yao M, Nguyen TV, and **Pike CJ** (2007) Estrogen prevents β-amyloid peptide induced neuronal death by regulating Bcl-w and Bim expression. *Journal of Neuroscience*, **27**: 1422-1433.

d. Carroll JC, Rosario ER, Villamagna A, and **Pike CJ** (2010) Continuous and cyclic progesterone differentially interact with estradiol in the regulation of Alzheimer-like pathology in female 3xTg-AD mice. *Endocrinology* 151 (6): 2713-2722. PMCID: PMC2875823 ['Must Read' by *Faculty of 1000 Biology*]

e. Yin F, Yao J, Sancheti H, Feng T, Melcangi RC, Morgan TE, Finch CE, **Pike CJ**, Mack WJ, Cadenas E, and Brinton RD (2015) Perimenopause is a bioenergetic transition of female brain characterized by deficits in glucose metabolism and synaptic function. *Neurobiology of Aging* **36** (7): 2282-2295. PMCID: PMC4416218

4. Obesity and Alzheimer's disease. Obesity during middle-age is a significant risk factor for the development of Alzheimer's disease in old age. Emerging research efforts in my lab aim to understand how adiposity and type 2 diabetes promote Alzheimer's neuropathology. Areas of particular focus include interactions with age-related losses in testosterone and estrogen, modulation by the genetic risk factor *APOE4*, and translational approaches to mitigate the effects of obesity by controlling inflammation.

a. Barron AM, Rosario ER, Eltereifi R, and **Pike CJ** (2013) Sex-specific effects of high-fat diet on indices of metabolic syndrome in 3xTg-AD mice: Implications for Alzheimer's disease. *PLoS ONE* **8** (10): e78554. PMCID: PMC3810257

b. Jayaraman A, Lent D and **Pike CJ** (2014) Diet-induced obesity and low testosterone increase neuroinflammation and reduce neuron survival. *Journal of Neuroinflammation* **11**(1): 162. PMCID: PMC4190446

c. Barron AM, Garcia-Segura LM, Caruso D, Jayaraman A, Lee JW, Melcangi RC, and **Pike CJ** (2013) Ligand for translocator protein reverses pathology in a mouse model of Alzheimer's disease. *Journal of Neuroscience* **33** (20): 8891-8897. PMCID: PMC3733563

d. Christensen A and **Pike CJ** (2015) Menopause, obesity and inflammation: Interactive risk factors for Alzheimer's disease. *Frontiers in Aging Neuroscience.* **7**: 130. Doi 10.3389/fnagi.2015.00130 PMCID: PMC4493396

e. Moser VA and **Pike CJ** (2016) Obesity and sex interact in the regulation of Alzheimer's disease. *Neuroscience & Biobehavioral Reviews.* 67:102-118. PMCID: PMC4912955

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/christian.pike.1/bibliography/40353252/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 AG034103, CJ Pike (PI) 09/01/11-08/31/17
NIH/NIA
Interactions between Testosterone and Type 2 Diabetes in Alzheimer's Disease Pathogenesis
This project investigates the relationships between androgens, obesity, and type 2 diabetes and their roles as interactive risk factors in the development of Alzheimer's pathology.
Role: PI

P01 AG26572, R Brinton (PI) 06/01/06-05/31/21
NIH/NIA
Perimenopause in Brain Aging and Alzheimer's Disease
This Program Project investigates relationships between the perimenopause transition and the development of at-risk phenotypes for Alzheimer's disease with a mechanistic emphasis on bioenergetics, neuroinflammation, and obesity/metabolic syndrome.
Role: Project Leader (Project 2)

R01 AG051521, C Finch (PI)
NIH/NIA 09/01/15 – 08/31/20
Amyloid and Inflammation: Modulation by ApoE, Gender, Air Pollution, and Drugs
This project investigates novel inflammation-gene-environment (IGE) interactions during brain aging and Alzheimer disease (AD) for apoE alleles and gender.
Role: Co-Investigator

SAGA-17-419408, CJ Pike (PI) 11/01/16-10/31/19
Alzheimer's Association
Interactions between Sex, APOE4, and Neuroinflammation in AD Pathogenesis
This project evaluates relationships how sex and *APOE* genotype affect neuroinflammation and the development of Alzheimer-related neuropathology in experimental models of the disease.
Role: PI

Completed Research Support (Last 3 Years)

P50 AG005142, H Chui (PI)
NIH/NIA
Alzheimer's Disease Research Center 04/01/10 – 03/31/15
This project will assess the ability of select compounds to function as neuroprotective mimetics for estradiol and testosterone in experimental models of Alzheimer's disease.
Role: Co-Project Leader (Project 2: *Novel NeurosERMs and NeurosARMs for Protection Against Alzheimer Pathology*)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Elizabeth A. (Lianne) Sheppard

POSITION TITLE: Professor and Assistant Chair

eRA COMMONS USER NAME (credential, e.g., agency login): LSHEPPARD

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Johns Hopkins University, Baltimore, MD	B.A.	06/1979	Psychology
Johns Hopkins University, Baltimore, MD	Sc.M.	01/1985	Biostatistics
University of Washington, Seattle, WA	Ph.D.	06/1992	Biostatistics

A. Personal Statement

My research interests focus on statistical methods for understanding the health effects of environmental and occupational exposures; they include study design, measurement error, exposure modeling and estimation, and estimation of environmental exposure effects with application to a wide range of health outcomes. The current proposal, which is focused on air pollution exposure and risk of Alzheimer's disease and dementia, is strongly related to several projects in my past and current portfolio. I am co-PI of the study Air Pollution, the Aging Brain and Alzheimer's Disease. Early in my career I worked on Alzheimer's disease research reflected in 3 citations below. As a biostatistician jointly appointed in two departments, I actively collaborate with many different principal investigators on multiple projects in the environmental and occupational health sciences. I have over 145 peer-reviewed publications; most pertain to statistics in the environmental and occupational health sciences. These include biostatistical methods papers (see Section C), numerous publications from the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air) study (including a May 2016 paper in *The Lancet* showing the relationship between air pollution exposure and increased progression of subclinical cardiovascular disease), and a publication in the *New England Journal of Medicine* estimating the risk in post-menopausal women of long-term air pollution exposure on cardiovascular disease incidence from the Women's Health Initiative cohort. In addition, I have extensive training and mentoring experience. In my role as Assistant Chair I actively support the development of our junior faculty. I direct a training grant to advance quantitative training in the environmental health sciences, BEBTEH (Biostatistics, Epidemiologic and Bioinformatic Training in Environmental Health) and an undergraduate research experience program to enhance diversity in the environmental health sciences, SURE-EH (Supporting Undergraduate Research Experience in Environmental Health). I am a Fellow of the American Statistical Association, a member of the HEI Review Committee, and a member of the Editorial Board of Epidemiology. I am one of seven statutory Clean Air Scientific Advisory Committee (CASAC) members, I have served on various CASAC Special Panels, I have served on two of EPA's Scientific Advisory Board Panels: Ethylene Oxide, and Toxicological Review of Libby Amphibole Asbestos, and I am currently serving on the EPA FIFRA Scientific Advisory Panel evaluating the carcinogenic potential of glyphosate.

1. Tsuang D, Kukull W, **Sheppard L**, Barnhart R, Peskind E, Edland S, Schellenberg G, Larsen E, Raskin M: Impact of sample selection on APOE E4 allele frequency: A comparison of two Alzheimer's disease samples. *J Am Geriatrics Soc*, 1996, 44:704-707. PMID: 8642164
2. Bowen J, Malter A, **Sheppard L**, Kukull W, McCormick W, Teri L, Larson E: Predictors of mortality in patients diagnosed with dementia of the Alzheimer's type. Neurology, 1996, 47:433-439. PMID: 8757016
3. O'Meara ES, Kukull WA, **Sheppard L**, Bowen JD, McCormick WC, Teri L, Pfanschmidt, Thompson JD, Schellenberg GD, Larson EB: Head injury and risk of Alzheimer's disease by apolipoprotein E genotype. American Journal of Epidemiology 1997, 146:373-384. PMID: 9290497
4. Adar SD, D'Souza J, Mendelsohn-Victor K, Jacobs DR, Cushman M, Thorne PS, **Sheppard L**, Thorne PS, Burke GL, Daviglus ML, Szpiro AA, Diez-Roux AV, Kaufman JD, Larson TV. Markers of inflammation and

coagulation after long-term exposure to coarse particulate matter: A cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis. *EHP*, 2015,123(6): 541–548. PMCID: PMC4455582

Positions and Honors

Positions and Employment

1993-2001 Research Assistant Professor, Department of Biostatistics & Department of Environmental and Occupational Health Sciences (DEOHS), University of Washington
2001-2006 Research Associate Professor, Department of Biostatistics & DEOHS, University of Washington,
2005-8,10,11 Member, EPA Clean Air Scientific Advisory Committee Special Review Panel for Ozone
2006-2009 Research Professor, Department of Biostatistics & DEOHS, University of Washington
2007-2009 Member, EPA Clean Air Scientific Advisory Committee Special Review Panels for NOx and SOx
2009-present Professor, Departments of Biostatistics & EOHS, University of Washington
2009-present Member, Health Effects Institute Review Committee
2011-2013 Member, EPA SAB Panel for Toxicological Review for Libby Amphibole Asbestos
2013-present Member, EPA Clean Air Scientific Advisory Committee Special Review Panel for NOx
2014-present Member, EPA Clean Air Scientific Advisory Committee Special Review Panel for SOx
2014-2015 Member, EPA SAB Chemical Assessment Advisory Committee for Ethylene Oxide
2014-present Assistant Chair for Research and Faculty Engagement, Dept. EOHS, University of Washington
2015-2017 Member, Statutory EPA Clean Air Scientific Advisory Committee

Other Experience and Professional Memberships

1995 Member, HEI Review Committee for RFA 94-2: Particulate Air Pollution and Daily Mortality
1996 Member EPA Review Panel for Intra-individual Variation in Human Susceptibility to Cancer
2001 Member, NIOSH Safety and Occupational Health Study Section
2006-2009 Consultant to the HEI Review Committee for the Public Health and Air Pollution in Asia (PAPA)
2007-2009 Member, NRC Committee on Contaminated Drinking Water at Camp Lejeune
2009 NIEHS Special Emphasis Panel for the Children's Env Health & Disease Prevention Centers
2009 Consultant, EPA Human Studies Review Board
2009-2012 Member: External Advisory Committee of the USC Children's Health Study
2010-present Member: Epidemiology Editorial Board
2010 External Reviewer: Emory University Graduate School's Proposed Doctoral Program in Environmental Health Sciences
2014-present Member, External Advisory Committee for the University of California, Berkeley-Stanford Children's Environmental Health Center

Honors

1991 Outstanding Student Award, University of Washington School of Public Health
2000 Nominee: Distinguished Graduate Mentor Award (UW)
2006 Elected Fellow, American Statistical Association
2009-2010 Genentech Endowed Professor of Biostatistics, University of Washington
2010 Distinguished Faculty Lecture, UW School of Public Health

B. Contributions to Science

Statistical Methods

1. Early in my career I collaborated with Ross Prentice to develop statistical methods for aggregate data studies. These studies are a substantial improvement upon ecological studies focused on the association between an exposure such as dietary intake and a health outcome such as breast cancer. They give better estimates of the health effect parameter targeted in individual-level studies while using a group-level study design and analysis coupled with some limited sampling of exposure data from individuals.

a. Prentice RL, **Sheppard L**: Aggregate data studies of disease risk factors. Biometrika 82:113-125, 1995.
b. **Sheppard L**, Prentice RL: On the reliability and precision of within and between population estimates of relative rate parameters. *Biometrics* 51:853-863, 1995. PMID: 7548704
c. **Sheppard L**, Prentice RL, Rossing MA: Design considerations for estimation of exposure effects on disease risk using aggregate data studies. *Stat in Med* 15:1849-1858, 1996. PMID: 8888477

d. **Sheppard L**: Insights on information and bias in group-level studies. Biostatistics 4:265-278, 2003. PMID: 12925521

2. Together with Adam Szpiro and other colleagues we have made important contributions on developing measurement error correction methods for inference about health effects for application to air pollution cohort studies. This work has fundamentally changed the perspective on measurement error. In these studies pollution is modeled using a statistical model. The measurement error induced has classical-like and Berkson-like components. These advances are critical to appropriate inference about air pollution health effects.

a. Szpiro AA, **Sheppard L**, Lumley T. Efficient measurement error correction with spatially misaligned data. *Biostatistics*, 2011, 12:610-23. PMCID: PMC3169665
b. **Sheppard L**, Burnett RT, Szpiro AA, Kim S-Y, Jerrett M, Pope CA III, Brunekreef B. Confounding and exposure measurement error in air pollution epidemiology, *Air Quality, Atmosphere & Health*, 2011, Jun;5(2):203-216. PMCID: PMC3353104
c. Szpiro AA, Paciorek C, **Sheppard L**. Does more accurate exposure prediction necessarily improve health effect estimates? *Epidemiology*, 2011, 22:680-685. PMCID: PMC3195520
d. Bergen S, **Sheppard L**, Sampson PD, Young-Kim S, Richards M, Vedal S, Kaufman JD, Szpiro AA. A national prediction model for components of PM2.5 and measurement error corrected health effect inference. *Environmental Health Perspectives*, 2013 Sep;121(9):1017-25. PMCID: PMC3764074

3. I have a strong interest in quantitative exposure modeling for application to epidemiological studies. This body of work spans both occupational and environmental epidemiology applications to include noise and air pollution exposures. Recently our team has significantly advanced spatial and spatio-temporal modeling methods for air pollution exposures.

a. Seixas N, **Sheppard L**: Maximizing accuracy and precision using individual and grouped exposure assessments. Scand J Work and Env 22:94-101, 1996. PMID: 8738886
b. Szpiro AS, Sampson PD, **Sheppard L**, Lumley T, Adar SD, Kaufman J. Predicting intra-urban variation in air pollution with complex spatio-temporal dependencies. *Environmetrics*, 2009, 21: 606–631. PMCID: PMC4029437
c. Lindström J, Szpiro AA, Sampson PD, Oron A, Richards M, Larson T, **Sheppard L**. A flexible spatio-temporal model for air pollution with spatial and spatio-temporal covariates. *Environmental and Ecological Statistics*, 2014, 21:411–433. PMCID: PMC4174563
d. Keller JP, Olives C, Kim SY, **Sheppard L**, Sampson PD, Szpiro AA, Oron A, Vedal S, Kaufman JD. A unified spatiotemporal modeling approach for predicting concentrations of multiple air pollutants in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. *Environmental Health Perspectives*, 2015, Apr;123(4):301-9. PMCID: PMC4384200

4. We have solved the challenge of referent selection and proper analysis of the case-crossover study design for air pollution epidemiology. This is reflected in the accompanying editorial to our 2005 paper (Janes et al 2005b) which claimed “the issue of how to sample referent periods in case-crossover studies of air pollution is clearly answered and a standard approach is available that can easily be implemented using standard software tools. Now it is time for the field to move on to minimizing other, potentially much larger sources of bias in studies of the short-term effects of air pollution.”

a. Levy D, Lumley T, **Sheppard L**, Kaufman J, Checkoway H: Referent selection in case-crossover analyses of health effects of air pollution. *Epidemiology* 12:186-192, 2001. PMID: 11246579
b. Janes H, **Sheppard L**, Lumley T. Overlap bias in the case-crossover design, with application to air pollution exposures. *Statistics in Medicine*, 2005a, 24:285-300. PMID: 15546133
c. Janes H, **Sheppard L**, Lumley T. Case-crossover analyses of air pollution exposure data: Referent selection strategies and their implications for bias. *Epidemiology*, 2005b, 16:717-26.

Applications

5. I have made major contributions to studies of air pollution, noise exposure, manganese exposure, and other environmental and occupational exposures. Selected contributions include:

a. Miller KA, Siscovick DS, **Sheppard L**, Shepherd K, Sullivan JH, Anderson G, Kaufman JD. Long-term exposure to fine particulate matter air pollution and cardiovascular events in women, *New England Journal of Medicine*, 356:447-458, 2007. PMID: 17267905
b. Seixas NS, Neitzel RL, Stover B, **Sheppard L**, Feeney P, Mills D, Kujawa SG. 10-year prospective study of noise exposure and hearing damage among construction workers. *Occupational and Environmental Medicine*, 2012, 69:643-50. PMCID: PMC4570847
c. Adar SD, D’Souza J, **Sheppard L**, Kaufman JD, Hallstrand TS, Davey ME, Sullivan JR, Jahnke J, Koenig J, Larson TV, Liu LJS. Adopting clean fuels and technologies on school buses: Pollution and

health impacts in children. *Am J Respir Crit Care Med*. 2015 Jun 15;191(12):1413-21. [Epub ahead of print Apr 13]. PMCID: PMC4476560

d. Kaufman JD, Adar SD, Barr RG, Budoff M, Burke GL, Curl CL, Daviglus ML, Diez Roux AV, Gassett AJ, Jacobs, Jr DR, Kronmal R, Larson TV, Navas-Acien A, Sampson PD, **Sheppard L**, Siscovick DS, Stein JH, Szpiro AA, Watson KE. Air Pollution and Acceleration of Coronary Artery Calcification: The Multi-Ethnic Study of Atherosclerosis and Air Pollution. *The Lancet*, 2016, Aug 13;388(10045):696-704. [Epub 2016 May 24] PMCID: PMC5019949

Complete List of Published Work in MyBibliography (of >145 original articles published or accepted)**:**
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/44484767/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

1R01ES026187-01A1 REV Li (Multi-PI), Sheppard (Multi-PI) 09/30/2016-07/31/2021
Air Pollution, the Aging Brain and Alzheimer's Disease
This study provides an opportunity to develop comprehensive insights into the effects of air pollution on the aging brain, including the effects of air pollution on cognition, the risk of Alzheimer's disease, and various potential mechanisms of neurodegenerative disease and cerebrovascular disease. The proposed study seeks to advance fundamental research using state-of-the-art exposure science. A deeper understanding of these environmental exposures will contribute significantly to the identification of disease mechanisms and improvements in public health, particularly because these exposures can be modified by changes in regulations and individual behaviors.
Role: Multi Principal Investigator

1R25ES025503-01 Sheppard (PI) 07/01/2015-06/30/2020
Supporting Undergraduate Research Experience in Environmental Health (SURE-EH)
SURE-EH will provide environmental health science research experience and educational opportunities to at least four traditionally underrepresented undergraduate students per year from across the UW.
Role: Principal Investigator

T32ES015459 Sheppard (PI) 07/01/2009-06/30/2019
Biostatistics, Epidemiologic and Bioinformatic Training in Environmental Health (BEBTEH)
The purpose of this training program is to improve quantitative science expertise in the environmental health sciences (EHS) by producing quantitative science researchers with strong EHS skills and EHS researchers with strong quantitative science skills. The fundamental innovation of this program is its unified structure to bridge EHS with bioinformatics and biostatistics.
Role: Principal Investigator and Director

RD-83479601 Vedal (Center PI), Sheppard (Core PI) 12/01/2010-11/30/2017 (NCE)
UW Center for Clean Air Research
This center examines near-roadway pollution, a multi-pollutant atmosphere, consists of vapor and gas phase components that vary by vehicle emission source, road surface, extent of physical aging and the type and degree of atmospheric processing and photochemical reactions. The immediate aim of the UW CCAR is to disentangle features of this complex mixture to provide insight into those that are especially toxic to the cardiovascular system. The ultimate aim is to identify the specific near-roadway emission sources and interactions that produce the greatest toxicity.
Role: Project PI and Co-Investigator

1R01ES021488-01 Racette (PI) 12/1/2012-11/30/2017
Imaging Biomarkers of Neurotoxicity in Welders
The major goals of this project are to determine the relations between functional neuroimaging results and clinical signs and symptoms of parkinsonism among welders exposed to manganese. The project will also examine dose-response relations for neuroimaging results and neurobehavioral test findings.
Role: UW subcontract PI and Co-Investigator

R01ES025991 Racette (PI) 8/1/2015 – 4/30/2020
Motor and Cognitive Health Outcomes in a Mn-Exposed African Communities

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Sioutas, Constantinos

eRA COMMONS USER NAME (credential, e.g., agency login): SIOUTAS

POSITION TITLE: Fred Champion Professor of Civil and Environmental Engineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Aristotelian University of Thessaloniki, Greece	B.S.	1986	Mechanical Engineering
University of Minnesota	M.S.	1988	Mechanical Engineering
University of Minnesota	M.S.	1989	Aerospace Engineering
Harvard University	Sc.D.	1994	Environmental Engineering

A. Personal Statement

Airborne particulate matter (PM) in urban areas, derived from vehicle emissions and other sources, have well documented adverse effects with direct impact on life expectancy and quality of life. According to WHO, outdoor PM air pollution in both urban and rural areas was estimated to cause 3.7 million premature deaths worldwide per year in 2012; this mortality is due to exposure to small particulate matter of 10 microns or less in diameter (PM10), which causes cardiovascular and respiratory disease as well as cancers. In contrast to multiple health risks from toxic airborne PM for cardiopulmonary conditions, the direct involvement of the nervous system is less appreciated, even though the brain may be as vulnerable to toxic airborne PM as the heart and lungs. There is, therefore, an urgent need to define the links between PM exposure and onset and progression of neurodegenerative diseases, and to identify possible preventive or therapeutic interventions, which is the goal of the proposed research. My earlier work has focused almost exclusively on respiratory and cardiovascular effects of air pollution. In limited studies published recently in collaboration with the group of Drs. Finch, Morgan, Forman and Mack (see list below), we demonstrated the role of urban PM on neuro-inflammation. Our ground-breaking work has been showcased in a feature article at Science in January 2017 (http://www.sciencemag.org/news/2017/01/brain-pollution-evidence-builds-dirty-air-causes-alzheimer-s-dementia). Since inflammatory events have been associated with neurodegenerative processes, it is possible that extended exposure to PM may aggravate events connected to the progression of these disorders. The work described in this proposal, represents natural continuation- expansion of my research in this area, as these novel studies will establish the direct link between urban airborne ultra-fine particles (UFP) and neurodegenerative disease.

1. Zhang H., Liu H, Davies K.J.A., Sioutas C., Finch C.E., Morgan T.E and Forman H.J. “Nrf2-regulated phase II enzymes are induced by chronic ambient nanoparticle exposure in young mice with age-related impairments”. *Free Radical Biology,* 9: 2038-2046, 2012
2. Davis D.A., Bortolato M., Godar S.C., Sander T.K., Iwata N,, Pakbin P., Shih J.C., Berhane K., McConnell R., Sioutas C., Finch C.E. and Morgan T.E “Prenatal Exposure to Urban Air Nanoparticles in Mice Causes Altered Neuronal Differentiation and Long-term Depression-like Responses”. *PLOS One,* 8(5): e64128.doi:10.1371, 2013
3. Cheng H, Davis DA, Hasheminassab S, Sioutas C, Finch CE. Urban traffic-derived nanoparticulate matter reduces neurite outgrowth via TNFα in vitro. J Neuroinflamm. 13(1) doi: 10.1186/s12974-016-0480-3. PMID: 26810976, 2016

4. Woodward NC, Levine MC, Haghani A, Shirmohammadi F, Saffari A, Sioutas C,Morgan TE, and Finch CE. Toll-like receptor 4 in glial inflammatory responses to air pollution in vitro and in vivo. J Neuroinflammation. 14;14(1):84. doi:10.1186/s12974-017-0858-x. PubMed PMID: 28410596, 2017

B. Positions and Employment

1989-1992 Advanced Product Development Engineer, 3M Company
1992-1994 Doctoral Candidate/Aerosol Engineer, Harvard University
1994-1997 Assistant Professor of Aerosol Science, Harvard University
1997-2003 Associate Professor of Civil Engineering, University of Southern California
2004- Fred Champion Professor of Civil and Environmental Engineering, University of Southern California

Other Experience and Professional Memberships

2008- Editor in Chief: Aerosol and Air Quality Research
2009- Editorial Board: Atmospheric Environment

Honors

1981-1985 Fellow of the Greek National Institute of Scholarships
1986-1987 Fulbright Foundation Fellow
1991 3M Circle of Technical Excellence Recipient
2000 Faculty Research Award, University of Southern California
2001- Member of the Air Quality Advisory Committee of the State of California on Particulate Matter
2011 Haagen-Smit Prize winner for best publication in *Atmospheric Environment*
2011 2010 Scientific and Technological Achievement Award, US EPA
2012 Top cited article 2011-2012; *Atmospheric Environment*
2013- Trustee, Aristotle University of Thessaloniki, Greece
2013 USC School of Engineering, Senior Faculty Research Award
2014 David Sinclair Award, American Association for Aerosol Research

C. Contribution to Science

1. My research has followed an integrated approach to the problem of the well-publicized and significant effects of aerosols produced from anthropogenic processes on health and the environment. My group at the University of Southern California, and a significant number of my collaborating colleagues from many institutions in the US and Europe, have worked collectively to investigate the underlying mechanisms that produce the health effects associated with exposure to air pollutants generated by a variety of combustion sources, such as traffic, harbor and airport operations, power plants, and photo-chemically induced atmospheric reactions. We have conducted extensive studies examining the oxidative properties of urban coarse, fine, and ultrafine particles (UFPs). We showed that UFPs were most potent particulates in the urban environment toward inducing cellular heme oxygenase-1 (HO-1) expression and depleting intracellular glutathione. HO-1 expression, a sensitive marker for oxidative stress, is directly correlated with the high organic carbon and polycyclic aromatic hydrocarbon (PAH) content of UFPs. Because the small size of UFPs allows better tissue penetration, we used electron microscopy to study subcellular localization. UFPs and, to a lesser extent, fine particles, localize in mitochondria, where they induce major structural damage. This may contribute to oxidative stress. Our studies demonstrated that the increased biological potency of UFPs is related to the content of redox cycling organic chemicals and their ability to damage mitochondria. Moreover, we showed that mice exposed to UFP exhibited significantly larger early atherosclerotic lesions than mice exposed to $PM_{2.5}$ or filtered air. Exposure to ultrafine particles also resulted in an inhibition of the anti-inflammatory capacity of plasma high-density lipoprotein and greater systemic oxidative stress as evidenced by a significant increase in hepatic malondialdehyde levels and upregulation of Nrf2-regulated antioxidant genes.
    a. Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Froins J. & Nel, A. “Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage”. *Environ Health Perspectives.; 111(4): 455–460, 2003,* PMC1241427
    b. Xia, T., Kovochich, M., Brant, J., Hotze, M., Sempf, J., Oberley, T., Froines JR. Sioutas C and Nel, A. E. “Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular

toxicity according to an oxidative stress paradigm". *Nano Letters*, *6*(8), 1794-1807, 2008, PMC 16895376

c. Künzi L., Manuel Krapf M., Daher N., Dommen J., Jeannet N., Schneider S., Platt S., Slowik J.G., Baumlin N., Salathe M., Prévot A.S.H., Kalberer M., Sioutas C., Baltensperger U. and Geiser M. "Adverse effects of atmospherically aged gasoline exhaust particles on airway epithelia". *Nature, Scientific Reports* 5, doi:10.1038/srep11801, 2015, PMC4484354

d. Li R., Navab K., Hough G., Pakbin P., Mittelstein D., Saffari A., Sulaiman D., Beebe T., Wu L., Wine E., Araujo J., Fogelman A., Sioutas C., Navab M., Hsiai T. "Effect of Exposure to Atmospheric Ultrafine Particles on Production of Free Fatty Acids and Lipid Metabolites in the Mouse Small Intestine". *Environmental Health Perspectives*, 123(1): 34-41, 2015, PMC3566535

2. I have been the principal investigator in the design and development of state-of-the-art particle concentrator technologies that have enabled the assessment of the relative toxicity of particulate pollution sources using for the first time in the literature realistic atmospheres in in vivo and in vitro studies in multimillion-dollar research centers funded by the US EPA, NIH and CARB in Southern California. Several of these technologies are also being used by agencies such as the U.S. EPA, as well as a host of international institutes in Europe and Asia. These technologies have been used in a number of major toxicological investigations funded by EPA, NIH and CARB, exploring the linkages between PM emitted from traffic and cardiovascular, respiratory and neurological health effects.

   a. Kim, S., Jaques, P. A., Chang, M., Froines, J. R., & Sioutas, C. "Versatile aerosol concentration enrichment system (VACES) for simultaneous in vivo and in vitro evaluation of toxic effects of ultrafine, fine and coarse ambient particles Part I: Development and laboratory characterization". *Journal of Aerosol Science*, *32*(11), 1281-1297, 2001

   b. Cho, A. K., Sioutas, C., Miguel, A. H., Kumagai, Y., Schmitz, D. A., Singh, M., Froines, J. R. "Redox activity of airborne particulate matter at different sites in the Los Angeles Basin". *Environmental Research*, *99*(1), 40-47,2005

   c. Morgan T.E, Davis D.A, Iwata N., Tanner J.A, Snyder D., Ning Z., Kam W., Hsu Y.T, Winkler J.W, Chen J.C, Petasis N.A, Baudry M., Sioutas C., and Finch C.E "Glutamatergic Neurons in Rodent Models Respond to Nanoscale Particulate Urban Air Pollutants in Vivo and in Vitro". *Environmental Health Perspectives,* 119 (7): 1003-1009 DOI: 10.1289/ehp.1002973, 2011, PMC3222976

   d. Zhang H., Liu H, Davies K.J.A., Sioutas C., Finch C.E., Morgan T.E and Forman H.J. "Nrf2-regulated phase II enzymes are induced by chronic ambient nanoparticle exposure in young mice with age-related impairments". *Free Radical Biology,* 9: 2038-2046, 2012, PMC3342863

3. I have also led the design and development of innovative and effective personal monitoring devices that were used by epidemiologists in many parts of the world to measure population exposures to air pollutants and determine how this exposure relates to lung growth, exacerbation of early asthma and cardiopulmonary inflammation. These technologies, combined with novel source apportionment techniques, have also been instrumental in major NIH- funded panel studies in demonstrating for the first time stronger associations between systemic inflammation and primary (i.e. directly emitted) than secondary (photo-chemically formed) PM in elderly populations in the LA Basin. On the other hand, the same studies demonstrated stronger association between respiratory inflammation and secondary organic aerosols than primary PM, thereby establishing for the first time a clear distinction of the effects of primary vs secondary emissions in the LA basin. These findings are very important in the promulgation of air quality control strategies, as they underscore for the first time differential health outcomes associated with primary vs secondary PM.

   a. Misra, C., Singh, M., Shen, S., Sioutas, C., & Hall, P. M. "Development and evaluation of a personal cascade impactor sampler (PCIS)." *Journal of Aerosol Science*, *33*(7), 1027-1047, 2002

   b. Delfino, R. J., Sioutas, C., & Malik, S. "Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health". *Environmental Health Perspectives*, 934-946, 2005

   c. Delfino, Ralph J., Norbert Staimer, Thomas Tjoa, Andrea Polidori, Mohammad Arhami, Daniel L. Gillen, Micheal T. Kleinman et al. "Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with primary combustion aerosols in subjects with coronary artery disease." *Environ Health Perspect* 116, no. 7: 898-906, 2008, PMC2453185

   d. Delfino, Ralph J., Thomas Tjoa, Daniel L. Gillen, Norbert Staimer, Andrea Polidori, Mohammad Arhami, Larry Jamner, Constantinos Sioutas, and John Longhurst. "Traffic-related air pollution and

blood pressure in elderly subjects with coronary artery disease." *Epidemiology:* 21(3): 396-404, 2010, PMC3872093

4. My group at USC has conducted in the past 18 years the most extensive physical, chemical and toxicological characterization of PM in the Los Angeles Basin (LAB) to-date. These data were crucial in understanding the physico-chemical evolution of PM in the LA Basin and formed the basis for the design of hypotheses-based toxicological and panel studies using our LA Basin as a real-world laboratory. We have studied the formation and dynamics of traffic- generated air pollutants near freeways and demonstrated the increased (more than 10-fold) exposures to these toxic pollutants near freeways and busy thoroughfares. These studies have produced highly cited publications, (including the Hagen-Smit award of Atmospheric Environment for seminal paper) and have been used as a criterion for placing schools near roadways as part of the state of California Senate Bill 25 (SB25).
    a. Zhu, Y., Hinds, W. C., Kim, S., Shen, S., & Sioutas, C. "Study of ultrafine particles near a major highway with heavy-duty diesel traffic". *Atmospheric Environment*, *36*(27), 4323-4335, 2002. (paper awarded the Hagen-Smit prize)
    b. Saffari, A., Daher, N., Shafer, M. M., Schauer, J. J., & Sioutas, C. "Global Perspective on the Oxidative Potential of Airborne Particulate Matter: A Synthesis of Research Findings". *Environmental Science & Technology*, 2014, PMC24873754
    c. Verma, V., Pakbin, P., Cheung, K. L., Cho, A. K., Schauer, J. J., Shafer, M. M., & Sioutas, C. "Physicochemical and oxidative characteristics of semi-volatile components of quasi-ultrafine particles in an urban atmosphere". *Atmospheric Environment*, *45*(4), 1025-1033, 2011, PMC N/A
    d. Hasheminassab, S., Daher, N., Ostro, B. D., & Sioutas, C. "Long-term source apportionment of ambient fine particulate matter (PM2.5) in the Los Angeles Basin: A focus on emissions reduction from vehicular sources". *Environmental Pollution*, *193*, 54-64, 2014, PMC 25005887

Complete List of Published Work in MyBibliography (*see also ISI Web of Science:* 294 refereed publications, H Index: 64, 15,769 citations): http://www.ncbi.nlm.nih.gov/pubmed/?term=sioutas

D. Additional Information: Research Support

Ongoing Research Support

Award # 1P01ES022845-01
Award # 83544101 Rob McConnell (PI) 07/01/13 – 06/30/18
National Institutes of Health & Environmental Protection Agency
Southern California Children's Environmental Health Center (SC-CEHC)
The objectives of the SCCEHC are to define the role of air pollution in the development and progression of childhood obesity and it associated metabolic and inflammatory consequences, and to translate the emerging science to key groups to facilitate science-based interventions to prevent and reduce the consequences of childhood obesity and metabolic abnormalities. / Role: Key Personnel

Award # 1R01ES024936-01 William Mack (PI) 01/15/15 – 11/30/19
National Institutes of Health
Neurotoxicity of Airborne Particles: Role of Chronic Cerebral Hypoperfusion
The proposed research program seeks to determine the impact of particulate matter (PM) exposure on white matter injury and neurocognitive decline. These associations are further examined in the setting of underlying cerebrovascular disease (chronic cerebral hypoperfusion). / Role: Co-Investigator

Award # 6051-S10 Constantinos Sioutas (PI) 06/22/15 – 06/30/17
Westat, Incorporated
Measurement of Outdoor Ambient Ultrafine Particulates for a Study of Lung Cancer Risk in California
The proposed research is to support the mobile ambient monitoring campaign in a study of ultrafine particles and lung cancer risk among NIH-AARP study participants in southern California. / Role: PI

Award # 6051-S10 Caleb Finch (PI) 09/30/15 – 08/31/20
National Institutes of Health
Amyloid and inflammation: modulation by apoE, gender, air pollution, and drugs

Description/ Role: we examine environmental factors that affect PM neuro-toxicity, including: PM source and location, atmospheric aging and photochemical transformation, and changes in particle size and chemical composition; the collected PM will be used to address the two most critical unanswered questions in this field: (a) what are the PM components most responsible for PM neurodevelopmental and neurodegenerative health effects? and (b) what are the biological mechanisms by which they affect human health? / Role: Co-Investigator

Award # 1R21AG050201-01A1 Caleb Finch (PI) 04/01/16 – 03/31/18
National Institutes of Health
Air Pollution Nano-Particulate Matter, APP Processing, and Glutamate Receptors
Description/ Role: We address key gaps in how urban traffic-derived PM impacts synaptic functions: We will know the strength of covariations in individual mice between memory tasks and cell and molecular parameters (synaptic responses, GluA1 levels, LTP and Abeta). We also propose to characterize the reciprocal links between APP processing and AMPA receptor activation, which have not been previously brought into discussion of air pollution neurotoxicity. / Role: Co-Investigator

Completed Research Support

Award # ES012243 Ralph Delfino (PI) 04/15/11 – 01/31/17
UC Irvine (Prime: NIEHS) - Award 2011-2611
Transcriptomatic, Oxidative Stress, and Inflammatory Response to Air Pollutants
The objective of this proposal is to study the associations of circulating biomarkers of effect with exposure to PM, associations of biomarkers of airway inflammation with exposure to PM, the magnitude of associations of circulating and airway biomarkers of effect with exposure to aerosols and inverse associations of erythrocyte antioxidant enzyme activities with exposure to aerosols, especially ultrafine and diesel exhaust particles. / Role: Co-Investigator

Award # 5R01AI065617-15 Chatila Talal (PI) 09/01/11 – 11/30/16
Children's Hospital Boston (National Institutes of Health)
Genetic and Epigenetic Programming of Allergic Airway Inflammation
The objective of this proposal is collecting and characterizing ambient particulates emitted from mobile sources for proposed inhalation experiments as well as for using our state of the art technologies for creating the exposure atmospheres in these experiments. / Role: Co-Investigator

Award # 25138-1397-01 Arezoo Campbell (PI) 11/01/13 – 04/01/16
Western University of Health Science (AQMD)
Evaluation of Ambient Particulate Matter Neurotoxicity Using Primary Human Brain Cells
To better understand how size and composition modulates neurotoxic potential of particulates, PM samples in three size fractions (coarse, fine, and ultrafine) will be collected from an urban site in Los Angeles. We have shown that exposure to ambient PM activates inflammatory responses in rodent brains. We propose to use a novel multi-cell culture system, consisting of primary human brain cells, to understand the mechanisms underlying PM-induced neurotoxicity. / Role: Co-Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Nicholas L. Smith

POSITION TITLE: Professor of Epidemiology

eRA COMMONS USER NAME (credential, e.g., agency login): nlsmith

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Irvine	BA	06/1985	French Literature
University of California, Irvine	BA	06/1987	Psychology
University of California, Berkeley	MPH	05/1993	Epidemiology and Biostatistics
University of Washington, Seattle	PhD	06/1997	Epidemiology

A. Personal Statement

I am Director of the Seattle VA Epidemiologic Research and Information Center (ERIC) of the VA Cooperative Studies Program (CSP) and hold an academic appointment as Professor of Epidemiology at the University of Washington. The Seattle ERIC is one of 4 CSP epidemiologic sites that serve the VA community by conducting large-scale observational epidemiologic research among Veterans. We have expertise in managing large VA datasets and retrieving, managing, and analyzing information from these resources. We work collaboratively with VA-funded investigators and consult regularly on epidemiologic issues related to study design, conduct, and interpretation. I also serve as Director of the Vietnam Era Twin (VET) Registry, which includes over 14,000 male twins who served during the Vietnam era and selected twin offspring and offspring mothers.

B. Positions and Honors

Positions

1992	Intern, Cardiovascular Health Studies Branch of the National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA
1992-1993	Intern, Human Populations Laboratory of the California State Department of Health Services, Berkeley, CA
1993-1997	Research Assistant, Cardiovascular Health Research Unit, University of Washington, Seattle,
1995-1997	Pre-doctoral Fellow, Health Services Research and Development, Seattle Veterans Affairs Medical Center, Seattle, WA
1997-2000	Investigator, Cardiovascular Health Research Unit, University of Washington, Seattle, WA
1997-2006	Staff Epidemiologist, Seattle Epidemiology and Information Resource Center VA Puget Sound Health Care System, Seattle Division, Seattle, WA
2000-2005	Research Assistant Professor, Department of Epidemiology, University of Washington, Seattle, WA
2005-2010	Associate Professor, Department of Epidemiology, University of Washington, Seattle, WA
2006-	Director, Seattle Epidemiology Research and Information Center (ERIC) and the Vietnam Era Twin (VET) Registry, VA Puget Sound Health Care System, Seattle Division, Seattle, WA
2010-	Affiliate Investigator, Group Health Research Institute, Group Health Cooperative
2010-	Professor, Department of Epidemiology, University of Washington, Seattle, WA

Honors

1984-1987	Dean's Honor List, University of California

1987	Phi Beta Kappa Society
1992	Health Net Wellness Scholarship
1995-1997	Pre-Doctoral Fellowship in Health Services Research and Development, Department of Veterans Health Administration, Veterans Affairs
1997	Epidemiology Outstanding Student Award and Scholarship

C. Contribution to Science

1. Veteran Health
My contribution to a better understanding of the health and well-being of Veterans has been through my role as director of 1 of 4 epidemiologic centers with the VA Office of Research and Development Cooperative Studies Program. As director of the Epidemiologic Research and Information Center, I have had the opportunity to collaborate with investigators with a wide spectrum of interests, all of which focused on improving the health and health care of U.S. Veterans.

a. Goldberg J, Magruder KM, Forsberg CW, Kazis LE, Ustün TB, Friedman MJ, Litz BT, Vaccarino V, Heagerty PJ, Gleason TC, Huang GD, **Smith NL**. The association of PTSD with physical and mental health functioning and disability (VA Cooperative Study #569: the course and consequences of posttraumatic stress disorder in Vietnam-era Veteran twins). *Qual Life Res*. 2013;23(5):1579-91.
b. Walsh TJ, Shores MM, Fox AE, Moore KP, Forsberg CW, Kinsey CE, Heckbert SR, Zeliadt S, Thompson ML, Smith NL, Matsumoto AM. Recent trends in testosterone testing, low testosterone levels, and testosterone treatment among Veterans. *Andrology*. 2015;3(2):287-92. [PMCID pending]
c. Goldberg J, Magruder KM, Forsberg CW, Friedman MJ, Litz BT, Vaccarino V, Heagerty PJ, Gleason TC, Huang GD, **Smith NL.** Prevalence of Post-Traumatic Stress Disorder in Aging Vietnam-Era Veterans: Veterans Administration Cooperative Study 569: Course and Consequences of Post-Traumatic Stress Disorder in Vietnam-Era Veteran Twins. *Am J Geriatr Psychiatry*. 2016;24:181-91.
d. Magruder KM, Goldberg J, Forsberg CW, Friedman MJ, Litz BT, Vaccarino V, Heagerty PJ, Gleason TC, Huang GD, **Smith NL.** Long-Term Trajectories of PTSD in Vietnam-Era Veterans: The Course and Consequences of PTSD in Twins. *J Trauma Stress*. 2016;29:5-16.

2. Comparative Safety of Commonly used Medications
High-quality, observational pharmacoepidemiologic research can provide important insights into the unanticipated adverse health effects of established and newly marketed medications. Of particular interest to me and my colleagues have been hormones and cardiovascular medications. Observational epidemiology can also further our understanding of within-class effects, both beneficial and harmful. I had led several NIH-funded studies investigating comparative-safety of commonly used hormone therapy in peri and postmenopausal women. Our research, which has taken advantage of formulary changes at Group Health Cooperative to conduct a quasi-experimental design, demonstrated that oral conjugated equine estrogens (marked at Premarin and Prempro) are less safe than other oral hormones, including estradiol and esterified estrogen. Relative to estradiol, conjugated equine estrogens are associated with a marked increased risk of venous thrombosis. Using blood from controls in our case-control study, we demonstrated that women using conjugated equine estrogens had a higher propensity to clot than those using the other oral estrogens.

a. Shores MM, **Smith NL**, Forsberg CW, Anawalt BD, Matsumoto AM. Testosterone treatment and mortality in men with low testosterone levels. *J Clin Endocrinol Metab* 2012;97:2050-8.
b. **Smith NL**, Blondon M, Wiggins KL, Harrington LB, van Hylckama Vlieg A, Floyd JS, Hwang M, Bis JC, McKnight B, Rice KM, Lumley T, Rosendaal FR, Heckbert SR, Psaty BM. Lower risk of cardiovascular events in postmenopausal women taking oral estradiol compared with oral conjugated equine estrogens. *JAMA Intern Med.* 2014;174:25-31. [NIHMSD: 720007; PMCID pending]
c. Floyd JS, Blondon M, Moore KP, Boyko EJ, **Smith NL**. Validation of methods for assessing cardiovascular disease using electronic health data in a cohort of Veterans with diabetes. *Pharmacoepidemiol Drug Saf*. Pending print.

d. **Smith NL**, Harrington LB, Blondon M, Wiggins KL, Floyd JS, Sitlani CM, McKnight B, Larson EB, Rosendaal FR, Heckbert SR, Psaty BM. The Association of Statin Therapy with the Risk of Recurrent Venous Thrombosis. *J Thromb Heamost*. 2016.

3. Genetic Risk Factors for Cardiovascular Endo and Clinical Phenotypes
I have contributed to a better understanding of the biology underlying common cardiovascular conditions by conducting statistically-informed research into the genetic predictors of cardiovascular clinical phenotypes and their endophenotypes. Part of my contributions has come from my leadership in 3 settings where I co-convene international working groups addressing the genetics of venous thrombosis, hemostasis, and heart failure. Our work has been cutting edge and has provided the larger biologic research community with validated results. As a recent example, we conducted the largest meta-analysis of a genome-wide investigation of venous thrombosis and identified 9 genome-wide significant hits: 6 were previously validated, 2 replicated in 3 different populations, and 1 did not replicate. The 2 genes newly associated with venous thrombosis have never been suspected, have no known role in coagulation or fibrinolysis yet account for 25% (2 of 8) of the validated findings.

a. Germain M, Chasman DI, de Haan H, […], Trégouët DA, **Smith NL**, Morange PE. Meta-analysis of 65,734 Individuals Identifies TSPAN15 and SLC44A2 as Two Susceptibility Loci for Venous Thromboembolism. *Am J Hum Genet*. 2015; 96(4):532-42. [PMC4385184]
b. Huffman JE, de Vries PS, Morrison AC, […],Reiner AP, O'Donnell CJ, **Smith NL**. Rare and low-frequency variants and their association with plasma levels of fibrinogen, FVII, FVIII, and vWF. *Circulation* 2015. [Epub ahead of print] [PMCID pending with journal]
c. Baumert J, Huang J, McKnight B, […], Strachan DP, Peters A, **Smith NL**. No evidence for genome-wide interactions on plasma fibrinogen by smoking, alcohol consumption and body mass index: results from meta-analyses of 80,607 subjects. *PLoS One*. 2014;9:e111156. [PMCID pending]
d. **Smith NL**, Heit JA, Tang W, Teichert M, Chasman DI, Morange PE. Genetic variation in *F3* (tissue factor) and the risk of incident venous thrombosis: meta-analysis of 8 studies. *J Thromb Haemost* 2012;10:719-22. (PMC3397243)

D. Research Support

Ongoing Support

5R01 HL103612 (Psaty: PI) 8/10/11 – 5/31/16
National Heart, Lung, and Blood Institute, NIH
Prospective Meta-Analyses of Drug-Gene Interactions: CHARGE GWAS Consortium
For common variants, the project will accelerate the discovery of drug-gene interactions that may affect a variety of unintended therapeutic effects.
Role: Co-Investigator

R21 HL121414 (Smith: PI) 7/15/14 - 4/30/16
National Heart, Lung, and Blood Institute, NIH
The Association of Vasomotor Symptoms with Thrombosis in Postmenopausal Women
In the setting of the Women's Health Initiative Hormone Therapy (WHI-HT) trials data, we examine the association of vasomotor symptoms in postmenopausal women with intermediate thrombotic phenotypes and venous thrombosis disease endpoints.

VA 97-010 (Smith: Director) 8/1/06 – 09/30/16
Cooperative Studies Program, Department of Veteran Affairs
Epidemiologic Resource and Information Center (ERIC)
The ERIC serves as a resource center supporting VA research and as a VA Cooperative Study coordinating center for observational studies in veteran populations. The ERIC is also the administrative home for the Vietnam Era Twin (VET) Registry.

Recently Completed Support

2R01 HL073410 (Smith: PI) 8/27/09 – 5/31/15
National Heart, Lung, and Blood Institute, NIH
Estrogens and pharmacogenetic risks of venous thrombosis in postmenopausal women
This study is an extension of our population-based, case-control study in post-menopausal women to examine pharmaco-genetic risks of venous thrombosis associated with oral estradiol and other estrogens.

5R01 HL095080 (Smith: PI) 9/26/08 – 7/31/15
National Heart, Lung, and Blood Institute, NIH
Pharmacologic and Pharmacogenetic Associations with Recurrent Venous Thrombosis
The primary aim of this study is to address a series of pharmacoepidemiologic hypotheses related to recurrent venous thrombosis in an adult population. Using a population-based inception cohort of 2,100 incident venous thrombosis patients on whom we will have complete baseline and longitudinal follow-up of clinical characteristics and pharmaceutical treatments, we test hypotheses related to β-blocker therapy and statin therapy use.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Thompson, Paul M.

eRA COMMONS USER NAME (credential, e.g., agency login): THOMPSONP2

POSITION TITLE: Professor of Neurology, Psychiatry, Engineering, Radiology, Pediatrics and Ophthalmology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Oxford University, England	B.A.	06/1991	Greek/Latin Languages
Oxford University, England	B.A. / M.A.	06/1993	Mathematics
University of California, Los Angeles	Ph.D.	06/1998	Neuroscience

A. Personal Statement

I direct the 35-member USC Imaging Genetics Center and am PI and Co-founder of the ENIGMA Consortium, which conducts the largest brain imaging studies in the world (e.g., Hibar et al., *Nature*, 2015; *Nature Communications*, 2017). I direct the ENIGMA Center for Worldwide Medicine, Imaging and Genomics, an NIH Center of Excellence that brings together over 800 scientists to study 18 major brain diseases, from schizophrenia, bipolar illness and depression to HIV, and most recently, stroke recovery. ENIGMA's work has been covered in *Science, Nature*, and *MIT Technology Review* and honored (Kent Innovations in Academia First Prize, 2015) as an innovative approach for "crowd-sourcing" science. ENIGMA's work led to the discovery of common genetic variants that affect the brain in over 30,000 people scanned with MRI and genome-wide scans. I was trained as a mathematician and later received a Ph.D. in neuroscience. In 2013, my team and I moved to the University of Southern California, to create the USC Imaging Genetics Center (http://igc.ini.usc.edu) and the USC Stevens Institute for Neuroimaging and Informatics, a new facility in a dedicated 5-floor building, with the staff and expertise to coordinate and assist hundreds of worldwide neuroimaging collaborations. I am a Professor of Neurology, Psychiatry, Engineering, Radiology, Pediatrics, and Ophthalmology at USC. Our Center's research focuses on the neuroscience, mathematics, software engineering and clinical aspects of neuroimaging and brain mapping. My group of 35 trainees develops new methods to analyze brain images. We track how diseases spread in the brain over time, often before symptoms begin, and how medications resist them. We specialize in multi-site neuroimaging efforts on an unprecedented scale, such as the ENIGMA Consortium, whose 30 working groups analyze data from over 35 countries (Thompson + 287 authors, 2013; Hibar et al., Nature, 2015). I serve as PI for numerous NIH grants on large-scale brain MRI and DTI studies, and have organized Summer Schools for over 200 students on Mathematics in Brain Imaging (2004, 2008). Over 20 researchers visit our Center annually to learn brain image analysis. In my 23+ years in neuroimaging, I published over 1,879 publications, including over 800 peer-reviewed journal papers, now cited over 80,000 times (h-index=132). I am on the board of editors for 7 journals in engineering and neuroscience. Our Center's mathematical and computational tools are used in over 100 national and international collaborations, with research teams in drug companies and other universities. Recent work, reported in *Nature, Nature Genetics, New England Journal of Medicine, Nature Neuroscience, and PNAS*, has mapped dynamic (4D) processes in brain development and in clinical populations, and factors that influence them. I manage many large-scale image analysis studies. All our analysis methods are available to all interested investigators, and have already been used to initiate collaborations with many investigators interested in medical imaging.

B. Positions and Honors

Positions and Employment

1993 - 1998 Fulbright Scholar, U.S. - U.K. Fulbright Commission, London, England
1993 - 1998 Fellow, Howard Hughes Medical Institute
1993 - 1998 Ph.D. in Neuroscience, Laboratory of Neuro Imaging, UCLA School of Medicine
1998 - 2007 Assistant then Associate Professor of Neurology, UCLA School of Medicine
2007 - 2013 Professor of Neurology & Psychiatry, UCLA School of Medicine
2013 - present Adjunct Professor of Neurology & Psychiatry, UCLA School of Medicine
2013 - present Professor of Neurology, Psychiatry, Engineering, Radiology, Pediatrics & Ophthalmology, USC
2013 - present Associate Director, USC Institute for Neuroimaging & Informatics
2013 - present Director, USC Imaging Genetics Center
2014 - present Director and PI, NIH Center of Excellence for "Big Data to Knowledge" (BD2K): ENIGMA Center for Worldwide Medicine, Imaging & Genomics

Other Experience and Professional Memberships

- Associate Editor or Editorial Board Member for: IEEE Transactions on Medical Imaging (until 2010), Human Brain Mapping, Medical Image Analysis, Cerebral Cortex, Current Medical Imaging Reviews, Inverse Problems and Imaging, Translational Neuroscience
- Study Sections and Technical Evaluation Group, National Library of Medicine; Small Business Innovation Research (SBIR/STTR) Grants Program; Alzheimer's Disease Association Grants Programs; Study Sections and Site Visitor, National Institute for Child Health and Development (NICHD), NLM, NCRR, NSF, NIMH; PhD Committee member for **>80** Graduate Students and chair for over 30 (between 1999-2017)
- Program Committee member for: Mathematical Methods in Biomedical Imaging; SPIE, IEEE International Symposium on Biomedical Imaging; European Conference on Computer Vision, MICCAI

Honors

1989-1991 Oxford University Scholar in Classical Languages; 1991-1993 Oxford University Scholar in Mathematics; 1997 SPIE Medical Imaging Award, Best Paper; 1998 Di Chiro Outstanding Scientific Paper Award; Eiduson Award for Neuroscience Research; 1998 Outstanding Graduate Student of 1998, UCLA; Chancellor's Service Award; 2003 Turken Prize for Alzheimer's Disease Research, Turken Endowment; 2008 Wiley Young Investigator Award, Organization for Human Brain Mapping; 2014, 2015, 2016 Thomson-Reuters "Highly Cited Researcher" in Neuroscience; 2015 Kent International Innovations in Academia Award, First Prize; 350th most highly cited researcher worldwide: http://www.webometrics.info/en/node/58

C. Contribution to Science

For 23 years I have developed new neuroimaging methods and approaches to study brain development, aging, neurological disease and psychiatric disorders. This effort led to **over 700 publications with over 1,000 co-authors. I now lead a worldwide alliance of scientists, called the ENIGMA Consortium, which brings together 800 scientists from 35 countries to study 18 major brain diseases.** Thomson Reuters lists me as a Top Cited Researcher in Science (h-index=132; 700+ publications).

1. **ENIGMA Consortium and Imaging Genetics**. I co-founded and lead the ENIGMA Consortium (http://enigma.ini.usc.edu) – a global alliance of 800 scientists - with all their vast biomedical data and expertise - discovering factors that help or harm the brain. ENIGMA published the largest neuroimaging studies to date of **Major Depressive Disorder** (Schmaal 2016a,b; N=10,105), **Schizophrenia** (van Erp 2016; N=4,568), **Bipolar Disorder** (Hibar 2016; N=4,304), **Obsessive Compulsive Disorder** (Boedhoe 2017; N=3,589), and **Attention-Deficit/Hyperactivity Disorder** (Hoogman 2017; N=3,242) combining MRI data from >20,000 people. Papers on epilepsy, addictions, HIV, and neurogenetic disorders (22q deletion syndrome) are in preparation (Whelan 2017; Mackey 2017; Fouche 2017; Villalon 2017). **We study 18 major brain diseases in over 30 countries worldwide – schizophrenia, major depression, bipolar illness, ADHD, anorexia, anxiety, HIV/AIDS, addictions, autism and 22q deletion syndrome, OCD and epilepsy. ENIGMA performs the largest-ever genomic screens of brain measures, involving GWAS in >31,000 people with brain MRI scans and clinical data.** A dynamically evolving community - attracting new members, projects, and opportunities - ENIGMA's sites work 24/7 on computational projects in harmonizing, integrating, and relating distributed biodata worldwide. Our algorithms analyze brain maps, measures and signals, and relate them to genomic, environmental and epidemiological data, and clinical outcomes. With worldwide alliances tackling major brain diseases, and different imaging techniques, ENIGMA's 30 Working Groups study medication effects, clinical symptoms, and brain connectivity. A network

of concurrent subprojects share information dynamically. Together, we discovered 8 genetic loci that affect brain volumes in over 30,000 people (Hibar +287 authors, *Nature*, Jan. 2015; Adams et al., *Nature Neurosci*., 2016; Hibar et al., *Nature Communications*, 2017; Stein +207 authors, *Nature Genetics*, 2012). *The Lancet* noted this effort as "Crowd sourcing meets neuroscience". The *New York Times* noted that ENIGMA "gives us a source of power we have not had". I am the PI for the NIH-funded ENIGMA Center of Excellence ($11M U54 grant), and I am senior author on large-scale neuroimaging projects with labs and consortia worldwide.

a. Stein JL, …(205 authors)… & **Thompson PM** 2012 Identification of common variants associated with human hippocampal and intracranial volumes. Nature Genetics, 44(5):552-61. PMCID: PMC3635491
b. Hibar D, …(285 authors)… & **Thompson PM** 2015 Common genetic variants influence human subcortical brain structures. Nature, 520(7546):224-9. PMCID: PMC4393366
c. Medland SE, Jahanshad N, Neale BM & **Thompson PM** 2014 Whole genome analyses of whole brain data: working in an expanded search space. Nature Neuroscience, 17(6):791-800. PMCID: PMC4300949
d. **Thompson PM**, …(+287 ENIGMA Consortium authors)… & ENIGMA Consortium 2013 The ENIGMA consortium: large-scale collaborative analyses of neuroimaging and genetic data. Brain Imaging & Behavior - Special Issue on Imaging Genetics, 8(2):153-82. PMCID: PMC4008818

2. **Alzheimer's Disease, HIV/AIDS, and Methamphetamine Use**. I and my team developed the first method to **track the dynamic progression of Alzheimer's disease in the living brain** (Thompson et al., J. Neuroscience, 2003), creating dynamic time-lapse movies of disease progression. I developed mathematical innovations in a method called tensor-based morphometry (Thompson et al., Nature, 2000) to measure growth and tissue loss in the living brain, and profiles of tissue loss in the dementias. We published over 100 papers discovering factors that affect brain aging –from exercise and diet, obesity (Ho et al., PNAS 2010), blood markers of pathology, genetics, and drug treatments. In the New England Journal of Medicine, we reported evidence that brain tissue loss is faster in carriers of a TREM2 mutation, leading to an approach to boost power in drug trials. I was first author on a paper mapping methamphetamine effects on the brain, picked by *Discover Magazine* as one of the "Top 100 Discoveries in Science" (2004).
   a. **Thompson PM**, Hayashi KM, de Zubicaray G, Janke AL, Rose SE, Semple J, Herman D, Hong MS, Dittmer SS, Doddrell DM & Toga AW 2003 Dynamics of Gray Matter Loss in Alzheimer's Disease. Journal of Neuroscience, 23(3):994-1005.
   b. **Thompson PM**, Hayashi KM, Simon SL, Geaga JA, Hong MS, Sui Y, Lee JY, Toga AW, Ling W & London ED 2004 Structural Abnormalities in the Brains of Human Subjects who use Methamphetamine. Journal of Neuroscience, 24(26):6028-36.
   c. **Thompson PM**, Dutton RA, Hayashi KM, Toga AW, Lopez OL, Aizenstein HJ & Becker JT 2005 Thinning of the cerebral cortex in HIV/AIDS reflects CD4+ T-lymphocyte decline. Proc Natl Acad Sci USA, 102(43):15647-52. PMCID: PMC1266080
   d. Rajagopalan P, Hibar DP & **Thompson PM** 2013 TREM2 Alzheimer risk gene carriers lose brain tissue faster, letter to the editor. New England Journal of Medicine, 369(16):1568-9. PMCID: PMC4024453
3. **Mapping Childhood Development**. In *Nature* (2000), I was first author on a paper reporting a **new method to track brain growth rates in children**, using continuum mechanics and covariant partial differential equations from physics. We applied this with collaborators worldwide to study autism, fetal alcohol syndrome, and neurogenetic disorders (Fragile X, Williams syndrome). My most highly cited paper (2704 citations) tracked the sequence of development of the human cerebral cortex from age 4 to 21 (Gogtay et al., 2001). We used this same method to suggest how some antipsychotics might slow brain changes in schizophrenia.
   a. **Thompson PM**, Giedd JN, Woods RP, MacDonald D, Evans AC & Toga AW 2000 Growth Patterns in the Developing Brain Detected By Using Continuum-Mechanical Tensor Maps. Nature, 404(6774):190-193.
   b. **Thompson PM**, Vidal C, Giedd JN, Gochman P, Blumenthal J, Nicolson R, Toga AW & Rapoport JL 2001 Mapping Adolescent Brain Change Reveals Dynamic Wave of Accelerated Gray Matter Loss in Very Early-Onset Schizophrenia. Proc. Nat. Acad. Sciences USA, 98(20):11650-5. PMCID: PMC58784
   c. Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, Nugent TF 3rd, Herman DH, Clasen LS, Toga AW, Rapoport JL & **Thompson PM** 2004 Dynamic Mapping of Human Cortical Development During Childhood and Adolescence. PNAS, 101(21):8174-9. PMCID: PMC419576
   d. **Thompson PM**, Cannon TD, Narr KL, van Erp T, Poutanen VP, Huttunen M, Lönnqvist J, Standertskjöld-Nordenstam CG, Kaprio J, Khaledy M, Dail R, Zoumalan CI & Toga AW 2001 Genetic Influences on Brain Structure. Nature Neuroscience, 4(12):1253-8.
4. **Brain Connectomics**. I have been PI for NIH grants developing methods for high-angular resolution diffusion imaging, and diffusion imaging at ultra-high magnetic fields. With Dr Neda Jahanshad in my group

(Jahanshad et al. PNAS), we developed a method to screen the genome and all the detected connections in the brain's anatomical network to discover specific genetic variants that affect brain connectivity:

a. Jahanshad N, Rajagopalan P, Hua X, Hibar DP, Nir TM, Toga AW, Jack CR Jr, Saykin AJ, Green RC, Weiner MW, Medland SE, Montgomery GW, Hansell NK, McMahon KL, de Zubicaray GI, Martin NG, Wright MJ & **Thompson PM**; Alzheimer's Disease Neuroimaging Initiative 2013 Connectome-wide genome-wide search discovers SPON1 gene variant influencing dementia severity. PNAS, 110(12):4768-73. PMCID: PMC3606977

5. **Mentoring and Training**. I mentored over 70 PhD students, 20 postdocs, and several K-awardees who went on to rewarding positions in academia and private industry; many are our long-term collaborators. I am K award primary mentor for Emily Dennis (on brain trauma) and Sook Lei Liew (on stroke recovery). Our Center hosts over 20 invited lectures per year; our ENIGMA international exchange program hosts ~30 visitors annually to learn neuroimaging methods, who visit and bring new ideas. **I give >30 invited lectures a year** on new directions in imaging and genetics; some are **online at our ENIGMA site: http://enigma.ini.usc.edu**

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/pubmed?term=Thompson%2C%20Paul%20M%5BAuthor%5D

D. Research Support

Ongoing Research Support

U54 EB020403 (Thompson) 09/29/14-09/30/18
NIH
ENIGMA Center for Worldwide Medicine, Imaging & Genomics
The ENIGMA Center for Worldwide Medicine, Imaging and Genomics is an unprecedented global effort uniting 307 scientists from 185 institutions and all their vast biomedical data, to work on 10 major human brain diseases: schizophrenia, bipolar disorder, major depression, ADHD, OCD, autism, 22q deletion syndrome, HIV/AIDS, epilepsy and addictions. ENIGMA integrates images from multiple modalities, genomes, connectomes and biomarkers on an unimaginable scale. New computations integrate, cluster, and learn from complex biodata types.
Role: PI

RF1 AG041915 Thompson (PI) 09/15/14-08/31/19
NIH
Growth factors, neuroinflammation, exercise, and brain integrity
We will evaluate in three large populations how growth factors, inflammation, and exercise work together to protect or harm the human brain - identifying promising directions for treatment, prevention, and a mechanistic understanding of diseases and disorders such as Alzheimer's disease, schizophrenia, and drug addiction.
Role: PI

R01AG040060 Thompson (PI) 09/01/11-05/31/17
NIH/NIA
Alzheimer's disease risk analyzed using population imaging genomics
We will use MRI measurements of this connectivity as an AD risk proxy to better characterize how known AD risk genes affect the brain, helping researchers to improve treatment focus. We will also use this proxy to identify new possible AD risk genes, allowing researchers to assess more homogeneous samples of people.
Role: PI

R01 NS080655 Thompson/Navia (MPIs) 08/01/12-07/31/17 (NCTE)
NIH
Predicting Brain Changes in HIV/AIDS
Building on our recent discoveries, this project greatly advances our ability to map, and predict, brain changes in people living with HIV/AIDS. HIV/AIDS is perhaps the greatest threat to public health worldwide in the 21st century. 40 million people are HIV-infected – a shocking 1 out of every 100 people aged 18-45 - and 40% have some neurological or cognitive impairment.
Role: Contact PI

University of Michigan Thompson (Subaward PI) 06/01/16-05/31/20
Prime: NIH RF1AG051710 Ye/Thompson/Wang (MPIs)
Multi-Source Sparse Learning to Identify MCI and Predict Decline
The goal of this subcontract is to evaluate and analyze the genetic effects on brain structure in over 800 people with Alzheimer's disease, mild cognitive impairment, and normal elderly people.
Role: Subaward PI

University of Texas Thompson (Subaward PI) 09/12/12-06/30/17
Prime: NIH R01MH085667 Hatch/Soares/Thompson (MPIs)
Searching for Endophenotypes of Bipolar Disorder
This study will examine the role of heritability on key brain abnormalities involved in causation of BD. If our hypotheses are confirmed, this will indicate that abnormalities in fronto-limbic brain regions in patients with BD are heritable and could be utilized as “endophenotypes” to guide future research on the specific genes involved.
Role: Subaward PI

U54EB020406 Toga (PI) 09/29/14-09/30/18
NIH
Big Data for Discovery Science
The overarching goal of our BDDS Center is to ease the management and organization of biomedical big data and accelerate data-driven discovery by eliminating or reducing three distinct barriers to effective discovery science: complexity with respect to physical distribution and heterogeneity, scalability of analysis, and ease of access and interaction with big-data and associated analytic methods.
Role: Investigator

P41EB015922 Toga (PI) 08/01/12-07/31/17
NIH/NCRR
Laboratory of Neuro Imaging Resource (LONIR)
We develop, validate and disseminate powerful and user-friendly tools and biomedical analysis protocols for studies of various neurological disorders. All LONIR data, analysis protocols, computational resources and research findings are openly shared online, enhancing research efforts of a wide community.
Role: Investigator

P50-AG05142-31 Chui (PI) 04/01/15-03/31/20
NIH/NIA
Alzheimer Disease Research Center (ADRC)
To promote interdisciplinary research in Alzheimer disease, by providing human subjects, brain tissue, education, statistical support, funding for pilot and R01 projects. The USC ADRC has three unique overarching goals: 1) elucidate vascular contributions to Alzheimer’s disease (AD); 2) catalyze local research in AD at USC (especially Phase I/Phase II clinical trials); and 3) contribute expertise in vascular disease and imaging to national collaborative initiatives.
Role: Investigator

P01AG052350 (Zlokovic/Toga) 09/01/16-08/31/21
NIH/NIA
Vascular contributions to dementia and genetic risk factors for Alzheimer’s disease
Program project to study imaging and molecular biomarkers of neurovascular dysfunction in individuals at genetic risk for AD both familial and sporadic.
Role: Co-Investigator

Completed Research Support

UCSF Thompson (Subaward PI) 05/18/10-01/31/16
Prime: NIH R01MH089722 Valcour (PI)
Neurodevelopment and imaging among HIV-infected Children from the PREDICT study
The proposed work will determine the brain impact of deferring antiretroviral therapy until there is immunosuppression, as currently recommended by WHO guidelines.
Role: Subcontract PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Tu, Xin Ming

eRA COMMONS USER NAME (credential, e.g., agency login): xinmtu

POSITION TITLE: Professor of Biostatistics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Fudan University, Shanghai, China	B.S.	06/1982	Mathematics
Duke University, Durham, NC	M.S.	06/1986	Statistics
Duke University, Durham, NC	Ph.D.	06/1989	Statistics

A. Personal Statement

I am Professor of Biostatistics in the Division of Biostatistics and Bioinformatics, Department of Family Medicine and Public Health, and Director of Biostatistics in the Institute of Aging, UC San Diego. With more than 20 years of experience in biostatistical and psychosocial research and over 200 publications, I am well versed in causal inference, longitudinal data analysis, cluster randomized designs, structural equation models, semi-parametric models, functional response models, and their applications to biomedical and psychosocial research. More relevant to the current proposal, I have been working with the PIs and their team on multiple projects and have applied related causal inference and longitudinal methods to these studies. With over 20 years of experience on statistical consulting, collaboration and methodological research, especially with the focus on psychosocial research, I am well positioned to work together with the PIs and others in the study team to apply/develop statistical models most appropriate for the proposed aims.

a. Wu, P., Gunzler, D., Lu, N., Chen, T., Wyman, P. and **Tu, X.M**. Causal inference for community-based multi-layered intervention study. Statistics in Medicine, **33**(22): 3905-3918, 2014. DOI: 10.1002/sim.6199 and PMCID: PMC4156555.
b. Wu, P., Han, Y., Chen, T., and **Tu, X.M.** Causal inference for Mann-Whitney-Wilcoxon rank sum and other nonparametric statistics. Statistics in Medicine, **33**(8):1261-1271, 2014. This publication is a featured article of 2014 (2% of all publications that year). PMID: 24132928.
c. Gunzler, D., Tang, W., Lu, N., Wu, P. and **Tu, X.M**. A class of distribution-free models for longitudinal mediation analysis. Psychometrika, **79**(4):543-568, 2014. PMID: 24271505.
d. Chen, T., Wu, P., Tang, W., Zhang, H., Feng, C., Kowalski, J. and Tu, X.M. Variable selection for distribution-free models for longitudinal zero-inflated count responses. Statistics in Medicine, 35: 2770-2785, 2016. .

B. Positions and Honors

Positions and Employment

1990-1992	Postdoctoral Fellow, Dept. of Biostatistics, Harvard School of Public Health, Boston, MA.
1992-1998	Assistant Professor, Dept. of Statistics and Dept. of Psychiatry, Univ. of Pittsburgh, PA.
1994-1998	Co-Director, Methodology Core, Mental Health Clinical Research Center, Western Psychiatric Institute and Clinic
1998-1998	Associate Professor, University of Pittsburgh, Pittsburgh, PA.
1998-2003	Associate Professor, Dept. of Biostatistics and Epidemiology, University of Pennsylvania, School of Medicine, Philadelphia, PA.
2003-present	Professor, Dept. of Biostatistics and Computational Biology, and Dept. of Psychiatry, University of Rochester, Rochester, NY.
2007-2015	Associate Chair, Dept. Biostatistics and Computational Biology, University of Rochester, Rochester, NY.

2003-present	Director, Statistical Consulting Service, Dept. Biostatistics and Computational Biology, University of Rochester, Rochester, NY.
2007-2016	Director, Division of Psychiatric Statistics, Dept. of Biostatistics and Computational Biology, University of Rochester, Rochester, NY.
2016-present	Professor, Division of Biostatistics and Bioinformatics, Dept. of Family Medicine and Public Health, University of California in San Diego, San Diego, CA.

C. Contribution to Science

a. I have made important contributions to pooled testing and its applications to screening for infections of HIV and other rare diseases.

   a. **Tu, X.M**., Litvak, E., Pagano, M. On the informativeness and accuracy of pooled testing in estimating prevalence of a rare disease: application to HIV screening. Biometrika, **82**:287-297, 1995.
   b. **Tu, X.M**., Litvak, E. and Pagano, M. Issues in HIV screening programs. American Journal of Epidemiology, **136**:244-255, 1992.
   c. Litvak, E., **Tu, X.M.,** and Pagano, M. Screening for the presence of a disease by pooling sera samples. Journal of the American Statistical Association, **89**:424-434, 1994
   d. **Tu, X.M**., Litvak E. and Pagano M. Screening Tests: Can We Get More by Doing Less. Statistics in Medicine, **13**:1905-1919, 1994.

b. I have made significant contributions to models for disease latency and survival and its applications in estimation of prevalence and incidence of HIV infection and incidence of deaths due to AIDS

   a. **Tu, X.M.,** Meng, X. and Pagano, M. The AIDS epidemic: estimating survival after AIDS diagnosis from surveillance data. JASA, **88**:26-36, 1993.
   b. Pagano, M., **Tu, X.M.,** DeGruttola, V., MaWhinney, S. Analysis of censored and truncated data: estimating the reporting delay distribution and current AIDS incidence. Biometrics, **50**:1203-1214, 1994.
   c. **Tu, X.M.,** Meng, X. and Pagano, M. Survival differences and trends in patients with the acquired immunodeficiency syndrome in the United States. Journal of Acquired Immune Deficiency Syndromes, **6**:1150-1156, 1993.
   d. DeGruttola, V., **Tu, X.M.** and Pagano, M. Pediatric AIDS in New York City: estimating the distributions of infection, latency and reporting delay and projecting future incidence. Journal of the American Statistical Association, **87**:633-640, 1992.

c. I have made significant contributions to both parametric and semi-parametric models for longitudinal data with informative missing and their applications to HIV/AIDS and mental health research.

   a. DeGruttola, V. and **Tu, X.M.** Modeling progression of CD4-lymphocyte count and its relationship to survival time. Biometrics, **50**:1003-1014, 1994.
   b. **Tu, X.M.,** Zhang, J., Kowalski, J., Shults, J., Feng, C., Sun, W. and Tan, W. Power analyses for longitudinal study designs with missing data. Statistics in Medicine, **26**:2958-2981, 2007.
   c. Zhang, H., Yu, Q., Feng, C., Gunzler, D., Wu, P. and **Tu, X.M**. A new look at the difference between GEE and GLMM when modeling longitudinal count responses. Journal of Applied Statistics, **39**(9):2067-2079, 2012. DOI: 10.1080/02664763.2012.700452
   d. Zhang, H., Lu, N., Feng, C., Thurston, S., Xia, Y. and **Tu, X.M**. On fitting generalized linear mixed-effects models for binary responses using different statistical packages. Statistics in Medicine, **30**(20):2562-2572, 2011. DOI: 10.1002/sim.4265

d. I have developed a broad of class of regression models that extend longitudinal models to a wide range of non-regression analysis such as correlation, structural equation models and causal inference for rank outcomes.

a. Ma, Y., Tang, W., Feng, C. and **Tu, X.M.** Inference for Kappas for longitudinal study data: Applications to sexual health research. Biometrics, **64**(3):781-789, 2008. PMID: 18047535 [PubMed – indexed for MEDLINE]
b. Chen, T., Wu, P., Tang, W., Zhang, H., Feng, C., Kowalski, J. and **Tu, X.M**. Variable selection for distribution-free models for longitudinal zero-inflated count responses. Statistics in Medicine, **35**: 2770-2785, 2016.
c. Chen, T., Kowalski, J., Chen, R., Wu, P., Zhang, H., Feng, C. and **Tu, X.M**. Rank-preserving regression for longitudinal data with missing responses. Statistics in Medicine, **35**: 3333-3346, 2016.
d. Wu, P., Han, Y., Chen, T., and **Tu, X.M.** Causal inference for Mann-Whitney-Wilcoxon rank sum and other nonparametric statistics. Statistics in Medicine, **33**(8):1261-1271, 2014. This publication is a featured article of 2014 (2% of all publications that year). PMID: 24132928.

e. I have contributed to medical, especially psychiatric, research by collaborating with medical investigators, and have published more than 100 papers in peer-reviewed journals including the Journal of the American Medical Association and American Journal of Psychiatry.

a. March, J., Foa, E.B, Gammon, P., Chrisman, A., Curry, J., Fitzgerald, D., Sullivan, K., Franklin, M., Huppert, J., Rynn, M., Zhao, N., Zoellner, L., Leonard, H., Garcia, A., Freeman, J. and **Tu, X.M.** Cognitive-behavior therapy, sertraline, and their combination for children and adolescents with obsessive-compulsive disorder --- The pediatric OCD treatment study (POTS) randomized controlled trial. Journal of the American Medical Association, **292**(16):1969-1976, 2004.
b. Zubenko, G., Mulsant, B.H., Rifai, A.H., Sweet, R.A., Pasternak, R.E., Marino, L.J., and **Tu, X.M.** Impact of acute psychiatric inpatient treatment on major depression in late life and prediction of response. American Journal of Psychiatry, 151:987-994, 1994.
c. Lyness, J.M., Yu, Q., Tang, W., **Tu, X.M.** and Conwell, Y. Risks for depression onset in primary care seniors: Potential targets for preventive interventions. American Journal of Psychiatry, **166**(12):1375-1383, 2009. PMCID: PMC2982671
d. Wyman, P.A., Brown, H.C., LoMurray, M., Schmeelk-Cone, K., Petrova, M., Yu, Q., Walsh, E., **Tu, X.M**. and Wang, W. An outcome evaluation of the sources of strength suicide prevention program delivered by adolescent peer leaders in high schools. American Journal of Public Health, **100**(9):1653-1661, 2010. PMCID: PMC2920978

Complete List of Published Work in [SciENcv] [MyBibliography]:
https://www.urmc.rochester.edu/biostat/people/faculty/documents/Xin-Tu-CV.pdf

D. Research Support
Ongoing Research Support

R01AG022381 (Kremen) 07/01/15 – 03/31/20
NIH/NIA
The VETSA Longitudinal MRI Twin Study of Aging
The goal of this study is to characterize heterogeneity of brain and cognitive aging trajectories in a genetically-informative study using structural MRI. We will also examine influences of biomedical, psychosocial, stress, and personality factors on brain and cognitive aging.
Role: Biostatistician

R01AG050595 (Kremen) 09/01/15 – 05/31/19
NIH/NIA
The VETSA Longitudinal Twin Study of Cognition and Aging (VETSA 3)
The goal of this study is to determine genetic and environmental influences on cognitive, personality/psychosocial, and health/medical measures in middle aged twins. The study will examine the inter-relationships among these domains and their ability to predict cognitive and adaptive aging.
Role: Biostatistician

UL1TR001442 (Firestein) 08/13/15 – 03/31/20
NIH/NCATS

San Diego Clinical and Translational Research Institute (CTRI)
The CTRI provides infrastructure support and educational opportunities at UC San Diego.
Role: Biostatistician

Completed Research Support

Ul1 Feng (PI) 10/01/13 – 09/31/14
UR-CTSI
Allowance for Center Effects in the Analysis of Randomized Clinical Trial with Time-to-Event Outcomes
Compare relative efficiency of SLRT & ULRT under 2 different scenarios & obtain optimal linear combination.
Role: Biostatistician

5 U10 EY017387-05 Kieburtz (PI) 02/10/09 – 01/31/15
NIH
Data Coordination and Biostatistics Center for the NORDIC Network
Research network entitled “Neuro-Ophthalmology Research Disease Investigator Consortium” (NORDIC) that will conduct multicenter clinical research studies in neuro-ophthalmology.
Role: Biostatistician

C007864/2 Wyman (PI) 01/01/13 – 12/31/15
NYS Mental Health
Integrated Youth Suicide Prevention Program for Underserved and Rural Communities in NY State
Role: Biostatistician

1 R34 MH096854-01A1UR Knox (PI) 09/19/12 – 01/31/16
NIH
Outcomes in Callers to the VA’s 24/7 Veterans Crisis Line
Role: Biostatistician

UL1 Lu (PI) 07/01/14 – 06/30/15
UR-CTSI
Modeling Human Interactions in Social Networks
To understand how interactions of socially connected humans will change behavioral & health outcomes.
Role: Biostatistician

5 R01 GM108337-03 He (PI) 07/01/13 – 03/31/16
NIH
Moving beyond description: Statistical and causal inference for social media data
Develop & apply new class of statistical & causal inference models for human interactions & their impacts on health/health-related behavioral studies that integrate online social media information to understand roles of human interaction on disease spread, mental health in hard-to-reach population, presence & natural helping promoting wellness & reducing norms supporting violence in low-income urban neighborhoods.
Role: Biostatistician

UL1 Feng (PI) 01/05/15 – 12/31/16
UR-CTSI
Role: Co-investigator
Statistical Issues of Composite Time-to-Event Outcomes in Clinical Trial

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Vanos, Jennifer Kristin

eRA COMMONS USER NAME (credential, e.g., agency login): JVANOS

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Guelph, Guelph, Ontario, Canada	B.S. (Env)	04/2008	Environmental Sciences
University of Guelph, Guelph, Ontario, Canada	Ph.D.	02/2012	Atmospheric Sciences
Health Canada, Ottawa, Ontario, Canada	Postdoctoral	12/2012	Environmental Health Sciences

A. Personal Statement

I have a unique background and expertise in the fields of meteorology and human health, which although seemingly distinct, they are interconnected and important to study in concert. This background has allowed me to push forward into new frontiers concerning the relationships of the state of the atmosphere on large and smaller scales with human health, specifically extreme heat and air pollution. I focus on the physical process of dangerous environmental conditions, and how these conditions affect human health in time and space. At the microclimate scale, I focus on the biophysical causes and impacts extreme heat and air pollution on human health under dynamic conditions, such as in complex urban environments and/or during physical activity. My research areas include human heat balance modeling, extreme heat climatology, urban bioclimatic design and instrumentation, children's heat health, and air pollution epidemiology.

My interdisciplinary expertise in these areas, leadership and organizational skills, and intrinsic motivation to use my skills to optimize health will allow me to successfully carry out my tasks on this project related to the managing air pollution data, providing guidance on the application of the given data in conjunction with relevant environmental connections, and guiding the team on manuscript preparation and reporting of air quality information. I am currently successfully administering multiple projects and guiding graduate students (e.g. staffing, research organization and tasks, budgeting), continue to collaborate in interdisciplinary research across institutions and countries, and produce numerous peer-reviewed publications each year. The proposed research project builds logically on my prior work. I also bring a dimension of working across disciplines in a joint interdisciplinary position at UC San Diego (Climate, Atmospheric Science, Physical Oceanography and Family Medicine and Public Health) to bridge the gap between the atmosphere and human health.

B. Positions and Honors

Positions and Employment

2011–2012 Postdoctoral Researcher, Environment Health Sciences, Health Canada
2013 Visiting Research Scholar, Dept. of Geography & Regional Studies, University of Miami
2013–2016 Assistant Professor, Atmospheric Science, Dept. of Geosciences, Texas Tech University
2013–2016 Climate Science Center Faculty Associate, Texas Tech University
2015–2016 National Wind Institute Affiliated Faculty, Texas Tech University
2017– Assistant Professor, Climate, Atmospheric Science, and Physical Oceanography, Scripps Institution of Oceanography; Family Medicine & Public Health, School of Medicine, UC San Diego

Other Experience and Professional Memberships

2010 Selected Participant, International Research Institute Climate & Health Course, Columbia University
2010 Member, International Society of Biometeorology
2010 Member, American Meteorological Society
2012 Member, American Association of Geographers (Climate Specialty Subgroup)
2013–2016 Seminar Series Organizer, Climate Science Center, Texas Tech University
2013–2014 Session Co-Chair, Weather, Climate, and Health, American Association of Geographers. 2014–2016 Research Associate, 'HEAT' project: A Pufendorf Initiative towards a Multidisciplinary Heat Research Collaboration of humans and ecosystems at Lund University, Sweden
2014–2016 Course Lecturer & Facilitator, Climate Change & Health Research Methods, Umeå Center for Global Research, Umeå University, Sweden. June 2014; June 2015; June 2016
2014– Editorial Advisory Board, International Journal of Biometeorology
2014–2017 Chair, Students and New Professional Group, International Society of Biometeorology
2014 Project Co-Lead and Workshop Organizer, 1st International SNP Workshop on Biometeorology and Applied Synoptic Climatology, Umeå Sweden, June 2014
2014 Member, International Association for Urban Climate.
2015– Research Affiliate, Preventing Obesity by Design (POD) Research Group, Texas Tech University
2015 Invited Speaker, HEAT Project, Pufendorf Institute, Lund University, Sweden, May 2015
2015– Board Member & Planning Chair, American Meteorological Society, Board on Environment & Health
2015– Session Planning Co-chair, Environment & Health Sessions, 2016, 2017, 2018 American Meteorological Society Annual Meetings
2015 Member, International Society of Exposure Sciences
2016 International Workshop Organizer & Facilitator, 2nd International SNP Workshop, "Enhancing the Teaching and Learning of Biometeorology in Higher Education", Old Dominion University
2016 Invited Keynote Speaker, "Promotion of Urban Health and Environmental Equity in a Changing Climate, Conference: "Behind the Perspective — Environmental Equity, Justice, & Sustainability in Underserved Communities", Texas Tech University, November 2016
2016 Workshop Organizer & Steering Committee, Developing integrated heat-health information for long-term resilience, and early warning, for the El Paso-Juarez, Las Cruces, July 2016.
2017– Co-Director, Technology and Health, Institute for Public Health, UC San Diego
2017 Invited Speaker, Protecting Population Health from Climate Variability and Change, University of Washington Center for Health and the Global Environment, January 2017.
2016– Steering Committee, Developing an Integrated Heat-Health Information System for Long-term Resilience to Climate and Weather Extremes in the El Paso-Juárez-Las Cruces Region
2016 Member, International Society of Environmental Epidemiology

Honors

2011 Course Scholarship, Summer Institute for Climate and Health, International Research Institute for Climate and Society, CIPHA Columbia University, Earth Institute
2011 Dean's Graduate Scholarship, School of Environmental Sciences, University of Guelph
2011 Alexander Graham Bell Canada Graduate Scholarship, National Science and Engineering Research Council (NSERC)
2012 NSERC Visiting Fellowship at a Canadian Government Laboratory, Health Canada, Environmental Health Science Research Bureau
2014 Invited Participant, National Academies Keck Futures Initiative
2014 Ralph E. POWE Junior Faculty Enhancement Award, Oak Ridge Associated Universities

C. Contributions to Science

1. Early research throughout my Ph.D. focused on urban micrometeorology and addressed issues related to human heat balance modeling for thermal comfort in outdoor environments. My research completed in this area comprehensively examined gaps in the scientific literature pertaining to human physiology connected to fine-scale weather features during exercise, including mechanisms of heat formation and dissipation, heat stress on the body, the importance of skin temperature monitoring, and clothing effects. I also applied heat wave and climate projections to urban parks and heat balance modeling into the future. This work

further improved upon the ability to model outdoor thermal comfort of humans performing physical activity and applied findings to health, emergency heat stress preparedness, and urban bioclimatic design—a topic prominent in European city design, yet minimal in North America. Linking these various disciplines provided me with a strong interdisciplinary foundation from which to grow, and further provided a critical foundation for microclimatologists and biometeorologists in the completion and comprehension of climate research involving human physiology. The four publications below built upon one another to jointly demonstrate the viability and potential uses of outdoor energy balance models.

a. **Vanos, JK.,** Warland, JS., Gillespie, TJ., Kenny, NA. (2010). Review of the physiology of human thermal comfort while exercising in urban landscapes and implications for bioclimatic design. International Journal of Biometeorology. 54(4): 319–334. PMID: 20155515
b. **Vanos, JK.,** Warland, JS., Gillespie, TJ., Kenny, NA. (2012). Improved predictive ability of climate-human-behaviour interactions with modifications to the COMFA outdoor energy budget model. International Journal of Biometeorology. 56(6), 1065-1074. PMID: 22350422
c. **Vanos, JK.,** Warland, JS., Gillespie, TJ., Kenny, NA. (2012). Thermal comfort modelling of body temperature and psychological variations of a human exercising in an outdoor environment. International Journal of Biometeorology. 56(1): 21-32. PMID: 21188424
d. **Vanos, JK**., Warland, JS., Gillespie, TJ., Slater, GA., Brown, RD., Kenny, NA. (2012). Human energy budget modeling in urban parks in Toronto, ON and applications to emergency heat stress preparedness. Journal of Applied Meteorology & Climatology. 51(9): 1639–1653. doi: http://dx.doi.org/10.1175/JAMC-D-11-0245.1

2. Work completed during my Postdoctoral Degree at Health Canada involved air pollution and applied synoptic climatology studies assessing the synergistic impacts of synoptic-scale air masses (large-scale weather systems) and air pollution on human health. Air pollution is a significant contributor to illness and death, yet understanding the confounding and interactive factors of various pollutants and meteorological combinations (e.g. heat wave 'types') proves difficult, and the effects also vary across different subpopulations. Work that I led in this position addressed air pollution-related mortality in various ages of the population, particularly the elderly, and cause-specific mortality (cardiovascular and respiratory) in the full population of all large Canadian cities. Findings demonstrated that mortality risks due to air pollution exposure differ by weather type (hot or cold, dry or moist), with increased accuracy obtained when accounting for interactive effects through adjustment for dependent pollutants using a new distributed lag nonlinear model (DLNM). My Post-Doctoral work culminated in assisting with investigations of the modifying effects of socioeconomic in connecting respiratory health with air pollution and traffic related air pollution exposure within 200m of busy roadways in Windsor, Ontario.

   a. **Vanos, JK**., Cakmak, S. Bristow, C., Brion, V., Tremblay, N., Martin, SL., Sheridan, SC. (2013). Synoptic weather typing applied to air pollution mortality among the elderly in 10 Canadian cities. Environmental Research. 126, 66–75. PMID: 24012249
   b. **Vanos, JK.,** Cakmak, S., Kalkstein, LK., Yagouti, A. (2014). Association of weather and air pollution interactions on daily mortality in 12 Canadian cities. Air Quality, Atmosphere, & Health. 8 (3), 307–320. PMCID: PMC4449933
   c. **Vanos, JK.,** Hebbern, C., Cakmak, S. (2014) Risk assessment for cardiovascular and respiratory mortality due to air pollution and synoptic meteorology in 10 Canadian cities. *Environmental Pollution*. 185, 322–332
   d. Cakmak, S., Hebbern, C, Cakmak, JD, **Vanos, JK.** (2016). The modifying effect of socioeconomic status on the relationship between traffic, air pollution, and respiratory health in elementary schoolchildren. Journal of Environmental Management. 177, 1–8. PMID: 27064731

3. My most recent research endeavors synthesize my Doctoral and Post-Doctoral training to link environmental design, physiology, and behavior with exposures of heat, ultraviolet B radiation (UVB), and air pollution at a personal scale, particularly in children. Personal monitoring is an important future research avenue, underscoring two research thrusts: 1) the need to for personal assessments of exposures to solve issues at finer scales rather than generalized population outcomes, and 2) children are among the population groups disproportionately affected by ambient extremes and climate change, yet minimally studied. Hence, I am working towards developing real-world observations using personalized instruments in urban and schoolyard microclimates for risk exposure assessments. Initial findings indicate that more

substantive evidence is needed for applicable child-specific policies and guidelines to protect children from the acute consequences of ambient environmental stressors to effectively limit heat injury and burns. Select new and current projects involve multi-scalar and remote sensing analyses in urban parks and playgrounds, mobile air pollution sensing with low cost monitors, and new spatially innovative instrumentation methods for collecting personal air temperature, UVB, and physiological data with miniaturized sensors.

a. **Vanos, JK.** (2015). Children's Health and Vulnerability in Outdoor Microclimates: A Comprehensive Review. *Environment International*. 76, 1–15.
b. McKercher, GR, Salmond, JA, **Vanos, JK**. (*2017).* Characteristics and Applications of Small, Mobile Air Pollution Monitors: A Review. Environmental Pollution. *In press.* 10.1016/j.envpol.2016.12.045.
c. **Vanos, JK.**, McKercher, GR., Naughton, K., Lochbaum, M. Schoolyard Shade and Sun Exposure: Assessment of Personal Monitoring During Children's Physical Activity. (*In Press*). *Photochemistry and Photobiology*, Manuscript ID PHP-2016-10-RA-0251*.*
d. **Vanos, JK.,** Middel, A., McKercher, GR., Kuras, ER., Ruddell, BL. (2016). A Multiscale Surface Temperature Analysis of Urban Playgrounds in a Hot, Dry City. *Landscape & Urban Planning* 146,29-42.

Complete List of Published Work:
https://www.ncbi.nlm.nih.gov/sites/myncbi/1bq3yXtGabCkH/bibliography/51929710/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

University of California San Diego — Vanos (PI) — 04/01/2017–03/31/2018
Institute for Public Health
Children's Exposure to Ultraviolet Radiation during Physical Activity: Influence of Sensor Type and Site Design
Major Goals*:* To validate new bio-sensors for performance after modularization and the ability to accurately measure variables connected to children's heat exposure-response.
Role: PI

Tromp Foundation/
International Society of Biometeorology — Vanos (PI) — 02/01/2016–09/01/2017
Enhancing the Teaching and Learning of Biometeorology in Higher Education
Major Goals: To gain critical insight into the prospects of the implementation of biometeorology topics into undergraduate courses and curricula worldwide, and the best practices with which to do so.
Role: PI

1444758 — Mazdak (PI) — 04/15/2016–04/14/2020
National Science Foundation
Sustainability Research Networks: The Urban Water Innovation Network (U-WIN): Transitioning Toward Sustainable Urban Water Systems
Major Goals: To create a network of 14 institutions that advance the fundamental knowledge, build capacity, and forge collaborations needed to find technological and behavioral solutions that promote sustainable urban water systems.
Role: Senior Personnel

Completed Research Support

Texas Tech University
Transdisciplinary Research Academy — Vanos (PI) — 04/01/2016–03/31/2017
The Value of Greening Urban Environments
Major Goals: To measure and demonstrate the microclimate and carbon budget impacts of transforming parking lots and impermeable surfaces into areas of urban greenspace with trees and healthy vegetation.
Role: PI

National Wind Institute | Bruning/Vanos (Co-PIs) | 04/01/2015–12/1/2015
Establishment of a Joint Atmospheric Sciences-National Wind Institute Research Electronics Lab at Texas Tech University
Major Goals: Refurbished a storage space to become a new space for atmospheric science instrumentation projects.
Role: Co-PI

5P20MD000516 | Juarez (PI) | 12/15/2014–11/30/2015
National Institute on Minority Health and Health Disparities
Linking Climate, Air Pollution, and Housing Conditions to Develop Strategies to Reduce Racial Disparities in Infant Mortality.
Major Goals: Identify modifiable environmental factors (air quality, temperature) to focus interventions in the policy environment, where they have the greatest potential to reduce rates of infant mortality and its undue burden on black infants.
Role: Senior Personnel

Tromp Foundation/
International Society of Biometeorology | Vanos (Co-PI) Gosling & Hondula (Co-PIs) | 01/01/2014–31/12/2015
Extending the Application of Climate and Health Research Tools into Distinct Climate Regimes in Russia, India, & New Zealand.
Major Goals: To hold a major international workshop in Umea, Sweden, bring together students and new professionals in climate and health fields of biometeorology to advance research and applications of synoptic bioclimatology throughout the world.
Role: Co-PI

Oak Ridge Associated Universities | Vanos (PI) | 04/01/2014–31/03/2016
Real-Time Intra-Urban Air Quality Monitoring Through the Use of Mobile Platforms
Major Goals: To measure and quantify gaseous ambient air pollutants at a fine scale for better understanding of urban exposures to human health.
Role: PI

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME:Zhang, Hongqiao

eRA COMMONS USER NAME (credential, e.g., agency login):HONGQZ

POSITION TITLE: Research Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Shandong Medical University, Shandong, China	B.M	07/1993	Preventive Medicine
Chinese Academy of Preventive Medicine, Beijing, China	MPH	07/1996	Environmental Health Science
University of Alabama, Birmingham, AL	Ph.D.	07/2005	Environmental Health Science
University of California, Merced	Postdoctoral	09/2006	Molecular Toxicology

A. Personal Statement

I have a broad educational background in medicine, epidemiology, toxicology, and molecular biology. These backgrounds and nearly 20 years of research experience enable me to provide necessary supports for the successful accomplishment of the proposed project. My research has been focusing on the regulation of antioxidant and phase II detoxifying enzymes, and inflammatory mediators, i.e., the basal and inducible expression of these genes in response to environmental stimuli. Currently my research focuses on two directions: 1) the molecular mechanism of age-related decline of Nrf2-EpRE signaling-regulated antioxidant and detoxifying capacity; and 2) the different antioxidant and inflammatory responses to airborne nanoparticles in alveolar macrophages and bronchial airway epithelial cells from young and aged people. I will be primarily responsible for designing and carrying out experiments measuring the expression level of inflammatory cytokines and mediators of interest in tissues of animals.

B. Positions and Honors

1996-2000, Assistant Researcher, Institute of Environmental Health Monitoring, Chinese Academy of Preventive Medicine, Beijing, China
2006-2010, Assistant Biology Researcher, Univ. California, Merced
2010- 2016, Senior Research Associate in Gerontology, Univ. Southern California
2016-present, Research Assistant Professor in Gerontology, Univ. Southern California

C. Contributions to Science

Publications relevant to the current application (in chronological order)

1. Dickinson, D.A., Iles, K.E., Zhang, H., Blank, V. and Forman, H.J. Curcumin alters EpRE and AP-1 binding complexes and elevates glutamate-cysteine ligase gene expression. FASEB J.17 (3): 473-5,2003. PMID: 12514113 [PubMed - indexed for MEDLINE]

2. Zhang, H., Dickinson, D.A., Liu, R. -M., and Forman, H.J. 4-Hydroxynonenal increases gamma-glutamyl transpeptidase gene expression through mitogen-activated protein kinase pathways. Free Radic Biol Med. 38(4): 463-478, 2005. PMCID: PMC2801023
3. Chinta, S.J., Kumar J., Zhang, H., Forman, H.J. and Andersen, J.K. Increase of gamma-glutamyl transpeptidase activity following GSH depletion has a compensatory rather than inhibitory effect on mitochondrial complex I activity: implications for Parkinson's disease. Free Radic Biol Med. 40(9): 1557-63, 2006. PMCID: PMC2804072
4. Zhang, H., Liu, H., Iles, K.E., Liu, R-M, Postlethalwait, E.M., Laperche, Y. and Forman, H.J. 4-Hydroxynonenal induces rat gamma-glutamyl transpeptidase through mitogen-activated protein kinase-mediated electrophile response element/nuclear factor erythroid 2-related factor 2 signaling. Am. J. Respir. Cell Mol. Biol. 34 (2): 174-81,2006. PMCID: PMC2696200
5. Zhang, H., Liu, H., Dickinson, D.A., Liu, R-M, Postlethalwait, E.M., Laperche, Y. and Forman, H.J. Gamma-glutamyl transpeptidase is induced by HNE via EpRE in rat epithelial type II cells. Free Radic Biol Med. 40(8): 1281-92, 2006. PMCID: PMC2702664
6. Liu, H., Zhang, H., Iles, K.E. and Forman, H.J. The ADP-stimulated NADPH oxidase activates the ASK-1/MKK4/JNK pathway in alveolar macrophages.Free Radic Res. 40(8):865-74, 2006. PMCID: PMC2713795
7. Liu, H., Zhang, H., Iles, K.E. and Forman, H.J. Silica induces macrophage cytokines through phosphatidylcholine-specific phospholipase C with hydrogen peroxide. Am J Respir Cell Mol Biol. 36 (5): 594-9, 2007. PMCID: PMC1899332
8 Zhang, H., Court, N. and Forman, H.J. Submicromolar concentrations of 4-hydroxynonenal induce glutamate cysteine ligase expression in HBE1 cells. Redox Rep. 12 (1): 101-6, 2007. PMCID: PMC2730489
9. Zhang, H. and Forman, H.J. Acrolein induces heme oxygenase-1 through PKC-delta and PI3K in human bronchial epithelial cells. Am J Respir Cell Mol Biol. 38(4): 483-90, 2008. PMID: 18048804 [PubMed - indexed for MEDLINE]
10. Zhang, H. Shi, A. Rinna, A. and Forman, H.J. Resveratrol and 4-hydroxynonenal act in concert to increase glutamate cysteine ligase expression and glutathione in human bronchial epithelial cells. Arch Biochem Biophys. 481(1): 110-115,2009. PMCID: PMC2692270
11. Zhang, H. and Forman, H.J. Reexamination of the electrophile response element sequences and context reveals a lack of consensus in gene function. Biochimica et Biophysica Acta - Gene Regulatory Mechanisms 1799(7): 496-501, 2010.
12. Zhang H, Shih A, Rinna A, Forman HJ. Exacerbation of tobacco smoke mediated apoptosis by resveratrol: an unexpected consequence of its antioxidant action.Int J Biochem Cell Biol.43(7):1059-64, 2011. PMCID: PMC2891685
13. Zhang H and Forman HJ. Glutathione synthesis and its role in redox signaling. Semin Cell Dev Biol. 23 (7): 722-8, 2012. PMCID: PMC3422610 [Available on 2013/9/1]
14. Zhang H, Liu H, Davies KJ, Sioutas C, Finch CE, Morgan TE, Forman HJ. Nrf2-regulated phase II enzymes are induced by chronic ambient nanoparticle exposure in young mice with age-related impairments. Free Radic Biol Med. 1;52(9):2038-46, 2012. PMCID: PMC3342863
15. Zhang H, Liu H, Borok Z, Davies KJ, Ursini F, Forman HJ. Cigarette smoke extract stimulates epithelial-mesenchymal transition through Src activation. Free Radic Biol Med. 15;52(8):1437-42, 2012. PMCID: PMC3312989
16. Zhang H and Forman HJ. TGFbeta1 rapidly activates Src through a non-canonical redox mechanism. Free Radic Biol Med. 75 Suppl 1:s4. 2014.
17. Zhang H, Kelvin JA Davies, and Henry Jay Forman.TGFbeta1 rapidly activates Src through a non-canonical redox signaling mechanism. Arch Biochem Biophys. 568:1-7. 2015
18. Zhang H, Davies KJ, and Forman HJ. Oxidative stress response and Nrf2 signaling in aging. Free Radic Biol Med. 88, Pt B:314-36. 2015.
19. Zhang H and Forman HJ.. 4-Hydroxynonenal activates Src through a non-canonical pathway that involves EGFR/PTP1B. Free Radic Biol Med. 89:701-7.2015.
20. Zhang H and Forman HJ.. Signaling by 4-Hydroxy-2-Nonenal: Exposure Protocols, Target Selectivity and Degradation. Arch Biochem Biophys 617, 145-154. 2016
21. Zhang H and Forman HJ.. 4-hydroxynonenal-mediated signaling and aging. Free Radic Biol Med.*in press*, 2016.
22. Zhang H, Zhou L, Yuen J, Birkner N, Leppert V, O'Day PA, and Forman HJ. Delayed Nrf2-regulated antioxidant gene induction in response to silica nanoparticles. Free Radic Biol Med. PMID: 28389405, 2017.

D. Additional Information: Research Support and/or Scholastic Performance

R01 ES023864-01, Forman (PI), 2/9/15-10/30/20
Human models of the particulate-induced inflammatory/antioxidant axis in aging
This grant focuses on the effect of aging on the balance between pro-inflammatory and anti-oxidant defenses in response to air pollution nanoparticles.
Role: designing and performing experiments, data analysis and presentation as publications.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME	POSITION TITLE
Zlokovic, Berislav V.	Professor and Chair, Dept. Physiology & Biophysics Director, Zilkha Neurogenetic Institute Univ. of So. California, Keck School of Medicine, Los Angeles
eRA COMMONS USER NAME (credential, e.g., agency login) BZlokovic	

EDUCATION/TRAINING *(Begin with baccalaureate include postdoctoral training and residency training if applicable)*

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Belgrade College of Sciences	B.S.	07/70	Science & Mathematics
School of Medicine, Belgrade	M.D.	12/75	Medicine
Queen Elizabeth College, London	Graduate	12/82	Transport Biology
University of Belgrade	Ph.D.	06/83	Physiology
King's College, London	Postdoctoral	12/85	Neuroscience
Medical Board of California	MD certified	08/95	Med License A053853

A. Personal Statement

I have a career long focus studying the role of cerebral blood vessels in the pathogenesis and treatment of neurologic disorders such as Alzheimer's disease, and related disorders and stroke. Using animal models and studying human brain, my laboratory has shown that dysfunction in the blood-brain barrier (BBB) and brain microcirculation can accumulate before neuronal dysfunction and contribute to the onset and progression of neurodegeneration and cognitive impairment in Alzheimer's disease. My research team has identified the cellular and molecular mechanisms in cerebral blood vessels causing disruption of the neurovascular unit and BBB breakdown, which leads to neuron loss in models of Alzheimer's disease, pericyte-deficient rodents and stroke. We have developed novel neuroimaging and molecular biomarkers of the neurovascular unit and BBB integrity in the living human brain. My group has identified molecular mechanisms at the BBB that maintain clearance of Alzheimer's toxin amyloid-beta from the brain into the circulation, and its influx or re-entry from the circulation into the brain, reflecting an important physiologic function of the BBB in maintaining amyloid-beta homeostasis. Discoveries of my research team have contributed to the development of Phase 2 and 3 studies in *Alzheimer's disease* patients based on amyloid-beta clearance and/or inhibition of its re-entry into the brain, respectively, and Phase 2 studies in *stroke* patients based on activated protein C as a neuroprotective agent.

B. Positions and Honors

CURRENT EMPLOYMENT

- Professor and Chair, Department of Physiology and Biophysics, and Director, Center for Neurodegeneration and Regeneration, Zilkha Neurogenetic Institute, University of Southern California (USC), Keck School of Medicine (KSOM), Los Angeles, California, Dec 2011- present
- Director, Zilkha Neurogenetic Institute, USC, KSOM, Los Angeles, California, July 2012 - present

CURRENT BIOTECHNOLOGY POSITIONS

- Scientific Director and Founder, ZZ Biotech L.L.C., 2007-present

POSTDOCTORAL FELLOWSHIPS/RESIDENCIES

- Research Fellow in Transport Biology, Queen Elizabeth College, London 1978-1981
- Senior Welcome Trust Research Fellow in Neurobiology and Blood-Brain Barrier Transport, King's College London (with Hugh Davson) 1982-1984
- Senior Research Fellow British Council and Welcome Trust in Neurobiology and Transport Physiology, St. Thomas's Hospital London and Marine Biological Association Plymouth 1984-1986
- Resident in Clinical Physiology (Neurology/Intensive Care), Sch Med, University of Belgrade 1986-1989

PRINCIPAL PREVIOUS EMPLOYMENT

- Professor of Neurosurgery and Neurology, University of Rochester Medical Center (URMC), Rochester, New York 2000-2011
- Dean's Professor, University of Rochester New York, 2005-2011
- Director, Center for Neurodegenerative and Brain Vascular Disorders, URMC New York, 2007-2011
- Director, Interdisciplinary Program in Dementia Research, URMC New York, 2009-2011

- Professor of Neurosurgery, Physiology & Biophysics, Univ. So. California (USC) Sch Med, Los Angeles, California, 1992-2000
- Associate Professor of Neurosurgery, Physiology & Biophysics, USC Sch Med, Los Angeles, CA 1989-91
- Director, Laboratory for Neurological Surgery, USC School of Medicine, Los Angeles, CA 1989-2000
- Associate Professor of Medical Physiology, School of Medicine, Belgrade 1986-1989

NATIONAL LEADERSHIP POSITIONS, SOCIETIES AND BOARDS

- NIH Neuroscience Study Section NLS3/BDCN3, regular member 1995- 2000
- Chair NIH Study Section: Brain Disorders BDCN-3 2003
- Chair NIH Study Section: Cell Death and Injury in Chronic Neurodegeneration 2004-2005
- NIH Distingusihed Neuroscience Editor for Challenging Grants 2009, 2010
- Editorial Board: Neurosurgery 1996-present
- Editorial Board: Neurobiology of Aging 2000-2005
- Ediotorial Board: Molecular Neurodegeneration, Associate Editor 2005-present
- Editor, Advanced Drug Delivery Reviews 2002
- Chair Keystone Meeting “Neuronal & Vascular Injury in Alzheimer’s Disease” 2001
- Member, Society for Neurosciences (SFN) 1989- present
- Chair SFN 2009 Symposium "The role of blood-brain barrier and non-neuronal cells in neurodegeneration"
- Chair, NIH Study Section: Brain Injury and Neurovascular Pathologies, 2005-2007 and 2012-2014
- Co-Chair, NINDS Workshop: Vascular Dementias and AD Dementia, 2013

Honors and Awards

- British Council Award for Medical Research 1988
- James Zumberg Innovation Award, University of So. California 1991
- Shannon Carreer Development Award, NIH 1991
- Herbert Jr Hoover Award for Medical Research, Los Angeles 1993
- Serbian National Academy of Sciences & Arts, Foreign Member 1997
- *MERIT Award* from the National Institute on Aging “in recognition of sustained contribution to aging and leadership and commitment to the field” 2004
- Dean’s Professor – Distinguished Faculty University of Rochester 2005
- *ISOA/Elan Award* for “novel approaches to drug discovery for Alzheimer’s disease” 2006
- *MetLife Award* for Medical Research for “significant contributions to our understanding of Alzheimer’s disease and for bringing us closer to a cure” 2006
- *Javits Award* National Institute on Neurological Disorders and Stroke for “distinguished record of substantial contributions in a field of neurological sciences” 2007
- *Potamkin Prize* from the American Academy of Neurology “in recognition of outstanding achievements in research on Alzheimer’s and related neurodegenerative diseases” 2009
- *McCune Award* from the Alzheimer's Association Dallas Texas “which is given to a researcher who has made a difference in Alzheimer’s disease” 2009
- *Presidential Citation* (University of Rochester) for “pioneering approach to Alzheimer’s disease” 2009
- *Inaugural Zilkha Senior Scholar* (University of So. California) for “sustained high level research on the role of vasculature in Alzheimer’s disease, related neurodegenerative disorders and stroke” 2012
- *Fellow, the American Association for Advancement of Science (AAAS)* 2013
- *SFN Presidential Lecture* – Blood-brain barrier and Neurodegeneration 2013
- *Mary Hayley and Selim Zilkha Chair in Alzheimer's Disease Research* 2013
- *Thomson Reuters, ‘The World’s Most Influencial Scientific Minds’* 2014 – ranking among the top 1 percent of the most cited authors in the filed of *Neurosciences* over the last 11 years (2002-2012)
- *The Chancellor’s Award Lecture in Neurosciences* Louisiana State University Health Sciences Center 2014
- *The Dana Alliance for Brain Initiative (DABI),* Member, 2014
- *The European Academy of Sciences (Academia Europeae) - the Life Sciences Class,* Member, 2015
- *Thomson Reuters List, ‘The World’s Most Influential Scientific Minds’* 2016 – the most cited authors in the field of *Neurosciences and Behavior* in 2016
- *Asked by the Nobel Assembly at Karolinska Institutet, The Nobel Committee to nominate one or more candidates for the Nobel Prize in Physiology and Medicine 2017*

C. Contribution to science (i-10 index: 210; H-index: 80; 350 publications; >25,300 citations (Google Scholar, 2017); complete list in Google Scholar: https://goo.gl/2Npcj2

1. Our studies in different models of neurological disorders and humans addressed the role of the *neurovascular unit* (NVU) and *blood-brain barrier* (BBB) in the pathogenesis of Alzheimer's disease, dementia, ALS and stroke. We found that a chronic BBB breakdown leads to neuronal dysfunction, injury and secondary neurodegeneration. Our recent study in the living human brain found an age-dependent BBB breakdown in the hippocampus, a region critical for learning and memory, which worsens with mild dementia and correlates with injury to pericytes, a BBB-associated cell. Listed below are recent original contributions and two reviews that cite several of our primary papers in this area. I served as the primary investigator in these studies.

Zlokovic BV 2008 Blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron*, 57(2):178-201.

Zhong Z, Deane R, Ali Z, Parisi M, Shapovalov Y, O'Banion MK, Stojanovic K, Sagare A, Boillee S, Cleveland DW & **Zlokovic BV** 2008 ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration. *Nature Neuroscience*, 11(4):420-42. PMCID: PMC2895310

Zlokovic BV 2011 Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nature Reviews Neuroscience*, 12(12):723-738. PMCID: PMC4036520

Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, Toga AW, Jacobs RE, Liu CY, Amezcua L, Harrington MG, Chui HC, Law M, **Zlokovic BV** 2015 Blood-brain barrier breakdown in the aging human hippocampus *Neuron* 85(2):296-302. PMCID: PMC4350773

Winkler EA, Nishida Y, Sagare AP, Rege SV, Bell RD, Perlmutter D, Sengillo JD, Hillman S, Kong P, Nelson AR, Sullivan JS, Zhao Z, Meiselman HJ, Wenby RB, Soto J, Dale Abel E, Makshanoff J, Zuniga E, De Vivo DC **Zlokovic BV** 2015 GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration *Nature Neuroscience* 18(4):521-530. NIHMSID: NIHMS684079

Zhao Z, Nelson AR, Betsholtz C, **Zlokovic BV** 2015 Establishment and dysfunction of the blood-brain barrier *Cell* 163, 1064-1078 NIHMSID: NIHMS734996

Kisler K, Nelson A, Montagne A, **Zlokovic BV** (2017) Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer's disease. Nature Reviews Neuroscience (in press)

2. Our studies of vascular brain mural cells have demonstrated that pericytes control key neurovascular functions including regulation of the BBB permeability, cerebrovascular integrity and cerebral blood flow that is necessary for normal neuronal structure and function. We also showed that aberrant signal transduction between endothelial cells, pericytes and astrocytes leads to BBB breakdown and CBF dysregulation causing secondary neurodegenerative changes and neuronal loss. I was the primary investigator in these studies.

Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R & **Zlokovic BV** 2010 Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron*, 68(3):409-427. PMCID: PMC3056408

Winkler EA, Bell RD & **Zlokovic BV** 2011 Central nervous system pericytes in health and disease. *Nature Neuroscience*, 14(11):1398-1405. PMCID: PMC4020628

Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, Holtzman DM, Betsholtz C, Armulik A, Sallstrom J, Berk BC & **Zlokovic BV** 2012 Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature*, 485(7399):512-516. PMCID: PMC4047116

Sagare AP, Bell RD, Zhao Z, Ma Q, Winkler EA, Ramanathan A & **Zlokovic BV** 2013 Pericyte loss influences Alzheimer-like neurodegeneration in mice. *Nature Communications*, 4:2932. PMCID: PMC3945879

Sweeney MD, Ayyadurai S & **Zlokovic BV** 2016 Pericytes of the neurovascular unit : key functions and signaling pathways. *Nature Neuroscience*, 19 (6), 771-783.

Kisler K, Nelson AR, Rege SV, Ramanathan A, Wang Y, Ahuja A, Lazic D, Tsai PS, Zhao Z, Zhou Y, Boas DA, Sakadžić S, **Zlokovic BV** (2017) Pericyte degeneration leads to neurovascular uncoupling and limits oxygen supply to brain. *Nature Neuroscience*, 20, 406-416.

3. In my earlier research, we found that *peptide bond* prevents peptides from using rapid amino acid transport systems at the BBB and that neuropeptides (e.g., enkephalins, vasopressin, DSIP, TRH, leptin) have limited access to the brain, but can still be transported slowly across the BBB via specific "peptidergic" carriers and/or receptors. This work led to discovery of RAGE and LRP1 as major influx and efflux receptors for Aβ at the BBB,

respectively, that control Aβ levels in the brain. These studies led to a concept that **i)** peripheral circulating Aβ contributes to Aβ levels in the brain and **ii)** clearing Aβ from brain to blood limits Alzheimer's cerebral β-amyloidosis. Both findings contributed to development of Aβ-lowering clinical trials. I was the primary investigator in these studies.

Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, Welch D, Manness L, Lin C, Yu J, Zhu H, Ghiso J, Frangione B, Schmidt AM, Armstrong DL, Arnold B, Liliensiek B, Nawroth P, Hofman F, Kindy M, Stern D & **Zlokovic BV** 2003 RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. *Nature Medicine*, 9(7):907-913.

Deane R, Wu Z, Sagare A, Davis J, Du Yan S, Hamm K, Xu F, Parisi M, LaRue B, Hu HW, Spijkers P, Guo H, Song X, Lenting PJ, Van Nostrand WE & **Zlokovic BV** 2004 LRP/amyloid β-peptide interaction mediates differential brain efflux of Aβ isoforms. *Neuron,* 43(3):333-344.

Sagare A, Deane R, Bell RD, Johnson B, Hamm K, Pendu R, Marky A, Lenting PJ, Wu Z, Zarcone T, Goate A, Mayo K, Perlmutter D, Coma M, Zhong Z & **Zlokovic B V** 2007 Clearance of Alzheimer's amyloid-β by circulating lipoprotein receptors. *Nature Medicine*, 13(9):1029-1031. PMCID: PMC2936449

Deane R, Singh I, Sagare AP, Bell RD, Ross NT, Larue B, Love R, Perry S, Paquette N, Deane RJ, Thiyagarajan M, Zarcone T, Fritz G, Friedman AE, Miller BL & **Zlokovic BV** 2012 A multimodal RAGE-specific inhibitor reduces amyloid β-mediated brain disorder in a mouse model of Alzheimer disease. *Journal of Clinical Investigation*, 122(4):1377-1392. PMCID: PMC3314449

4. We are also interested to better understand the underlying mechanisms by which genes that influence the risk for AD affect the brain vascular system (i.e., *APOE4, PSEN1, CLUSTERIN, PICALM*), as well as how vascular-specific transcription factors such as *MEOX2* homeobox gene and SRF/myocardin influence neurovascular function in AD patients and animal models. In these studies I served as the primary investigator.

Zlokovic BV, Martel CL, Matsubara E, McComb JG, Zheng G, McCluskey RT, Frangione B & Ghiso J 1996 Glycoprotein 330/megalin: Porbable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer's disease amyloid β at the blood-brain and blood-cerebrospinal fluid barriers *Proceedings of the National Academy of Sciences USA*, 93(9):4229-4234. PMCID: PMC39517

Wu Z, Guo H, Chow N, Sallstrom J, Bell R, Deane R, Brooks AI, Kanagala S, Rubio A, Sagare A, Liu D, Li F, Armstrong D, Gasiewicz T, Zidovetzki R, Song X, Hofman F & **Zlokovic BV** 2005 Role of the MEOX2 homeobox gene in neurovascular dysfunction in Alzheimer disease. *Nature Medicine*, 11(9):959-965.

Deane R, Sagare A, Hamm K, Parisi M, Lane S, Finn MB, Holtzman DM & **Zlokovic BV** 2008 apoE isoform-specific disruption of amyloid β peptide clearance from mouse brain. *Journal of Clinical Investigation*, 118(12):4002-4013. PMCID: PMC2582453

Bell RD, Deane R, Chow N, Long X, Sagare A, Singh I, Streb JW, Guo H, Rubio A, Van Nostrand W, Miano JM & **Zlokovic BV** 2009 SRF and myocardin regulate LRP-mediated amyloid-β clearance in brain vascular cells. *Nature Cell Biology*, 11(2):143-53. PMCID: PMC2654279

Zhao Z, Sagare AP, Ma Q, Halliday MR, Kong P, Kisler K, Winkler EA, Ramanathan A, Kanekiyo T, Bu G, Owens NQ, Rege SV, Si G, Ahuja A, Zhu D, Miller CA, Schneider JA, Maeda M, Maeda T, Sugawara T, Ichida JK, **Zlokovic BV** 2015 Central role for PICALM in amyloid-β blood-brain barrier transcytosis and clearance *Nature Neuroscience*, 18(7):978-987. PMCID: PMC4482781

5. We started studies on protein C/activated protein C (**APC**) pathway in the CNS with therapeutic implications for stroke and neurological disorders with an overall goal to advance basic knowledge to translation to the clinic. These studies discovered **i)** direct vasculoprotective, BBB-stabilizing, neuroprotective, anti-inflammatory, pro-neurogenic and pro-angiogenic activities of APC and its cytoprotective-selective mutants using human and rodent cellular models, and rodent models of stroke, brain trauma and ALS; and **ii)** receptors and intracellular signaling pathways mediating cytoprotective effects of APC and its cell-signaling analogs. Our progress was translated in 2012 into clinical trials for ischemic stroke of 3K3A-APC, a cytoprotective-selective APC mutant with >90% loss of anticoagulant activity. 3K3A-APC is currently being evaluated in Phase 2 studies in 115 stroke patients (> 90% enrolled by 11/16). I served as the primary investigator in these studies.

Cheng T, Liu D, Fernández JA, Griffin JH, Castellino F, Rosen E, Fukudome K & **Zlokovic BV** 2003 Activated protein C blocks P53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. *Nature Medicine*, 9(3):338-342.

Guo H, Liu D, Gelbard H, Cheng T, Insalaco R, Fernández JA, Griffin JH, **Zlokovic BV** 2004 Activated protein C prevents neuronal apoptosis via protease activated receptors 1 and 3. *Neuron*, 41(4):563-572.

Liu D, Cheng T, Guo H, Fernández JA, Griffin JH, Song X & **Zlokovic BV** 2004 Tissue plasminogen activator neurovascular toxicity is controlled by activated protein C. *Nature Medicine*,10(12):1379-1383.

Cheng T, Petraglia AL, Li Z, Thiyagarajan M, Zhong Z, Wu Z, Liu D, Maggirwar SB, Deane R, Fernández J, LaRue B, Griffin JH, Chopp M & **Zlokovic BV** 2006 Activated protein C inhibits tissue plasminogen activator-induced brain hemorrhage *Nature Medicine*, 12(11):1278-1285.

Zhong Z, Ilieva H, Hallagan L, Bell R, Singh I, Paquette N, Thiyagarajan M, Deane R, Fernandez JA, Lane S, Zlokovic AB, Liu T, Griffin JH, Chow N, Castellino FJ, Stojanovic K, Cleveland DW & **Zlokovic BV** 2009 Activated protein C slows ALS-like disease by transcriptionally inhibiting SOD1 in motor neurons and microglial cells. *Journal of Clinical Investigation*, 119(11):3437-3449. PMCID: PMC2769191

Winkler EA, Sengillo JD, Sagare AP, Zhao Z, Ma Q, Zuniga E, Wang Y, Zhong Z, Sullivan JS, Griffin JH, Cleveland DW & **Zlokovic BV** 2014 Blood-spinal cord barrier disruption contributes to early motor neuron degeneration in ALS model mice. *Proceedings of the National Academy of Sciences USA*, 111(11):E1035-E1042. PMCID: PMC3964055

Wang Y, Zhao Z, Rege SV, Griffin JH, Goldman SA & **Zlokovic BV** 2016 3K3A-APC stimulates post- ischemic neuronal repair by human neural progenitor cells in mice. *Nature Medicine,* 22 (9), 1050-1055.

D. Research Support

2R01AG023084-11 Zlokovic (PI) 12/01/03-03/31/20 ACTIVE
Cerebrovascular β-Amyloidosis: Aβ CNS Transport Pathways
The role of PICALM in Aβ blood-brain barrier clearance and neuronal toxicity using murine and iPSC models.

2R01NS034467-17 Zlokovic (PI) 09/01/95-03/31/19 ACTIVE
Alzheimer's Aβ, Apolipoproteins and Blood-brain barrier
To determine the roles of apolipoproteins E2-E4 in Aβ vascular clearance in endothelium and pericytes.

1R01AG039452-4 Zlokovic (PI) 08/01/11-07/31/17 ACTIVE
The role of pericytes in the adult and the aging brain
To study how brain pericytes regulate neurovascular and neuronal functions.

9R01NS090904-17 Zlokovic (PI) 09/1/14-09/1/19 ACTIVE
Activated Protein C in Stroke Models
To develop 3rd generation of improved APC mimetic agents/biologics for stroke

5P50AG005142-30 ADRC Chui (PI) 04/01/2015-03/31/20 ACTIVE
Project 1: *Neurovascular Factors in AD* Zlokovic, Leader
Neurovascular function in participants with no or mild cognitive impairment with vascular risk profiles.

1P01AG052350-01 Zlokovic (PI) 9/30/16-5/31/21 ACTIVE
Vascular contributions to dementia and genetic risk factors for Alzheimer's disease
Multi-institutional program to test the neurovascular hypothesis of AD in individuals with genetic risk for AD (*APOE4*) and early autosomal AD (*PSEN1* mutations) and the rat model of ADAD (*APP; PSEN1* mutation).

Fondation Leducq Zlokovic (North American Coordinator);Wardlow (European Coordinator)
Transatlantic Networks of Excellence – 01/01/2017-01/01/22 ACTIVE
Understanding the role of the perivascular space in cerebral small vessel disease

1RO1NS100459-01 Zlokovic (PI) 9/30/16-10/01/21 ACTIVE
The role of pericytes in white matter disease
New models of pericyte ablation, mutiparametric MRI, viral-based connectomics, behavior and tissue analysis.

ALZHEIMER'S ASSOCIATION Zlokovic (PI) 11/1/16-11/30/21 ACTIVE
Vascular Contributions To Dementia and Amyloid and Tau Lesions in APOE4 Carriers Strategic 509279 Grant

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Southern California
Duns Number:	0729333930000
Street1*:	3720 South Flower Street
Street2:	
City*:	Los Angeles
County:	Los Angeles
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90089-0701
Project/Performance Site Congressional District*:	CA-037

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ● No

If YES, check appropriate exemption number: 1 2 3 4 5 6 If

NO, is the IRB review Pending? ● **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number 00007099

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3518-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Overall_Abstract_Final.pdf
8. Project Narrative*	Overall_Narrative_Final.pdf
9. Bibliography & References Cited	Overall_Bibliography_Final.pdf
10.Facilities & Other Resources	
11.Equipment	

Our overarching goal is to further resolve the neurodegenerative role of traffic-related air pollutants (TRAP), a ubiquitous exposure in urban areas where most older Americans reside. Our recently published epidemiologic data *showed strong association between elevated $PM_{2.5}$ (particulate matter <2.5μm) and increased dementia risk in women >65 years, with a bias for ApoE4 homozygotes (JC Chen in Cacciottolo et al 2017, PMID 28140404). In this same report, experimental studies of female mice from the Finch-Sioutas Labs showed that exposure to nPM (a nano-sized subfraction of TRAP) was pro-amyloidogenic with an ApoE4 bias.* Other changes include attrition of hippocampal CA1 neurites and myelin, which model selective damage in AD and cerebral ischemia. In a mouse stroke model, nPM exposure exacerbated cerebral ischemic damage (*William Mack: Liu et al 2016, PMID 27071057*). Differences by sex and ApoE alleles suggest sources of heterogeneity in human responses to TRAP.

We propose four projects: epidemiological studies of two nationwide cohorts of women (Project 1, JC Chen: Women's Health Initiative Memory Studies; WHIMS) and men (Project 2, C Franz and W Kremen: Vietnam Era Twin Study of Aging, VETSA) and two experimental studies of air pollution exposure (Project 3, Finch: mouse models of aging and AD; Project 4 Wm Mack, chronic ceebral hypoperfusion). These projects address a common set of questions: (1) What is the AD risk imposed by TRAP and does the associated risk vary by sex, life stage, and APOE/other alleles? (2) What neurodegenerative changes are induced by TRAP and what is the resulting risk for early cognitive decline of AD? (3) Which *brain pathways* are most susceptible to TRAP neurotoxicity? (4) Do shared mechanisms (e.g., amyloidogenesis, cerebrovascular damage and hypoperfusion, and neuroinflammation) predispose to premature cognitive decline and an increased AD risk? Two supporting Cores provide population neuroinformatics, neuroimaging of blood-brain-barrier (BBB) and myelinated tracts, large-scale air pollution modeling and epidemiology, inhalation exposure assessment and neurotoxicology. Neuroimaging Core B1 provides human brain imaging harmonized across sites and mediation analyses (Projects1-2). B2 provides high-resolution imaging of BBB and tractography for mouse models (Projects 3-4). Core C Environmental Exposure and Neurotoxicology subcore C1 harmonizes population exposure estimates for WHIMS and VETSA (Projects1-2); C2 provides inhalation exposure of mice *for studies of sex and ApoE allele responses (Project 3) and of chronic cerebral hypoperfusion (Project 4)*; C3 analyzes brain inflammatory protein responses to TRAP. For P01 integration, the Administrative Core builds on the infrastructure of AirPollBrain (led by Finch& Chen), a USC-funded collaborative network since 2010. Results of this program will advance understanding of TRAP contributions to AD risk and accelerated cognitive decline, and provide a rationale for preventive intervention in the environmental neurotoxicology of AD.

Relevance to Public Health [*revised***]**

The brain is vulnerable to air pollution. A multi-disciplinary and multi-institutional team from the University of Southern California *and their collaborators* will study how traffic-related air pollution (TRAP) from metropolitan areas where most older Americans reside *contributes to accelerated brain aging and risk of dementia*. In the next 5 years, the team will study human populations and experimental models to examine the brain pathways that are specific for Alzheimer disease for *gender and age differences in vulnerability to* TRAP. *These studies address the neglected role of common environmental neurotoxins in the development of Alzheimer disease and interactions with Alzheimer risk genes.*

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Caleb Middle Name E. Last Name*: Finch Suffix: Ph.D

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Street2: Department of Contracts & Grants
City*: Los Angeles
County: CA
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Zip / Postal Code*: 90089-0191

Phone Number*: 213-740-1758 Fax Number: 213-740-0853

E-Mail*: cefinch@usc.edu

Credential, e.g., agency login: cefinch

Project Role*: PD/PI Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1969

Attach Biographical Sketch*: File Name: Finch_NIH_bio_AirPoll_P01_2017.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Jiu-Chiuan Middle Name Last Name*: Chen Suffix: M.D.

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Organization Name*: University of Southern California
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Credential, e.g., agency login: JC_Chen

Project Role*: PD/PI Other Project Role Category:

Degree Type: MD,SCD,MPH Degree Year: 1992

Attach Biographical Sketch*: File Name: JC_Chen_New_Format_Biosketch_0523-2017.pdf

Attach Current & Pending Support: File Name:

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OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	Overall_Intro_Final.pdf
Research Plan Section	
2. Specific Aims	Overall_Aims_Final.pdf
3. Research Strategy*	Overall_ResStrat_Final.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	MultiplePILeadershipPlan_Final.pdf
12. Consortium/Contractual Arrangements	8315_Franz_Final_UCSD_Consortium.pdf
13. Letters of Support	LETTERS_OF_SUPPORT.pdf
14. Resource Sharing Plan(s)	
15. Authentication of Key Biological and/or Chemical Resources	
Appendix	
16. Appendix	

INTRODUCTION TO THE OVERALL PROGRAM

1. Integration across human versus rodent projects: shared hypotheses; mechanisms. See 'Overall Program A3 and Approach, Summary of Scientific Projects C1'. *We hypothesize that the adverse effects of traffic- related air pollutants (TRAP) on cognitive impairment and increased risk for MCI/AD are mediated through multiple convergent neuropathological pathways (cerebrovascular pathology; amyloid pathology; tauopathy) that are modulated by APOE4 and sex. Traffic-related air pollutants (TRAP)-associated cerebrovascular injuries will be evaluated by the volume of white matter hyperintensities measured by structural brain MRI in populations (Projects 1&2). For mouse studies (Projects 3&4), cerebrovascular and white matter tract responses to TRAP exposure will be assessed by in vivo multiphoton imaging for regional CBF and BBB permeability and angiography. DTI-MRI (7.0T) brain imaging at 80μm isotropic spatial resolution will assess white matter connectivity in fractional anisotropy maps.*

2.Dose-exposure data:

a.for human projects: variation of air pollution exposure data across different regions in the country and different time/place for the study populations.

The proposed approaches will account for the variation in air pollution exposure present in both the modeled environmental processes and constructed individual residential histories. The standard MESA-AIR models follow a "regionalized" framework combining temporal basis functions with spatially-varying coefficients and spatiotemporal residuals[1] (see Core C1). This approach captures both regional- and local-scale exposure variability. The WHIMS prospective design allows the Project-1 to account for time-varying exposure levels due to relocation since enrollment, and we could estimate the residence-specific exposures (weighted by the duration at each location) already used in the published work.[2-4] This approach will be applied to Project-2 built on VETSA.

b.for animal projects: much higher doses than the estimated exposures in humans; further definition and justification of the exposure dosing in animal studies may be needed. See Project 3 Methods and Core C2. *The mouse exposure to PM0.2 at 300 ug/m^3 for 150 h (1% of the lifespan) delivers 2.25 mg/kg. For humans, exposure to PM0.2 at 30 ug/m^3 for 1% of lifespan yields 2.5 mg/kg, assuming inhalation rates: mouse, 0.025 L/min; human, 12 L/min.*

3.Harmonization of neuroimaging for human projects

ADNI, WHIMS, and VETSA scans will be analyzed separately. We will adjust for scanner strength and site effects in all within-cohort analyses that contain multiple sites or scanner strength. To analyze cross-site data within VETSA, we will use random effects meta-analyses, which avoids the need to use the same scan protocol across sites and gives more robust effects than aggregated mega-analysis. We have related expertise leading ENIGMA (http://enigma.ini.usc.edu/), in which we pool scans from >60 sites to detect genetic, epigenetic, and disease-related brain effects.

4.Distinction between mouse Projects 3 & 4. See Project 4 Introduction. *To maximize connections between the projects and increase the overall interaction of the program grant, Project 4 employs similar exposure paradigms (nPM, age, mouse strain) and outcome assessments as Project 3. Project 4 is designed to assess the effects of nPM in an experimental paradigm that isolates an exclusively vascular influence/ risk factor for AD and cognitive decline (CCH). While the exposure paradigm is intentionally shared, the scientific questions are independent and different. Project 4 examines synergistic relationships between nPM exposures and CCH. As stated, critical vascular endpoints in Project 4 examine effects of age in nPM/ CCH exposed mice. Project 4 hypothesizes that nPM-CCH will not show a "ceiling effect" seen in mice exposed to only nPM and described in Project 3. Additionally, Project 4 Aim 3 now examines TLR4 and C5 interplay as a central mechanism of injury in nPM/CCH mice.*

5.New institutional support from three USC deans. See Administrative Core 2.A.2. The development of this new program of environmental neurosciences has received continuing support from the Provost's Research Office since 2010. *For the P01 resubmission, new support is committed from USC Vice President, Prof. J Randolph Hall, for $20,000/year (see Support letter); from the Keck School of Medicine for 3% of Indirects/yr for 5 years (email documentation from Keck Dean Rohit Varma and Tom Buchanan, Vice-Dean for Research); from the Leonard Davis School of Gerontology for $40,00/year (support letter from Dean Pinchas Cohen); and from the Viterbi School of Engineering for $5,000/yr for 5 years (support letter from Vice Dean Moghaddam). Total institutional support is $401,200 for the entire program period.*

OVERVIEW OF PROGRAM SPECIFIC AIMS

The long-term goal of this program is to resolve individual risk, heterogeneity, and biological basis of Alzheimer's disease (AD) associated with exposure to ambient air pollution in aging populations. The development of this new P01 follows recommendations by the 2015 AD Research Summit for conducting "*Interdisciplinary Research to Understand the Heterogeneity and Multifactorial Etiology of Disease.*" [1, 2]

Older adults are most susceptible to the adverse effects of ambient air pollution[3, 4] that is well known to impact respiratory, immune, and cardiovascular systems. Increasing evidence from the environmental health sciences (EHS) literature has pointed to the neurotoxic effects of outdoor air pollution, including pollutants from regional emissions and local sources such as vehicular exhausts. Our investigative team is empowered by the USC-funded AirPollBrain Network to develop the collaborative network at the frontier of environmental neurosciences in air pollution and brain aging. We focus on traffic-related air pollutants (TRAP), because traffic is the biggest source of urban air pollution, accounting for 25% of ambient $PM_{2.5}$ (particulate matter with aerodynamic diameters <2.5μm) across the world.[5] In the US, TRAP has become a major public health concern,[6] because of its association with a wide range of adverse health outcomes. With NIA's critical support from NIA (RF1AG051521, R21AG040683, R21AG040753, R21AG050201), USC investigators have started translating the new EHS knowledge into studying the contribution of TRAP to brain aging in experimental models.[7-12] Since 2014, three epidemiologic studies[13-15] in non-US populations have linked regional air pollutants (including $PM_{2.5}$) with an increased risk for dementia. NIA support (R01AG033078; R21AG051113) also helped established the first epidemiologic evidence for association of increased dementia risk with regional $PM_{2.5}$ exposure in an US-wide cohort of older women which has ApoE4 bias (Fig.2, section A4). However, whether TRAP increases AD risk in both women and men remains unclear.

This new P01 proposes to further resolve the role of TRAP, a ubiquitous exposure in metropolitan areas where over 75% of older Americans reside. Specifically, this application assembles four projects, including two community-based cohort studies and two neurotoxicological experimental projects. These projects are cohesively designed and integrated to address a common set of four fundamental questions:

Q1: What is the AD risk imposed by TRAP and does the associated risk vary by sex, life stage, APOE and other alleles?

Q2: What neurodegenerative changes are induced by TRAP and what is the resulting risk for early cognitive decline of AD?

Q3: Which brain regions are most susceptible to TRAP neurotoxicity?

Q4: Are there shared underlying mechanisms predisposing to premature cognitive decline and an increased AD risk?

We formulate these questions, based on a conceptual framework which combines new data from air pollution epidemiology and neurotoxicology with state-of-the-knowledge in cognitive neurosciences, clinical neuroimaging and neuropathology as related to the life course and mechanisms of AD. The two population studies are designed cost-efficiently, each built on a geographically-diverse cohort of women (from the Women's Health Initiative Memory Studies [WHIMS]; Project-1) and men (from the Vietnam Era Twin Study of Aging [VETSA]; Project-2). The proposed studies on WHIMS and VETSA examine putative associations of TRAP with early neuroanatomic biomarkers predictive of increased risks for AD, mild cognitive impairment, and preclinical AD. We will assess whether/how exposure before and/or during late life influence the neuropsychological processes; elucidate the brain structure/neuropathology affected by TRAP; and assess the biological susceptibility to adverse TRAP effects on brain aging. Projects 1 and 2 innovate by bringing together sophisticated spatio-temporal air pollution modeling and novel neuroimaging to advance our knowledge in population neurosciences of TRAP and brain aging. In Projects 3 and 4, mouse models are exposed to precisely defined air pollution for neurotoxicological responses of AD-specific pathways. Cutting-edge mouse brain imaging studies are integrated in mouse models (AD transgenic mice; chronic cerebral hypoperfusion; inducible microglial knockout of TLR4) to address mechanistic links of TRAP to amyloidogenesis, cerebrovascular damage, and neuroinflammation. Each project leverages expertise and unique resources from two Supporting Cores equipped with state-of-the-art tools and analyses in environmental epidemiology, exposure sciences, air pollution neurotoxicology, population neuroinformatics, and white matter and neurovascular brain imaging. Findings from the proposed research will improve our understanding of the critical and greatly understudied role of environmental contributions to AD and related dementia[16-18]: With our novel environmental neuroscience approaches, this PO1 can provide new impetus for preventive intervention and deciphering the environmental neurobiology of AD.

P01 OVERVIEW

A. Significance [*revised*]

A1. Public Health Significance of Studying Traffic-Related Air Pollutants and ADRD Risk [*revised*]

Our recent epidemiologic findings show strong associations of elevated $PM_{2.5}$ (particulate matter <2.5μm) with white matter loss[4] and increased dementia[1] risk in a US-wide cohort of older women (>65 years) from the Women's Health Initiative Memory Study (WHIMS) (Fig.2 below, R01AG033078; PI: Chen). Correspondingly, experimental studies show that nPM (a nano-sized subfraction of $PM_{2.5}$) exposure in urban environments induces oxidative stress with selective attrition of hippocampal neurites and glutamate receptors and also increases amyloid deposits in AD transgenic female mice *with ApoE4 preference* [1] (Fig. 3 below; RF1AG051521; PI: Finch). This revised P01 application proposes to further resolve the role of traffic-related air pollutants (TRAP),[23] a ubiquitous exposure in metropolitan areas where >75% of older Americans reside.[24] Since 2014, seven studies[1, 17-19, 25-27] reported an increased risk for dementia associated with exposures to regional air pollutants (including $PM_{2.5}$)[1, 17-19, 26] or TRAP.[25, 27] *Using the medical claims data from 2.2 million adults aged 55-85 years who resided in Ontario, Canada,[25] investigators found that the dementia risk increased by 7%, comparing people living less than 50 m from a major traffic road versus >300 m. This could translate to 7-11% of dementia cases among those with high exposure to traffic; the elevated risk remained (~5%) even after accounting for regional air pollutants. However, their approach did not allow for ascertaining the clinical AD cases.* We recently expanded our analyses of WHIMS outcome data and showed that high $PM_{2.5}$ exposure (exceeding the US EPA standard of 3-year average >12 μg/m^3) in 1999-2010 increased AD risk by 71% (see section A4). Traffic accounted for 15%-50% of source-related $PM_{2.5}$ across the US, depending on regions and differences in primary vehicular sources.[28-31] If the hypothesized causal link is substantiated by the proposed research in both sexes, long-term exposure to TRAP could account for 5-16% of incident AD cases.[32]

A2. Ambient Air Pollution – a Novel Environmental Determinant of Brain Aging [*revised*]

Growing evidence supports the emerging concept that exposures to ambient air pollutants are a novel environmental determinant of brain aging.[33] In the last few years, cross-sectional studies reported relatively low performance in various tests of cognitive function among adults and older people residing in places with higher levels of ambient air pollutants (including $PM_{2.5}$ [34-36] and ozone[36, 37]) or in proximity to major roadways.[38, 39] Adverse PM effects on cognitive declines were also shown in most longitudinal studies.[40-43] *Of the seven reports [1, 17-19, 25-27] of increased risks for dementia associated with ambient air pollution, only three non-US studies examined TRAP. However, there were notable limitations in their exposure characterization. One study only measured the proximity to major roadways,[25] the second study relied on aggregated exposure estimates[26] prone to ecological biases, or the third study using a spatial model towards the end of study follow-up to estimate the NO_x exposure in earlier years,[27] which obscured the temporality of the reported association.*

We identify **six critical knowledge gaps in the current literature on air pollution-neuroepidemiology of pathological brain aging, which are addressed in Projects 1 and -2**. (1) Dearth of prospective cohort data linking individual-level exposure estimates with increased ADRD risks in US-based populations (except our recent findings;[1] and preliminary data from Project-1, section A4). (2) No studies have examined the putative exposure effects on early biomarkers predictive of AD. (3) The existing evidence linking air pollution with cognitive aging was not specific to understanding the exposure effects of early cognitive decline predictive of AD. (4) No study has examined mid-life (e.g., aged <60 y) TRAP exposure effects on brain aging. (5) Very few neuroimaging studies elucidated the vulnerable target areas in adult brains and mechanistic pathways underlying the neurotoxicity of air pollution. (6) We lack longitudinal data to examine individual susceptibility to and gene-environment interactions in neurotoxic effects of air pollution on ADRD risk (except our recent findings;[1] see Fig.2 below in Section A4).

Mechanisms in air pollution neurotoxicology are proposed in **Projects 3-4**, using the same mouse genotypes and paradigm for exposure to TRAP, to **address six critical knowledge gaps**. (1) How closely do neurodegenerative pathways in AD match the brain regions responding to air pollution; (2) How do sex and age alter neurodegenerative and white matter vulnerability to TRAP. (3) How do human ApoE3 and E4 alleles alter vulnerability. (4) How does cerebral hypoperfusion alter vulnerability. (5) Does TRAP exposure alter the blood-brain barrier. (6) *What is the role of microglial TLR4 in neurodegenerative responses to TRAP.*

A3. Overall Framework of Proposed P01 Application [*revised*]

Figures 1 depicts the overall conceptual framework of this program project linking exposure to TRAP with neuropathology, adverse subclinical neurotoxicity, and risk for AD in both populations and experimental models. Based on this framework, we posit that the adverse effects of TRAP on cognitive impairment and

increased risk for MCI/AD are mediated through multiple convergent neuropathological pathways (cerebrovascular pathology; amyloid pathology; tauopathy) that are modulated by APOE4 and sex. The

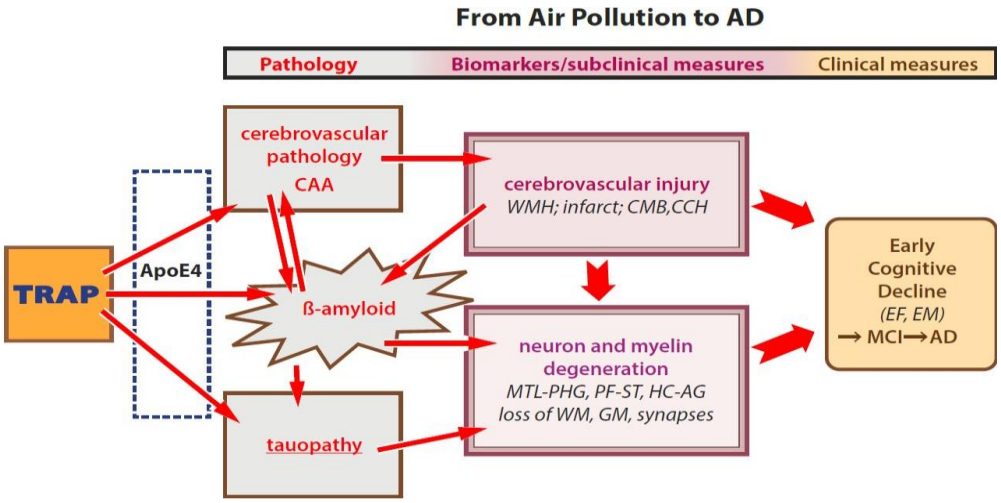


Figure 1: Schematic outline of neurodegenerative pathways from air pollution to AD. ApoE4, the AD-risk apolipoprotein E4 isoform; CAA, cerebral artery amyloid; CMB, cerebral microbleeds; CCH, chronic cerebral hypoperfusion; EF, executive function impairments; EM, episodic memory impairments; GM, grey matter; HC-AG, hippocampus-amygdala; MTL-PHG, medial temporal lobe-parahippocampal gyrus; PF-ST, prefrontal-striatal; WMH, white matter hyperintensities.

cerebrovascular hypothesis is increasingly recognized, as highlighted in editorials by JC Chen (Co-PI of P01).[44, 45] Extensive literature documents that regional air pollutants, especially the airborne particles, increase the risks of coronary heart disease and stroke.[46-48] Epidemiologic data from recent cohort studies further pointed to the important role of TRAP contributing to the increased risk and mortality of cerebrovascular disease.[48-50] These putative TRAP-associated cerebrovascular injuries will be evaluated by the volume of white matter hyperintensities measured by structural brain MRI in populations (Projects1 & 2). Project-2 will also use extant data on arterial spin labeling to assess cerebral hypoperfusion in men. Projects 1 and 2 will examine the TRAP-induced neurodegeneration by investigating cortical thinning and GM/WM volume loss measured by structural MRI. For mouse studies in Projects 3&4, blood-brain barrier (BBB) integrity after TRAP-nPM exposure will be assessed by 7.0T MRI brain imaging with gadolinium tracer. Recently published data of Project 3 showed TRAP-nPM induced hippocampal CA1 neurite atrophy [1, 51] and *CA1 subregional myelin degeneration*[51] that model the greater vulnerability of CA1 neurons to AD and cerebral ischemia. TRAP-induced cerebrovascular hypoperfusion affecting myelin and neuron degeneration (Project 4; in collaboration with Project 3) *will be examined in hippocampal subregions by histochemistry* and by Western blots for myelin and neuron proteins. The proposed studies of these putative brain structural neurotoxicity will focus on the medial temporal lobe-hippocampus memory system [52] and the frontostriatal-executive system, [53] vulnerable neural networks affected in brain aging and in AD.[54]

A4. Environmental Neurosciences of Brain Aging

USC investigators have led new discoveries in the emerging field of environmental neurosciences of ADRD in human populations[1, 3, 4, 36] and experimental studies[1, 11, 13, 14, 16, 51, 55-57] with critical NIA support. Our recent report [1] (R01AG033078, PI: Chen), showed that elevated $PM_{2.5}$ (3-year average >12 μg/m^3) was associated with 1.65-fold higher risk of accelerated cognitive decline and 1.92-fold increased risk of dementia (Fig.2A), with potentially greater impact in ApoE ε4 carriers (Fig.2B). We employed a conventional spatiotemporal modeling approach[58, 59] to estimating $PM_{2.5}$ and examined its adverse effects using Cox models to estimate the hazard ratios (HRs) for global cognitive decline (>8-point loss in modified mini-mental state exam) and dementia risk among non-Hispanic white women (N=3647 with ε3 or ε4; aged 70.3±3.7) in 1999-2010. Our results showed that residing in high $PM_{2.5}$ locations (exceeding the US EPA standard of 3-year average >12 μg/m^3) increased the incidence of global cognitive decline and all-cause dementia, and these observed adverse $PM_{2.5}$ effects were greater among women of ε4/4. The overall incidence rate of accelerated global

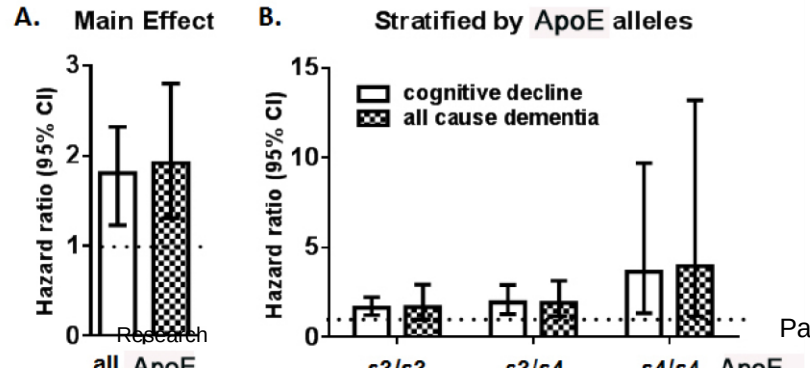


Figure 2. Adverse effects of $PM_{2.5}$ exposure on pathological brain aging, stratified by APOE alleles, in Cox models. Hazard ratios for adverse outcomes were estimated with high (3-year averages $PM_{2.5}$ >12 μg/m^3) versus low exposure, all adjusting for age, geographic region, spatial random effect, years of education, household income, employment status, lifestyle factors (smoking; alcohol use; physical activities), and clinical characteristics (use of hormone treatment; depression; body mass index; hypercholesterolemia;

cognitive decline increased by 81% (hazard ratio or HR=1.81; 1.42-2.32), comparing older women residing at locations with high versus low $PM_{2.5}$ (Fig.2A). The incidence of all-cause dementia in older women with high $PM_{2.5}$ exposure was 92% higher (HR=1.92; 1.32-2.80) than the incidence with low exposure (Fig.2B). Incidence rate for all-cause dementia associated with high $PM_{2.5}$ exposure increased by 68% (HR=1.68; 0.97-2.92), 91% (HR=1.91; 1.17-3.14), and 295% (HR=3.95; 1.18-13.19) respectively in ε3/3, ε3/4, and ε4/4 carriers. High $PM_{2.5}$ exposure was also associated with greater incidence of global cognitive decline by 65% (HR=1.65; 1.23-2.23), 93% (HR=1.93; 1.29-2.90), and 264% (HR=3.64; 1.36-9.69) in women of ε3/3, ε3/4, and ε4/4 alleles. Incidence rate of AD increased by 71% (HR=1.71; 1.18-2.48) associated with residing in location with high $PM_{2.5}$ exposure in the full sample (n=134 AD in the final adjusted model; N=6128). The relatively small number of AD cases limited our further assessment of interaction with APOE alleles.

Studies with mice showed that airborne ultrafine particulate matter from urban TRAP is proamyloidogenic[1] (R01AG051521, PI: Finch). EFAD mice (transgenic for human ApoE alleles and Familial Alzheimer disease mutations) showed *sex-ApoE differences in cerebral cortex amyloid deposits after chronic exposure to a nPM, which is a nano-sized subfraction of $PM_{2.5}$.*[16] *Female E4FAD showed the strongest response, while male EFAD did not respond. The increase of fibrillary amyloid deposits in nPM-exposed E4FAD mice (Fig.3A; unpublished) is the first experimental evidence for sex differences in response to TRAP components for brain amyloid deposition and is consistent with greater risks for ADRD associated with high $PM_{2.5}$ in older women ε4/4 carriers (Fig.2B).* Wildtype mice also responded to chronic nPM, with a pro-amyloidogenic shift in processing of the endogenous murine APP towards Aβ production, with an increased ratio of sAPPb:a (Fig. 3B). These novel findings extend prior reports on wild-type rodents, two with inhaled exposure to

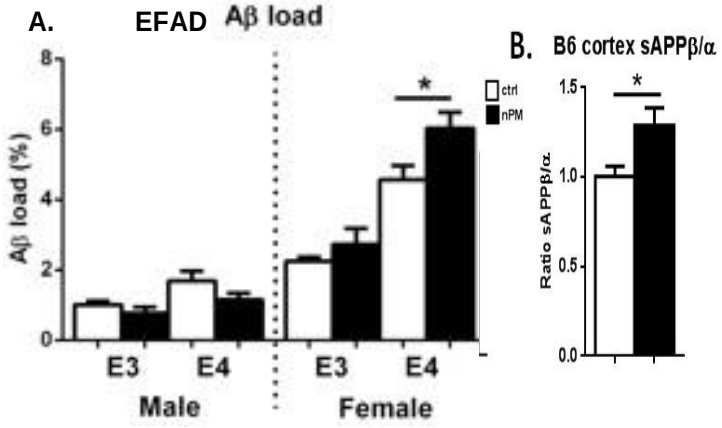


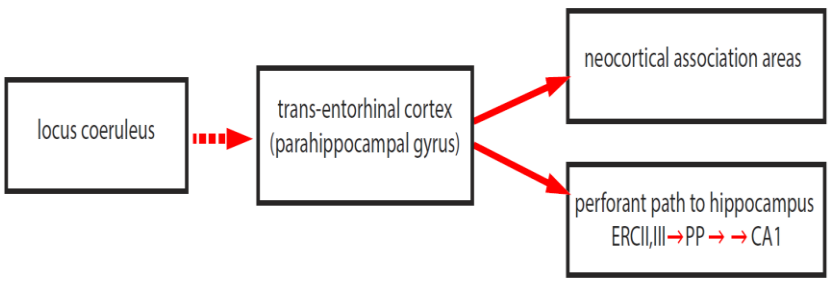
Figure 3. TRAP-nPM is proamyloidogenic for EFAD and C57BL/6 mice. (A). Female E4FAD mice chronically exposed to nPM had robust increase of Aβ42 deposits in cortex (immunoreactive area), while male EFAD did not respond. (B). Male C57BL/6 mice ('B6', the background strain of EFAD) with chronic nPM had a pro-amyloidogenic shift of APP processing (an increased ratio of the sAPPb:a peptides by Western blot). N=7-9, Mean+/-SEM, *p<0.05

diesel particles [60, 61] and others using nickel-nanoparticle enriched ambient air[62] or concentrated $PM_{2.5}$, [63] all with increased endogenous soluble Aβ peptide. Wildtype rodents have never shown fibrillary Aβ deposits during aging, possibly because the endogenous rodent Aβ peptide has less aggregation to neurotoxic forms because of three amino acid differences from the human,[64]. Together, these findings support the hypothesis that **TRAP-nPM accelerates cognitive decline and increases dementia risk by synergizing with AD pathway-specific neurodegenerative mechanisms**. A precedent for these air pollution ApoE4 interactions is the greater vulnerability of ApoE4 carriers to an increased AD risk associated with pesticide exposure.[65, 66] Downstream effects of TRAP-nPM include degeneration of hippocampal CA1 myelinated neurons that mediate episodic memory and that are selectively vulnerable to AD and ischemia. [67]

A5. Novel Mechanistic Pathways of Air Pollution Neurotoxicity [*revised*]

We propose an overarching neuropathogenetic hypothesis that TRAP exposure accelerates cognitive decline and increases the risk of AD by promoting *AD-specific neurodegenerative pathways* (**Figure 4**), which spread progressively from the entorhinal cortex to the hippocampus, with extensive white matter deterioration. The locus coeruleus is especially vulnerable to toxins and likely the earliest site of tauopathy.[68] The propagation of

Figure 4: Schema of AD neurodegenerative pathways. The locus coeruleus shows the earliest tauopathy, followed by the trans-entorhinal cortex, and thence to other neocortical and subcortical regions. Neurons of entorhinal cortex (ERC) layers II and III project via the perforant path (PP) to hippocampal neurons of the dentate gyrus, CA3 and CA1.



AD neuropathologies follows a pathway of neurodegeneration from the entorhinal cortex in the medial temporal lobe (MTL) to the hippocampus via the myelinated perforant path (PP).[67, 69] These postmortem findings are further supported by brain imaging MR studies, which show a sequence of neurodegeneration in the MTL posterior parahippocampal gyrus white matter (PWM) that spreads via the PP to the hippocampus. Consistent data from human brain imaging studies have shown the atrophy of MTL-parahippocampal gyri (MTL-PHG) and hippocampal-amygdala (HC-AG) subfields as the early neuroanatomic biomarkers of AD.[70-73] For instance, clinical AD brains had smallest PMW volumes, while amnestic MCI had intermediate PWM loss.[74, 75] Moreover, cognitively normal elderly with initially smaller PWM volumes incurred accelerated memory decline and increased AD risk.[76] Because of the shared vulnerability of hippocampal CA1 neurons to AD and to cerebral ischemia,[67] TRAP exposure may promote convergent neurodegenerative processes from cerebrovascular and neuronal interactions. Aspects of this overarching hypothesis are addressed by each project (Table C below). *Project-2 also has unique data on pupillary responses reflecting the activation of locus coeruleus[77] where the AD pathology may first appear*, as suggested by neuropathology in young adults with "pre-tangle" tauopathy propagating rostrally to the trans-entorhinal region of the MTL. [69, 78, 79] Together the four projects will assess WM neurodegeneration as an early signature of TRAP neurotoxicity.

A6. Traffic-Related Air Pollutants Urban-dwelling populations, especially those living in close proximity to the roadways, are potentially exposed to high levels of traffic-related air pollutants (TRAP) from motor vehicle emissions. TRAP represents a complex mixture of the EPA-regulated criteria air pollutants (e.g., carbon monoxide; nitrogen dioxides [NO_2]; PM) and other hazards. Despite the improving air quality in the past two decades,[80] with both the vehicle miles traveled[81 82] and size of urban-dwelling population continue to grow, the TRAP and its contribution to PM exposure will remain a major public health concern. A recent CDC report linked the 1999-2008 National Health and Nutrition Examination Surveys (NHANES) to the National Highway Planning Network,[83] showed that more than 40% of the geocoded locations were within 500m of one or more major roads, which is often considered the TRAP exposure zone.[84] For this P01 application, we focus on TRAP primarily from automobile exhaust near roadways for two main reasons. First, epidemiological studies have linked exposure to automobile exhaust with a wide range of adverse health outcomes (e.g., cancers, heart disease, asthma, preterm birth, autism).[10, 84] However, the impact of long-term TRAP exposure on brain aging remains unclear (as reviewed in Project-1&-2). Second, the literature in air pollution neurotoxicology[33, 85-88] points to the critical role of smaller-sized airborne particles in TRAP. Although there is interest in assessing health risks from brake-wear, tire-wear or road-dust suspension,[89-92] the neurotoxicity of these "non-exhaust" emissions is not well defined. The mixture of TRAP can be difficult to measure and model, because it is inherently dynamic in place and time. For the epidemiologic studies, TRAP will be characterized by the estimated ambient levels of NO2 (a gaseous surrogate), elemental carbon (EC) component of $PM_{2.5}$ (a marker of diesel exhaust),[93-96] and predicted source profiles of $PM_{2.5.}$ After obtaining the geocoded information over time, we will conduct the individual-level, residence-specific exposure estimation of these TRAP exposures, using state-of-the-art spatiotemporal modeling approaches (Core C1). Studying exposure to ambient air pollutants near residences is particularly relevant to aging Americans, because elderly people may spend most of their time on core activities in residential neighborhoods and local areas.[97, 98] For experimental studies, mice are exposed to a nano-sized (nPM) subfraction of $PM_{2.5}$ collected near a local urban freeway in Los Angeles.[16] Although primary emissions from diesel engines are used as a model TRAP pollutant, we prefer TRAP-nPM from near-roadway air samples as more representative of real-world exposure (see Core C2).

B. Innovation [*revised*]

The following features define the innovation of this PO1 application:

(1) The proposed interdisciplinary research program follows a cohesive conceptual framework to investigate the impact of TRAP on neuropathology, biomarkers/subclinical measures of neurodegeneration and cerebrovascular damage, and long-term consequence on early cognitive decline predictive of AD.

(2) In Projects 1&2, state-of-the-art spatiotemporal air pollution models are brought together with the novel population neuroimaging approaches to advancing our knowledge in population neurosciences of TRAP and brain aging.

(3) In Projects 3 & 4, air pollution inhalation toxicological approach and cutting-edge mouse brain imaging studies are integrated with mouse experimental models: EFAD transgenic mice*; chronic cerebral hypoperfusion and a new inducible microglial targeted TLR4-knockout mouse to probe mechanisms* of TRAP neurotoxicity.

(3) The design of our P01 population studies is cost-efficient by building on two large, unusually well-characterized, geographically diverse cohorts of women and men with unique clinical, neuropsychological, and structural brain MRI data NIA had invested for more than ten years.

C. Approach [*revised*]

Four scientific projects are designed cohesively and integrated to address a common set of four fundamental questions. In the Table C, we summarized the respective contribution of each project, including the key design feature and methodological approaches.

Table C: Four Scientific Projects Address a Common Set of Fundamental Questions *[revised]*

Q1: What is the AD risk imposed by TRAP and does the associated risk vary by sex, life stage, and APOE/other alleles?
Q2: What neurodegenerative changes are induced by TRAP and what is the resulting risk for early cognitive decline of AD?
Q3: Which brain regions are most susceptible to TRAP neurotoxicity?
Q4: Are there shared underlying mechanisms predisposing to premature cognitive decline and an increased AD risk?

	Project-1	Project-2	Project-3	Project-4
	Traffic-related air pollutants and AD: risk, susceptibility, and mechanisms in women	Urban air pollution and pathological brain aging: a nationwide twin study in men	Age-sex-ApoE allele interactions in myelinated neuron vulnerability to air pollution	Urban air pollution and chronic cerebral hypoperfusion (CCH): aging and sex Influences
Population Context /Experimental Models	WHIMS: a joint cohort of post---menopausal & older women, 48 states	VETSA: a cohort of mid---aged (51---60) male twins, 49 states	Mice: Wildtype; EFAD (human ApoE & AD genes), *i-m TLR4-ko*	Mice: Wildtype; EFAD (human ApoE & AD genes); *i-mTLR4-ko*
Primary exposure measures/focus	NO_2; EC---$PM_{2.5}$; traffic $PM_{2.5}$	NO_2; EC---$PM_{2.5}$; traffic PM2.5	TRAP---nPM	TRAP---nPM & CCH
Basic Questions				
Q1 AD risk imposed by TRAP by sex, life stage, and APOE				
TRAP→→ AD risk	predicted risk scores for preclinical AD/MCI/AD; incident MCI	predicted risk scores for preclinical AD/MCI/AD	Aβ deposits; neuron atrophy	Aβ deposits; neuron atrophy
by sex	in women	in men	male + female	male + female
Exposure by life stage	Aged 50---54 & > 65	aged 30---40 & 50---60	young and middle aged	young and middle aged
by genetic factors	ApoE4	ApoE4; genetic risk scores	ApoE4	ApoE4
Q2 What neurodegenerative changes are induced by TRAP				
Neurodegenerative changes	risk for Aβ(+) brain change; risk for tau---related brain change	risk for Aβ(+) brain change; risk for tau---related brain change	corpus callosum myelin hippocampal CA1 neurites and myelin.	corpus callosum myelin; hippocampal CA1 neurites and myelin
Early cognitive decline predictive of AD	episodic memory; executive functions	episodic memory; executive functions; pupillometry---based risk indices	spatial working memory and other cognitive impairments	spatial working memory and other cognitive impairments
Q3 Which brain regions are most susceptible				
TRAP→→ vulnerable brain regions	HC; MTL; fronto---striatal	Hippocampus; MTL; prefrontal cortex; frontal WM	corpus callosum myelin; perforant path; hippocampal CA1 neurons and myelin	corpus callosum myelin; perforant path; hippocampal CA1 neurons and myelin
Q4 Shared underlying mechanisms				
GM damage	cortical thinning; reduced HC and MTL volumes	cortical thinning; reduced HC and MTL volumes	Aβ production; neuron atrophy/ death;	Aβ production; neuron atrophy/death;
WM damage	regional WM loss; MTL WM loss	regional WM loss; MTL WM loss; reduced WM integrity	regional WM loss; myelin damage; WM tract integrity	regional WM loss; myelin damage; WM tract integrity
Cerebrovascular injuries	increased WMH	increased WMH; cerebral hypoperfusion	cerebral blood flow; BBB leakage, CAA & cerebral microbleeds	cerebral blood flow; BBB leakage, CAA & cerebral microbleeds
Inflammation	no direct measures	no direct measures	*i-m TLR4-ko* and EFAD inflammatory responses	activation of complement; induction of TLR4

BBB: blood-brain barrier; CCH: chronic cerebral hypoperfusion; EFAD, mice carrying human ApoE3-4 alleles and familial Alzheimer Disease genes; HC: hippocampus; *i-m TLR4-ko, inducible microglial targeted TLR4-knockout mouse;* MTL: medial temporal lobe; WMH: white matter hyperintensities.

Summary of Scientific Projects

Project-1 aims to: (1) determine the impact of TRAP on early biomarkers predictive of increased risks for AD, MCI, and preclinical AD in older women; (2) examine the associations of cognitive decline reflecting early AD with TRAP exposure before/during late life; (3) elucidate the brain structure and neuropathology mediating the TRAP effects on pathological brain aging, using both targeted and agnostic approached with high-dimensional neurocomputation; and (4) evaluate the contribution of ApoE4 and vascular brain injuries to the individual susceptibility to neurotoxic TRAP effects. Extended the NIA-funded study (R01AG033078), Project-1 is built on the joint cohort of mid-aged and older women in the WHIMS of Younger Women (WHIMS-Y; n=1326, inception age 50-54) and WHIMS-MRI (n=1403, inception age 65-80) followed annually since 1996, both with comparable longitudinal assessments of neuropsychological functions.

Complementary to Project-1 in women, Project-2 is built on the VETSA (Veterans Twin Study of Aging), a nationwide prospective study designed to identify early biomarkers of late-life cognitive impairment by following a twin cohort of men starting at late midlife (age 51-60). Project-2 will (1) assess TRAP effects on AD-related brain signature risk scores; (2) assess the impact of TRAP on cognitive and brain aging; and (3) examine gene-environment interactions. The twin design of VETSA plus the proposed MZ co-twin analyses provides major methodological advantages to control for potential confounding by early life environment and genetic factors. Project-2 unique data include Diffusion Tensor Imaging, arterial spin labeling, and pupillary-based cognitive responses as biomarkers of early cognitive decline reflecting locus coeruleus dysfunction.

Projects 3 and 4 use mouse models of TRAP exposure to probe age and sex differences in AD risk and mechanisms of neurotoxicity. Project 3 defines the progression of neuron and white matter degeneration and cerebrovascular disturbances in wildtype and EFAD mice exposed to TRAP for age and sex differences in vulnerability. The new microglial-targeted TLR4-ko mouse (i-m TLR4) will test microglial TLR4 roles in neurodegenerative responses to air pollution. Project 4 introduces interactions of TRAP with chronic cerebral hypoperfusion. Because ApoE4 carrying women have greatest AD risk, mice carrying human ApoE alleles (EFAD) will be studied for sex interactions with TRAP.

Summary of Supporting Cores

Administrative Core A: (1) provides the integrated program leadership and coordination; (2) strengthen existing partnerships and facilitate new collaborations across USC and affiliate institutes; (3) ascertain scientific progress of the program by joint monthly meetings, quarterly work-in-progress meetings, and annual meetings with External Advisory Committee; and (4) provide fiscal management and administrative services and monitor resources- expenditures; and (5) carry out the joint dissemination to the broader scientific community

Neuroimaging Core B includes two subCores: B1 (Population Neuroimaging) to provide high-quality human MRI brain image analysis in support of Projects 1 & 2; and B2 (Mouse Brain Imaging) to acquire and analyze mouse brain MRI scans in support of Projects 3 & 4.

Environment Exposure & Neurotoxicology Core C has three subCores: C1 (Environmental Data Support) to provide harmonized measures of the residence-specific exposure estimates in populations; C2 (Inhalation Exposure) to collect TRAP-nPM for standardized inhalation exposure in animal models; and C3 (Neurotoxicology) to analyze the brain protein responses to TRAP in mice from Projects 3 &4.

Environment: USC is becoming a national leader in building basic, clinical, and translational neurosciences. During the past 5 years, USC recruited leading investigators: Arthur Toga and Paul Thompson (Lab of Neuroimaging), and Berislav Zlokovic (Zilkha Neurogenetics Institute). Thompson and Zlokovic are part of this P01 team. Thompson also serves on the Executive Committee of AirPollBrain (PIs: Finch & Chen), a USC-funded collaborative network since 2010. AirPollBrain Network has created a unique intellectual environment, interdisciplinary research infrastructure, educational resources and mentoring capacities. In addition to supporting this P01, AirPollBrain helped several junior faculty (R00AG039528; R01ES023780) and trainees (F31ES025080; F31ES027340) compete for NIH funding to support careers in environmental neurosciences.

Investigators: This P01 assembled USC faculty scientists with complementary expertise in neurobiology of AD (Finch; Pike; Morgan), population & clinical neuroimaging (Thompson; Braskie; Liu), mouse brain imaging (Zlokovic; Thompson), neuroinformatics (Thompson; Braskie) and high-dimensional data analyses (Thompson; Millstein); brain vascular biology (Zlokovic; Mack), clinical neurology (Liu) and neurosurgery (Mack), cognitive neurosciences and neuropsychology (Gatz; Nation, Petkus), latent structure modeling (Petkus), epidemiology of AD (Gatz; Chen), air pollution/environmental-neuroepidemiology (Chen), inhalation exposure assessment and neurotoxicology (Sioutas; Forman). Besides collaboration with WHIMS investigators, through NIH-funded collaborative initiatives, we extended this “team science” approach to working with the MESA-AIR group (subcontract PI: Kaufman) and the VETSA investigators (Kremen & Franz). Together, we will address the fundamental research questions in the frontier of environmental neurosciences in brain aging.

Multiple Principal Investigator Leadership Plan

Rationale and Justification of Leadership by Multiple Principal Investigators (PI)

This program integrates multi-disciplinary approaches of four projects and three cores to address a common set of fundamental questions. The two community-based cohort studies and two neurotoxicological experimental projects are supported by resources and expertise from the Neuroimaging Core and the Environmental Exposure & Neurotoxicology Core. This research involves the translation and integration of different concepts and approaches in several scientific fields, including neurobiology of AD; population neuroimaging; mouse brain imaging, neuroinformatics and high-dimensional data analyses; brain vascular biology, clinical neurology and neurosurgery; cognitive neurosciences and neuropsychology; latent structure modeling; epidemiology of AD, environmental-neuroepidemiology; air pollution modeling for large cohort populations; inhalation exposure assessment and neurotoxicology. Thus, the multiple-PI (MPI) mechanism is most appropriate for this application to ensure equal input from each PI. The governance and organization of this multidisciplinary research team falls naturally into the areas of expertise brought to the project by Finch (contact PI) and Chen.

This P01 application represents a collaboration of environmental neurosciences initiatives across USC. In 2010, Co-PIs Finch & Chen initiated the AirPollBrain (APB) Network with support from USC Vice-Provost for Research Dr. Randolph Hall and the USC Collaboration Fund. The APB is a scientific network of multidisciplinary USC faculty from multiple schools, with its primary mission to develop an interdisciplinary research and educational/ training program in *Environmental Neurosciences of Neurodevelopment and Brain Aging in Urban-Dwelling Populations*. The APB group represents 15 faculty and about 30 trainees, from undergraduates to postdoctoral levels. Since 2011, Drs. Finch and Chen have worked together and helped the APB faculty obtained more than 10 federal grants supporting their effort to better understand whether and how exposure to ambient air pollutants affect human brain development and aging across life course. These new grants include awards to junior faculty (R00AG039528; R01ES024936 [ONES Award 2015]; R01ES023780) and trainees (F31ES025080; F31 ES027340). The APB group also works closely with the USC Neurosciences graduate program to develop training in Environmental Neurosciences and mentoring for potential careers in this underdeveloped but critical are in basic, clinical, and translational neurosciences.

Leadership Roles and Responsibilities

Finch and Chen initiated planning for this P01 application at the APB annual retreat in May 2015. During the past year, Finch and Chen engaged Project PIs/Core Directors and their team members to develop the P01 scope, with sound research plans. Finch will serve as the contact PI for communications between the multi-disciplinary research team and NIH.

Caleb Finch, PhD. is trained in molecular biology and has worked on the neurobiology of aging since his PhD research, begun in 1964. He has had continuous funding from NIH or NSF since 1969. In 1984, Finch founded the USC Alzheimer Disease Center, which he led until 2005 and currently serves as Associate Director. Finch also developed and led an NIA-P01 on sex steroids and neurodegeneration (1995-2005) and a T32 Training in the Neurobiology and Endocrinology of Aging, 1985-2011. For this project, Finch will maintain close contact within the experimental team and the cores, who are also collaborators and co-authors on recent publications. Finch and Chen also communicate frequently as part of the AirPollBrain group to nurture synergies between investigative teams' grad students and postdocs across experimental and epidemiological domains.

Jiu-Chiuan Chen, MD, MPH, ScD, is a physician-epidemiologist with formal training in internal medicine (with a subspecialty in environmental and occupational medicine), epidemiologic methods, environmental health sciences and toxicology (clinical; environmental; occupational). Chen is the PI of the *"Environmental Determinants of Cognitive Aging in the WHI Memory Study"* (R01AG033078), "*Neurodevelopment in Urban Environments: Role of Exposure to Ambient Air Pollution*" (R21ES022369), and "*Regional Neurotoxicity & Early Biomarkers of Air Pollution Effects on Brain Aging*" (R21AG051113). For this MPI project, Chen will lead the USC-based Population Environmental Neurosciences team members who have been working on the NIA-funded R01 in the last 5 years. Chen will continue leading this team to contribute their collective expertise in epidemiology of dementia, vascular neurology and neuroimaging in dementia, geriatric neuropsychology, cognitive psychometrics, and quantitative psychology to study the neurotoxic effects of air pollution on brain aging. As the environmental epidemiologist PI with expertise in translational environmental health research,

Chen will also contribute his expertise in environmental health sciences, spatial epidemiology, and development/application of large-scale spatiotemporal modeling.

Communication Plan

The two PIs will exchange emails (or phone conversations as needed) with a progress note on a weekly basis. This MPI will lead bimonthly meetings including four projects, quarterly meetings of the Executive Committee consisting of Project PIs and Core Directors, and annual meetings with the External SAB. The annual meeting will invite the Internal Advisory Committee (see below) to join. These large joint group meetings will take place alternating between USC University Park Campus (UPC) and Health Science Campus (HSC). Additional meetings between the two PIs and/or for joint group meetings will be arranged for specific needs, e.g., cross-project integrated analyses; planning for joint publications and presentations.

Process for Making Decisions on Scientific Direction

Drs. Finch and Chen will share decision-making responsibility for issues related to the scientific direction and conduct of the study. Finch and Chen have complementary expertise in the above mentioned areas for appropriate decisions on scientific direction

Conflict Resolution Procedures

For this application, both PIs have participated in defining the fundamental research questions, developing the integrated projects, and preparing the scientific proposals with all project PIs and core directors. The scientific research and project management plans contain no conflicts. If a conflict arises between the 2 PIs, attempts will be made to resolve the conflict through discussion and negotiation. In principle, the PI with the most relevant expertise will make the final decision. As needed we will consult with our Internal USC advisors (see below). If this should not prove successful, the PIs will seek mediation via joint discussions with their respective division directors and department chairs.

Internal Advisory Committee

In case of remaining conflict that cannot be resolved, the two PIs will seek guidance from the *Internal Advisory Committee* and. The Internal Advisory Committee members include:

(1) Helena Chui, M.D., Director, Alzheimer's Disease Research Center/Memory-Aging Center, Keck School of Medicine, Chair of Neurology; expertise in clinical neurosciences and neuroimaging of AD

(2) Pinchas Cohen, M.D., Executive Director, Ethel Percy Andrus Gerontology Center; expertise in translational neurobiology of aging

(3) Rob McConnell, M.D., Director of Southern California Children's Environmental Health Center, Deputy Director of the Southern California Environmental Health Sciences Center, AirPollBrain Executive Committee Member; expertise in air pollution health effects and toxicology

(4) Jonathan Samet, M.D., M.S., Chair Dept. of Preventive Medicine, Keck School of Medicine; expertise in air pollution health effects/toxicology, and integrated environmental health sciences.

The Internal Advisors will also attend the annual progress report meeting

Change in PI Location

If one of the PIs moves, the relevant portion of the grant will be transferred to the new institution. In the event that a PI cannot maintain full participation, a new PI will be recruited in consultation with the Internal Advisors and, subject to the approval of the Institution.

Data Sharing and Publication Procedures

The PIs will share data analyses in conjunction with the P01 biostatisticians. We will establish a Central Program Data Repository for Project 3&4 databases, with data dictionary for password-protected access (Core A, Research Strategy; http://envneurosci.usc.edu/APB/)).

Authorship rank in publications will be based on the relative scientific contributions of the PIs/core directors, and other contributing research personnel. Authorship disputes will be considered by the Internal Advisors Committee.

Budget Allocation and Fiscal Management

Finch will be responsible for budget allocation and resources in consultation with Chen. Budget allocation and distribution of resources to specific project/supporting core of the proposed P01 have already been agreed upon by all project PIs and core directors. When the need to re-budget and/or re-allocate the P01 resource, Finch will work with Chen and the respective project PI/core director to develop a revised budget with detailed categorical items, written as line-by-line, year-by-year USC budget that reconciles with proposed budget and scientific scope of work in line with this P01 application.

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Form Approved Through 10/31/2018 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

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Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: PAR-13-258 Title: NIA Program Project Applications (P01)

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. eRA Commons User Name
Franz, Carol, E.	PhD	CEFRANZ

3c. POSITION TITLE
Associate Professor

3d. MAILING ADDRESS *(Street, city, state, zip code)*
9500 Gilman Drive, MC 0738
La Jolla, CA 92093-0738

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Psychiatry

3f. MAJOR SUBDIVISION
School of Medicine

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 858-822-1793 FAX: 858-822-5856

E-MAIL ADDRESS:
cfranz@ucsd.edu

4. HUMAN SUBJECTS RESEARCH	4a. Research Exempt	If "Yes," Exemption No.
☐ No ☒ Yes	☒ No ☐ Yes	

4b. Federal-Wide Assurance No.	4c. Clinical Trial	4d. NIH-defined Phase III Clinical Trial
FWA00004495	☒ No ☐ Yes	☒ No ☐ Yes

5. VERTEBRATE ANIMALS ☒ No ☐ Yes — 5a. Animal Welfare Assurance No

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
04/01/18	03/31/23	$180,247	$266,508	$876,394	$1,288,043

9. APPLICANT ORGANIZATION
Name The Regents of the Univ. of Calif., U.C. San Diego
Address
University of California, San Diego - UCSD
9500 Gilman Drive, MC 0041
La Jolla, CA 92093-0041

10. TYPE OF ORGANIZATION
Public: ☐ Federal ☒ State ☐ Local
Private: ☐ Private Nonprofit
For-profit: ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
1956006144A1
DUNS NO.804355790 Cong. District CA-049

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name Ann Tseung
Title Senior Contract and Grant Officer
Address UCSD/OCGA
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La Jolla, CA 92093-0934
Tel: 858-822-5805 FAX: 858-534-0280
E-Mail: atsueng@ucsd.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name Evelyn Olaes
Title Senior Grant Analyst
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9500 Gilman Drive, MC 0041
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Tel: 858-822-4109 FAX: 858-822-0834
E-Mail: vchsgrants@ucsd.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE *(In ink. "Per")* 13. DATE
EOlaes
04/26/17

UNIVERSITY OF CALIFORNIA, SAN DIEGO UCSD

BERKELEY • DAVIS • IRVINE • LOS ANGELES • MERCED • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • SANTA CRUZ

DEPARTMENT OF PSYCHIATRY
9500 GILMAN DRIVE (MC 0603)
LA JOLLA, CALIFORNIA 92093-0603

Dr. Caleb Finch and Dr. Jiu-Chiuan Chen
Davis School of Gerontology
University of Southern California
Los Angeles, CA 90089-1061

Re: UCSD R01 as part of P01 “Urban Air Pollution and Alzheimer’s Disease: Risk, Heterogeneity, and Mechanisms”

April 24, 2017

Dear Tuck and JC,

Bill Kremen and I are enthusiastic about our participation in your P01 “Urban Air Pollution and Alzheimer’s Disease: Risk, Heterogeneity, and Mechanisms” (4/01/2018-3/31/2023) through our R01 proposal. We believe that this collaborative multidisciplinary examination of the effects of urban air pollution on cognitive and brain outcomes in both humans and rodents has potential to make major contributions to our understanding of environmental risk factors for Alzheimer’s disease and their mechanisms of action.

We look forward to continuing to work with you and the rest of the P01 team over the next five years. We will participate in scientific planning, data collection of the historic geolocation data for the VETSA twins, data analyses, and manuscript preparation. With our all-male VETSA sample, we will provide a valuable addition to age-related research the effects of air pollution, which has been conducted on primarily older women.

This collaborative project is truly unique and the extensive data from these different studies will doubtless yield many valuable insights; the work you are proposing to do is very important and is poised to address critical public health issues in our rapidly growing aged population. We and our colleagues look forward to working with you on this exciting project.

Sincerely yours,

Carol E. Franz, PhD
Associate Professor
University of California San Diego

William S. Kremen, PhD
Professor
University of California, San Diego

Scope of Work

University of California San Diego

(Franz and Kremen, PIs)

The primary scope of work of the UCSD investigators and staff will be:

1) To obtain address histories from VETSA participants from 1994 to current time (Vietnam Era Twin Registry, subaward). And to conduct geolocation based on these address histories.

2) To provide P01 Core B with VETSA 1 and VETSA 2 MRI data, as needed, so Core B can create MRI-based brain risk scores for Alzheimer's disease and Mild Cognitive Impairment (MCI).

3) To provide geolocation data to Core C, as needed, so Core C can index cumulative traffic related air pollution (TRAP) for the VETSA sample.

4) To conduct data analyses, focused on the specific aims, of associations between TRAP, brain and cognitive aging, utilizing data from the VETSA study.

4) To participate in weekly conference calls, ongoing data analysis, literature reviews, manuscript preparation, and presentation of scientific findings at academic conferences.

From: Buchanan, Thomas [mailto:buchanan@med.usc.edu]

Sent: Tuesday, May 23, 2017 5:53 PM

To: Jiu-Chiuan Chen <jcchen@usc.edu>; Caleb E Finch <cefinch@usc.edu>

Cc: Budge, Ted <budge@med.usc.edu>; Rohit Varma <rvarma@usc.edu>

Subject: Cost Share P01 AG005567 “Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms

Drs. Finch and Chen

The Keck School commits to providing institutional support for the referenced P01 that is equal to 3% of the indirect costs for components of an award that come to the Keck School.

Let us know if you have any additional questions or need additional verification of this cost share.

Good luck with the application!

Tom Buchanan

Vice Dean for Research

USC Leonard Davis
School of Gerontology

PINCHAS COHEN, M.D.
Dean, USC Leonard Davis School of Gerontology
Executive Director, Ethel Percy Andrus Gerontology Center
William and Sylvia Kugel Dean's Chair of Gerontology
Professor of Gerontology and Medicine

Wednesday, May 24, 2017

Caleb Finch, PhD
Professor of Gerontology
USC Leonard Davis School of Gerontology

Dear Tuck,

I wish to provide specific support for your P01 AG005567 "*Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms.*". The reviews are very encouraging for resubmission. You and JC Chen have built an outstanding program on air pollution. Your efforts have already recruited at least four other Gerontology faculty into research on air pollution, including demographers Jennifer Ailshire and Eileen Crimmins, nematode biologist Sean Curran, and several scientists in the mitochondrial space.

For institutional support of your P01 and related projects, I am authorizing the Leonard Davis School of Gerontology to develop a new *Center for Environmental Gerontology* (CEG). The mission of the CEG is to catalyze and support faculty and trainee research on air pollution and other environmental factors that impact human aging processes. It seems like that most processes of aging are exacerbated by air pollution. The vulnerability of ApoE4 carriers to air pollution in your studies anticipates other gene-environment interactions in precision medicine, and as targets for intervention.

The School of Gerontology will provide initial support to *CEG* of $40,000/year. These funds will include (a) $15,000 for support for a protected portion of your salary and for Todd Morgan's salary; (b) $5,000 for support for an annual symposium on the topic of the medical implications of air pollution; (c) $10,000 for vouchers for air pollution investigators to use in the gerontology core facilities, including the SeaHorse-Core, The Biomarkers-Core, and the Mouse-Core; and (d) $10,000 for support for graduate and postdoc attendance at National meetings.

I would like you to be the founding Director of the *CEG*.
We anticipate that you will continue to work closely with the USC Alzheimer Center and with the AirPollBrain Network in expanding environmental research across USC. We also expect that other Schools participating in your P01 will give commensurate support.

Sincerely,

Pinchas Cohen, M.D.

University of Southern California
http://www.usc.edu/faculty/Cohen/
3715 McClintock Ave, Los Angeles, California 90089-0191 • Tel: 213 740 1354 • E-mail: hassy@usc.edu

USC Viterbi
School of Engineering

OFFICE OF THE DEAN

22 May 2017

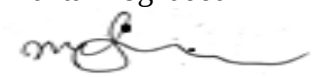
Dear Professor Finch,

The Viterbi School of Engineering wishes to provide enthusiastic support for your P01 AG005567 "Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms." The reviews are very encouraging for resubmission. Your collaborations with Professors Costas Sioutas of the Viterbi School of Engineering and JC Chen of the Keck Medical School have built an outstanding program on air pollution. We are pleased that the leading journal Science specifically recognized these contributions in their Perspective The Polluted Brain (Jan 27, 2017). In the Viterbi School, other faculty, including George Ban-Weiss, have developed studies on air pollution.

When the P01 is funded, the Viterbi School of Engineering will provide institutional support of $5,000 per year to the Administrative Core for its 5 year duration. We expect these funds to support Viterbi School pre- and post-doctoral trainee attendance at off-campus meetings and for publication costs. We are aware that the very tight P01 budget could not include these important budget lines.

We anticipate that you will continue to work closely with Professors Sioutas and Chen, and with the AirPollBrain Network in expanding environmental research across USC. We also expect that other Schools participating in your P01 will give commensurate support.
If you have any questions or need additional information, please feel free to contact me.

Sincerely,
Mahta Moghaddam



Vice Dean for Research (AY 2016-17)
USC Viterbi School of Engineering

University of Southern California
3650 McClintock Avenue, Los Angeles, California 90089-1450 • Tel: 213 740 7832 • Fax: 213 740 8493

UNIVERSITY OF CALIFORNIA, SAN DIEGO UCSD

BERKELEY • DAVIS • IRVINE • LOS ANGELES • MERCED • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • SANTA CRUZ

DEPARTMENT OF PSYCHIATRY
9500 GILMAN DRIVE (MC 0603)
LA JOLLA, CALIFORNIA 92093-0603

Dr. Caleb Finch and Dr. Jiu-Chiuan Chen
Davis School of Gerontology
University of Southern California
Los Angeles, CA 90089-1061

Re: UCSD R01 as part of P01 “Urban Air Pollution and Alzheimer’s Disease: Risk, Heterogeneity, and Mechanisms”

May 15, 2017

Dear Tuck and JC,

Bill Kremen and I are enthusiastic about our participation in your P01 “Urban Air Pollution and Alzheimer’s Disease: Risk, Heterogeneity, and Mechanisms” (4/01/2018-3/31/2023) through our R01/Project 2 proposal. We believe that this collaborative multidisciplinary examination of the effects of urban air pollution on cognitive and brain outcomes in both humans and rodents has potential to make major contributions to our understanding of environmental risk factors for Alzheimer’s disease and their mechanisms of action.

We look forward to continuing to work with you and the rest of the P01 team over the next five years. We will participate in scientific planning, data collection of the historic geolocation data for the VETSA twins, data analyses, and manuscript preparation. With our all-male VETSA sample, we will provide a valuable addition to age-related research the effects of air pollution, which has been conducted on primarily older women.

This collaborative project is truly unique and the extensive data from these different studies will doubtless yield many valuable insights; the work you are proposing to do is very important and is poised to address critical public health issues in our rapidly growing aged population. We and our colleagues look forward to working with you on this exciting project.

Sincerely yours,

Carol E. Franz, PhD
Associate Professor
University of California San Diego

William S. Kremen, PhD
Professor
University of California, San Diego

USC
UNIVERSITY
OF SOUTHERN
CALIFORNIA

May 20, 2017

Dear Committee, Caleb Finch and William Mack,

I am pleased to confirm my commitment to serve as a collaborator on the experimental research proposed in Projects 3 & 4 for the P01 entitled "Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms". I have reviewed and collaborated with your teams on the study design and statistical approaches, and will be actively involved in data analysis and interpretation. I have a long-standing collaboration with Professor Finch in P01 AG026572-011 Brinton (PI) 09/21/16-09/20/20, Progesterone in Brain Aging and Alzheimer's Disease and we have co-authored several articles, e.g., Yin et al 2015, PMID25921624. I have also worked with Dr. Bill Mack extensively as a co-investigator on your ongoing study (Mack; R01ES024936 Neurotoxicity of Airborne Particles: Role of Chronic Cerebral Hypoperfusion) that utilizes similar analysis methods.

Keck School of Medicine

Department of Preventive Medicine

Division of Biostatistics

In addition, I will advise P01 Projects 3 and 4 in year 1 on the development of a common REDCap (Research Electronic Data Capture) database for data entry and management. I will provide statistical consultation in project years 2-5 for analysis and reporting of the common dataset.

As you know, I am the director of the Biostatistics Resources core of the Southern California CTSI (of which Dr. Bill Mack is a KL2 scholar) as well as the Biostatistics and Data Management Core of the USC Alzheimer's Disease Research Center (of which Professor Finch is a Co-Director and Dr. Bill Mack is a pilot grant awardee). Your work is highly relevant to both of these institutes and biostatistics and data resources from these institutes will be available.

Your pilot data demonstrate a female and apoE4 bias in amyloid-beta load in response to air pollution exposure (project 3) and pilot data from Project 4 suggests a role for air pollution in white matter injury and the progression of acute ischemic stroke. I have worked with you in developing the analysis sections of the current grant proposal; the proposed research is very exciting. It incorporates a well-designed study plan to assess the influence of age and sex on the joint influence of particulate matter and underlying cerebrovascular disease. I look forward to working with you to address this topic, and heartily express my support for and commitment to collaborate with you on this research.

Sincerely,

Wendy Mack

Wendy Mack, PhD
Professor
Department of Preventive Medicine
Keck School of Medicine
University of Southern California

Keck School of Medicine of the University of Southern California
2001 N. Soto St., SSB202Y
Los Angeles, California 90033
Tel: 323 442 1810
Fax: 323 442 2993

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0729333930000

Legal Name*: University of Southern California
Department: Contracts and Grants
Division:
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Mr.	Steven		Misuraca	

Position/Title: Contracts and Grants Officer
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701
Phone Number*: 213-740-8207 Fax Number: 213-740-6070 Email: misuraca@research.usc.edu

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Administrative Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
04/01/2018	03/31/2023

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Southern California
Duns Number:	0729333930000
Street1*:	3720 South Flower Street
Street2:	
City*:	Los Angeles
County:	Los Angeles
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90089-0701
Project/Performance Site Congressional District*:	CA-037

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: 1 2 3 4 5 6 If

NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	CoreA_Abstract_Final.pdf
8. Project Narrative*	
9. Bibliography & References Cited	
10.Facilities & Other Resources	
11.Equipment	

The Administrative Core supports this interdisciplinary and multi-university team of investigators. The P01 program has three performance sites: the USC University Park Campus (Core C, Projects 3); USC Health Science Campus (Core B, Projects 1 & 4); and University of California, San Diego (Project 2). The proposed program includes an established group of collaborative scientists with expertise in neurobiology of AD, population neuroimaging, mouse brain imaging, brain vascular biology, clinical neurology, cognitive neurosciences, neuropsychology, epidemiology of AD, large-scale air pollution modeling, environmental epidemiology, inhalation exposure assessment and neurotoxicology.

The specific aims of the Administrative Core are:

1) Provide the integrated program leadership and coordination;
(2) Strengthen existing partnerships, facilitate new collaborations *and foster the career development of junior investigators/trainees* across USC and participating institutes;
(3) Assess scientific progress of the program, through joint group meetings per month, work-in-progress meetings per quarter, and annual meetings *of the project-core leaders and key personnel* with the External Advisory Committee;
(4) Provide fiscal management and administrative services and monitor resources and expenditures;
(5) Carry out the joint dissemination of study results to the broader scientific community.

The Administrative Core will provide leadership, oversight, planning, and coordination to facilitate scientific integration and synthesis across projects, promote cross-disciplinary interactions, and support multi-site operation of proposed program activities. The collaborative infrastructure of the proposed P01 is the product of the AirPollBrain (PIs: Finch & Chen), a collaborative network funded by USC since 2010. Over the last few years, the AirPollBrain has developed partnerships with several USC-based Centers/Institutes in brain sciences (e.g., ADRC/Memory and Aging Center, Zilkha Neurogenetic Institute, Mark and Mary Stevens Neuroimaging and Informatics Institute; Institute of Developing Mind) and also helped create the new Research Program on Neurological Effects of Environmental Exposure for the latest renewal of Southern California Environmental Health Sciences Center (P30 ES007048-20). The Administrative Core is well positioned to leverage these institutional resources and maximize the impact of the proposed P01 research.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Caleb Middle Name E. Last Name*: Finch Suffix: Ph.D

Position/Title*: Professor
Organization Name*: University of Southern California
Department: Davis School of Gerontology
Division:
Street1*: 3715 McClintock Avenue
Street2:
City*: Los Angeles
County: CA
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 90089-0191

Phone Number*: 213-740-1758 Fax Number: 213-740-0853

E-Mail*: cefinch@usc.edu

Credential, e.g., agency login: cefinch

Project Role*: Other (Specify) Other Project Role Category: Core Lead

Degree Type: Ph.D. Degree Year: 1969

Attach Biographical Sketch*: File Name:

Attach Current & Pending Support: File Name:

Tracking Number:

Funding Opportunity Number: PAR-16-359 . Received Date:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Jiu-Chiuan Middle Name Last Name*: Chen Suffix: M.D.

Position/Title*: Associate Professor
Organization Name*: University of Southern California
Department: Preventive Medicine
Division: Keck School of Medicine
Street1*: 2001 N. Soto Street, MC 9237
Street2:
City*: Los Angeles
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 90089-9237

Phone Number*: (323) 442-2949 Fax Number: (323) 442-3272
E-Mail*: jcchen@usc.edu

Credential, e.g., agency login: JC_Chen

Project Role*: Other (Specify) Other Project Role Category: Core Co-Lead
Degree Type: M.D. Degree Year: 1992

Attach Biographical Sketch*: File Name:
Attach Current & Pending Support: File Name:

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ❍ Yes ● No

*Agency-Defined Phase III Clinical Trial? ❍ Yes ❍ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ❍ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

❍ Yes ❍ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	CoreA_Intro_Final.pdf
Research Plan Section	
2. Specific Aims	CoreA_Aims_Final.pdf
3. Research Strategy*	CoreA_ResStrat_Final.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	
13. Letters of Support	
14. Resource Sharing Plan(s)	
15. Authentication of Key Biological and/or Chemical Resources	
Appendix	
16. Appendix	

INTRODUCTION TO THE ADMINISTRATIVE CORE

The review panel considered the Administrative Core (Core A), led by Drs. Finch and Chen, an integral and important component of the PPG. At the First Stage Review highlighted several major strengths, including "*its outstanding investigative team*," "*access to extensive resources and expertise of USC in TRAP, AD and neurotoxicology research,*" "*involvement of multiple renowned programs at USC and three external participating sites,*" and the "*leadership and communications facilitated by the existing AirPollBrain Network*" which engages the PIs and senior co-investigators at USC. This Core's overall strengths (*high to moderate*) were somewhat distracted by several weaknesses, as parsed below in ***italic*** and followed by brief summaries of our responses (in the specific Sections). There were no notable weaknesses at the Second Stage Review of Core A. Accordingly, the Section Subheadings with submitted changes in *text (Georgia font)* are labeled as **[***revised***], [***new***]** or otherwise left unchanged in the A1 proposal.

1. "lack of a well described dissemination plan"

➢➢ To correct this omission, we have added more details of the dissemination plan, under the revised **Specific Aim 5:** Carry out the joint dissemination of study results to the broader scientific community.

2. "absence of mentoring plans for faculty and trainees"

➢➢ We have revised the Specific Aim 2 ("Strengthen existing partnerships, facilitate new collaborations, *and foster the career development of junior investigators/trainees* across USC and participating institutes") to correct this omission. The proposed approach was described in Section 2B, under the Specific Aim 2.

3. "lack of …plans about data management, data quality monitoring, data security and medical informatics"

➢➢ For this P01, this critical function is not designed within the Administrative Core. Detailed description of the scientific expertise and facility was given in the respective list of personnel biosketch and environments/facilities section for each Research Project/Supporting Core. Given the complexity of this P01, we believe this arrangement is more cost-efficient, since it leverages the wide range of expertise and resources across USC and participating institutes.

4. "inadequate communications with participating sites, given only quarterly videoconferencing"

➢➢ One new section was added under Specific Aim 1, where we described the ***Systems for External Information Flow.*** We also clarified the frequency of regular meetings that will involve the other participating sites outside USC.

Systems for External Information Flow* [*new*]. *AirPollBrain will benefit from communications of findings with collaborating institutes.

The Core will organize *monthly* joint group meetings of all project and core leaders; *quarterly* meetings of the entire project teams, with videoconferencing for non-USC members; and *annual* meetings of project-core leaders with External Advisory Committee (EAC). The Internal Advisory Committee (IAC) will attend the quarterly and annual EAC meetings.

5. "limited institutional commitment"

➢➢ The development of this new program of environmental neurosciences has received continuing support from the Provost's Research Office since 2010. *For the P01 resubmission, new support is committed from USC Vice President, Prof. J Randolph Hall, for $20,000/year (see Support letter); from the Keck School of Medicine for 3% of Indirects/yr for 5 years (email documentation from Keck Dean Rohit Varma and Tom Buchanan, Vice-Dean for Research); from the Leonard Davis School of Gerontology for $40,00/year (support letter from Dean Pinchas Cohen); and from the Viterbi School of Engineering for $5,000/yr for 5 years (support letter from Vice Dean Moghaddam). Total institutional support is $401,200 for the entire program period.*

6. "lack of adequate staff, including a full-time PPG administrator"

➢➢ The revised budget increases Core A support to include a full-time Program Project Coordinator.

CORE A. ADMINISTRATIVE CORE

1. SPECIFIC AIMS [*revised*]

The Administrative Core, Co-Directors Caleb Finch and Jiu-Chiuan Chen, will support this interdisciplinary team for the overarching goal to better characterize the individual risk, heterogeneity, and mechanisms of AD associated with exposure to ambient air pollution in aging populations. The P01 program has three performance sites: the USC University Park Campus (Core C, Projects 3); USC Health Science Campus (Core B, Projects 1 & 4); and University of California, San Diego (UCSD, Project 2). Core C1 Environmental Exposure Data Support receives input from the University of Washington. Project 1 has a subcontract with Wake Forest School of Medicine.

The proposed program includes an established group of collaborative scientists with expertise in neurobiology of AD, population neuroimaging, mouse brain imaging, brain vascular biology, clinical neurology & neurosurgery, cognitive neurosciences, neuropsychology, epidemiology of AD, large-scale air pollution modeling, environmental epidemiology, inhalation exposure assessment and neurotoxicology. Given the complexities in its scientific activities, the primary objective of the Administrative Core is to provide leadership, oversight, planning, and coordination to facilitate scientific integration and synthesis across projects, promote cross-disciplinary interactions, and support multi-site operation of proposed program activities.

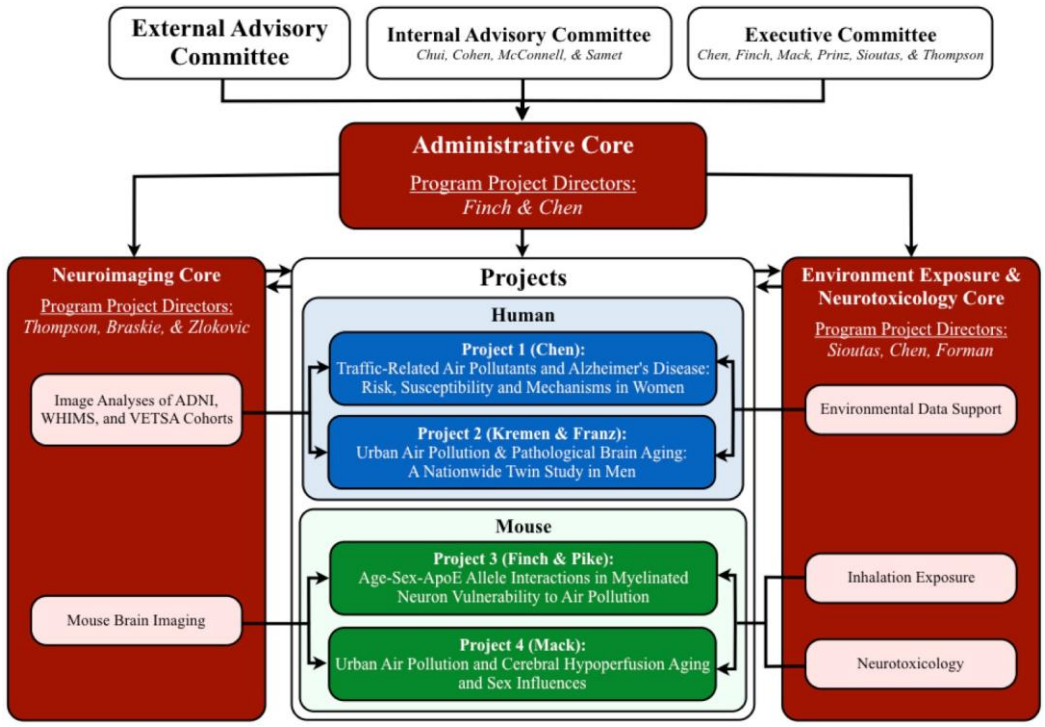
The collaborative infrastructure of proposed P01 is the product of the AirPollBrain Network (PIs: Finch & Chen), a collaborative network funded by USC since 2010 that has recruited 15 USC faculty to the topic. Over the last few years, the AirPollBrain has built partnerships with several USC-based leading Centers/Institutes in brain sciences (e.g., ADRC/Memory and Aging Center, *Zilkha Neurogenetic Institute*, Mark and Mary Stevens Neuroimaging and Informatics Institute; Institute of Developing Mind) and also helped create the new Research Program on Neurological Effects of Environmental Exposure for the latest renewal of Southern California Environmental Health Sciences Center (P30 ES007048-20). The Administrative Core is well positioned to leverage these institutional resources and maximize impact of the proposed P01 research. The operation of Administrative Core will be guided by the External Advisory Committee bringing expertise from other institutions, and also receive input from the Internal Advisory Committee of senior USC faculty. The specific aims of the Administrative Core are to:

1. Provide the integrated program leadership and coordination;
2. Strengthen existing partnerships, facilitate new collaborations, *and foster the career development of junior investigators/trainees* across USC and participating institutes;
3. Assess scientific progress of the program, through *monthly* joint group meetings of all four projects and core leaders; *quarterly* meetings of the entire project teams, with videoconferencing for non-USC members; and *annual* meetings *of the project-core leaders and key personnel* with External Advisory Committee.
4. Provide fiscal management and administrative services and monitor resources and expenditures; and
5. Carry out the joint dissemination of study results to the broader scientific community.

2. RESEARCH STRATEGY (CORE A. ADMINISTRATIVE CORE) [*revised Figure 1*]

2.A. SIGNIFICANCE

Figure 1. Program Project Team Organizational Structure



Environmental risk factors in Alzheimer's disease and related disorders (ADRD) are poorly defined. This Core supports an ambitious program of research to address major gaps in air pollution epidemiology and neurotoxicology to characterize the individual risk, heterogeneity, and biological basis of AD associated ambient air pollution in aging populations. With critical support from NIA (R01AG033078, RF1AG051521, R21AG040683, R21AG040753, R21AG051113, R21AG050201), USC is emerging as a national leader in this frontier of environmental neurosciences of brain aging. Our interdisciplinary leadership is comprised of a core group of experienced scientists with well-defined roles to facilitate the Administrative Core support of overall vision and aims. The Core will organize *monthly* joint group meetings of all project and core leaders; *quarterly* meetings of the entire project teams, with videoconferencing for non-USC members; and *annual* meetings of project-core leaders with External Advisory Committee (EAC). The Internal Advisory Committee (IAC) will attend the quarterly and annual EAC meetings.

CE Finch, JC Chen, TE Morgan, C Sioutas, CJ Pike, and Wm Mack have worked together since 2010. The External Advisory Committee for the program will include recognized leaders in urban air pollution, ADRD, and neuroimaging, details below). Core A draws from the AirPollBrain Network (PI: Finch & Chen), a USC-funded collaboration network since 2010 (details below), as well as institutional resources from USC's Department of Preventive Medicine at the Keck School of Medicine; the Leonard Davis School of Gerontology; the ADRC/Memory and Aging Center; the Zilkha Neurogenetic Institute; the Mark and Mary Stevens Neuroimaging and Informatics Institute; and, the Southern California Environmental Health Sciences Center.

2.A.1. Governance and Organizational Structure [*revised*]

Four Scientific Projects and three Supporting Cores constitute the proposed P01: Fig. 1 shows components and interactions. Project 1 (Human), JC Chen: Traffic-Related Air Pollutants and Alzheimer's Disease: Risk, Susceptibility and Mechanisms in Women (subcontract to WF SOM). Project 2 (Human), W Kremen & C Franz (UCSD): Urban Air Pollution and Pathological Brain Aging: a Nationwide Twin Study in Men. Project 3 (Mouse), Finch-Morgan-Pike: Age-Sex-ApoE Allele Interactions in Neuronal and White Matter Vulnerability to Air Pollution. Project 4 (Mouse), Wm Mack: Urban Air Pollution and Cerebral Hypoperfusion Aging and Sex Influences. Core A, Administration: CE Finch (contact PI) and JC Chen. Core B, Neuroimaging Core: P Thompson, *SubCore1*, Population Neuroimaging: M Braskie. *SubCore2*, Mouse Brain Imaging: B Zlokovic. Core C, Environmental Exposure & Neurotoxicology: C Sioutas, *SubCore1*, Environmental Data Support: JC Chen (with subcontract to UW). *SubCore2*, Collection and Characterization of TRAP-nPM: C Sioutas. *SubCore3*, Brain cell responses to TRAP-nPM: H Forman.

Core A is designed to coordinate collaboration and communication across projects and cores, and to identify and support new research opportunities. The Administrative Core is crucial to P01 objectives to characterize the individual risk, heterogeneity, and biological basis of AD-associated air pollution in aging populations. Core A provides the structure (Fig.1) to facilitate administrative and scientific oversight, and effective links to projects and cores.

Core A will be led by Program Project Co-Directors, Caleb Finch and Jiu-Chiuan Chen from USC. Finch and Chen bring extensive expertise in administering large research programs, which translate environmental health sciences knowledge (air pollution epidemiology; neurotoxicology) and approaches (spatiotemporal modeling; inhalation exposure sciences) into study the risk, heterogeneity and mechanism of AD, integrated with state-of-the-art neuroimaging and neuroinformatics.

Program Project Co-Director: Caleb Finch PhD is ARCO/William F. Kieschnick Professor in the Neurobiology of Aging at USC's Leonard Davis School of Gerontology. Dr. Finch's major research interest is basic mechanisms in human aging with a focus on inflammation. He was the founder of the NIA-funded Alzheimer Disease Research Center in 1984 and continues as Associate-Director. A new research area is the effect of air pollution on brain development and aging, which he is developing through a USC-wide network.

Program Project Co-Director: Dr. Jiu-Chiuan (JC) Chen is an Associate Professor of Preventive Medicine in the Division of Environmental Health at USC's Keck School of Medicine. Dr. Chen is a physician-epidemiologist with formal training in Internal Medicine, Environmental and Occupational Medicine, Environmental Health Sciences, and Epidemiology (Clinical, Environmental, and Occupational). He brings extensive knowledge in medicine and toxicology together with skills in quantitative methods to study environmental health and chronic disease epidemiology. He is applying the translational epidemiologic approach to understanding the neurocardio-physiological and inflammatory responses to environmental hazards in workplace and ambient environment, concerning how environmental stressors affect the development and progression of chronic diseases (e.g., cardiovascular and neurological diseases), with primary focus on the elderly population.

Executive Committee and *Internal Advisory Committee*. Oversight of the P01 resides with the Executive Committee, composed of Project and Core Directors: CE Finch (USC), J Chen (USC), Wm Kremen (UCSD), Wm Mack (USC), P Thompson (USC), and C Sioutas (USC). The Executive Committee will also consult with the Internal Advisory Committee (IAC), a group of senior USC faculty representing critical research areas:

(1) Helena Chui, M.D., Director, Alzheimer's Disease Research Center/Memory-Aging Center, Keck School of Medicine, Chair of Neurology; expertise in clinical neurosciences and neuroimaging of AD
(2) Pinchas Cohen, M.D., Executive Director, Ethel Percy Andrus Gerontology Center; expertise in translational neurobiology of aging
(3) Rob McConnell, M.D., Director of Southern California Children's Environmental Health Center, Deputy Director of the Southern California Environmental Health Sciences Center (SCEHSC), AirPollBrain Executive Committee Member; expertise in air pollution health effects and toxicology.
(4) Jonathan Samet, M.D., M.S., Chair Dept. of Preventive Medicine, Keck School of Medicine; expertise in air pollution health effects/toxicology, and integrated environmental health sciences.

External Advisory Committee (EAC)**.** We will recruit four authorities from the scientific community to form an EAC. The EAC will include expertise in translational environmental health sciences, air pollution inhalation toxicology, clinical neurology and pathogenesis of AD, state-of-the-art neuroimaging of brain aging, and mouse models for AD, and for neurotoxic environmental factors. EAC candidates are typically senior researchers with strong records of NIH funding. The EAC will approve changes to the P01 structure or responsibilities.

2.A.2. **Existing Infrastructure and Resources [***revised***]**

Department of Preventive Medicine at USC Keck School of Medicine. Since its founding in 1977, the Department of Preventive Medicine has evolved to a world-class research platform with 131 faculty members carrying out a wide range of research with educational programs. Unique in its collaborative nature and transdisciplinary approach to education and research, the Department takes a broad and encompassing focus, reflecting expansive scope of population health and preventive medicine. The Department is a recognized leader with major contributions to public health and disease etiology, treatment and prevention. Its cutting-edge research is consistently ranked at the top of NIH funding among comparable medical school departments.

Leonard Davis School of Gerontology. Founded in 1975, USC's Leonard Davis School of Gerontology is the oldest school of its type. Rich research opportunities in The Leonard Davis School faculty address a broad range of human development to aging, biology, psychology, sociology, policy, and aging services offer a unique, multidisciplinary curriculum. School faculty have trained several generations of leaders in biogerontology.

ADRC/Memory and Aging Center. The Alzheimer Disease Research Center (ADRC) focuses on reducing Alzheimer and Vascular Contributions to Cognitive Impairment in Diverse Populations. Founded in 1984 by P01 Co-Director CE Finch, the ADRC provides accurate diagnoses, comprehensive care plans and access to leading-edge clinical trials. USC-ADRC has built an interdisciplinary team to bridge basic science to clinical

trials. ADRC's current research focuses on these overarching goals: 1) Clarify the pathological and phenotypic interactions between AD and CVD, 2) Increase recruitment and retention of minority subjects from LALES, CHES, and the surrounding neighborhood, 3) Promote clinical trials and translational Research in memory and aging at USC, and 4) Continue active participation in national initiatives, including National Alzheimer Coordinating Center (NACC), Alzheimer Disease Cooperative Study (ADCS), Alzheimer Disease Neuroimaging Initiative (ADNI), and Genome Wide Association Study (GWAS). The ADRC is part of USC's Memory and Aging Center, which provides advanced diagnostic services and care for memory disorders.
Zilkha Neurogenetic Institute (ZNI). ZNI was established at the Keck School of Medicine in 2003 with a gift from Selim Zilkha. ZNI is a leader for basic neuroscience research, with a multi-disciplinary team of outstanding faculty focused on the causes of and therapies for neurodegenerative disease and psychiatric disorders. Berislav Zlokovic, a leading expert on the blood brain barrier, directs the ZNI and Core B2.
Mark and Mary Stevens Neuroimaging and Informatics Institute (INI). USC benefactors Mark and Mary Stevens donated $50 million to endow and name INI. The visionary gift translation of basic research into new therapies, preventions and cures for brain injury and disease, including AD, schizophrenia and traumatic brain injury. INI is composed of the Laboratory of Neuro Imaging (LONI), the Imaging Genetics Center (IGC, led by P01 member Paul Thompson), and the Center for Image Acquisition (CIA). We are leaders in the development of advanced computational algorithms and scientific approaches for the comprehensive and quantitative mapping of brain structure and function. IGC is dedicated new approaches for worldwide analysis of brain data and genomic data. Opened in late 2016, CIA has a fully operative Siemens Magnetom Prisma, a 3 Tesla MRI scanner, and a Siemens Magnetom 7T MRI scanner.
Air Pollution Brain Network (AirPollBrain Network): **[***revised***]** In 2010, CE Finch and JC Chen established the AirPollBrain Network to organize and promote research across USC on interactions of brain development and aging with air pollution, (http://envneurosci.usc.edu/APB/) with continuing support from the Provost's Research The primary mission of AirPollBrain is to develop an interdisciplinary research and educational/ training program in *Environmental Neurosciences of Neurodevelopment and Brain Aging in Urban-Dwelling Populations*. The APB group represents 15 faculty and 30 trainees, from undergrads to postdocs. At bimonthly meetings, our students discuss our own research and meet with invited speakers from across the US.

Institutional Support: The development of this new program of environmental neurosciences has received continuing support from the Provost's Research Office since 2010. *For the P01 resubmission, new support is committed from USC Vice President, Prof. J Randolph Hall, for $20,000/year (see Support letter); from the Keck School of Medicine for 3% of Indirects/yr for 5 years (email documentation from Keck Dean Rohit Varma and Tom Buchanan, Vice-Dean for Research); from the Leonard Davis School of Gerontology for $40,00/year (support letter from Dean Pinchas Cohen); and from the Viterbi School of Engineering for $5,000/yr for 5 years (support letter from Vice Dean Moghaddam). Total institutional support is $401,200 for the entire program period.*

Preliminary Studies Our team has a strong history of funding from NIA and other NIH agencies for research related to this proposal. **Table 1** (below) outlines prior work, which enabled this proposal with areas of expertise. Not shown are other awards for Center grants and T32's for training of pre- and postdocs.

2.B. APPROACH

Specific Aim 1: Provide the integrated program leadership and coordination.

Leadership and Oversight. Program Project Co-Directors Finch and Chen will work closely to discuss the Project's administrative, research, and dissemination objectives. Finch and Chen meet weekly in person, or by teleconference as needed to direct the Project. Issues that they cannot resolve or needing further input will be discussed *ad hoc* with the Executive Committee, for further consideration at the monthly meetings of project and core directors. The communication links between the Co-PIs and the Project and Core Directors are well established through prior collaborations and the AirPollBrain Network. Several members of the APB Executive Committee are Co-PIs and senior co-investigators of this P01: Chen, Gatz, Finch, Sioutas, Thompson.

The Administrative Core will be seated in the Leonard Davis School of Gerontology on USC's University Park Campus. This campus is also the site of the laboratories of CE Finch, H Forman, CJ Pike, and C Sioutas. ***A full-time Project Coordinator*** will be hired based on accounting and administrative experience, and will have permanent office space in the Leonard Davis School, close to the labs of CE Finch. This position will coordinate meetings make travel arrangements, process invoices, input and maintain accounting data from the Projects and Cores, including subcontracts to University of California San Diego (UCSD), University of Washington (UW), and Wake Forest School of Medicine (WF SOM). Lastly, the Project Coordinator will create monthly P01-wide action items, timelines, and is responsible for all financial records.

Systems for Internal Information Flow. Internally, the Administrative Core will ensure that all Project and Core Directors and staff have access to Program Project-wide and Project- and Core-specific activities and data, such as research results, data on progress toward goals and timelines, etc.

Table 1. The Program Project Draws Scientific Expertise and Resources from NIH-Funded Studies

Team Member(s)	Project Name	Neurobiology of AD	Population neuroimaging	Brain vascular biology	Clinical neurology & Neurosurgery	Cognitive neurosciences	Neuropsychology	Epidemiology of AD	Large-scale air pollution modeling	Environmental epidemiology	Inhalation exposure assessment	Neurotoxicology
Finch CE, *2017-18*	*Air pollution nano-particulate matter, APP processing, and glutamate receptors (R21 AG050201)*	X		X		X					X	X
Finch 2015-20	*Inflammation in Brain Aging: Modulation by ApoE Alleles, Gender, and Air Pollution (NIA R01 AG051521)*	X		X		X					X	X
Finch 2013-18	*Pre-and postnatal exposure of mice urban traffic derived air pollution particles for effects on adult behavior and neuron structure. P01 ES022845; R McConnell PI),*	X									X	X
Finch 2017-2021	*Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System". PD160021P1 (US Dept Army; P Brunden, PI)*	X				X					X	X
Finch, 2011-2013	*Air Pollution and vulnerability to Alzheimer-like neurodegeneration in transgenic mice (NIA R21 AG-040683)*	X									X	X
Finch, 2011-2013	*Aging and sensitivity to traffic-generated air pollutants in male and female mice (NIA R21 AG-040753)*	X									X	X
Mack, Wm	*Neurotoxicity of Airborne Particles: Role of Chronic Cerebral Hypoperfusion (NIEHS R01ES024936)*	X		X	X	X					X	X
Pike CJ	*Interactions between Testosterone and Type 2 Diabetes in Alzheimer's Disease Pathogenesis (NIA R01 AG034103)*	X										X
Pike	*Perimenopause in Brain Aging and Alzheimer's Disease (NIA P01 AG26572, R Brinton PI)*	X										X
Chen, JC 2015-2017	*Regional Neurotoxicity & Early Biomarkers of Air Pollution Effects on Brain Aging (NIA R21 AG051113)*		X		X	X	X	X	X	X		
Chen 2011-2015	*Environmental determinants of cognitive aging in WHI Memory Study (NIA R01 AG033078)*		X		X	X	X	X	X	X		
Chen 2016-2021	*Environmental Determinants of Pathological Brain Aging in the WHI Memory Studies (NIEHS R01ES025888)*		X		X	X	X	X	X	X		
Chen 2013-2015	*Neurodevelopment in Urban Environments: Role of Exposure to Ambient Air Pollution (NIEHS R21 ES022369)*					X	X		X	X		
Chen 2016-2021	*Alzheimer's Disease & Related Dementias: Geography, Environments & Mechanisms (NIA RF1AG054068)*		X		X	X	X	X	X	X		
Gatz, M	*Gene-Environment Interplay of Social Contexts and Aging-Related Outcomes (NIA R01 AG037985)*					X	X	X				
Kremen, W	*The VETSA Longitudinal MRI Twin Study of Aging (NIA R01 R01AG022381)*		X			X	X					
Franz, C	*Archiving the Vietnam Era Twin Studies of Aging (VETS): New Uses for Old Data (NIA R03AG046413)*		X			X	X					

The Administrative Core will maintain communication and integration between the Cores and Projects as well as the public, stakeholders, and other related NIA-funded Program Projects (including in terms of dissemination). Specifically, Co-Directors Finch and Chen will be responsible for submitting annual progress reports and ensuring compliance with relevant grant policies and regulations; overseeing preparation and publication of scientific manuscripts from the research projects and pilot studies; and communicating and implementing programmatic changes as set out by the NIA.

In person meetings and interactions: Co-Directors Finch and Chen will work closely to discuss progress on the P01 objectives. They will meet with the Executive Committee and Internal Advisory Committee

monthly to discuss and direct the program and resolve any issues. Any issues of disputes that the Co-Directors cannot resolve alone will be discussed and resolved with input from the Executive Committee, and subsequently NIA if still unresolved. The Co-Directors will meet biweekly (including via Skype for personnel who are unavailable in person) with Core and Research Project Directors to ensure satisfactory progress of Center and Core/Project goals. The Project Coordinator will assist Co-Directors in the coordination of meetings, archiving discussions and action points, and the resolution of action points.

Website and Communications: Core A will utilize the existing AirPollBrain website (http://envneurosci.usc.edu/APB/) to facilitate internal communication among cores and projects, as well as external communication with stakeholders and the general public. The AirPollBrain website will have both public and private components. Internally the website will be password protected for each project. Each core will have its own link for posting study protocols and findings for internal review. Some areas of the website will be password protected and managed by the Administrative Core.

The Project Coordinator will prepare and make available meeting notes from Project and Core Directors, and EAC meetings. All data will available to Program Project members through Drop Box, already established for the APB Network, with password access protected for specific projects and investigative subgroups.

Systems for External Information Flow* [*new*]. *AirPollBrain has a history of jointly sponsored projects with WF SOM (R01ES025888; RF1AG054068; R21AG051113; R01AG033078) and UW (R01ES025888). Chen was instrumental to the development of the ABCD-E (Adolescent Brain Cognitive Development & Environments) Consortium, which involves UCSD and was supported by AirPollBrain shortly before the ABCD cohort was funded by the NIDA in 2016. The P01 team will continue to develop resources of our multiple institutions. We will respond to the evolving needs and priorities of our AD research community. The AirPollBrain website will provide forums for registered users to post questions or provide answers in a publicly available web space. Wiki pages, blogs, RSS feeds, forums, and mailing lists allow user groups to share, organize information, and collaborate from open web browsers. Mailing lists will be provided for targeted communication between members of the P01 team. Shared calendars will publicize events or track meetings.

Specific Aim 2 [*revised*]: Strengthen existing partnerships, facilitate new collaborations, *and foster the career development of junior investigators/trainees* across USC and participating institutes**.** We employ a multidisciplinary approach to characterize the individual risk, heterogeneity, and mechanisms of ADRD associated with exposure to ambient air pollution in aging populations. Operationally, the Administrative Core will organize the monthly and quarterly group meetings of Project and Core Directors to address new results and identify new research opportunities. These meetings will be coordinated with the *AirPollBrain* bimonthly meetings. This integrated approach will enhance collaboration with other groups at USC.

Over the last few years, the AirPollBrain Network has created a unique intellectual environment, interdisciplinary research infrastructure, educational resources and mentoring capacities that have helped junior faculty compete for NIH grants (R00AG039528; R01ES024936 [ONES Award 2015]; R01ES023780). *Dr. Rob McConnell (IAC member), Director of the SCEHSC Career Development Program (https://scehsc.usc.edu/career_development_program.php), has agreed to provide our young investigators full access for these mentoring resources and training opportunities. The Executive Committee will also ensure that junior faculty and trainees are appropriately matched with senior investigators. Senior faculty will provide junior faculty and trainees guidance on research projects, compliance issues, and data analyses, and as appropriate, reviewing papers, grant proposals, presentations, and professional/career development, community engagement in research, and dissemination activities to the scientific community, the community at large, and policy and decision-makers, all with an eye toward supporting the development of independent investigators. This pairing ensures that junior faculty/trainees obtain critical insights in these projects. In 2015, Chen developed a training model for predoctoral research in population environmental neurosciences of neurobehavioral development (ES025080; 1/5 NIEHS F31s in 2015), which was subsequently replicated for two other trainees (F31ES027340; F31ES026482). This predoctoral training model can be extended to studying environmental neurosciences of brain aging in human populations and experimental models.*

Specific Aim 3: Ascertain scientific progress of the program through joint group meetings per month, quarterly work-in-progress meetings, and annual meetings with External Advisory Committee.

The Administrative Core will assess scientific progress of the program, through *monthly* joint group meetings of all four projects and core leaders; *quarterly* meetings of the entire project teams, with videoconferencing for non-USC members; and *annual* meetings of the project-core leaders with External Advisory Committee, with participation of key personnel. A P01-specific subsite will be established on the AirPollBrain home page to give Project members for access to project specific data and for general communication across projects and cores.

This mechanism will ensure daily connections across all members at USC, UCSD, WF SOM, and UW, to enhance monitoring the scientific progress. Additionally, *in-person* interaction will be supported by joint Project and Core meetings alternating between at the Leonard Davis School on the University Park Campus and the Dept. of Preventive Medicine on the Health Sciences Campus, which have been the sites of the AirPollBrain meetings since 2010. These meeting rooms are equipped for video-conferencing for participating researchers at UCSD, UW, and WF SOM. The quarterly 'Work-in-progress' meetings will summarize findings across Projects, including Cores, as relevant. Finally, the EAC will meet annually at USC with the P01 Co-Directors to assess progress of each project and Core, but also to identify new scientific opportunities from other findings in this growing field. The External Advisory Committee (EAC) will be responsible for:

- Advising P01 Co-Directors on needs of the broader scientific community;
- Strategic counsel to the Executive Committee on implementing the Program Project's vision; and
- Review progress of the P01 Projects and Cores and suggest new approaches and priorities as needed.

Specific Aim 4: Provide fiscal management and administrative services and monitor resources and expenditures. P01 Co-Directors Finch and Chen have overall fiscal responsibility and will provide oversight of the Project and Core budgets to ensure that all Projects and Cores are meeting their objectives and are spending appropriately. Project and Core Directors will be responsible for managing individual budgets (as satellite accounts), with regular oversight by the P01 Co-Directors. As noted above, the Program Coordinator will assist the P01 Co-Directors in fiscal management, administrative oversight, and monitoring of resources.

Specific Aim 5 [*revised***]:** Carry out the joint dissemination of study results to the broader scientific community. *We will oversee efforts to communicate its progress with multiple audiences, both academic and community-based, to promote the conduct of research related to environmental risk factors in ADRD within and beyond the USC community. These may include researchers, community and academic scholars, health care providers, and the public health community. Data will be disseminated via webinars and conferences. Scientific Information will be translated to public health and clinic providers. Information relevant to policy and decision-makers will be promoted to engage policy makers, key opinion leaders and decision makers. The AirPollBrain personnel will integrate and disseminate emerging research findings: providing speakers for public organizations, e.g. the Alzheimer's Association Southland Chapter; ADRC Community Outreach activities; and SCEHSC Town hall meetings. Investigators also anticipate participating in nationwide calls of the NIA programs on AD as new findings emerge.*

We are committed to collaborative research and to the NIH Grant Policy on Sharing of Unique Research Resources including the Sharing of Biomedical Research Resources Principles and Guidelines for Recipients of NIH Grants and Contracts. **Table 2** shows dissemination products and strategies to target audiences external to our Program Project.

Table 2. Summary of Audiences, Products, and Dissemination Strategies.

Audience	Examples of Audience	Products	Dissemination Strategies
Researchers	Epidemiology, Neuroscience, Neurology, Gerontology, Toxicology, Urban Geography	• Working papers and journal articles • Conference presentations • Videos of presentations	• Website of AirPollBrain • Academic conferences
Policymakers	NIA, Congress, State and local officials and other government agencies	• Papers and reports • Policy briefs • Brochures • Briefings, videos, and podcasts	• Website • Individual meetings, testimony to state legislature, Congress
Practitioners	Organizations that conduct work in Medicine, Epidemiology, Neuroscience, Neurology, Gerontology, Toxicology	• Papers and reports • Policy briefs • Guides and other educational products • Videos	• Website • Innovative distribution channels
Students	Undergraduates Graduates students Postdocs Early-career researchers Sabbatical visitors	• Papers and reports • Short policy briefs • Brochures and posters • Electronic newsletters • Briefings and podcasts	• Website • Campus outreach • Social media: YouTube, Facebook, and Twitter

We will be responsive to inquiries from the research community. A platform for communication and collaboration will be the P01's presence on the AirPollBrain website (http://envneurosci.usc.edu/APB/). The public and the research community can access this site for updates on our research, including new publications; participant and staff contact information; project milestones; and public meetings and events. The public space of the website will showcase the scientific progress, publications, press releases, and the like. The website will be a key modality to disseminate research findings in 'lay language' for the broader public.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0729333930000

Legal Name*: University of Southern California
Department: Contracts and Grants
Division:
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Mr.	Steven		Misuraca	

Position/Title: Contracts and Grants Officer
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: CA
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701
Phone Number*: 213-740-8207 Fax Number: 213-740-6070 Email: misuraca@research.usc.edu

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Neuroimaging Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
04/01/2018	03/31/2023

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Southern California
Duns Number:	0729333930000
Street1*:	3720 South Flower Street
Street2:	
City*:	Los Angeles
County:	Los Angeles
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90089-0701
Project/Performance Site Congressional District*:	CA-037

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: 1 2 3 4 5 6 If

NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Core_B_Abstract_2017_FINAL.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Core_B_Bibliography_2017_FINAL.pdf
10.Facilities & Other Resources	Core_B_Facilities_Resources_2017.pdf
11.Equipment	Core_B_Equipment_2017.pdf

Abstract Core B

The Imaging Core B (Paul Thompson, Director) provides brain image analysis for Projects 1-4, which analyze neurodegenerative responses to air pollution. Sub-core B1 (M. Braskie) supports Projects 1 and 2 on human brain MR images. Sub-core B2 (B. Zlokovic) supports Projects 3 and 4 on mouse brain.

Sub-core B1 Human Neuroimaging

Aim 1: Perform standardized structural image analyses in the ADNI, WHIMS and VETSA cohorts to assess voxelwise cortical thickness, volumes of selected Alzheimer's disease (AD)-relevant subcortical structures (hippocampus and amygdala), and regional white matter using FreeSurfer software. We will also assess white matter hyperintensity volumes. In ADNI and VETSA we will analyze diffusion tensor imaging scans.

Aim 2: Use machine learning to generate structural MRI cortical signatures and associated predicted risk scores related to risk for preclinical AD, mild cognitive impairment, and AD.

Aim 3: Support Projects 1 & 2 in their proposed mediation analyses using both targeted approach in structural equation models (SEM) and agnostic approach with high-dimensional analyses.

Sub-Core B2, Mouse brain imaging.

Aim 1. Perform *in vivo* mouse brain imaging to assess cerebral blood flow, blood brain barrier (BBB) permeability, vascular angiography, and MRI tractography.

Aim 2. Postmortem studies of the neurovascular unit & BBB by confocal microscopy for immunofluorescence.

Aim 3. Ultra-highfield MRI to detect mouse connectivity changes through diffusivity and tractography maps & quantification of BBB permeability by dynamic contrast enhanced (DCE)-MRI.

Aim 4 Analyze mouse data from Projects 3 and 4.

FACILITIES & OTHER RESOURCES
CORE B1:

UNIVERSITY OF SOUTHERN CALIFORNIA
MARK AND MARY STEVENS NEUROIMAGING AND INFORMATICS INSTITUTE

The USC Mark and Mary Stevens Neuroimaging and Informatics Institute is housed in a state-of-the-art facility designed specifically to accommodate our research. The overall academic environment is a remarkable specially designed modern building of 35,000 gross sq ft. called Stevens Hall for Neuroimaging (SHN) (**Figure 1**) on the Health Sciences Campus. The facility will houses a data center, a state-of-the art theater, offices, and workspaces. The allocation of significant campus funding to create this Institute, and its facilities, creates the opportunity to establish a broad and rich multidisciplinary expertise. The Keck School of Medicine has dedicated significant campus resources, capital infrastructure and space, to LONI and the Stevens Neuroimaging and Informatics Institute.

Figure 1. The newly renovated Stevens Hall for Neuroimaging (SHN)

SHN houses nearly 100 faculty, postdocs, students and staff in a variety of seating configurations from private offices to shared offices to open workspaces designed for collaboration. Great care was taken to design the people space to facilitate ongoing discussion and collaboration while providing a peaceful work environment for all.

The Institute provides an extensive infrastructure designed and operated to facilitate modern informatics research and support for hundreds of projects including several multi-site national and global efforts. We have redundancies built in to all equipment, and a completely secure facility to protect equipment and data. The resources described below provide networking, storage and computational capabilities that will ensure a stable, secure and robust environment. It is an unprecedented test bed to create and validate big data solutions. Because these resources have been designed, built and continuously upgraded over the years by our systems administration team, we have the appropriate expertise and operating procedures in place to use these resources to their maximum benefit.

Data Center Infrastructure. The data center is approximately 3,000 square feet and was designed using cutting-edge high density cooling solutions and high density bladed compute solutions. A total of 48 racks are dedicated to research use. Of the 48, 10 racks are reserved for core services. The core services are on separate, dedicated, redundant power to ensure continuous operation. The data center includes a Powerware 9395 UPS system providing two 750kW/825kVA UPSs in an N+1 configuration for non-core racks and two 225kW/250kVA in a 2N configuration for core services racks. The UPS sends conditioned power to 300kVA Power Distribution Units (PDUs) located inside the data center. The PDUs feed 400A rated Track Power Busways mounted above rows of racks providing an “A” bus and a “B” bus for flexible overhead power distribution to the racks. There are VRLA batteries with 9 minutes of battery run time for the core services UPS and 6 minutes of battery run time for the non-core UPS (note that the generator requires less than 2 minutes of battery run time in order to fully take over the load in the event of an outage). A 750kW/938kVA diesel emergency generator located in a weatherproof sound attenuated enclosure adjacent to the building will provide at least 8 hours of operation before needing to be refueled.

Data Center Physical Site Security. The datacenter is secured by three levels of physical access to ensure HIPAA compliance for data security. The facility is secured 24/7 with card and biometric access control devices. The interior of the datacenter contains glass break and motion detection censors that report events directly to the campus Department of Public Safety (DPS). Only authorized personnel are allowed in, and guests are permitted only after checking in, and only during business hours. The datacenter itself is additionally secured by a second layer of proximity card access. Only authorized staff are permitted to enter the datacenter facility. All racks are secured, allowing the functional presumption that any system might

contain sensitive data, ensuring all systems receive the same level of protection, minimizing the physical attack surface for the entire datacenter.

Computational and Storage Resources. Rapid advancements in imaging and genetics technology have provided researchers with the ability to produce very high-resolution, time-varying, multidimensional data sets of the brain. The complexity of the new data, however, requires immense computing capabilities.

The compute infrastructure within the datacenter boasts 4,096 cores and 38 terabytes of aggregate memory space. This highly available, redundant system is designed for demanding big data applications. Blades in the Cisco UCS environment are easy to replace. A failing blade sends an alert to Cisco where a replacement ticket is generated automatically. Upon arrival, the new blade can go from the shipping box to being fully provisioned and in production in as little as 5 minutes.

The Cisco UCS blade solution described above allows the Institute to run the services of a much larger physical infrastructure in a much smaller footprint without sacrificing availability or flexibility. Each Cisco chassis hosts 8 server blades and has 160 gigabits of external bandwidth available per chassis. Each of the 48 racks can hold up to 6 chassis plus requisite networking equipment (4 fabric extenders). Thus, the new data center has adequate rack space to accommodate this project.

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Network Resources. Service continuity, deterministic performance and security were fundamental objectives that governed the design of the network infrastructure. The Institute's intranet is architected using separate edge, core and distribution layers, with redundant switches in the edge and core for high availability. While ground network connectivity is entirely Gigabit, server data connectivity is nearly all 10 Gigabit fiber and Twinax connected to an array of Cisco 9372 distribution switches, numerous Cisco Nexus 6628 switches, and Cisco Nexus 2248 fabric extenders. For Internet access, the Institute is connected to the vBNS of Internet2 via six fiber optic Gigabit lines using a variety of route paths to ensure that the facility's external connectivity will be maintained in the case of a single path failure.

The facility has two next generation firewall appliances providing network security and deep packet inspections. The Stevens Neuroimaging and Informatics Institute has also implemented virtual private network (VPN) services using SSLVPN and IPsec services to facilitate access to internal resources by authorized

users. A VPN connection establishes an encrypted tunnel over the Internet between client and server, ensuring that communications over the Web are secure.

Furthermore, the Institute has an extensive library of communications software for transmitting data and for recording transaction logs. The library includes software for monitoring network processes, automatically warning system operators of potential problems, restarting processes that have failed, or migrating network services to an available server. For instance, the laboratory has configured multiple web servers with Linux Virtual Server (LVS) software for high-availability web, application and database service provisioning as well as load balancing. A round-robin balancing algorithm is currently used such that if the processing load on one server is heavy, incoming requests, be it HTTP, JSP or MySQL, are forwarded to the next available server by the LVS software layer. Listeners on one virtual server monitor the status and responsiveness of the others. If a failure is detected, an available server is elected as master and it assumes control and request forwarding for the entire LVS environment.

Offsite and Onsite Backup Resources. All critical system data and source code is backed up regularly to local nearline storage and cloned to LTO6 magnetic tape for offsite archival with Iron Mountain. Offsite backup data is kept in current within one week to enable rapid redeployment of all services with a view of operations and data as of a reasonable time frame. The EMC Isilon nodes retain snapshots of one month's worth of data to offer the most rapid, but least disaster-resilient restoration. Snapshot retention allows rapid restoration of unintentionally overwritten or deleted data and does not require retrieval from archived tape. Nearline-stored backup data provides similarly rapid restoration of the prior year's on-site data. Offsite archived magnetic media, while the last resort, can be recalled the same day and offers retrieval of data from a date range as recent as the prior week to as old as the initial archival series of tapes over one year old. Onsite datasets grow rapidly, constantly and require a flexible tape backup solution. Clones are pushed to tape by way of a pair of EMC backup accelerators to an expandable 34 LTO6 drive Quantum i6000 tape array. The array provides the backup parallelism the expansive data collection requires for offsite archival on such an aggressive schedule.

Virtualized Resources. Due to the rate that new servers need to be provisioned for scientific research, the Institute deploys a sophisticated high availability virtualized environment. This environment allows systems administrators to deploy new compute resources (virtual machines or VM's) in a matter of minutes rather than hours or days. Furthermore, once deployed these virtualized resources can float uninhibitedly between all the physical servers within the cluster. This is advantageous because the virtualization cluster can intelligently balance virtual machines amongst all the physical servers, which permits resource failover if a virtual machine becomes I/O starved or a physical server becomes unavailable. The net benefit for the Institute is more software resources are being efficiently deployed on a smaller hardware footprint, which results in a savings in hardware purchases, rack space and heat expulsion.

The software powering the virtualized environment is VMware's ESX 6. This is deployed on eight Cisco UCS B200 M3 servers and eight Cisco UCS B200 M4 servers. The B200 M3 servers each have sixteen 2.6/3.3 GHz CPU cores and 128GB of DDR3 RAM. The B200 M4 servers each have 64 2.3/3.6 GHz CPU cores and 512GB of DDR4 RAM. These sixteen servers reside within a number of Cisco UCS 5108 blade chassis with dual 8x 10 Gigabit mezzanine cards providing a total of 160 Gigabits of available external bandwidth per chassis. Storage for the virtualization cluster is housed on the aforementioned Isilon clusters, XtremeIO, and VNX storage arrays. The primary bottleneck for the majority of virtualization solutions is disk I/O and these storage arrays more than meet the demands of creating a highly available virtualized infrastructure whose capabilities and efficiency meet or greatly exceed those of a physical infrastructure. A single six rack unit (6RU), eight blade chassis can easily replicate the resources of a 600+ server physical infrastructure when paired with the appropriate storage solution such as the Isilon storage cluster.

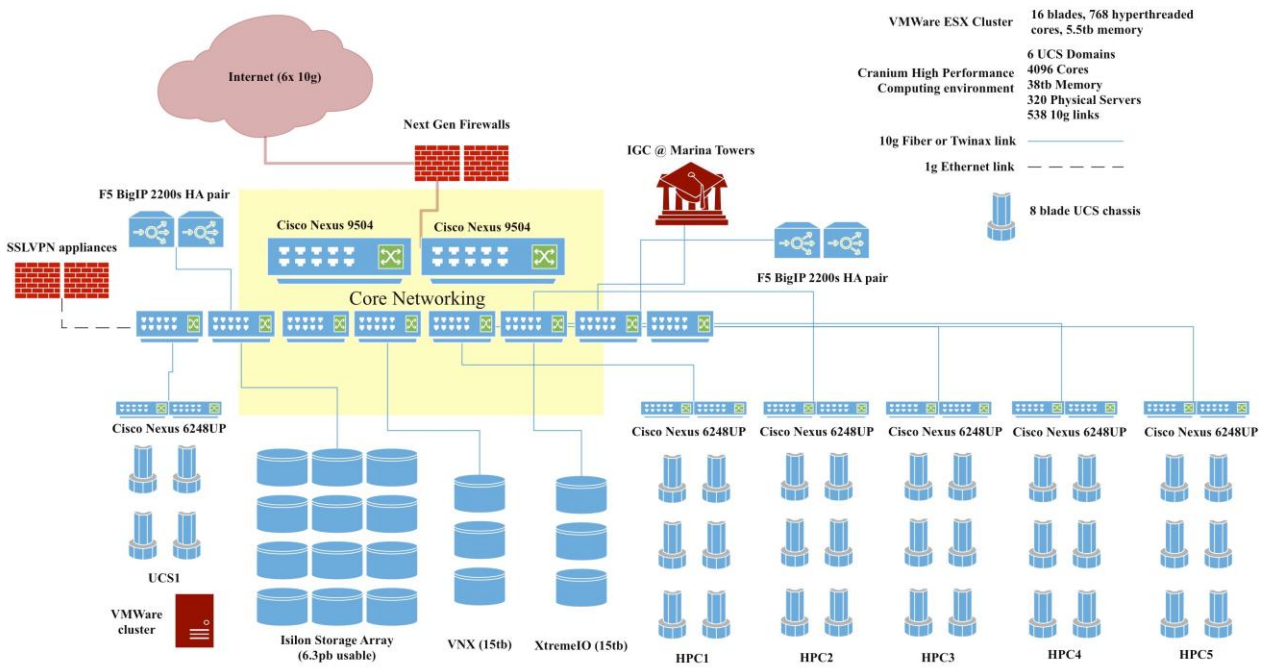

Figure 2. Stevens Neuroimaging and Informatics Institute network infrastructure and supercomputing environment.

Workflow Processing. To facilitate the submission and execution of compute jobs in this compute environment, various batch-queuing systems such as SGE (https://arc.liv.ac.uk/trac/SGE) can be used to virtualize the resources above into a compute service. A grid layer sits atop the compute resources and submits jobs to available resources according to user-defined criteria such as CPU type, processor count, memory requirements, etc. The laboratory has successfully integrated the latest version of the LONI Pipeline (http://pipeline.loni.usc.edu) with SGE using DRMAA and JGDI interface bindings. The bindings allow jobs to be submitted natively from the LONI Pipeline to the grid without the need for external scripts. Furthermore, the LONI Pipeline can directly control the grid with those interfaces, significantly increasing the operating environment's versatility and efficacy, and improving overall end-user experience. See **Figure 3** for a screenshot of the latest version of the pipeline.

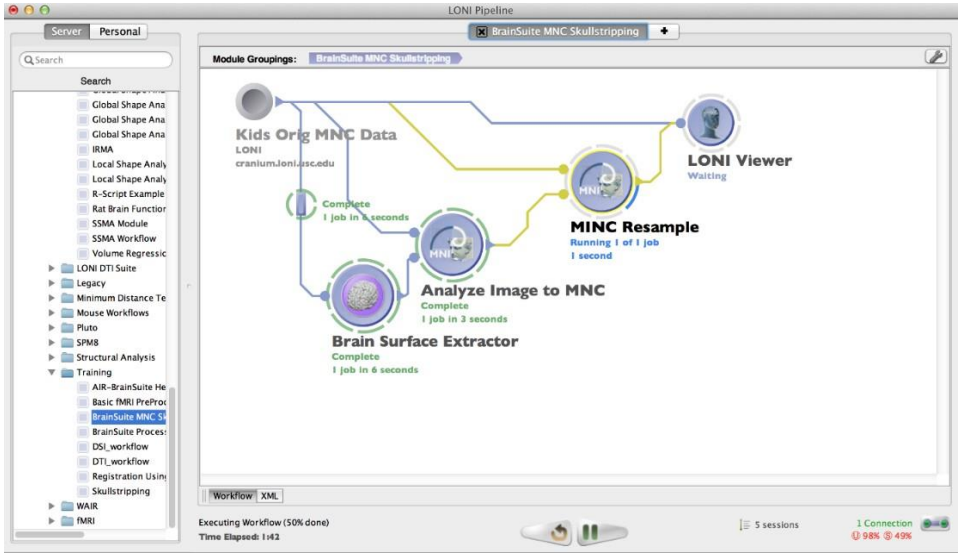

Figure 3. The LONI Pipeline Execution Environment

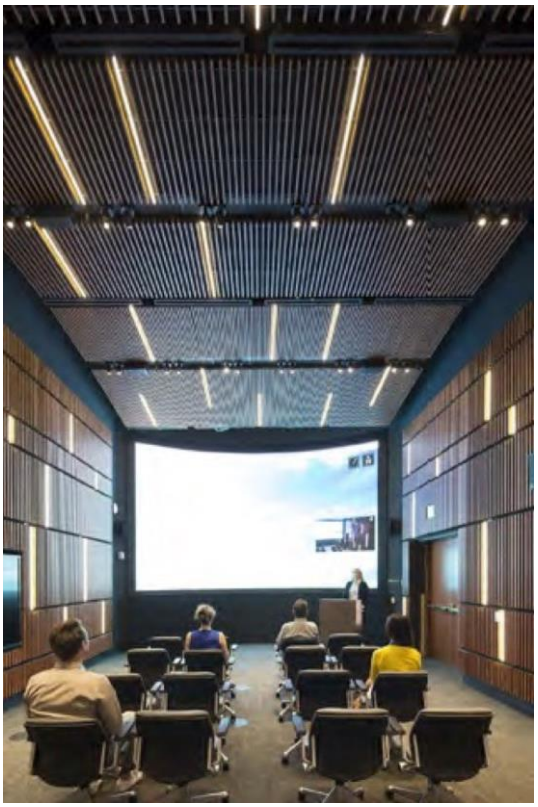

Figure 4. Data Immersive Visualization Environment (DIVE)

In addition to a data center, SHN houses a 50-seat high definition theater – the Data Immersive Visualization Environment (DIVE) (Figure 4). The prominent feature of the DIVE is a large curved display that can present highly detailed images, video, interactive graphics and rich media generated by specialized research data. The DIVE display features a dominant image area, with consistent brightness across the entire display surface, high contrast, and 150° horizontal viewing angle. The display resolution target is 4k Ultra HD, 3840x2160 (8.3 megapixels), in a 16:9 aspect ratio. The DIVE is designed to facilitate research communication, dissemination, training and high levels of interaction.

Adjoining the SHN on the north side is a brand new MRI facility. The Center for Imaging Acquisition houses a Siemens Magnetom Prisma 3 Tesla MRI scanner and a Siemens Magnetom Terra 7T MRI scanner. The MAGNETOM Prisma 3T includes simultaneous 80mT/m @ 200T/m/s gradients, a new, high end gradient system that delivers high gradient amplitudes and fast switching capabilities in a combination that is currently truly innovative. The Siemens Magnetom Terra 7T MRI system is an investigational device. Both MRI scanners are at the leading edge for neuroimaging

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The Human Connectome Project is already working on methods for improving mapping the Connectome such as Multi-shell HARDI reconstructed with a Fiber Orientation Density methodology. This will be enhanced having the gradient performance of the 3T Prisma.

The USC Siemens Terra 7T MRI consists of a magnet system which generates the ultrahigh magnetic field. The electrical and mechanical shim are integrated in the gradient coil. The magnet comprises the superconducting magnet including the system for cooling (interface for helium fill/refill, cold head), energizing (current probe) and monitoring the magnet during operation. In addition, it includes the cabling up to the point

where the external lines are connected. The system consists of an actively shielded highly homogeneous superconducting magnet (7.0 Tesla) housed in a 830 mm horizontal room temperature bore, low-loss helium cryostat and zero helium boil-off technology. Field shimming is primarily accomplished using superconducting shim coils. Final shimming is performed with a small number of passive shims. The system is supplied with a helium level monitor and an emergency quench heater control unit. A two-stage 4.2 K cryo refrigerator cooling system is employed, consisting of two independent cryocooler systems that eliminate the static cryogenic consumption.

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Siemens Magnetom Terra 7T system. The Siemens 7T is an actively shielded whole body highly homogeneous superconducting magnet. Construction has begun and the delivery date is slated for Oct 2016. The 7T Terra system is designed for future clinical use with planned clinical clearances, and can be switched to clinical tasks with Dual Mode.

The CIA is also equipped with a variety of patient monitoring and stimulus equipment. Patient physiologic monitoring is achieved with either a BIOPAC MP150 system or a Phillips Invivo Expression Patient Monitor (MR400). Stimulation equipment includes a Cambridge Research Systems BOLDscreen LCD display for fMRI and a Current Designs 4-button response box pad. Contrast is delivered via a Medrad Spectris Solaris EP Injector.

Additional Office Spaces. Additional office spaces for faculty, staff and students is available in Suite 200 of the Marina Towers in Marina Del Rey, CA. This building houses other high tech research groups like the Information Sciences Institute and serves as a West LA hub for the Stevens Neuroimaging and Informatics Institute. Suite 400 is nearly 5,200 sq ft with 14 faculty and shared offices, 2 director's offices, 2 conference rooms, a student workroom with 8 workstations and a large kitchen.

Core B2: FACILITIES AND RESOURCES-MOUSE IMAGING SUBCORE

The Center for Neurodegeneration and Regeneration is situated in the Zilkha Neurogenetic Institute at the University of Southern California Keck School of Medicine. The collaborative atmosphere and integrated academic infrastructure make this a unique and technologically advanced facility to conduct scientific research. In addition to the resources readily available in our laboratory facilities, see description below, other facilities and experts are available to assist and enrich our research at a multitude of levels, including a skilled biostatistics and computational biology department, expert vivarium and Division of Laboratory Animal Medicine staff, various core facilities included below, and frequent visiting distinguished professors. As a result, the Center for Neurodegeneration and Regeneration is a focal point in which intellectual ideas drive and effectively utilize the resources available to this laboratory.

Laboratory:

Dr. Zlokovic's Center for Neurodegeneration and Regeneration is located in the Zilkha Neurogenetic Institute at the USC Keck School of Medicine. There are dedicated suites/rooms for specialized procedures and techniques: (**A**) A neurovascular imaging suite containing one multi-photon microscope (Zeiss LSM 5MP) coupled to a Mai Tai DeepSee Ti:Sapphire and HeNe 543 laser and one multi-photon/confocal microscope (Zeiss LSM 510) coupled to a Mai Tai DeepSee Ti:Sapphire, Argon 488, HeNe 543, and HeNe 633 laser. Suite also contains a picospritzer and stimulator for analysis of CBF response to whisker stimulation. (**B**) TissueCyte 1000 Whole Mount Tissue Scanner (Tissue Vision) for serial two-photon tomography and 3D reconstruction of

brain connectomes and angiograms. (**C**) A microscopy suite containing a Keyence BZ-9000 fluorescence microscope, an Olympus AX70 Research microscope equipped with a motorized stage and both bright field and epifluorescent capabilities, an Inverted Nikon Eclipse T2000-U microscope, and a Nikon TE2000-S microscope with a temperature-controlled chamber for real time cell culture imaging. (**D**) A blood flow suite containing laser Doppler flow meter (Transonic Systems Inc.) for quantification of CBF response to brain activation, quantitative autoradiography (MCID™ Autoradiography) for quantification of regional resting CBF, a custom-designed laser speckle imaging apparatus (Thor Laser Speckle Flowmetry) for visualization of pial blood vessels and blood flow, and a custom-designed intrinsic optical signal mapping apparatus for visualization of regional brain activation. (**E**) Imaging suite for neuronal (cortical activation) by voltage sensitive dye (VSD) imaging and electrophysiological recordings. (**F**) A behavioral suite containing a Barnes maze, rotar-rod, novel object location, novel object recognition, wire grip, beam balance, remote fear memory recall, cued contextual fear conditioning, 8-arm radial maze, complex running wheel, foot fault, open field, tube dominance and burrowing test apparatus to study murine cognitive function. (**G**) A cell culture room containing 4 CO_2 incubators for normoxic studies, 2 CO_2 chambers for hypoxic studies, two SterilGard laminar-flow hoods, and three liquid nitrogen storage tanks for cell storage (**H**) Adjacent molecular biology suites containing two Zeiss Palm Microbeam laser capture microscopes for single cell isolation and a multitude of molecular techniques, including centrifugation, sonication, RNA extraction, EMSA, DNA analysis, Western and Southern blot analysis, PCR thermal cyclers, immunoprecipitation of proteins and spectrophotometric protein determination. (**I**) A protein purification suite containing both FPLC (Biorad) and HPLC (Shimadzu) systems. (**I**) A radioisotope labeling suite specially designed for preparation of radiolabeled proteins, including ^{125}I-, ^{14}C-, ^{3}H-. Suite also contains a Gamma Wallac Wizard 1470 and Beckman Coulter LS6500 multipurpose counters for subsequent analysis of radioactivities. (**I**) An electrophysiology recording suite. (**J**) The Biomarker Core Facility is located in Dr. Zlokovic's laboratory space in Zilkha Neurogenetic Institute (ZNI) room 301 at University of Southern California (USC), Keck School of Medicine. The Biomarker Core has been using the state-of-the-art, ultrasensitive electrochemiluminescent Meso Scale Discovery (MSD) multiplex platform technology since 2013 to conduct simultaneous measurements of different neurovascular unit (NVU) biomarker categories in cerebrospinal fluid (CSF) for all pilot data analyses in *APOE4* and *PSEN1* mutation carriers and non-carriers and TgF344-AD rats and controls, and has determined subjects' *APOE* genotype. The Core is working closely with MSD scientific staff members to develop custom MSD assays, which includes converting existing enzyme-linked immunosorbent assays (ELISAs) to MSD assays and also original development of MSD assays for novel analytes of interest. These assay developments are being conducted for analyte measurement in both human and rat samples. MSD's V-PLEX product line is scientifically validated for state-of-the-art performance and quality. Surpassing FDA's analytical standards, V-PLEX assays guarantee lot-to-lot consistency to ensure reproducible and reliable results to advance scientific research. For the few biomarkers currently assayed with ELISA to provide a larger dynamic range of detection. In summary, the core is well equipped to provide services, resources and expertise on cerebrospinal fluid biomarkers for the proposed project. (**K**) A dedicated, access-restricted -80°C freezer (*Thermo Fisher Scientific*) is available for storage of human CSF biofluids. A separate -80°C freezer at ZNI room 212 to store backup aliquots. Samples will be diligently tracked in a password-protected database. MSD MESO QuickPlex SQ120 multiplex platform (Meso Scale Diagnostics, LLC. Rockville, Maryland) will be used for ultrasensitive electrochemiluminescent immunoassay detection and SpectraMax M2 Multimode Microplate Reader (Molecular Devices, LLC. Sunnyvale, California) will be used for reading ELISA plates. MSD and microplate readers are each connected to Dell laptops with the MSD Discovery Workbench software and SoftMax Pro software, respectively.

In the main laboratory, there is a dark room and cold room as well as a crytostat and vibratome for tissue sectioning and preparation. There are 12 laboratory benches for wet work, each focusing on specific procedures: immunofluorescent detection of brain pericytes, isolation of neurovascular cells (pericytes, endothelial, astrocytes, VSMC, microglia and neurons), cranial window preparation for multi-photon microscopy, proximal ligation assay to study protein-protein interaction and distribution of BBB transport proteins, ELISA development and applications, molecular cloning and transgenic mouse manipulation, molecular mechanisms of brain angiogenesis and receptor signaling, Aβ, apoE and apoJ *in vivo* brain clearance studies, protein chemistry, neuroprotective effects of activated protein C (APC), stroke modeling (MCAO, proximal, distal and embolic models, photothrombotic mini-stroke, confined focal white matter stroke and intrastriatal NMDA model), effects of APC treatment following various stroke models, neuroprotection and stem cell studies, Neurostar motorized ultra-precise small animal stereotaxic instrument (Model 963SD), stereological studies and neuronal spinogenesis, blood-brain barrier mediated clearance and

pharmacokinetics, in situ brain vascular perfusion, *in vitro* blood-brain barrier studies, glucose metabolism/transport in AD murine models, and cholesterol and copper metabolism in AD murine models.

In addition to the Zlokovic' lab and Center for Neurodegeneration and Regeneration the members of the lab regularly use the University Core Facilities for different projects including: Biomolecular Interaction Laboratory, Electron Microscope Research Core, Flow Cytometry Core, Functional Genomics Center, Gene Targeting and Transgenic Core, High Throughput Screening Core, Molecular Imaging Core, Proteomics Center, and Small Animal MRI Imaging Core Facility. This proposal does not anticipate using any of the other core facilities for the proposed research in the present application.

Figure 1. MR Solutions Preclinical MRI 7.0T

Zilkha Neurogenetic Institute – MR Solutions Preclinical MRI 7.0T/24cm, PET System. The MR Solutions Preclinical MRI System (**Figure 1**) is 24 cm in diameter and is designed for mice and rats for both *in vivo* and *ex vivo* applications. The system is based on the revolutionary and advanced cryogen-free superconducting magnet technology. The unit is also equipped with a preclinical PET imaging system with 2 rings for simultaneous imaging. This allows simultaneous imaging of the whole body of a mouse and rat. Additionally, the PET module can be removed from the MRI and used as a stand-alone unit. This new PET module offers a major breakthrough in high performance functional imaging technology. Furthermore, the unit has a motorized imaging translation support which is a horizontal translation stage for scanning animals in the prone position for simultaneous or sequential multimodality imaging. Also, the unit is novel in that it has a variable field magnet allowing the field of the magnet to be adjusted from 1-7 Tesla. Zilkha Neurogenetics Institute is in the process of acquiring this new magnet and it will be available for use by the end of 2016. The magnet will be housed in room 115C of the ZNI building and the room is prepped and ready for the unit to be installed.

Animal: The Vivarium at the ZNI has been accredited under AAALA and follows NIH guidelines for care and use of laboratory animals.

Computer: 16 IBM compatible PC's (pentium) will be available in the Center for Neurodegeneration and Regeneration. These computers have internet access for Medline searches, access to the Genebank and Protein Data Banks, and access to laser printers. Computers are used for bio-mathematical modeling and data analysis.

Office: Dr. Zlokovic has dedicated office space in the ZNI adjacent to the laboratory. He has a dedicated administrative support and a secretary to coordinate efforts on the project adjacent to his office. Dr. Zlokovic has access to a FAX machine, xerox machine and typewriter in the Administrative Offices in the Center for Neurodegeneration and Regeneration at the ZNI.

Equipment

CORE B1:

UNIVERSITY OF SOUTHERN CALIFORNIA
MARK AND MARY STEVENS NEUROIMAGING AND INFORMATICS INSTITUTE

Data Center Infrastructure. The data center is approximately 3,000 square feet and was designed using cutting-edge high density cooling solutions and high density bladed compute solutions. A total of 48 racks are dedicated to research use. Of the 48, 10 racks are reserved for core services. The core services are on separate, dedicated, redundant power to ensure continuous operation. The data center includes a Powerware 9395 UPS system providing two 750kW/825kVA UPSs in an N+1 configuration for non-core racks and two 225kW/250kVA in a 2N configuration for core services racks. The UPS sends conditioned power to 300kVA Power Distribution Units (PDUs) located inside the data center. The PDUs feed 400A rated Track Power Busways mounted above rows of racks providing an “A” bus and a “B” bus for flexible overhead power distribution to the racks. There are VRLA batteries with 9 minutes of battery run time for the core services UPS and 6 minutes of battery run time for the non-core UPS (note that the generator requires less than 2 minutes of battery run time in order to fully take over the load in the event of an outage). A 750kW/938kVA diesel emergency generator located in a weatherproof sound attenuated enclosure adjacent to the building will provide at least 8 hours of operation before needing to be refueled.

Data Center Physical Site Security. The datacenter is secured by three levels of physical access to ensure HIPAA compliance for data security. The facility is secured 24/7 with card and biometric access control devices. The interior of the datacenter contains glass break and motion detection censors that report events directly to the campus Department of Public Safety (DPS). Only authorized personnel are allowed in, and guests are permitted only after checking in, and only during business hours. The datacenter itself is additionally secured by a second layer of proximity card access. Only authorized staff are permitted to enter the datacenter facility. All racks are secured, allowing the functional presumption that any system might contain sensitive data, ensuring all systems receive the same level of protection, minimizing the physical attack surface for the entire datacenter.

Computational and Storage Resources. Rapid advancements in imaging and genetics technology have provided researchers with the ability to produce very high-resolution, time-varying, multidimensional data sets of the brain. The complexity of the new data, however, requires immense computing capabilities.

The compute infrastructure within the datacenter boasts 4,096 cores and 38 terabytes of aggregate memory space. This highly available, redundant system is designed for demanding big data applications. Blades in the Cisco UCS environment are easy to replace. A failing blade sends an alert to Cisco where a replacement ticket is generated automatically. Upon arrival, the new blade can go from the shipping box to being fully provisioned and in production in as little as 5 minutes.

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The USC Siemens Terra 7T MRI consists of a magnet system which generates the ultrahigh magnetic field. The electrical and mechanical shim are integrated in the gradient coil. The magnet comprises the superconducting magnet including the system for cooling (interface for helium fill/refill, cold head), energizing (current probe) and monitoring the magnet during operation. In addition, it includes the cabling up to the point where the external lines are connected. The system consists of an actively shielded highly homogeneous superconducting magnet (7.0 Tesla) housed in a 830 mm horizontal room temperature bore, low-loss helium cryostat and zero helium boil-off technology. Field shimming is primarily accomplished using superconducting shim coils. Final shimming is performed with a small number of passive shims. The system is supplied with a helium level monitor and an emergency quench heater control unit. A two-stage 4.2 K cryo refrigerator cooling system is employed, consisting of two independent cryocooler systems that eliminate the static cryogenic consumption.

Gradients & RF Receiver Technology. The gradient system essentially consists of a Gradient Power Amplifier (GPA) and a Gradient Coil (GC), both water cooled to sustain a high duty cycle. The Magnetom 7T uses the GPA model as is used on other Magnetom systems. The XR gradients are the same as Prisma and capable of 80mT/m @ 200T/m/s gradients. The gradient coil includes the full set of 5 second order electrical shim coils to adjust B0 homogeneity for patient and each measurement volume. The Siemens RF receiver technology has 64 channels receive and the USC 7T includes a Nova Medical 32 channel head coil. The system also has an 8–channel parallel RF transmit array (pTx). 3rd order shims provide four out of the seven possible 3rd order shims that built into the SC72 gradients, along with 2nd 4-fold SPS cabinet, wiring for 3rd order shim, electric infrastructure expansion, SW interface to drive additional 3rd order shims.

Siemens Magnetom Terra 7T system. The Siemens 7T is an actively shielded whole body highly homogeneous superconducting magnet. Construction has begun and the delivery date is slated for Oct 2016. The 7T Terra system is designed for future clinical use with planned clinical clearances, and can be switched to clinical tasks with Dual Mode.

The CIA is also equipped with a variety of patient monitoring and stimulus equipment. Patient physiologic monitoring is achieved with either a BIOPAC MP150 system or a Phillips Invivo Expression Patient Monitor (MR400). Stimulation equipment includes a Cambridge Research Systems BOLDscreen LCD display for fMRI and a Current Designs 4-button response box pad. Contrast is delivered via a Medrad Spectris Solaris EP Injector.

Core B2: FACILITIES AND RESOURCES-MOUSE IMAGING SUBCORE

(A) A neurovascular imaging suite containing one multi-photon microscope (Zeiss LSM 5MP) coupled to a Mai Tai DeepSee Ti:Sapphire and HeNe 543 laser and one multi-photon/confocal microscope (Zeiss LSM 510) coupled to a Mai Tai DeepSee Ti:Sapphire, Argon 488, HeNe 543, and HeNe 633 laser. Suite also contains a picospritzer and stimulator for analysis of CBF response to whisker stimulation.
(B) TissueCyte 1000 Whole Mount Tissue Scanner (Tissue Vision) for serial two-photon tomography and 3D reconstruction of brain connectomes and angiograms.
(C) A microscopy suite containing a Keyence BZ-9000 fluorescence microscope, an Olympus AX70 Research microscope equipped with a motorized stage and both bright field and epifluorescent capabilities, an Inverted Nikon Eclipse T2000-U microscope, and a Nikon TE2000-S microscope with a temperature-controlled chamber for real time cell culture imaging.
(D) A blood flow suite containing laser Doppler flow meter (Transonic Systems Inc.) for quantification of CBF response to brain activation, quantitative autoradiography (MCID^{TM} Autoradiography) for quantification of regional resting CBF, a custom-designed laser speckle imaging apparatus (Thor Laser Speckle Flowmetry) for visualization of pial blood vessels and blood flow, and a custom-designed intrinsic optical signal mapping apparatus for visualization of regional brain activation.
(E) Imaging suite for neuronal (cortical activation) by voltage sensitive dye (VSD) imaging and electrophysiological recordings.
(F) A behavioral suite containing a Barnes maze, rotar-rod, novel object location, novel object recognition, wire grip, beam balance, remote fear memory recall, cued contextual fear conditioning, 8-arm radial maze, complex running wheel, foot fault, open field, tube dominance and burrowing test apparatus to study murine cognitive function.
(G) A cell culture room containing 4 CO_2 incubators for normoxic studies, 2 CO_2 chambers for hypoxic studies, two SterilGard laminar-flow hoods, and three liquid nitrogen storage tanks for cell storage
(H) Adjacent molecular biology suites containing two Zeiss Palm Microbeam laser capture microscopes for single cell isolation and a multitude of molecular techniques, including centrifugation, sonication, RNA extraction, EMSA, DNA analysis, Western and Southern blot analysis, PCR thermal cyclers, immunoprecipitation of proteins and spectrophotometric protein determination.
(I) A protein purification suite containing both FPLC (Biorad) and HPLC (Shimadzu) systems. (**I**) A radioisotope labeling suite specially designed for preparation of radiolabeled proteins, including ^{125}I-, ^{14}C-, ^{3}H-. Suite also contains a Gamma Wallac Wizard 1470 and Beckman Coulter LS6500 multipurpose counters for subsequent analysis of radioactivities. (**I**) An electrophysiology recording suite.

(J) The Biomarker Core Facility is located in Dr. Zlokovic's laboratory space in Zilkha Neurogenetic Institute (ZNI) room 301 at University of Southern California (USC), Keck School of Medicine. The Biomarker Core has been using the state-of-the-art, ultrasensitive electrochemiluminescent Meso Scale Discovery (MSD) multiplex platform technology since 2013 to conduct simultaneous measurements of different neurovascular unit (NVU) biomarker categories in cerebrospinal fluid (CSF) for all pilot data analyses in *APOE4* and *PSEN1* mutation carriers and non-carriers and TgF344-AD rats and controls, and has determined subjects' *APOE* genotype. The Core is working closely with MSD scientific staff members to develop custom MSD assays, which includes converting existing enzyme-linked immunosorbent assays (ELISAs) to MSD assays and also original development of MSD assays for novel analytes of interest. These assay developments are being conducted for analyte measurement in both human and rat samples. MSD's V-PLEX product line is scientifically validated for state-of-the-art performance and quality. Surpassing FDA's analytical standards, V-PLEX assays guarantee lot-to-lot consistency to ensure reproducible and reliable results to advance scientific research. For the few biomarkers currently assayed with ELISA to provide a larger dynamic range of detection. In summary, the core is well equipped to provide services, resources and expertise on cerebrospinal fluid biomarkers for the proposed project.
(K) A dedicated, access-restricted -80°C freezer (*Thermo Fisher Scientific*) is available for storage of human CSF biofluids. A separate -80°C freezer at ZNI room 212 to store backup aliquots. Samples will be diligently tracked in a password-protected database. MSD MESO QuickPlex SQ120 multiplex platform (Meso Scale Diagnostics, LLC. Rockville, Maryland) will be used for ultrasensitive electrochemiluminescent immunoassay detection and SpectraMax M2 Multimode Microplate Reader (Molecular Devices, LLC. Sunnyvale, California) will be used for reading ELISA plates. MSD and microplate readers are each connected to Dell laptops with the MSD Discovery Workbench software and SoftMax Pro software, respectively.

In addition to the Zlokovic' lab and Center for Neurodegeneration and Regeneration the members of the lab regularly use the University Core Facilities for different projects including: Biomolecular Interaction Laboratory, Electron Microscope Research Core, Flow Cytometry Core, Functional Genomics Center, Gene Targeting and Transgenic Core, High Throughput Screening Core, Molecular Imaging Core, Proteomics Center, and Small Animal MRI Imaging Core Facility. This proposal does not anticipate using any of the other core facilities for the proposed research in the present application.

Figure 1. MR Solutions Preclinical MRI 7.0T

Zilkha Neurogenetic Institute – MR Solutions Preclinical MRI 7.0T/24cm, PET System. The MR Solutions Preclinical MRI System (**Figure 1**) is 24 cm in diameter and is designed for mice and rats for both *in vivo* and *ex vivo* applications. The system is based on the revolutionary and advanced cryogen-free superconducting magnet technology. The unit is also equipped with a preclinical PET imaging system with 2 rings for simultaneous imaging. This allows simultaneous imaging of the whole body of a mouse and rat. Additionally, the PET module can be removed from the MRI and used as a stand-alone unit. This new PET module offers a major breakthrough in high performance functional imaging technology. Furthermore, the unit has a motorized imaging translation support which is a horizontal translation stage for scanning animals in the prone position for simultaneous or sequential multimodality imaging. Also, the unit is novel in that it has a variable field magnet allowing the field of the magnet to be adjusted from 1-7 Tesla. Zilkha Neurogenetics Institute is in the process of acquiring this new magnet and it will be available for use by the end of 2016. The magnet will be housed in room 115C of the ZNI building and the room is prepped and ready for the unit to be installed.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

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Attach Biographical Sketch*: File Name: Thompson_Biosketch_Mar2017.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Degree Type: PHD,BBA Degree Year: 2006

Attach Biographical Sketch*: File Name: Biosketch_Braskie_2017_04.pdf

Attach Current & Pending Support: File Name:

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Degree Type: MD,PHD Degree Year: 1983/1995

Attach Biographical Sketch*: File Name: Zlokovic_NIH_Bio_4_11.pdf

Attach Current & Pending Support: File Name:

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Attach Biographical Sketch*: File Name: Montagne_NIH_Biosketch_4.26.17.pdf

Attach Current & Pending Support: File Name:

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Attach Biographical Sketch*: File Name: rjacobs_biosketch_05-2017.pdf

Attach Current & Pending Support: File Name:

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Degree Type: PHD,BAS,BS Degree Year: 2009

Attach Biographical Sketch*: File Name: Kisler_NIH_biosketch_May2017.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Degree Type: PHD Degree Year: 2013

Attach Biographical Sketch*: File Name: Nelson_Biosketch_051617_FIN.pdf

Attach Current & Pending Support: File Name:

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	Core_B_Intro_2017_FINAL.pdf
Research Plan Section	
2. Specific Aims	Core_B_Aims_2017_FINAL.pdf
3. Research Strategy*	Core_B_ResearchStrategy_2017_FINAL.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	CoreB_VertebrateAnimal.pdf
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	
13. Letters of Support	
14. Resource Sharing Plan(s)	Core_B_Resource_Sharing_Plan.pdf
15. Authentication of Key Biological and/or Chemical Resources	CoreB_Authentication_of_Key_Resources_Plan.pdf
Appendix	
16. Appendix	

CORE B - Introduction

UB-CORE B1 HUMAN IMAGING

Reviewers 1 & 2

1. ***Explain the MRI protocols, how data acquired on different scanners will be harmonized, and what quality control steps will be undertaken*** *(Crtq p11 & 12)**.***

Response: Our projects are from three different types of population, and were scanned using different scanners. Therefore, a harmonization of the data *acquisition* across projects will not be possible, and *differences* in the outcomes of the different analyses between projects must be interpreted cautiously. However, data from each cohort will be considered separately. Similarities of findings across studies with diverse populations will add support for TRAP-related effects that transcend between-subject variability. We have added the following sections to the grant to explain our data acquisition by cohort, quality control and harmonization procedures: *Table 1*, *Scan acquisition (Table 1) and harmonization*, and *Imaging quality control*.

Reviewer 1

2. ***How will the Core support the projects with their mediation analyses?*** *(Crtq p11)*

Response: Core B will perform the targeted Structural Equation Model (SEM) analyses, including testing mediations to help Projects 1 & 2 address their aims and hypotheses. For the Agnostic Mediation Analysis, we will provide Project 1 with summary brain measure values to be included. For both targeted and agnostic approaches, we will provide image visualization needed for publications and will assist in data interpretation.

3. ***What alternative strategies will be used if the AD risk score fails to be validated with the WHIMS data set*** *(Crtq p11)**.***

Response: We have added the following text to address this:

If we fail to validate the ADNI cortical thickness signature in WHIMS, we will refine the ADNI signatures by creating new cortical thickness signatures in WHIMS in the controls who progress to MCI or AD versus stable controls. We will then create an overlap map between the WHIMS and ADNI signatures. Finally, we will evaluate whether these refined maps predict cognitive decline from controls to MCI or AD in ADNI. If so, these refined maps may better indicate cognitive decline across cohorts. Patterns of atrophy may also differ by sex. To test this, we can generate sex-specific cortical thickness signatures in ADNI, and evaluate whether the female pattern is better validated in WHIMS relative to the initial signature pattern.

4. ***We are focused on AD, while other parts of PPG also included studies on cerebrovascular damage*** *(Crtq p11)**.***

Response: To enhance our consideration of cerebrovascular damage, in addition to including total white matter hyperintensity (WMH) volume in our SEMs, we will obtain WMH volume estimates both periventricularly and in deep white matter, which may have different histopathological correlates. We will test two models that evaluate whether brain measures mediate the relationship between TRAP and global cognition – one with total WMH, and one with periventricular and deep white matter hyperintensities considered separately.

Reviewer 2

5. ***Core B1 is understaffed for the amount of work*** *(Crtq p12)**.***

Response: We have added another graduate student, Brandalyn Reidel, to the budget. Her focus is Alzheimer's disease and brain imaging. She is an expert at using EPIC and heavily edited the initial computer program to refine and expand its capabilities.

Overall*:* We have now submitted a resource sharing plan.

SUB-CORE B2 MOUSE IMAGING

1. ***The imaging protocol was not well-articulated leaving the logistics (especially timing) in question*** *(Crtq p31)**.***

Response: We list the sequence of procedures on each mouse: 1. DCE-MRI…thru 7. Immunohistochemistry. Also see Table 4 Workplan.

New equipment installed in the Zilkha Neurogenetics Institute:

Multi-photon in vivo imaging: Nikon A1R combination confocal/ multiphoton microscope coupled to six lasers for in vivo imaging approaching 1 um depth in vivo at speeds up to 400 frames per second.

Ultra-highfield MRI: 7.0 Tesla cryogen-free superconducting magnet for mouse brain imaging at 80 µm isotropic spatial resolution, in vivo and ex vivo. Fig. 4 shows coronal sections of fiber spin density. We include an expanded description of the procedure and analyses (See Vertebrate Animal Section).

SPECIFIC AIMS – Core B

The primary mission of the Neuroimaging Core is to provide high-quality human MRI brain image analysis in support of Projects 1 & 2, and to acquire and analyze mouse brain MRI scans in support of Projects 3 & 4. Exposure to traffic-related air pollutants (TRAP) contributes to cognitive aging [1, 2], posing a pervasive threat to the aging American populations. The Neuroimaging Core B will provide the scientific expertise and technical support needed for the four scientific projects. We will help the projects to assess the impact of TRAP on predicted Alzheimer's disease (AD) risk and to better understand the underlying mechanisms in both human populations and experimental animal models. The Neuroimaging Core B will work with the scientific project PIs/co-investigators to examine whether TRAP exposure is associated with neurodegenenerative changes, measures of structural brain integrity, and cerebrovascular damage (e.g., disrupted blood-brain barriers), and also assess whether/how the resulting differences in of vulnerable brain regions and cerebrovascular injuries mediate the relationship between TRAP exposure and cognitive decline indicative of an increased AD risk as observed in human populations and tested in experimental animals

The Neuroimaging Core, which includes two subcores, will be led by **Dr. Paul Thompson**, a world-renowned scientist whose focus is developing novel computational strategies for analyzing brain images.

Dr. Meredith Braskie, whose research focus is to assess and understand AD risk through clinical and population neuroimaging, will lead The Population Neuroimaging Subcore (B1). **Dr. Berislav Zlokovic,** whose preeminent work focuses on Alzheimer's disease and blood brain barrier integrity will direct the Mouse Brain Imaging Subcore (B2).

Core B has the following specific aims:

Aim 1. To harmonize the structural brain MRI measures for population studies. We will perform standardized data analysis on already-collected structural MRI (sMRI) scans from ADNI, VETSA and WHIMS to assess vertex-wise cortical thickness, selected GM volumes (hippocampus, medial temporal lobe, amygdala), white matter hyperintensity (WMH) volume, and segmented white matter volume. In ADNI and VETSA we will calculate DTI mean diffusivity (MD), fractional anisotropy (FA), and axial and radial diffusivity (D_{ax} and D_{rad}). Project 2 will be analyzing their Arterial Spin Labeling scans independently.

Aim 2. To generate AD risk and related sMRI biomarker signatures and associated predicted risk scores for preclinical AD, mild cognitive impairment, and AD. To derive the predicted risk scores for AD and other associated early risk signatures, we will employ EPIC, a novel neuroimaging analysis tool that optimally partitions the cortex to classify a disease or other trait based on a neuroimaging measure, such as connectivity or cortical thickness [3, 4].

<u>The resulting Aim 2 products to be delivered to Projects 1 & 2 include the following predicted risk scores:</u>

Signature patterns of vertex-wise cortical thickness derived in ADNI that optimally classify higher versus lower AD risk based on: a) overall AD vs. controls; b) controls who progress to MCI vs. stable controls; c) preclinical AD [5] - amyloid + controls vs. amyloid- [6]; d) CSF tau+ controls vs. tau- [7]; e) overall MCI vs. controls; f) MCI amyloid+ vs. amyloid- controls [6]. For the overall predicted risk for AD and MCI, we will conduct a prospective validation: incident AD (~40 expected by 2017) and incident MCI (~150 expected by 2017) ascertained during the follow-up of 1313 WHIMS MRI participants who were cognitively normal at the time of brain MRI scanning.

Aim 3. Support Projects 1 & 2 in their proposed mediation analyses using both targeted approach in structural equation models (SEM) and agnostic approach with high-dimensional analyses. We will aid Projects 1 & 2 (VETSA and WHIMS cohorts) in performing their targeted structural equation modeling (SEM) to evaluate how **<u>a priori targeted AD brain regions</u>** (e.g., hippocampus, frontal cortex, or AD risk related cortical thickness patterns assessed in Aim 2) mediate the previously identified relationship between TRAP and cognition [1, 2], specifically global cognition, episodic memory, and executive function. We will evaluate how FreeSurfer-derived regional white matter volumes relate to TRAP exposure. In VETSA, we also will evaluate how voxelwise DTI MD relates to air pollution.

Mouse imaging SubCore

Aim1. Perform *in vivo* mouse brain imaging to assess cerebral blood flow, blood brain barrier (BBB) permeability, vascular angiography, and MRI tractography.

Aim 2. Postmortem studies of the neurovascular unit & BBB by confocal microscopy for immunofluorescence.

Aim 3. Ultra-highfield MRI to detect mouse connectivity changes through diffusivity and tractography maps & quantification of BBB permeability by dynamic contrast enhanced (DCE)-MRI.

Aim 4 Analyze mouse data from Projects 3 and 4.

NEUROIMAGING CORE RESEARCH STRATEGY

SIGNIFICANCE: Our Imaging Core has a novel, unified focus of characterizing the relationship between traffic-related air pollutants (TRAP) exposure and brain integrity and cognition in humans and mice. TRAP exposure may contribute to cognitive decline in humans and brain integrity disruptions in animal models [1-7].

POPULATION (HUMAN) NEUROIMAGING (SUBCORE B1)

SIGNIFICANCE: We will perform large-scale, high-quality human brain MRI scan analyses in support of Projects 1 & 2's aims to examine how TRAP relates to brain structure and connectivity, cerebrovascular damage, and Alzheimer's disease (AD)-related cognitive decline in two geographically-diverse, largely non-demented cohorts of women (Project-1; WHIMS MRI) and men (Project-2; VETSA). We will synchronize data analyses across three cohorts (ADNI; WHIMS; and VETSA) and will: (1) use ADNI scans to define sMRI-based signatures and associated risk scores of AD, MCI, and preclinical AD, and deliver these measures to WHIMS MRI and VETSA cohorts; and (2) provide technical and statistical support to Projects 1 & 2 for their proposed analyses linking TRAP to harmonized sMRI measures (brain volume; cortical surface; identified AD signature; WM hyperintensities [WMH]) and diffusion tensor imaging (DTI) measures. We will use structural equation modeling (SEM) to assess whether brain structure integrity in targeted regions (such as the medial temporal lobe), mediates the relationship between TRAP exposure and cognitive aging. To define the risk signatures and scores associated with diagnostic group and AD neuropathology pathology in older adults, we will apply an innovative machine learning approach to whole-brain cortical thickness at each vertex on the brain's surface. Our work will help us to better understand the effects of TRAP exposure on the brain and cognition in non-demented older adults. This may focus for future interventional research in at-risk older adults.

APPROACH:

Scan acquisition (Table 1) and harmonization: *ADNI scans are acquired at many sites, using standardized scan protocols and procedures (such as use of an MRI phantom) to minimize variability among sites. We will adjust for scanner strength (1.5 T or 3T) in all ADNI analyses except for DTI, which were collected only at 3T. Data for the WHIMS study were collected at 1.5 T across 14 sites, using a standardized protocol. VETSA scans were collected at two separate sites: at USCD on a GE 3T Discovery and at MGH using a Siemens 3T Tim Trio. Data from the two VETSA sites will be combined using random effect meta-analyses based on a tests ($p<0.05$). Each VETSA collection site will be weighted by the inverse of its variance and the between-studies variance With DTI, we use meta-analysis of the model coefficients, as the mean and variance of some DTI parameters (e.g. FA) depend on the voxel size, number of diffusion gradients, and the signal to noise ratio of the protocol. Meta-analysis avoids the need to use the same DTI protocol across sites and gives more robust effects than aggregated mega-analysis. We have related expertise leading ENIGMA, in which we pool scans from >60 sites [8] to detect genetic brain effects. We will adjust for site effects.*

Imaging quality control: *We will visually inspect all scans and will exclude any 1) subject having non-AD related anatomical anomalies, 2) scan with distortion or artifacts that prevent adequate co-registration or successful individual analysis, and 3) measure of interest that is >3 S.D. from the mean in either direction. For FreeSurfer analyses, we will visually assess segmentations slice by slice and will exclude from our statistics failed segmentations, defined as those whose partitions misestimate the correct hippocampal outline or gray-white matter-CSF boundaries on more than three consecutive sagittal slices. Research Staff will read training materials and complete relevant quality control training on practice sets before performing quality control on scans or analyses.*

Table 1.	*ADNI*	*VESTA1*	*VESTA-UCSD*	*VETSA-MGH*	*WHIMS*
T1-weighted	*MPRAGE/IR-SPGR (0.98x0.98x1.2 mm³)*	*MPRAGE – 2 repetitions (1x1x1.33 mm)*	*FSPGR (1x1x1.2 mm)*	*MPRAGE (1x1x1.2 mm)*	*T1-weighted gradient echo (1.16x1.16x1.5 mm³)*
DTI	*41 directions (b = 1000 s/mm²); 5 b0; 2.7x2.7x2.7 mm³*	*30 directions (b=500 s/mm2); 5 b0; 2x2x2 mm3; 2 repetitions with opposite phase-encode polarity*	*51 directions (b=1,000 s/mm²); 2 b0 with opposite phase-encode polarity; 2.5x2.5x2.5 mm³*	*2 repetitions; 30 directions (b=1,000 s/mm²);); 2 b0 with opposite phase-encode polarity; 2.5x2.5x2.5 mm³*	*N/A*
WMH	*T2 FLAIR*	*T2/PD double echo*	*T2/PD double echo*	*T2/PD double echo*	*T2 FLAIR*

FreeSurfer analysis: In Aim 1, we will harmonize structural data analysis across ADNI, WHIMS and VETSA, using FreeSurfer software (surfer.nmr.mgh.harvard.edu) to segment the brain by tissue class (gray, white, CSF). FreeSurfer automatically labels the gray matter and associated white matter regions (**Figure 1**) using standardized delineations based on the Desikan-Killiany brain atlas [9]. We will also use FreeSurfer to calculate

hippocampal volume, total white matter volume, and estimated intracranial volume (ICV). Hippocampal segmentations will be based on a standard protocol [10]. Hippocampal, WMH, and regional white matter volumes will be adjusted for intracranial volume. Of more than 3000 sMRI scans to be reprocessed, 2133 (including 730 with repeated measures) came from WHIMS MRI and 992 (including 350 repeated) from VETSA.

Creating and Validating Predicted Risk Scores and Identifying Cortical Thickness Signatures: Prior studies have created AD "signatures" that allow for a summary assessment of AD risk based on patterns of atrophy that best characterize AD vs. controls [11, 12]. Here, we will create similar topographic signatures in ADNI, but will combine the information based on both diagnosis and neuropathology (see ***AD Risk Categories*** below***)*** for use in Project 1 & 2 Aims***.*** AD-related pathology has been associated with cortical thickness in cognitively intact older adults previously using vertex-wise or AD-signature approaches in many [11, 13-15], but not all [16] studies. In our largely non-demented subject cohorts, we focus on adults having increased risk for AD, allowing us to isolate the earliest structural indicators of AD.

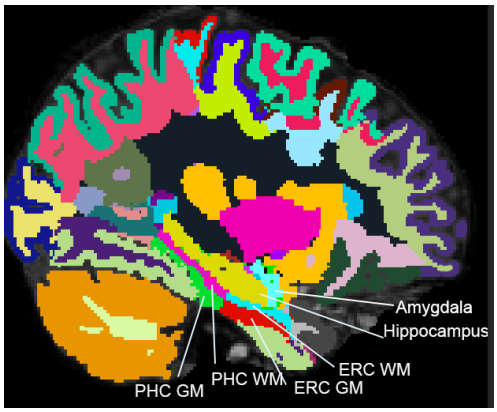


Figure 1. Example gray and white matter parcellation in FreeSurfer. PHC = Parahippocampal cortex; ERC = Entorhinal Cortex; WM= white matter; GM = gray matter

In neuroimaging studies, cortical thickness analyses are typically performed in one of two ways. Either statistics are run at every vertex or voxel in the brain, or statistics are performed on summary measures representing the mean cortical thickness across all the voxels or vertices within each standard region, such as those delineated in the Desikan-Killiany atlas [9]. These methods, while valuable, evaluate each brain region or vertex in isolation, rather than considering patterns of cortical thickness across the brain surface that may be representative of a disease or other phenotype. This increases the number of individual comparisons that must be made, and thus decreases the statistical power to detect effects. Averaging results across an anatomically defined region of interest additionally may obscure effects if cortical thinning is not uniform within what may be a somewhat arbitrarily defined region. In contrast, "Evolving Partitions to Improve Connectomics" (EPIC) [17] is a data-driven approach, which clusters or subdivides existing regions of interest to partition the cortex in a way that optimizes the classification of disease based on brain connectivity or cortical thickness [18].

For Aim 2, we will begin with vertex-wise cortical thickness measurements derived from FreeSurfer analyses of ADNI data to arrive at patterns of cortical thickness that best separate high risk versus low risk (See ***AD Risk Categories***). EPIC will use logistic regression as a classifier, and will perform leave-one-out validation. We will also perform external prospective validation within WHIMS on select comparisons (see ***AD Risk Validations***). Predicted risk scores indicate the probability of being considered high-risk based on a subject's cortical thickness pattern. As a result of Aim2 performance, we will generate mean cortical thickness values within the AD risk signatures and 6 categories of predicted AD/MCI Neuropathology risk scores that will be submitted to Projects 1 & 2 for the subsequent SEM (Aim3).

Table 2. ADNI - Assessing Biomarkers	N
Controls at baseline who also have 24 month diagnosis.	207
-Subset with AV45 amyloid PET	148
-Subset with CSF $A\beta_{42}$ & $ptau_{181}$	135
-Subset with CSF tau	133

AD Neuropathological Biomarkers – ADNI: AV45 amyloid positivity will be assessed using the mean positron emission tomography (PET) scan standardized uptake value ratio (SUVR) using the whole cerebellum as the reference region and a threshold of >1.11 [19]. Of the 266 total cognitively intact older adults who had MRI scans at baseline (**Table 2**), 207 had diagnoses available both at baseline and at a 24-month follow-up. Of those, 148 had AV45 amyloid PET scans at baseline, 135 had CSF $A\beta_{42}$, and $ptau_{181}$, and 133 had CSF tau available at baseline. Of the 207 controls, 13 had declined to a diagnosis of AD or MCI at 24 months. CSF total tau positivity (tau+; > 93 pg/ml [20]) and AV45 amyloid positivity (amyloid+; PET mean signal >1.11 [19]) were both significantly different between stable and declining older adults in this sample (p = 0.48 and p = 0.016, respectively). CSF $A\beta_{42}$ (<192 pg/ml) and $ptau_{181}$

Table 3. ADNI: Subject count by category. Values represent "baseline" scans unless otherwise noted.

	N	Neuropathology (Aβ or tau +/-)
Controls	266	
-subset with AV45 amyloid PET	207	60+/147-
-subset with CSF tau	188	36+/152-
-subset negative for CSF Aβ42 & tau	99	
MCI	377	
- subset with AV45 amyloid PET	350	187+/163-
AD	714	
Controls who progress to MCI *	66	

positivity (>23 pg/ml) [20] did not successfully distinguish stable cognition from decliners in this sample (p = 0.74 and p = 0.48, respectively). Although this is a small sample, this is consistent with a past model of AD biomarkers that CSF tau and amyloid PET may show detectable differences closer to an AD diagnosis compared with CSF Aβ, which changes earlier [21]. Thus CSF Aβ positivity, may be less informative when evaluating short term (24 month) decline. Here we focus on CSF tau+ and AV45 amyloid + as AD biomarkers

AD Risk Categories – ADNI, WHIMS, VETSA: We will use EPIC to partition cortical thickness patterns to best classify high versus low predicted risks for AD, MCI, and preclinical AD in ADNI (**Table 3**). We will define high versus low AD risk in six separate analyses in ADNI: 1) an overall risk score for clinical AD derived by comparing 714 AD versus 266 cognitively intact older adults (controls); 2) an overall risk score for *incident* MCI derived by comparing 66 controls who progress to MCI within two years versus 194 controls at baseline who are cognitively stable over two years; 3) a specific risk score for preclinical AD comparing 60 controls who are amyloid + assessed with an AV45 PET scan versus 147 amyloid- controls; 4) a risk score for increased tau pathology comparing 36 CSF tau+ controls versus 99 controls who are both CSF AB- and tau-; 5) an overall MCI risk score comparing 377 MCI versus 266 controls; 6) a specific risk score for amyloid+ MCI, comparing 187 AV45 amyloid+ MCI versus 147 amyloid- controls. Controls who progressed to MCI were not included in comparison 2 above if they first regressed from MCI or later regressed to control, as this suggests a less stable diagnosis. In WHIMS and VETSA, we will assess mean cortical thickness within these signatures and will provide Projects 1 & 2 with these values and predicted risk scores (the probability each subject has of being in the "high risk" category for each analysis). We will use linear regression to assess which of these signatures best predict a decline in cognitive domain scores or diagnosis in WHIMS and VETSA. See **Table 4** for subject population information in WHIMS and VETSA. The cortical thickness or predictive risk values from the best cognitive decline predictor in each cohort will be used by the projects in further analyses, including SEM.

AD Risk Validations – WHIMS: We will perform prospective validations using independent data in the WHIMS cohort on the optimal cortical partitions derived from the training. Specifically, we will validate the ADNI cortical thickness signature for AD versus control using a longitudinal validation of WHIMS 36-46 controls who progressed to AD in subsequent years. We will validate the ADNI cortical thickness signature for 150 controls who progressed to MCI versus cognitively stable controls using a longitudinal validation of WHIMS controls who progressed to MCI in subsequent years, both out of the 1313 subjects who were cognitively intact at the time of MRI scan 1. *If we fail to validate the ADNI cortical thickness signature in WHIMS, we will refine the ADNI signatures by creating new cortical thickness signatures in WHIMS in the controls who progress to MCI or AD versus stable controls. We will then create an overlap map between the WHIMS and ADNI signatures. Finally, we will evaluate whether these refined maps predict cognitive decline from controls to MCI or AD in ADNI. If so, these refined maps may better indicate cognitive decline across cohorts. Possibly, patterns of atrophy will differ by sex [22, 23]. To test this, we can generate sex-specific cortical thickness signatures in ADNI, and evaluate whether the female pattern is better validated in WHIMS relative to the initial signature pattern*

Table 4. WHIMS and VETSA: Subjects with sMRI

	VETSA	VETSA subset with DTI and WMH *	WHIMS sMRI and WMH *
Controls at MRI 1 (2005-6)	385	336 DTI only	1313
MCI by MRI 1 (2005-6)	62	55 DTI only	43
Controls at MRI 2 (2010-11)	486	303 DTI and WMH	718
MCI by MRI 2 (2010-11)	59	39 DTI and WMH	123
Incident AD 2005-6 to 2010	0	0	16
Incident AD expected 2010 to 2016	0	0	20-30

* WMH will be assessed using a FLAIR MRI in WHIMS and a T2/proton density double echo scan in VETSA.

Diffusion tensor imaging analyses – VETSA and ADNI: We will remove non-brain material and will perform eddy-correction before non-linearly aligning the diffusion scans to their associated T1-weighted image in template space. We will calculate scan-wide fractional anisotropy (FA), mean diffusivity (MD), and axial and radial diffusivity using FSL's DTIfit tool. We will evaluate MD, which is particularly sensitive to AD risk [24, 25] specifically within FreeSurfer-derived white matter parcellations adjacent to gray matter targeted as important to either episodic memory or frontal lobe-dependent function. These MD values will be included in our targeted structural equation models (**Aim 3**). We also will perform voxelwise statistics on MD within the total white matter to assess the white matter tracts most associated with TRAP exposure. In ADNI, we will compare DTI MD between 1) AD versus controls 2) MCI versus controls 3) amyloid+ controls versus amyloid- controls and 4) MCI amyloid+ versus controls for use in future analyses.

White matter hyperintensities: We will assess WMH using previously published methods based on a Bayesian segmentation approach applied in ADNI [26]. Each FLAIR or double echo image will be co-registered

to its own T1-weighted volume scan and to a common template. Inhomogeneity of our FLAIR or double echo scans will be based on a previously published local histogram normalization method [27]. We will then estimate WMH using a modified Bayesian probability structure using an established histogram fitting method. We will threshold these probabilities at 3.5 sd above the mean consistent with prior work to create a binary WMH mask [28], *and will also create separate masks of periventricular and deep WMH*.

Statistical Analyses: Single-sex based analyses will be performed separately in Projects 2 & 2 using WHIMS MRI and VETSA cohorts. Please see Projects 1 & 2 for covariates to be used for each cohort. We will use the false discovery rate (FDR) to control for voxel- and region-wise multiple comparisons.

Targeted Structural Equation Models (SEM): We previously used SEM to evaluate contributions to AD risk [29, 30]. Here, our targeted SEMs evaluate whether the integrity of *a priori* selected AD risk regions mediate the established relationship between TRAP and cognition to aid Projects 1 & 2 in their Aims. We will assess how well brain measures mediate an effect between TRAP and 1) **Global cognition (Figure 2)**. Hypothesized mediating brain measures are EPIC-derived cortical thickness AD risk signatures, hippocampal volume, *total versus periventricular/deep white matter WMH volumes*, and total white matter volume. 2) **Episodic memory ability (Figure 3).** Hypothesized mediating brain measures are mean entorhinal cortex (ERC) and parahippocampal cortex (PHC) thickness, white matter volume associated with both cortical regions, hippocampal volume, and (in VETSA), diffusion tensor imaging (DTI) mean diffusivity (MD) within the FreeSurfer-defined ERC and PHC-adjacent white matter. 3) **Frontal lobe-dependent function – specifically, processing speed and cognitive flexibility.** Hypothesized mediating brain measures are mean frontal lobe and anterior cingulate cortical thickness, frontal lobe white matter volume, WMH volume and (in VETSA), DTI MD within the FreeSurfer-defined frontal lobe gray and white matter.

Figure 2. Targeted SEM Global Cognition. See Projects 1 & 2 for description of covariates.

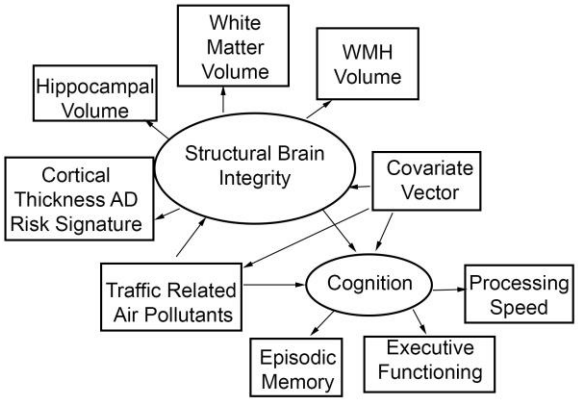
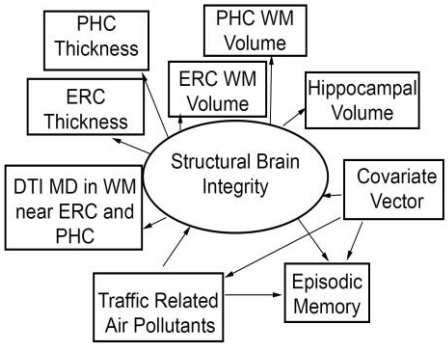


Figure 3. Targeted SEM Episodic Memory. See Projects 1 & 2 for description of covariates.



Agnostic Approach: In Project-1, we will work with Dr. Millstein to conduct mediation analysis with an agnostic approach to identifying regions of the brain that mediate effects of TRAP on brain aging. Specifically, the causal inference test (CIT),[31, 32] developed by Dr. Millstein, will be applied to the Project-1 proposed high-dimensional analyses (Aim3b). Two published studies [33, 34] on the mediation analyses of environmental neurotoxicity followed approaches closely related to the 30-year old Sobel test [35] that was not designed to distinguish causality from reverse causality. The CIT is uniquely suited to address this challenge, by providing a statistically powerful approach when multiple null hypotheses are false as has been demonstrated in simulation studies.[31, 32] In addition, to reduce the search space of mediators, the CIT-based framework could incorporate prior knowledge, such as the ADNI-defined signatures of cortical thinning that best differentiates AD from controls. In VETSA, we also will evaluate how voxelwise DTI MD relates to TRAP.

MOUSE IMAGING SUB-CORE B2

Core B2 assesses cerebrovascular and white matter tract responses to nPM exposure with *in vivo* multiphoton imaging for regional CBF and BBB permeability, and angiography. DTE-MRI, at 80 µm isotropic resolution provides white matter fractional anisotropy maps. BBB cellular integrity is assessed by confocal microscopy.

SIGNIFICANCE. The neurovascular unit (NVU)[1] links neurons with the cerebral vascular system and is comprised of vascular cells (endothelial cells, pericytes), glial cells (astrocytes, microglia, oligodendrocytes) and neurons [1–7]. Signal transduction between these cells maintains normal neurovascular integrity. *Endothelial cells* and *pericytes* form the BBB which limits entry of blood-derived toxic substances, pathogens and cells into the brain[1,2,8,9]. Dysfunction in each NVU cellular constituents (vascular, glia, neurons) is linked to AD progression in experimental, imaging, pathological, and epidemiological studies[1–8,10–14]. Vascular insults can initiate and exacerbate molecular neurodegenerative cascades to cognitive impairment and AD[1–7].

Urban Traffic Associated Air Pollution (TRAP) is hypothesized to be a major insult to the NVU that increases the risk of stroke, AD and AD-like brain pathologies, including to the BBB[15–17], which is exacerbated in *APOE4*

carriers[18]. In experimental studies, mixed vehicle exhaust can increase BBB permeability, decrease tight junction proteins (e.g. occludin and claudin-5) and elevate production of reactive oxygen species in *ApoE*$^{-/-}$ mice[19]. Endothelial *in vitro* studies show that PM2.5 increases BBB permeability and monocyte transmigration[20]. Furthermore, air pollution-derived fine and ultrafine PM stimulated increased Aβ42 production in wild-type mice: nPM from TRAP and nickel [21]. Additionally, nPM neurotoxicants cause hippocampal neuron atrophy and impaired glutamatergic functions[20] (Project 3) and exacerbate cerebral ischemia[22]. Preliminary data (Project 4) show major increase in BBB permeability with a concordant decrease in endothelial pericyte coverage in the corpus callosum after 3 days of experimental CCH. Furthermore, female EFAD mice have more Aβ positive vessels than males, with additive effects of *APOE4* (Project 3). Moreover, female E4FAD and APOE4-TR mice without FAD have microbleeds, which we showed to involve the CypA–nuclear factor-kB–matrixmetalloproteinase-9 pathway[23]. Sub-Core B2 will evaluate the impact of TRAP on the integrity of the NVU (including PWM: perforant path and CA1 neurons) and BBB in B6 and EFAD (Project 3) and in CCH mice (Project 4) allowing consistency and direct comparison between the findings of both projects.

Using cutting edge methodologies, we will employ multiphoton *in vivo* imaging to measure BBB permeability and angiography, ultra-high field MRI to detect connectivity changes through various diffusivity and tractography maps and for quantification of BBB permeability using dynamic contrast enhanced (DCE)-MRI, and post-mortem tissue immunofluorescent and fluorescent lectin staining. These methodologies will be used to test the hypothesis that TRAP components promote convergent neurodegenerative processes from cerebrovascular and neuronal interactions. The Mouse Imaging Sub-Core2 group has decades of experience and state-of-the-art equipment to measure BBB dysfunction and disruption of signaling between different cell types within the NVU[1,2,4,6,8,9,12,14,23–40]. The Zilkha Neurogenetics Institute has installed a new 7.0 Tesla MR for mice, which will enable ultra-high field MRI to detect connectivity changes through diffusivity and tractography and quantification of BBB permeability using dynamic contrast enhanced (DCE)-MRI. See Fig 1.

APPROACH: The Mouse Imaging Sub-Core is led by Dr. Berislav Zlokovic, a leader in the NVU and BBB in collaboration with Dr. Russel Jacobs on small animal MRI. Zlokovic lab members, Drs. Axel Montagne, Kassandra Kisler, Amy Nelson, and a technician will perform experiments.

Project 3 and 4 will provide mice for examination of *i*) *in vivo* BBB permeability using dynamic contrast enhanced (DCE)-MRI; *ii*) *in vivo* cerebral blood flow, BBB permeability and angiography measurements by 2-photon microscopy; *iii*) ultra-highfield diffusion tensor imaging (DTI)-MRI to detect connectivity changes through various diffusivity and tractography (fiber tracking) maps and *iv*) postmortem analysis of white matter tracks and BBB integrity using confocal microscopy. *The following procedures will all be performed in the same mouse, in sequence: 1.DCE-MRI, 2.Cranial window, 3.LDF, 4.BBB angiography and permeability; multiphoton, 5.Perfusion/tissue collection, 6. DTI-MRI, 7. Immunohistochemistry; confocal.*

Aim 1: *in vivo* cerebral blood flow, BBB permeability, & angiography by multi-photon microscopy

Multiphoton *in vivo* Imaging: *We recently acquired a new state-of-the-art Nikon A1R combination confocal/ multiphoton microscope coupled to six lasers spanning the visible spectrum and a dual-line Ti:sapphire laser (InsightDS+, Spectra Physics). This system is configured in an open framework gantry to enable in vivo imaging, can image at speeds up to 400 frames per second, and to depths of nearly 1um in vivo.*

Cranial window: Mice will be anesthetized (ketamine, 100 mg/kg and xylazine, 50 mg/kg), fixed in a stereotaxic frame (Kopf Instruments) and transitioned to isoflurane anesthesia. A circular cranial window will be drilled over the hindlimb region of somatosensory cortex (center at AP, -0.94 mm; L,1.5 mm). The window will be filled with 2% low melt agarose in artificial cerebrospinal fluid (aCSF) and covered with 3 mm coverslips.

Laser-doppler flowmetry (LDF). Cerebral blood flow (CBF) responses to hindlimb stimulation will be measured in anesthetized mice (~1% isofluorane) using laser-Doppler flowmetry measured through a cranial window [23,24,41]. The tip of the laser-Doppler probe (Transonic Systems Inc.) is stereotaxically placed above the cranial window over the somatosensory cortex hind-limb region. CBF will be measured at baseline and following 60 sec electrical stimulation of a hind-limb. The % increase in CBF due to stimulation will be obtained by subtracting baseline CBF from the stable maximum plateau during stimulus, averaged of 3 trials per mouse.

BBB permeability: Cortical cerebrovascular permeability will be determined with rapid *in vivo* multiphoton microscopy imaging as we described[25]. In brief, TMR-conjugated medium-size dextran (40,000 Da, Invitrogen) is injected via the left femoral vein. *In vivo* time-lapse images will be acquired every 2 min for 30 min. Images are subjected to threshold processing; the extravascular fluorescent intensity will be measured with the integrated density measurement function by a blinded investigator. The in vivo BBB permeability for TMR-dextran is estimated as the PS product[25–27] by the formula: PS = (1-Hct) 1/Iv V x dIt/dt, where Hct is hematocrit (45%), *Iv* is initial fluorescence intensity of the region of interest (ROI) within the vessel, *It* is the intensity of the ROI at time *t, V* is vessel volume, assuming 1 g of brain is equivalent to 50 cm^2.

BBB angiography: Fluorescein-conjugated mega-dextran (Invitrogen) is injected via the femoral vein[25]. Multiphoton Z stack images are immediately taken through the cranial window starting 50 mm below the cortical surface, continuing 500 mm deep thru cortical layers II-III at 1 µm intervals. Z stacks are reconstructed with ZEN software (Carl Zeiss Microimaging). The capillary length is analyzed by ImageJ software.

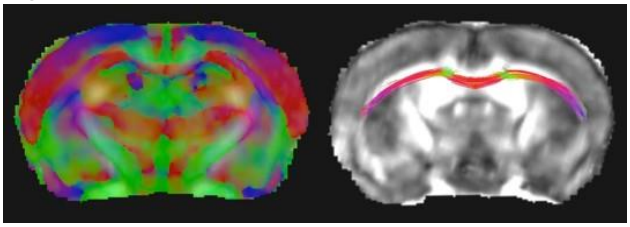
Aim 2: Postmortem analysis of NVU and BBB integrity using confocal microscopy.

Confocal microscopy: The Mouse Imaging Sub-Core has a confocal microscope (LSM 510) coupled to a Mai Tai DeepSee Ti:Sapphire, Argon 488, HeNe 543, and HeNe 633 lasers for up to four-color imaging. Tissue immunofluorescent and fluorescent lectin staining. Anesthetized mice are transcardially perfused with PBS-heparin. Brains are embedded in optimal cutting temperature (OCT) compound (Tissue-Tek) on dry ice and cryosectioned at 18 mm. Sections are blocked with 5% normal donkey serum for 90 min and incubated in primary antibody diluted in blocking solution overnight/4°C. Sections are washed in PBS and incubated with fluorophore-conjugated secondary antibodies. To visualize brain microvessels, sections are incubated with Alexa594-conjugated *L. esculentum* lectin. Primary antibodies: pericytes (PDGFRb, CD13), endothelial cells (CD31, lectin), neurons (SMI-312, NeuN), microglia (Iba1), astrocytes (AQP4, and GFAP), and MMP9, CypA, IgG and fibrin. Imaging will be quantified in corpus callosum, cerebral cortex and hippocampus.

Aim 3: Ultra-highfield MRI to detect mouse connectivity changes through various diffusivity and tractography maps & quantification of BBB permeability by dynamic contrast enhanced (DCE)-MRI.

Ultra-high field MRI in mice: Methods were developed to detect microstructural and connectivity changes through various diffusivity and tractography from diffusion weighted MR images (DTI)[28,42]. Quantification of BBB permeability changes using dynamic contrast enhanced (DCE)-MRI mouse protocols are adapted from methodologies developed in humans[29–31]. USC Zilkha Neurogenetics Institute has installed a 7.0 Tesla cryogen-free superconducting magnet for mouse molecular imaging, *in vivo* and *ex vivo* (Fig. 4).

Figure 4 ***(new)****. Mouse brain coronal slice, MRI 7.0T.* *Left panel: Deterministic streamline fiber tracts, as a function of fiber spin density; based on single and multi-shell diffusion weighted images (DWIs). Tracts are overlaid on maps of the fiber orientation distribution functions (ODF); 5-shell hybrid diffusion imaging (HYDI) was selected as 'ground truth' for comparison. Right: Fiber tract maps of corpus callosum (red), cingulum (green), and external capsule (purple) from DWI-MR of the left panel. White matter tracts are overlaid on fractional anisotropy (QA) maps. The 80 µm isotropic spatial resolution clearly maps cortico-callosal WM connectivity.*



DCE-MRI: The DCE-MRI protocol includes pre-contrast T1-values using a multi-time repetition (TR) spin-echo sequence (TR 5000, 3000, 1500, 800, 400 and 200 ms), followed by a series of T1-weighted images with identical geometry and temporal resolution of <5 sec. A bolus of Gd-DTPA (Gadolinium-diethylenetriamine pentaacetic acid, Magnevist®) is injected via tail vein at 5 min; DCE images are collected for 30 min postinjection. Tractography maps are performed on eddy-corrected DWI scans aligned to the Mori atlas by a deterministic method, FACT in Trackvis (http://trackvis.org/). Methods of Montagne et al will simultaneously study BBB permeability in hippocampus and other regions. Post-processing of DCE-MRI data uses in-house DCE software (*Rocketship*) in Matlab[29–31].

DTI-MRI: Fixed brains are kept within the skull and are soaked at 4°C in 5 mM ProHance (Bracco Diagnostics) for 4 d prior to scanning (**Fig. 2**[28]). For each scan, two intact fixed heads are secured in a Teflon® holder, submerged in perfluoropolyether (Fomblin®, Solvay Solexis) for imaging. Diffusion-weighted images (DWI) are acquired by conventional pulsed-gradient spin echo (PGSE). An optimized six point icosahedral encoding scheme is used for diffusion weighted acquisitions with a single un-weighted reference image for a total imaging time of ~24 hr. Imaging is performed on the entire brain at 80 µm isotropic resolution, which will allow measurement of white matter changes in corpus callosum, the perforant path, and CA1 neuron fields[28,42]. *Details on scanning, pre-, post-processing provided in the Vertebrate Animal Section & published reports*[28,42].

Table 4. Work plan Projects 3 and 4 for the Mouse Neuroimaging Sub-core over 5 years.

Year	Project		Genotype	Groups	Sex	Age	CCH/sham	nPM/Ctl	N/group	Total	Imaging/IHC
Y1-2	3	Aim1, Exp4	B6	2	2	1	0	2	10	40	40
Y3-4	4	Aim 2	B6	4	1	1	2	2	8	32	32
Y5	3	Aim2, Exp4	EFAD	2	1	1	0	2	10	20	20

CCH, chronic cerebral hypoperfusion

VERTEBRATE ANIMALS

1. Provide a detailed description of the proposed use of the animals in the work outlined in the Research Strategy section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.

C57BL/6 (B6)mice (male and female), EFAD transgenic (tg) mice (male and female) from Projects 3 and 4 will be examined by this Core. Experimental details are included in the Project's Vertebrate Animal Section and Research Design.

Year	Project		Genotype	Groups	Sex	Age	CCH/sham	nPM/Ctl	N/group	Total	Imaging/IHC
Y1-2	3	Aim1, Exp4	B6	4	2	1	0	2	10	40	40
Y3-4	4	Aim 2	B6	4	1	1	2	2	8	32	32
Y5	3	Aim2, Exp4	EFAD	2	1	1	0	2	10	20	20

CCH, chronic cerebral hypoperfusion

The following procedures will all be performed in the same mouse, in sequence: 1.DCE-MRI, 2.Cranial window, 3.LDF, 4.BBB angiography and permeability; multiphoton, 5.Perfusion/tissue collection, 6. DTI-MRI, 7. Immunohistochemistry; confocal.

DCE-MRI. The DCE-MRI protocol includes pre-contrast T1-values using a multi-time repetition (TR) spin-echo sequence (TR 5000, 3000, 1500, 800, 400 and 200 ms), followed by a series of T1-weighted images with identical geometry and temporal resolution of <5 sec. A bolus of Gd-DTPA (Gadolinium-diethylenetriamine pentaacetic acid, Magnevist®) is injected via tail vein at 5 min; DCE images are collected for 30 min postinjection. Tractography maps are performed on eddy-corrected DWI scans aligned to the Mori atlas by a deterministic method, FACT in Trackvis (http://trackvis.org/). Methods of Montagne et al will simultaneously study BBB permeability in hippocampus and other regions. Post-processing of DCE-MRI data uses in-house DCE software (*Rocketship*) in Matlab.

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BBB permeability. Cortical cerebrovascular permeability will be determined with rapid *in vivo* multiphoton microscopy imaging as we described. In brief, TMR-conjugated medium-size dextran (40,000 Da, Invitrogen) is injected via the left femoral vein. *In vivo* time-lapse images will be acquired every 2 min for 30 min. Images are subjected to threshold processing; the extravascular fluorescent intensity will be measured with the integrated density measurement function by a blinded investigator. The in vivo BBB permeability for TMR-dextran is estimated as the PS product by the formula: PS = (1-Hct) 1/Iv V x dIt/dt, where Hct is hematocrit (45%), *Iv* is initial fluorescence intensity of the region of interest (ROI) within the vessel, *It* is the intensity of the ROI at time *t, V* is vessel volume, assuming 1 g of brain is equivalent to 50 cm^2.

BBB angiography. Fluorescein-conjugated mega-dextran (Invitrogen) is injected via the femoral vein. Multiphoton Z stack images are immediately taken through the cranial window starting 50 mm below the cortical surface, continuing 500 mm deep thru cortical layers II-III at 1 µm intervals. Z stacks are reconstructed with ZEN software (Carl Zeiss Microimaging). The capillary length is analyzed by ImageJ software.

Perfusion. Animals are anesthetized by the inhalant isoflurane and blood collected through cardiac puncture using heparinized tubes. Animals are then be perfused with phosphate buffer, pH 7.4.

DTI-MRI. Fixed brains are kept within the skull and are soaked at 4°C in 5 mM ProHance (Bracco Diagnostics) for 4 d prior to scanning. For each scan, two intact fixed heads are secured in a Teflon® holder, submerged in perfluoropolyether (Fomblin®, Solvay Solexis) for imaging. Diffusion-weighted images (DWI) are acquired by conventional pulsed-gradient spin echo (PGSE). An optimized six point icosahedral encoding scheme is used for diffusion weighted acquisitions with a single un-weighted reference image for a total imaging time of ~24 hr.

Imaging is performed on the entire brain at 80 µm isotropic resolution, which will allow measurement of white matter changes in corpus callosum, the perforant path, and CA1 neuron fields.
A 7T MR scanner is used to acquire all diffusion weighted images (DWIs) of the mouse brains. Fixed brains are kept within the skull, all skin and cartilaginous tissue are removed, and brains are soaked at 4°C in 5 mM gadolinium contrast ProHance (Bracco Diagnostics, Inc., Princeton, NJ) for 4 days prior to scanning to minimize the T1 relaxation effect on the tissue. For each scan, two intact fixed heads are secured in a Teflon® holder, submerged in Galden® (perfluoropolyether with same magnetic susceptibility as water) (Fomblin®, Solvay Solexis, Inc., Thorofare, NJ). This ensures that no leakage will occur and that the signal will not change during acquisition. First, 3D-rapid acquisition with relaxation enhancement (RARE) anatomical images is acquired (TR/TE = 250/9 ms; RARE factor 8; 140x80x80 matrix; 28x16x16 mm FOV, 200 µm isotropic voxel size; 1 average). Then, DWIs is acquired using a conventional pulsed-gradient spin echo (PGSE) sequence (TR/TE = 300/16.2 ms, 350×200x200 matrix, 28x16x16 mm FOV, 80 µm isotropic voxel size, 1 average, δ = 3 ms, Δ = 8 ms, G_d = 1000 mT/m, nominal b-factor = 3000 s/mm^2). Six diffusion weighted images are acquired in addition to one volume with no diffusion sensitization using an optimized six points icosahedral encoding scheme for a total imaging time of 24 h.
To pre-process the raw *ex vivo* DWIs, we first correct for eddy current distortions using the “eddy correct” tool in FSL (www.fmrib.ox.ac.uk/fsl). Extra cerebral tissue is removed using the “skull-stripping” Brain Extraction Tool from BrainSuite (http://brainsuite.org/). All resulting volumes are visually inspected and manually edited as needed. Then, all images are linearly aligned using FSL’s “flirt” function with 12 degrees of freedom to allow for rotation, translation, scaling, and skewing in 3D. The gradient direction tables are rotated accordingly after each linear registration for the 6 diffusion volumes. Furthermore, each skull-stripped b_0 images are elastically registered to a minimum deformation template created using all linearly registered images for both groups. This is done to ensure that all scans are in the same space for further analysis.
We will then apply the DTI model using the FSL’s “dtifit” tool to compute fractional anisotropy (FA) maps. The diffusion tensor is computed using the eddy corrected and elastically registered DWI scans. A Gaussian low-pass filter with kernel size 3 (*i.e.*, 3x3x3 voxel) is applied to all maps. To test for group differences, a voxel-wise linear regression is run, with ***X*** *(experimental)* mice coded as 1 and *Y (control)* mice coded as 0. We run this for EFAD and CCH mice separately. A regional false discovery rate (FDR) correction will be used to correct for multiple comparisons across voxels. Additionally, searchlight-based multivoxel pattern statistics are performed on the resulting probabilistic *p*-value maps from the regression in all cohorts. As we published previously (Daianu SPIE and PlosOne), tractography maps are then performed on the eddy corrected DWI scans aligned to the Mori atlas using a deterministic fiber reconstruction method, FACT, in Trackvis (http://trackvis.org/).

2. Justifications: Provide justification that the species are appropriate for the proposed research. Explain why the research goals cannot be accomplished using an alternative model (e.g. computational, human, invertebrate, in vitro).
In vitro studies or computer/ mathematical models cannot satisfactorily reproduce the white matter/ neuronal injury and functional deficits resulting from nPM/ CCH exposures. Due to cost, ethical concerns, and reproducibility, mice are most appropriate for these studies. We choose mice because of our experience in the models being tested, availability of applicable antibodies/ assays, and potential for future investigations. C57BL/6 mice are the standard murine strain used by most aging researchers and are available through the NIA Aging Mouse Colony. Further, all previous nPM data has been collected using C57BL/6J mice. Altering background strains for the study would likely introduce strain specific differences in exposure responses and outcomes. This variation would increase sample sizes. Male and female mice are selected to study sex influences and comply with recent NIH initiatives to balance sex in animal studies. Power analyses are designed to limit sample size.

3. Minimization of Pain and Distress. Describe the interventions including analgesia, anesthesia, sedation, palliative care and humane endpoints to minimize discomfort, distress, pain and injury.
Discomfort, distress, pain, and injury will be minimized in all phases of the experiment. We will monitor behavioral changes, e.g., loss of appetite, grooming and activity. Moribund criteria include one or a combination of the following: >20% weight loss, sustained hunched posture, respiratory difficulty,

hypo/hyperthermia, inability of access food/water, inability to ambulate or make normal postural adjustments. Mice will be monitored for any signs of stress or pain as well as general criteria outlined by IACUC (Body Condition Score 2, BC2) and will be euthanized.

4. Euthanasia.
After completion of experimental procedures, all rodents will be euthanized by methods approved by the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

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Resource Sharing Plan

Data Sharing Plan: We will share data in accordance with NIH policy. Processed data will be made public along with publications, and will be distributed through supplementary materials or our own websites.

Sharing Model Organisms: not applicable

Genome Wide Association Studies: not applicable

All key resources for this proposal will be authenticated to enhance the reproducibility of our results, as appropriate and according to NIH policy. Key Biological Resources that will be utilized and validated in this proposal include:

Cell lines: None

Imaging of mouse strains (Project 3&4): C57BL/6 and EFAD.

C57BL/6 mice from NIA Aging Colony and Jackson Labs

EFAD (E3FAD and E4FAD) mice were generated by crossing 5XFAD to homozygous APOE3-, and APOE4-TR. 5xFAD mice are transgenic for 5 distinct FAD mutations (APP K670N/M671L+ I716V+ V717I and PS1 M146L+L286V). They are provided by University of Illinois at Chicago (Mary Jo LaDu, PhD).

Antibodies: Only commercially available antibodies will be used.

Other resources used in this proposal will be standard laboratory reagents. Should we need to generate or obtain additional unique resources in the course of this proposal, they will be authenticated using methods similar to those described above, as appropriate.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0729333930000

Legal Name*:	University of Southern California
Department:	Contracts and Grants
Division:	95-1642394
Street1*:	3720 South Flower Street
Street2:	
City*:	Los Angeles
County:	Los Angeles
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
ZIP / Postal Code*:	90089-0701

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Mr.	Steven		Misuraca	

Position/Title:	Contracts and Grants Officer
Street1*:	3720 South Flower Street
Street2:	
City*:	Los Angeles
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
ZIP / Postal Code*:	90089-0701

Phone Number*: 213-740-8207 Fax Number: 213-740-6070 Email: misuraca@research.usc.edu

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Environment Exposure & Neurotoxicology Core

12. PROPOSED PROJECT

Start Date*	Ending Date*
04/01/2018	03/31/2023

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of Southern California
Duns Number: 0729333930000
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: CA
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 90089-0701
Project/Performance Site Congressional District*: CA-037

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of Washington
DUNS Number: 6057994690000
Street1*: 4333 Brooklyn Avenue, NE, Box 359472
Street2:
City*: Seattle
County:
State*: WA: Washington
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 98195-9472
Project/Performance Site Congressional District*: WA-007

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: 1 2 3 4 5 6 If

NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Core_C_Abst_2017_FINAL.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Core_C_Bibliography_2017_FINAL.pdf
10.Facilities & Other Resources	Core_C_FACILITIES_AND_OTHER_RESOURCES.pdf
11.Equipment	Core_C_EQUIPMENT.pdf

Environmental Exposures and Neurotoxicology Core (Core C)

Director, C Sioutas, Co-Directors: JC Chen and H Forman

Abstract

The Environmental Exposures and Neurotoxicology Core C, directed by C. Sioutas, includes three supporting subcores to serve the proposed scientific projects, through the provision of the residence-specific exposure estimates in populations, standardization of the inhalation exposure experiments in animal models, and analyses of the neurotoxic responses to traffic-related air pollution (TRAP). SubCore C1, Environmental Data Support (coDir: JC Chen) provides Projects 1 and 2 with individual-level, location-specific estimates of long-term TRAP exposure, using state-of-the-art spatio-temporal modeling approaches harmonized across two human population-based cohorts. Subcore C2, Collection of TRAP-nPM (Dir: C.Sioutas) supplies experimental Projects 3 and 4 with well-characterized TRAP inhalation exposure atmospheres. TRAP are sampled near urban sites impacted by traffic emissions. SubCore C3, Brain Cell Responses to TRAP-PM (coDir: H. Forman) (i) tests individual batches of collected nPM using THP-1 cells for cytokine expression; and (ii) Western blot assay of brain protein changes to in vivo nPM exposure for Projects 3 & 4.

FACILITIES AND OTHER RESOURCES

1. University of Southern California Keck School of Medicine
Department of Preventive Medicine, Division of Environmental Health

The Department of Preventive Medicine has been one of the leading academic departments of preventive medicine and public health sciences for more than three decades. Ranked as the top academic department in preventive medicine with the largest NIH-sponsored researches, the department is home to worldwide authorities on chronic disease epidemiology (including cancer and cardiovascular disease), biostatistics, environmental medicine, health behavior, and prevention, detection, and treatment methods. The Division of Environmental Health (EH) is home to two federally funded regional research centers, including the Southern California Environmental Health Sciences Center (SCEHSC) and the Children's Environmental Health Center. The EH division is the operational house of the Children's Health Study, which is one of the most comprehensive cohort studies on the long-term health effects of air pollution among more than 15,000 school-aged children across 12 different communities in Southern California. Division faculty members have the scientific expertise in studying air pollution health effects in children. To support large-scale air pollution epidemiologic studies, the Division includes experienced staff scientists with the research capacities to conduct complex spatial epidemiologic analyses and monitor levels of the major components of smog—ozone gas, nitrogen dioxide, particulate matter and acid vapors—in population-based cohorts. The active research programs within the EH Division are almost entirely supported by extramural funds with approximately eight million dollars per year. Detailed description of the EH Division can be found at http://keck.usc.edu/en/Education/Academic_Department_and_Divisions/Department_of_Preventive_Medicine/Divisions/Environmental_Health.aspx

Intellectual Environment

The EH Division offers an excellent intellectual environment fostering innovative research in environmental epidemiology research and air pollution health effects sciences. EH Division faculty members study chronic health effects associated with environmental exposures, and many scientific products of their scholarly work have appeared in high-impact journals, such as the *New England Journal of Medicine*, the *Lancet*, and the *Proceedings of the National Academy of Sciences*. Of its 10 primary faculty members, two serve in the EPA's Clean Air Scientific Advisory Committee and one serves in the National Advisory Environmental Health Sciences Council.

Dr. JC Chen has been co-leading the USC AirPollBrain (APB) Network, a product of the program development project entitled ***"****Brain Health during Development and Aging in Urban Environments"* (co-PIs" Finch and Chen) funded by the USC Collaboration Fund (https://research.usc.edu/collaboration-fund-current-projects/) since 2010. This program brings together a network of USC faculty from Gerontology, the Viterbi School of Engineering, the College of Letters, Arts and Sciences, the Keck School of Medicine, School of Pharmacy, and Annenberg School for Communication and Journalism to better understand the scientific basis for the impacts of pollution on the brain, and to explore the health consequences. This program aims to promote the optimal neurodevelopment in humans and healthy brain aging by better understanding environmental effects of urban air pollution and gene environment interactions. Bimonthly meetings convene faculty and trainees on specific topics with a focus on developing new research projects and consortia. As of March 2016, the AirPollBrain has helped USC faculty obtained more than $10 millions of NIH funding to support the continuing development of research and education programs in environmental neurosciences studying ambient air pollution and neurodevelopment/brain aging.

The research program described in this application draws upon the expertise of faculty and staff scientists in the Department of Preventive Medicine at the University of Southern California and collaborating researchers from the NIEHS-sponsored Southern California Environmental Health Sciences Center (SCEHSC). The Division of Environmental Health is home to two federally funded regional research centers, including the SCEHSC and the Children's Environmental Health Center. The NIH-funded SCEHSC is a multi-university consortium of over 60 leading researchers across the region. The SCEHSC has several research initiatives, including *"Air Pollution, Neurodevelopment and Neurological Diseases,"* led by Dr. JC Chen (the USC PI).

The major goal of the initiatives has been to incubate new programs of EHS research by providing infrastructure and support to grow new research into mature programs supported by investigator-initiated grants, program projects and new centers.

Laboratories and Facilities

The project PI is a core member of the SCEHSC, which includes the Integrative Health Sciences Facility Core (IHSFC) and the Biostatistics Facility Core (BFC). The research team will thus benefit from consultation and technical support in areas needed for exposure assessment of air pollution including aerosol science, environmental chemistry, and geospatial modeling. Facilities involved in the performance of the proposed research include offices and laboratories, in particular, the BFC and GIS Research Laboratory, which is part of the IHSFC resources of the SCEHSC.

(1) GIS Research Laboratory

The GIS Research Laboratory is a university- and project-supported laboratory with a number of impressive software and hardware resources. It has its own server room with an IBM Blade Center System H Chassis, HS20 Blade Servers, Xiotech Storage Area Network, Cisco MDS 9000 Fiber Channel Switches, and Quantum PX-502 Tape Library. The servers are located behind a Sonicwall Pro 5060 firewall with stateful and deep packet inspection, potentially analyzing packets all the way up to the application level. Applications requiring high-performance are attached to high-speed fiber-channel drives, 15k RPM, RAID 10. Second tier storage for static data that does not require high performance is provided on 7200 RPM SATA drives, RAID 5. Data is backed up daily incrementally and full backups are completed once a week and once a month. Tapes are rotated off site to Iron Mountain weekly as well.

Software resources that are supported from this hardware platform include Microsoft Windows 2007-2010 (64 bit), .NET v.2.0-3.5, PHP 5, Microsoft IIS 6-7, Apache Tomcat, Microsoft SQL Server 2005-2008R2, the CommVault Data backup software, the ESRI web tools (ArcGIS Server, ArcIMS, ArcSDE, GeoPortal Toolkit, etc.), and Sentinel LM. The network resources include a Sonicwall Pro 5060 Firewall and Linksys 48-Port Gigabit Switches.

The lab administers the ESRI campus site license and provides the ESRI GIS, IDRISI GIS, and Trimble GPS software suites plus standard office, multimedia, and statistical analysis tools for the 60 or so faculty, graduate students, undergraduate research assistants, and staff that are affiliated with the GIS Research Laboratory and Geographic Information Science and Technology (GIST) graduate programs. All of the aforementioned computer facilities are supported by the College Information Technical Services Center and a dedicated computer services consultant housed in the GIS Research Lab itself.

(2) Biostatistics Facility Core of SCEHSC

The Biostatistics Facility Core (BFC) provides statistical support for planned and ongoing research projects by Center investigators. Core members assist in the development of research protocols from Center investigator who are requesting research grants or extramural support. This entails deciding on study design, calculating sample size or power, and reviewing proposed statistical methods. Core members also provide an interface between Center investigators and statistical resources by matching statisticians to investigators so that, to the maximum extent possible, Center projects will benefit from statisticians with particular expertise in the areas under investigation. Additionally, Core members stimulate collaboration between statisticians and other investigators by providing a focal point for statisticians to discuss issues with investigators from all the research cores. The Core also maintains a fast multi-processor computer that is accessible directly or through the campus network by all Center investigators. This resource provides a central repository for data, facilitates file sharing among Center investigators, and supports many commonly used software packages. The Core also assists Center member in the use of USC's High Performance Computing Cluster (HPCC), a university wide resource that provides thousands of processors for high-volume computing requirements. In addition to research support, the Core includes an educational component. This involves increasing the understanding of statistical issues among Center investigators through formal and informal consultations, broadening the awareness by statisticians of the many applications of statistical theory to environmental health problems, and enhancing graduate programs in biostatistics and epidemiology by providing a mechanism to assign graduate students to research problems in environmental health on which they can work under supervision. Core

members are also very active in working with the Center Outreach and Education Core to make presentations and to disseminate research results.

(3) Other Facilities & Resources

Computing/Software Resources: The USC Information Services Division provides access to an array of high performance computing resources, including a Sun Fire 15K and HPC-Master Linux Cluster. The large size of this computer allows virtually unlimited user access and data storage needed by major data sets Key personnel in this study have Pentium or compatible machines with statistical and word processing software; all machines have Ethernet access to the mainframe and library information systems. PCs are integrated through a network that links machines and provides access to a library of local web-based software programs to access major datasets and other materials, or even to conduct data management and analysis. Information Technology Services includes the Keck School of Medicine's "Keck Tech" routine services to update software and link computers and databases. Services include: Online forum, Listserve, Online Calendar, Cyberspace file center, and other modules to assist in inter-institutional and international project management and coordination. Data entry, online survey, phone survey and tracking, coding, and other modules enhance the efficiency of project operations, especially during the phase of data collection, management, and analysis. The design and construction of the system comply with NIH's recommendations to develop and utilize programs with open ended source code and develop scalable programs that would be more cost effective when need to be ported to other research institutions or environments. The IT services and resources are available to all personnel on this study. Database, data analysis and methodological research in the Department of Preventive Medicine are largely accomplished via personal PCs/microcomputers that are linked to secured fileservers. All PCs are networked with the University system and directly to the Internet. Software includes all standard statistical packages; (SAS, SPSS, SPLUS, STAT, nQuery Advisor, StatXact, etc.); some in-house packages (POWER, EPILOC); database managers (SQL, ACESS, DBASE), word processors; all major programming languages (FORTRAN, C++, Visual Basic); spreadsheet (EXCEL); and file transfer programs and graphics.

Office Space for the USC Department of Preventive Medicine: The Department of Preventive Medicine at KSoM numbers more than 90 fulltime faculty members who successfully compete for over $50 million in extramural support annually. The PI and co-investigators on this application are Department faculty. The Department includes internationally recognized programs of research in biostatistics, epidemiology of cancer and other acute and chronic diseases, genetic epidemiology, health behavior and occupational and environmental health. The Department is known for its strong research, excellence in teaching and commitment to promoting community health through education. Undergraduate, medical and graduate students are trained in the areas of biostatistics, epidemiology, health behavior research, public health and occupational and preventive medicine.

The newest vanguard in the expansion of the USC Health Sciences Campus is a 120,000 square-foot facility that is home to the Department of Preventive. This three-story building houses offices, laboratories, classrooms, a student and faculty fitness center and a café. The space also houses the PhD, Master's and MPH programs, and other trainees.

Personnel Resources:

The Department of Preventive Medicine has a talented staff of statistical programmers who have been working as lead analysts/programmers for the Children's Health Study and a variety of research projects on spatial analyses, air pollution cohort studies and chronic disease/cancer epidemiologic studies dealing with multisource/ multidimensional exposure and outcome data, all based in the SCEHSC at the EH Division, in the Statistical Consultation and Research Center at the Divisions of Biostatistics, and in the USC Norris Comprehensive Cancer Center on the Health Sciences Campus. These outstanding programmers have acquired familiarity with spatial epidemiologic studies of large geographically diverse populations, developed a pertinent array of programming code, and acquired requisite attention to detail needed to create and combine the multidimensional data files for complicated spatial epidemiologic analyses.

Health Sciences Libraries/Other USC Libraries: The Health Sciences Libraries (HSL) includes the Norris Medical Library and the Wilson Dental Library. Together, the libraries support the teaching, research, and patient care missions of the USC health sciences schools. The HSL is an independent library system of the university, but cooperates extensively with the other university libraries, including the nearly twenty libraries of

the Information Services Division, and the Law Library. Access to information is expanded through USC's membership in the Research Libraries Group, a national association of major universities and research institutions, and through membership in CALINET, a consortium of libraries at USC, UCLA, and Caltech. In addition, the USC libraries have developed reciprocal borrowing agreements with several local universities to improve faculty access to library resources.

The HSL, in cooperation with the Information Services Division, provides all USC users with access to a broad range of print and digital resources. The HSL licenses 14 databases including MEDLINE, EBM Reviews, and other health-related sources through Ovid; 160 electronic books and over 1600 electronic journals through Ovid and through individual publishers. The Norris Medical Library maintains a Web site that provides access to more than 1,700 biomedical information resources. The print collection includes 168,185 volumes and the Norris Medical Library receives 1,935 current periodicals. In addition, the Information Services Division licenses an additional 250 databases, approximately 2,500 electronic books, and nearly 2,200 electronic journals including over 250 in the biological sciences. The Norris Medical library has a Learning Resources Center which offers Internet, database searching, and software application workshops designed to meet the needs of USC health sciences students, faculty, and staff.

2. UNIVERSITY OF WASHINGTON DEPARTMENT OF ENVIRONMENTAL & OCCUPATIONAL HEALTH SCIENCES

Office:

Offices are available for the investigators and study staff in Dr. Kaufman's main laboratory, as well as in two suites of the University of Washington's Roosevelt Building (12 offices and 8 work stations). The Roosevelt building is within a quick shuttle ride of the UW Medical Center and the School of Public Health where the Departmental office is currently located.

Computer:

Computer resources are available to this project for data collection, data storage and management, and data analysis. Computers operate a mix of Intel/AMD workstations running Windows 7 along with a smaller number of Power PC and Intel Apple computers running OS X. Department computers run a standard set of productivity packages with UW-licensed statistical and other specialty packages (e.g. SAS, Stata, ArcGIS) installed on an as-needed basis. All are linked to the campus internet and network. Dr. Kaufman's office suites have secure servers with regular back-up regimens available for investigators.

Other:

The extensive UW campus facilities such as medical library, photocopying equipment, and medical illustration are available.

Research Environment:

The research environment provided by the Department of Environmental & Occupational Health Sciences will assist in a successful completion of the project. The project outlined in this application is an extension and expansion of collaborative work by the investigators of this application and the unique clinical/environmental interface in our institution. The institutional environment and infrastructure at the University of Washington enables the interaction, collaboration and synergy of multi-disciplinary experts skilled in air pollution and Alzheimer's disease epidemiology, biostatistics and environmental health sciences, essential for the success and implementation of this proposal.

C2&C3

The **University of Southern California** provides a rich scientific environment that offers both an intellectual community and infrastructure that will contribute to the success of the proposed research project. The work will be completed in the Keck School of Medicine, Dept of Preventative Medicine, the Viterbi School of Engineering and the Leonard Davis School of Gerontology at the University of Southern California.

The AirPollBrain Group at USC is an organized research group of scholars who do research on ways to promote optimal neurodevelopment in humans and healthy brain aging by better understanding environmental effects of urban air pollution and gene-environment interactions. The APB group developed from a core of USC's leadership in urban environmental health research in the Los Angeles Basin, with collaborating institutions. Taking a multi-disciplinary research, APB addresses effects of environmental pollution on the brain across the lifespan. APB is pursuing five main goals: I, Identify environmental risk factors impacting brain health in human populations with different environmental settings and genetic backgrounds; II, Develop experimental paradigms relevant to human studies and public health interventions; III, Integrate the existing environmental, neurosciences, and public health sciences programs at USC; IV, Provide the best sciences to support evidence-based regulations to protect public's health?; V, Create resources for training and education in interdisciplinary environmental health sciences from basic environmental biology to societal levels of analysis. Bimonthly meetings convene faculty and trainees on specific topics with a focus on developing new research projects and consortia.

USC has a number of research centers that provide a resource rich intellectual environment for the work proposed in this application:

NIA Center on Biodemography and Population Health: The USC/UCLA Center on Biodemography and Population Health (CBPH) aims to enhance understanding of biological and other processes that contribute to

population health at older ages and thereby contribute to more effective program and policy efforts to improve health and reduce health disparities. The CBPH promotes theory-based integration of biological measurement into population-based studies and on-going development and validation of biological measurement protocols. This center is directed by investigator Crimmins.

The USC Alzheimer's Disease Research Center (USC ADRC): The USC ADRC focuses on mild cognitive changes related to aging, Alzheimer disease (AD) and cerebrovascular disease (CVD) in multi-ethnic communities.. The ADRC integrates USC strengths in epidemiologic and longitudinal studies; in the molecular biology of aging and its diseases; and in genomics to determine basic and clinical and psychosocial approaches to the detection, cause, prevention, and treatment of early-stage cognitive impairments in human and in animal models. Research is also focused on normal brain aging processes and the transition to clinical stage dementias.

The Southern California Environmental Health Sciences Center: SCEHSC was established in 1996 to promote environmental health research in Southern California. The Center aims to more fully characterize environmental health hazards, understand the basis for personal vulnerability, and translate research into preventive action to reduce the burden of environmentally-related diseases. This Center has played an important role in the development of the air pollution and cognition initiative which is a focus of a significant program of research at USC.

The Statistical Consultation and Research Center (SCRC): The SCRC is an organized research unit within the Department of Preventive Medicine at the University of Southern California which integrates statistical, epidemiological and computing resources for researchers. There is also an organized Statistical Consulting Group across the campus consisting largely of social scientists. In addition, faculty in the Molecular and Computational Biology group provide collegial consulting in the approaches of molecular biology, computational biology and genetics.

Spatial Analysis Services: The GIS Research Laboratory has a permanent professional staff that administers campus-wide GIS infrastructure, collects and disseminates geospatial datasets, and provides training and consultation in the use of geospatial technologies. The GIS Research Laboratory supports research projects and courses needing geographic information science and spatial analysis inputs. In addition, the GIS Research Laboratory offers a large number of geospatial software solutions to the USC community and has developed a variety of web-based tools and extensions to help the USC community with their geoprocessing and spatial analysis needs.

Computer Facilities: The Leonard Davis School employs four full-time IT personnel to maintain the computing system in the building and access to computers and networks for researchers and graduate students. The Gerontology IT/Computing Services provides guidance, resources, and instruction to Gerontology faculty, staff, teaching assistants, research assistants, and students to enhance learning and teaching at the Andrus Gerontology Center through the effective use of digital and communications technology. It provides the faculty, staff and the students with the computing resources, hardware, and software. File servers, document servers and web servers which are securely hosted in the building to provide file sharing and web hosting services for the Leonard Davis School. The project personnel use IBM compatible computers with Microsoft Office, SAS, STATA, and internet productivity tools (software for e-mail and internet browsing). The computer lab of the Andrus Center, used in teaching classes, includes network desktop computers, wireless laptops, scanners, LCD projector and network printers. There are file servers, document servers and web servers which are securely hosted in the building to provide file sharing and web hosting services for the Center. Secure networks have been developed for users of large-scale population datasets such as the Health and Retirement Survey (HRS). There is also a secure room in the Leonard Davis School for both analysis of restricted data sets such as those linked to geographic indicators, medical records, Social Security records, or death records. This room has several computers, a large data storage capability but no internet access. Security is assured with special cabinets, door locks and alarm system. The office suite of the two investigators working with the HRS data contains two dedicated non internet connected computers for use with secure data.

Office Facilities: All project personnel in the USC Leonard Davis School of Gerontology have individual offices in the Leonard Davis School that are equipped with computers, hard lines, and wireless internet. The Leonard Davis School is housed in the Andrus Center, an entire building specifically for gerontology research and instruction. The 88,000 square foot structure houses several wet laboratories, a 250-seat auditorium, the gerontology library, offices, and multiple meeting rooms with video capabilities.

Libraries: The University of Southern California libraries, with the support of other parts of the Information Services Division, support many digital initiatives aimed at providing seamless access to information of every kind. The ultimate goal of developing and expanding digital access, and the ability of users to manage it, is to provide "one stop shopping," regardless of information format, for students, faculty and other researchers. The libraries maintain a large and ever-growing body of electronic resources, which include very large aggregator databases, reference tools, and discipline-specific resources for every area of study. The Integrated Document Delivery (IDD) team works with a global network of institutions to borrow, lend, and otherwise make available materials that support scholarly research for USC faculty, staff, and students. Interlibrary loan borrows books, dissertations, government documents, microforms and other loanable materials that are not owned by USC or are unavailable from USC's collection. Once received, articles are delivered online and physical items are made available at the Doheny Library Information Services desk. In addition, IDD services provides articles and documents owned by USC Libraries. The requested articles are scanned, converted to PDF format, posted on our server, and then delivered to the patron via a direct link in an email.

In addition to the substantial University Library, the Andrus Gerontology Center Library maintains one of the largest collections of scholarly, professional reference materials related to aging in the country, with a retrospective collection including over 15,000 monographs and more than 300 gerontology journals. Access to these materials is facilitated by a professional staff including a full-time librarian specializing in gerontology.

Resources For Web Casting, Webinars Etc: As part of the campus-wide Technology Enhanced Learning (TEL) and Distance Learning (DL) initiatives, the School of Gerontology has added two state-of-the-art Smart Classrooms and increased technological integration in the Leonard Davis Auditorium. The renovations provide top-of-the-line equipment including:

- Dual screen DTP projection
- Digital surround sound system
- Wireless handheld & lavaliere microphones
- Assistive hearing device system
- Document camera system & Virtual whiteboard
- Multiple cameras for webcasting complete with TV style control room
- Video teleconference unit

These facilities are staffed by two full time employees and two part time employees; both of whom are skilled at the production of video and web-based material.

USC Animal Facility: This is maintained by the Department of Animal Resources under the supervision of Don Casebolt, DVM. The Department employs 2 additional veterinarians, a manager of animal husbandry, 2 facility supervisors and a staff of 12 trained animal care workers (certified by the American Association of Laboratory Animal Science). USC has been accredited under the American Association for Accreditation for Laboratory Animal Care, since 1966. An Institutional Animal Care and Use Committee (IACUC) reviews all applications to ensure ethical and humane treatment of animals. Dr. Morgan is an active member of IACUC.

The Ray R Irani Hall Animal Facility was completed in 2007. The facility has a total capacity of 10,000 rodent cages within the 14,770 net square feet consisting of 20 animal holding rooms, 10 procedure rooms, 2 quarantine rooms, additional rooms for cage processing, bulk autoclave & storage, and office, locker & break rooms. The facility features a “suite” design concept of grouping 4 animal holding rooms with 2 procedure rooms into an individual security-controlled suite. Our group has exclusive use of 1 of these suites. The

individually-ventilated rodent cages ensures consistent air exchange rates of 50-80 air chages per hour within each cage to reduce ammonia and carbon dioxide levels. Incoming air is high-efficiency particulate arresting (HEPA) filtered for control of airborne particles. All components of the cages and racks are autoclaved and changed weekly. Wall-mounted supply air blowers and central exhaust plenums reduce noise and vibration. Automatic watering system is supplied to each cage.

Sioutas (C2):

Laboratory: Facilities at the USC Aerosol Lab (2,000 ft2) include state of the art instrumentation worth approximately $1.5M for testing and developing new sampling equipment. Additionally, an Engineering workshop is available, equipped with state-of-the-art computer-aided machining and design facilities. All samplers to be used in this study, i.e., virtual impactors-concentrators, PCIS samplers etc, are available at USC and will be dedicated to this work. Instrumentation necessary to conduct continuous and time-integrated particle measurement includes: electron microbalance (Kahn 30), gas analyzers (CO, NOx, O3). Details of the USC Aerosol lab can be found at the web page: http://www.usc.edu/aerosol.

Computers: The USC Aerosol Lab has several computers and lap tops, all equipped with necessary software for data analyses. All computers are networked to the central USC system, allowing easy access to scientific publications and other university resources. The computers are all connected to both inkjet color and black and-white and color laser printers access via network connections. Additionally, there is a shared network server for transferring and storing data files, and sharing information among the group members

Office: The PI has an office at Kaprielian Hall in the main USC campus

Forman (C3):

Laboratory: Dr. Forman has an 800 ft^2 of laboratory space on the 3rd floor of the Andrus Gerontology Center at the University of Southern California. His group also shares laboratories with Dr. Kelvin Davies comprised of separate cell culture facilities, a cold room, a "hot room" for radioactive labeling procedures, a dark room for photography and autoradiography, an equipment room, and regular "wet" laboratories. Dr. Forman also shares a combined 1500 ft^2 physiology and biochemistry laboratory and office suite at Children's Hospital Los Angeles (CHLA) with other investigators supported by a U54 grant for sickle cell disease research.

Computer: The laboratory is equipped with several PC computers that operate and receive data from instruments listed in the equipment section of this proposal. In addition, individual PC computers for running word processing, graphing, data analysis and other software are available on the desktops of laboratory personnel. Most computers have their own inkjet printers but can also use a networked HP Laserjet 5M. The PI uses a Mac computer that can either use dedicated inkjet and laser printers.

Office: PI occupies an 150 ft^2 office on the same floor as the laboratories in an 800 ft^2 office suite.

Other: One research associate and a postdoctoral fellow occupy a 150 ft^2 office on the same floor as the laboratories at USC. Fax machine, photocopier, and file space are located adjacent to PI's office at USC. A research specialist and technician, who work under Dr. Forman's supervision are in the CHLA laboratory and office suite. Dr. Forman has a Mac computer at CHLA where he spends an average of one day per week. The two laboratories are about 25 minutes away on a city bus line.

EQUIPMENT RELEVANT TO THIS PROPOSAL

Major Equipment at Sioutas Laboratories (C2):
Controlled temperature and relative humidity room with microgram sensitivity balances meeting US EPA standards, fume hoods, air and gas lines, etc.

- Concentrated Ambient Particle Systems (CAPS):
- High Volume Ultrafine Particle Collector
- Scanning mobility particle sizer (TSI Inc 3936)
- Micro orifice Uniform Deposit Impactors (MOUDI)
- Partisol Model 2300 Sequential Sampler (Thermoelectrom, Waltham MA)
- AE-14 Aethalometer (Magee Scientific Inc., Berkeley CA)
- Elemental and Organic Carbon Aerosol Analyzer (Sunset Laboratory)
- Nitrogen Dioxide Continuous Chemiluminesence Analyzer (Monitor Labs Model 8840)
- Gas-Phase Semivolatile Organic Compounds (SVOC)
- Modified Tisch 1202 sampler (34)
- Volatile Organic Compounds (VOC) SUMA Canisters
- Carbon Monoxide a Horiba model APMA-360
- Ozone UV photometer (Teledyne API model 400E)
- Non-methane Hydrocarbons
- NMHC Thermo Environmental Inc, Model 55
- Particle number, surface and volume size distribution (size range: 0.5–20um) Aerodynamic Particle Sizer (APS 3321, TSI Inc.)

Major Equipment at Forman Laboratories (C3):

Spectramax E3 Platereader; HPLC System #1: PE LC90uv Detector, PE 900 Series Interface, PE Series 410 LC Pump, Shimazu SIL-9A autoinjector and desktop PC, monitor, and printer ; HPLC System #2: PE LC240 fluorescence detector, PE Series 410 pump, PE Series 200 autosampler and desktop PC, monitor and printer; Baxter upright –80°C freezer; 3 Refrigerator/freezers; 2 upright –20°C freezers; Shimadzu UV-3000 spectrophotomer, Eppendorf 5417R refrigerated centrifuge; Hitachi F200 fluorescence spectrophotometer; PE LS-5 luminescence spectrophotometer; MJ Research minicycler with hot bonnet; Hybaid hybridization ovens (2); UV Stratalinker 1800; SpeedGel SG200 vacuum gel dryer; Speedvac™ concentrator, centrifuge and mechanical vacuum pump; UVP dual-intensity transilluminator; light box (uv/vis); Environ bacterial incubator/shaker; (2); Steri-cult cell culture incubators (2); dual tissue culture incubators; Clinical centrifuges; Fisher 60 Sonic dismembrator; organic solvent evaporator; fraction collector and collector drum; rotary evaporator; chromatography refrigerator; upright freezer; 3 picofuges ; 2 microfuges; spectrophotometric/ fluorescence platereader; electroporator; incubator (non-tissue culture); real-time PCR machine; liquid nitrogen tank; inverted microscope for tissue culture; luminometer w/printer; scintillation counter; ultracentrifuge with rotors ; fluorescence detector for gels; phosphoimager; X-ray developer; Eppendorf refrigerated centrifuge with rotors; Steri-cult CO_2 incubator with stand.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Constantinos Middle Name Last Name*: Sioutas Suffix:

Position/Title*: Professor
Organization Name*: University of Southern California
Department: Civil/EnvironmentalEngineering
Division:
Street1*: 3620 S. Vermont Avenue
Street2:
City*: Los Angeles
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 90089-2531

Phone Number*: 213 740 6134 Fax Number:

E-Mail*: sioutas@usc.edu

Credential, e.g., agency login: SIOUTAS

Project Role*: Other (Specify) Other Project Role Category: Core Lead

Degree Type: Sc.D. Degree Year: 1994

Attach Biographical Sketch*: File Name: Sioutas_NIH_Finch_Biosketch_2017.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: HENRY Middle Name Jay Last Name*: FORMAN Suffix: Ph.D

Position/Title*: Research Professor
Organization Name*: University of Southern California
Department: Davis School of Gerontology
Division:
Street1*: 3715 McClintock Avenue
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 900890191

Phone Number*: 818-288-1573 Fax Number:

E-Mail*: peroxideman@gmail.com

Credential, e.g., agency login: HFORMAN

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD,BA Degree Year: 1971

Attach Biographical Sketch*: File Name: Forman_biosketch_2017_final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Joel Middle Name Daniel Last Name*: Kaufman Suffix: M.D.

Position/Title*: Professor, EOHS, Epidemiology, Medicine
Organization Name*: University of Washington
Department: UW ENVIRONMENTAL & OCC HLTH SC
Division:
Street1*: 4225 Roosevelt Way NE, Suite 100
Street2:
City*: SEATTLE
County: King
State*: WA: Washington
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 981056099

Phone Number*: (206) 616-3501 Fax Number: (206) 897-1991

E-Mail*: JOELK@U.WASHINGTON.EDU

Credential, e.g., agency login: JOELKAUFMAN

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: MD,MPH,BA Degree Year: 1990

Attach Biographical Sketch*: File Name: Kaufman_Joel_biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Honqiao Middle Name Last Name*: Zhang Suffix: Ph.D

Position/Title*: Research Assistant Professor
Organization Name*: University of Southern California
Department: Davis School of Gerontology
Division:
Street1*: 3715 McClintock Avenue
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 90089-0191

Phone Number*: (626) 272-7968 Fax Number:

E-Mail*: hongqiaz@usc.edu

Credential, e.g., agency login:

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Ph.D. Degree Year:

Attach Biographical Sketch*: File Name: HZ_NIH_biosketch_2017_0516.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Elizabeth Middle Name A Lianne Last Name*: Sheppard Suffix: Ph.D

Position/Title*: Professor
Organization Name*: UNIVERSITY OF WASHINGTON
Department: DEPT OF BIOSTATISTICS
Division:
Street1*: Box 357232
Street2:
City*: SEATTLE
County:
State*: WA: Washington
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 981957232

Phone Number*: (206) 616-2722 Fax Number: (206) 543-3286

E-Mail*: sheppard@uw.edu

Credential, e.g., agency login: LSHEPPARD

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD,MS,BA Degree Year: 1992

Attach Biographical Sketch*: File Name: Sheppard_Biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Jiu-Chiuan Middle Name Last Name*: Chen Suffix: M.D.

Position/Title*: Associate Professor
Organization Name*: University of Southern California
Department: Preventive Medicine
Division: Keck School of Medicine
Street1*: 2001 N. Soto Street, MC 9237
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 90089-9237

Phone Number*: (323) 442-2949 Fax Number: (3232) 442-3272

E-Mail*: jcchen@usc.edu

Credential, e.g., agency login: JC_Chen

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: M.D./Sc.D. Degree Year: 1992, 2002

Attach Biographical Sketch*: File Name:

Attach Current & Pending Support: File Name:

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	Core_C_Intro_2017_FINAL.pdf
Research Plan Section	
2. Specific Aims	Core_C_Aims_2017_FINAL.pdf
3. Research Strategy*	Core_C_ResStrat_2017_FINAL.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	Core_C_Vertebrate_Animal_Section.pdf
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	UW_Sub_Docs(A124740).pdf
13. Letters of Support	
14. Resource Sharing Plan(s)	Core_C_Resource_Sharing_Plan.pdf
15. Authentication of Key Biological and/or Chemical Resources	CoreC_Authentication_of_Key_Resources_Plan.pdf
Appendix	
16. Appendix	

Tracking Number: Funding Opportunity Number: PAR-16-359 . Received Date:

Introduction to Core C: Response to Reviewers. Major text changes are *italicized in Research Strategy*

C1: Environmental Data Support for Projects 1 & 2 (JC Chen, co-Director)

Core C1 will provide harmonized measures of the residence-specific TRAP exposure estimates in geographically diverse populations, by refining/applying state-of-the-art spatiotemporal models. Core C1 is an integrated part of Core C, for which Reviewers (Stage-1) had recognized its multiple strengths ("*its organization, integration and synergies, the well-established team*") and were impressed with the "*overall leadership, the integration and synergy between the three sub-cores, and inclusion of innovative spatial-temporal models…*" Although not a major weakness, several technical issues were raised at two stages of reviews, as parsed below in ***bold*** and followed by brief summaries of our responses.

(Crtq p3) ***"…*weaknesses included validation of the actual environmental exposures over time."**
Response *Core C1 will implement exposure models focused on outdoor pollutant concentrations. All available models have been cross-validated [1-5] using out-of-sample actual monitoring data from the reference methods available at the time in question. For the pre-1999 models, we validate using older monitoring data.*

(Crtq p3) **"…historical data…subject to variability in terms of quality in different regions of the country…"** Response *Our historical $PM_{2.5}$ model is robust and captures available data sources,[3] using both the USEPA Federal Reference Method (FRM) network and the Interagency Monitoring of Protected Visual Environment (IMPROVE) network. Whereas FRM sites were located mostly in urban areas, IMPROVE sites monitor visibility and were located mostly in wilderness areas and national parks. We developed the model using both sets of data from 1999-2010, thus incorporating rural and urban data. We then validated the model using the available IMPROVE data from 1990-1998 as well as three other data sets from before 1999: California Air Resources Board dichotomous sampler monitoring, the Children's Health Study, and the Inhalable Particulate Network.*

(Crtq p3) **"People don't live in the same place for the entire lifespan**." Response *We were able to estimate residence-specific exposures since recruitment,[6-8] weighted by the duration at each location. The exposure time window of scientific interest covers both mid- to late life. Without high-quality monitoring data prior to 1970s, it was not possible to reliably estimate air pollution exposures in early life or young adulthood for nationwide cohorts like WHIMS/WHIMS-Y and VETSA (aged 65-80/50-54 and 51-60 years at the respective study inception in 1995-6 and 2002). We acknowledged this limitation in resubmission.*

The spatiotemporal modeling was "**very ambitious" and "data-fusion …methods [were] not specified."** Response *More technical details with appropriate references[5, 9] are given in the revised Core C1.*

C2: Collection and Characterization of TRAP-nPM and mouse exposure (C Sioutas, Director)

Crtq p14. ***focus on primary aerosols in Subcore C2 may not be consistent with exposure measures in Subcore C1, e.g., NO_2 from vehicles is largely a secondary pollutant; further justification is warranted**.
Response: *We focus on PM from primary aerosols that show consistent responses across decade of studies in vivo and in vitro. Inclusion of NO_2 or other trace gases is not feasible for the re-aerosolization of particles collected on filter because such oxidants would further change the activity of the collected particles in uncontrolled ways from contact with animals in the exposure and cage content.*

***Characterization of the collected PM appears limited. Sample storage and potential changes in sample integrity is not described**. Response*: The nPM show retention of chemical stability for ≥ 3 mo[10] and replicable dose responses for TNFa induction in batches from 2010 and 2015 (Fig. 3 of Cheng et al 2016[11]).*

C3: Brain Responses to TRAP-PM (HJ Forman, co-Director)

Crtq p15: ***C3 provides preliminary data that is not specific to the cell lines or proteins proposed. No one has mechanistically proven that neuroinflammatory effects of PM are due to direct toxicity; from a dosimetric standpoint, the levels of PM that access the brain are trivial, even via the olfactory bulb.**
Response *We agree and have discussed this issue in Cheng et al.[11]*

Crtq p15***Direct application of PM onto neurons/microglia is odd.** Response: *Studies with neurons/microglia are deleted.*

***Integration of Aim 3 with the toxicology projects seems absent. Will they investigate toxicity of PM from different sources?** Response *The proposed experimental studies are in continuity with a decade of work with nPM collected from this Los Angeles site. Cheng et al[11] shows in Fig 3 that the in vitro dose response of TNFa to nPM was identical in batches collected in 2010 and 2015, cited in the revision. The Sioutas group has shown seasonal differences of nPM batches in oxidative activity[12]. We describe collection in cooler months to minimize confounds from seasonal wildfires and secondary aerosols. Nonetheless, we will assess new nPM batches for dose-response induction of TNFa and IL1a with THP-1 cells.*

Environmental Exposures and Neurotoxicology Core (Core C)
Director, C Sioutas, Co-Directors: JC Chen and H Forman
Specific Aims

SubCore C1, Environmental Data Support characterizes ambient air pollution exposures in the geographically-diverse populations of Project 1 (Women's Health Initiative Memory Study) and Project 2 (VETSA: Vietnam Era Twin Study of Aging). Core C1 will: (1) support the design and conduct of environmental epidemiologic studies on large cohorts; (2) harmonize the spatiotemporal modeling approaches across two population studies; and (3) provide the P01 team with expertise in analyses and interpretation of air pollution-neuroepidemiologic data. The environmental data resource, exposure modeling tools, and collaborative infrastructure have been built from several NIH-funded air pollution-neuroepidemiologic projects based at USC. To harmonize the TRAP exposure characterization, Dr. Chen will coordinate the effort, through a subcontract with Dr. Joel Kaufman who leads the MESA-Air (Multi-Ethnic Study of Atherosclerosis and Air Pollution) team at the U. of Washington.

SubCore C2, Collection of TRAP-PM and Mouse Exposure is responsible for the collection and physico-chemical characterization of TRAP particulate material (TRAP-PM), and for carrying out the inhalation mouse exposures in Projects 3 &4. The main TRAP-PM studied is nano-sized particulate matter (nPM) of the ultrafine size range ($PM_{0.2um}$), which is collected at a well-characterized site in central Los Angeles , heavily impacted by traffic emissions . Given the focus of the study on capturing the fresh traffic-related nPM, the sampling period will be constrained to colder months to minimize the effects of ozone and the contribution of secondary organic aerosols (SOA). TRAP nano-size particulate matter is collected into ultrapure (milli-Q) water suspensions using highly innovative state-of the-art aerosol sampling technologies developed by the USC Aerosol group, led by prof. Sioutas. The nPM aqueous suspensions collected during the year are stored in the freezer, following protocols established by the US EPA, and subsequently analyzed for size and chemical composition, followed by re-aerosolization and exposure of mice. The nPM mass concentration and size distribution of the exposure atmospheres are continuously monitored throughout the animal exposure. We maintain average concentration of 250 (+/- 50) ug/m^3- roughly twice that of busy roadways, consistently with our prior and published work. nPM-bound Inorganic ions [ammonium (NH_4^+), nitrate (NO_3^-), sulfate (SO_4^{2-})] are analyzed by ion chromatography and nPM-bound metals/ trace elements are assayed by magnetic-sector inductively coupled plasma mass spectroscopy. Water-soluble organic carbon will be assayed by a GE-Sievers liquid analyzer (GE-Sievers, Boulder, CO).

SubCore C3, Brain Cell Responses to TRAP-PM (i) tests individual batches of collected nPM using THP-1 cells for cytokine expression; and (ii) performs Western blot assay of brain protein changes to in vivo nPM exposure for Projects 3 & 4. Core C3 will test individual nPM collections, using the differentiated THP-1 cell line. Core C3 also performs Western blot assays for TLR4, CD36, MyD88, TNFα, IL1α, IL6, IL10, TNFRI, C5, C5a, C5R.

Core C Environmental Exposure and Neurotoxicology Core is directed by C Sioutas with 3 SubCores: SubCore C1 (JC Chen), Environmental Data Support for Projects 1 & 2. SubCore C2, Collection of TRAP-PM and mouse exposure (C Sioutas); subCoreC3, Brain Cell Responses to TRAP-PM (H Forman). SubCores C2 and C3 support mouse exposure Projects 3 &4.

Subcore C1 Environmental Data Support (JC Chen, Co-Director)

The Environmental Data Subcore aims to: (1) support the study design and conduct of large-scale air pollution/spatial epidemiologic studies on geographically-diverse cohorts; (2) harmonize the spatiotemporal air pollution modeling approaches across two population studies (Project-1 & -2); and (3) provide the P01 team members access to expertise in the analyses and interpretation air pollution-neuroepidemiology of brain aging.

Significance: **[***revised***]** *For a multi-region longitudinal cohort study such as WHIMS and VETSA, estimating TRAP exposures requires a model with sufficient spatial and temporal resolution. In developing such spatiotemporal models, several challenges arise. These challenges include accounting for small-scale and intra-urban variation in TRAP concentrations, and developing comprehensive exposure predictions over time and space when the quality, availability and/or interpretation of relevant environmental data depends on regions and can vary from year to year. Existing methods for modeling NO_2 (gaseous surrogate) and other TRAP exposure indicators include land use regression (LUR), dispersion models, universal kriging and Bayesian hierarchical models. LUR incorporates geographic covariates to inform small-scale variation in unmonitored areas and has become a popular approach for modeling TRAP exposure. Recent simulation work[13] showed that LUR-spatiotemporal models would produce less biased estimates of chronic health effects compared with conventional geostatistical approaches. The MESA-AIR (http://deohs.washington.edu/mesaair/researchers) team, led by Dr. Joel Kaufman (subcontract PI) has developed a suite of well-validated spatiotemporal models[1, 4, 9, 14-19] to predict ambient outdoor concentrations of air pollutants. These approaches were successfully applied to other multi-region longitudinal cohorts including WHI,[20] the Sister Study,[21, 22] and the Cardiovascular Health Study.*

a. Approach

(1)Organization of Subcore: Dr. JC Chen will direct the Environmental Data Subcore. In addition to serving his role as the environmental/spatial epidemiology expert of the Core C based at USC, Chen will lead the coordinated efforts to ensure the quality and comparability of TRAP exposure estimates used in Projects-1 & -2. He will advise Project-2 PI and co-investigators in developing the questionnaires/survey tools to update/reconstruct the residential histories and selecting the optimal procedures for geocoding. To harmonize the TRAP exposure characterization, Core C will establish a subcontract with U. of Washington, where Dr. Joel Kaufman (subcontract PI) leads the MESA-Air team. Chen & Kaufman recently helped develop the location data and air pollution exposure estimates needed for a nationwide study on air pollution and risk of Parkinson's disease.[23] The subcontract supports Dr. Kaufman and team to apply relevant MESA-AIR spatiotemporal air pollution models and derive location-specific time-varying yearly TRAP exposure for this P01.

(2)Methods: The proposed extension/implementation of MESA-AIR approach represents state-of-the-art in modeling air pollution exposures for large cohort studies, as it incorporates the methodological advances and environmental data resources developed from the EPA's long-term investment in MESA-AIR (2006-onward).[24] The novel modeling approach integrates measurements from the U.S. EPA Air Quality System[25] with comprehensive *(n>800)* spatiotemporal covariates assembled in the MESA-AIR Exposure Assessment Core Database.[26] The MESA-AIR approach models pollutant concentrations as a linear combination of temporal basis functions with spatially-varying coefficients and spatiotemporal residuals.[18] The resulting exposure estimates therefore capture both regional- and local-scale variability.[4] In the context of WHIMS, Chen and Kaufman has been working on the R01ES025888 (2016-21), which will support the downscaling of yearly MESA-AIR models to estimate monthly outdoor concentrations (1990-2016 for NO_2;1999-2016 for $PM_{2.5}$) on the national-scale. Although downscaling of spatiotemporal models is computationally intensive, we have previously shown that exposure estimation processes starting with shorter time-scales followed by temporal aggregation is more preferable than directly modeling yearly exposure.[27] Figure 1.1 depicted the preliminary results of a downscaled MESA-AIR model of **monthly $PM_{2.5}$ in one climatic zone (the Southeastern US) in 1999-2013, with the model performance via 10-fold cross validation showing a high degree of estimation accuracy (R^2_{CV} = 0.73).** Also available to this P01 is a new spatiotemporal model of **historical annual $PM_{2.5}$ concentrations in the**

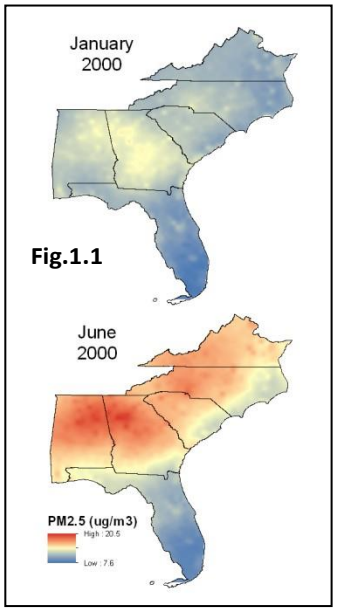

Fig.1.1

continental US from 1980-1998. This pre-1999 model performed well when validated using IMPROVE Network and USC-based Children's Health Study data (**R^2=0.84–0.91**).[3] We will also implement the national spatial exposure models[1] to estimate annual average concentrations of major $PM_{2.5}$ components, focused on elemental carbon (a marker of diesel exhaust particle). *In the Table 1 below, we applied the existing yearly models ($PM_{2.5}$[4]; NO_2[5]) to the WHI cohort and showed the desirable exposure contrasts in both urban (census tracts > 50% classified as urban; 87% of WHI locations) and rural areas.*

Table 1 Rural vs urban exposures

	1999 $PM_{2.5}$ Rural	**2011 $PM_{2.5}$ Rural**	**1999 NO_2 Rural**	**2011 NO_2 Rural**
Mean (SD)	11.4 (3.3) μg/m³	8.3 (1.7) μg/m³	7.3 (2.5) ppb	4.1 (1.5) ppb
Range	2.2 – 22.5 μg/m³	1.7 – 15 μg/m³	0.1 – 40 ppb	0.7 – 32 ppb
	1999 $PM_{2.5}$ Urban	**2011 $PM_{2.5}$ Urban**	**1999 NO_2 Urban**	**2011 NO_2 Urban**
Mean (SD)	13.7 (3.5) μg/m³	9.4 (1.6) μg/m³	17.2 (6.8) ppb	10.1 (4.4) ppb
Range	2.4 – 26.6 μg/m³	1.9 – 16.1 μg/m³	0.5 – 46 ppb	1.1 – 33 ppb

For this application, the P01 subcontract will support the MESA-Air team for continuing development of augmented exposure models*, including the extension of downscaled monthly NO_2 model (from the already validated yearly model for 1990-2012) to 1980s and refinement of $PM_{2.5}$ composition models/clustering analyses.*

The MESA-AIR team has already developed techniques for incorporating satellite measures and chemical transport model output,[5, 9] fusing data into a flexible spatiotemporal modeling approach implemented in the SpatioTemporal R package.[18, 28] Methodological development for characterizing PM composition (and hence sources) includes a novel clustering approach (based on predictive k-means and predictive sparse principal component analyses) recently applied to identify profiles of $PM_{2.5}$ composition from EPA's Chemical Speciation Network to predict the source profile labels at residential locations of the Sister Study.[2]

SubCore C2: Collection and Characterization of TRAP-nPM (Constantinos Sioutas, Director)

Significance During the past decade, using state-of-the-art aerosol sampling technologies[29] developed by the USC Aerosol lab directed by Professor Sioutas, we have collected and characterized TRAP-nPM from several freeways and urban sites in Los Angeles, impacted by a variety of air pollution sources and formation mechanisms, for experimental inhalation exposures of caged rodents in collaboration with USC faculty, including the present coPIs[11, 30, 31, 35, 59]. These collaborations since 2010 have yielded more than 10 co-authored publications and 6 funded NIH projects (Biosketches of Finch, Forman, Mack, Morgan, and Sioutas).

Air pollution and exposure to airborne particulate matter (PM) have received considerable attention from the health science community. Epidemiological and toxicological studies document short- and long-term effects of PM exposure on cardiovascular and respiratory systems[32-35] and neurodegenerative issues.[36, 37] Moreover, secondary species such as sulfate, nitrate and/or organics were associated with myocardial infarction.[38] *Response to Crtq p14. We focus on PM from primary aerosols that show consistent responses across decade of studies in vivo and in vitro. Inclusion of NO2 or other trace gases is not feasible for the re-aerosolization of particles collected on filter because such oxidants would further change the activity of the collected particles in uncontrolled ways from contact with animals in the exposure and cage content.*

The PM size critically influences bioactivities and the type and severity of the health effects. In comparison to larger particles, ultrafine nanoparticles (nPM), traditionally defined as PM with an aerodynamic diameter < 0.2 μm, have higher number concentration and surface area and therefore larger concentrations of adsorbed or condensed toxic air pollutants per unit mass.[39] The nPM are strongly linked to systemic oxidative stress and promote more atherosclerotic plaque formation than PM2.5 in mouse models.[40] nPM likely act as the most efficient particle delivery vehicles for toxic chemicals to the respiratory system and systemic circulation, causing adverse health outcomes.[41] These adverse associations linked to nPM have been extended to show the acceleration of cognitive decline of elderly community- based populations[42-45] and neurodevelopmental impairments of children.[46, 47] The causes of cognitive impairment were analyzed in rodent and cell models, which implicate neuro-inflammatory responses to urban air pollutants.[48-50] Specifically, we and others observed that rodents exposed to nPM had brain-wide activation of microglia, with induced TNFα and IL-1, among other inflammatory responses.[51-53] This evidence supports findings of increased microglial activation and white matter hyperintensities in small postmortem samples of children from a highly polluted Mexican city[46, 54] and in the association of white matter loss in older human adults in an MRI analysis of the WHIMS cohort of US women.[55]

b.Approach.

(i) Methods: *The sampling period will be constrained to colder months to minimize the effects of ozone, and the contribution from seasonal wildfires and secondary organic aerosols (SOA). Recent studies show that SOA contribution to nPM is about 50% less in colder vs warmer seasons, while ozone is roughly 3-fold lower.[56-58] The mouse exposure to PM0.2 at 300 ug/m³ for 150 h (1% of the lifespan) delivers 2.25 mg/kg. For humans, exposure to PM0.2 at 30 ug/m³ for 1% of lifespan yields 2.5 mg/kg, assuming inhalation rates: mouse, 0.025 L/min; human, 12 L/min.*

nPM collection*:* TRAP nano-size particulate matter (nPM) is collected as described in Morgan et al.[37] Briefly, urban nPM (aerodynamic diameter <200 nm) is collected at 400 L/min flow using a high-volume ultrafine particle sampler.[29] The sampler incorporates an ultrafine particle multiple rectangular (slit) geometry jet conventional impactor and an after-filter holder. The nPM are collected at a single site adjacent to the CA-110 highway in Los Angeles. These aerosols represent a mix of fresh ambient PM mostly from vehicular traffic.[12] The nPM is collected on Teflon filters (8x10", PTFE, 2 μm pore) and transferred to aqueous suspension by 30 min soaking of filters in Milli-Q deionized water; total organic compounds <10 ppb; particle free; endotoxin levels < 1 units/mL; endotoxin-free vials), followed by vortexing (5 min) and sonication (30 min) for resuspension. No endotoxin is detected in these suspensions (*Limulus* amoebocyte assay: LPS <0.02EU/ml). As control, fresh sterile filters are sham extracted. Aqueous nPM suspensions are pooled and frozen as a stock at –20°C, following US EPA recommended procedures. These nPM retain chemical stability for ≥ 3 mo[10] and *replicable dose responses for TNFa induction in batches from 2010 and 2015 (Fig. 3 of Cheng 2016[59]).*

For mouse exposure, the nPM are re-aerosolized by an atomizer using compressed particle-free filtered air (Fig.2). Passage through a silica gel will diffusion-dry the generated nPM; static charges are removed by passage over polonium-210 neutralizers. During mouse exposure, the particle size and concentration are continuously monitored by a scanning mobility particle sizer (SMPS model 3080; TSI Inc., Shoreview, MN) in parallel with mouse exposure chambers. Average concentration of 300 (+/- 50) ug/m^3- are ca. twice that of busy roadways.[50] From the total of 15 l/min of aerosol flow generated, the majority (10 l/min) is drawn through the exposure chamber. The remaining 5 l/min is diverted to filters for particle collection and characterization. Teflon and quartz filters sample aerosols during exposure. Inorganic ions [ammonium (NH_4^+), nitrate (NO_3^-), sulfate (SO_4^{2-})] are analyzed by ion chromatography; PM-bound metals/trace elements assayed by mass spectroscopy. Water-soluble organic carbon collected on quartz filters is assayed by a GE-Sievers liquid analyzer (GE-Sievers, Boulder, CO). For details of inorganic and organic contents of these samples, see [37]. Concentrations of gaseous co-pollutants (CO, NOx and O3) are monitored as in prior studies [37]. Fig.3 shows a typical chemical/ elemental composition of nPM from the Los Angeles site (Morgan et al.[37]).

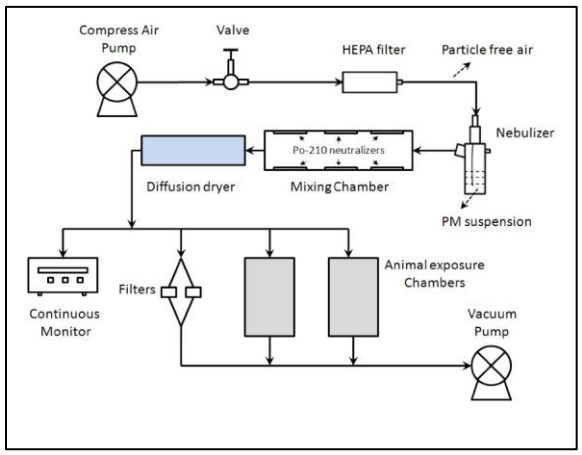

Fig.2 Schematic of the exposure apparatus

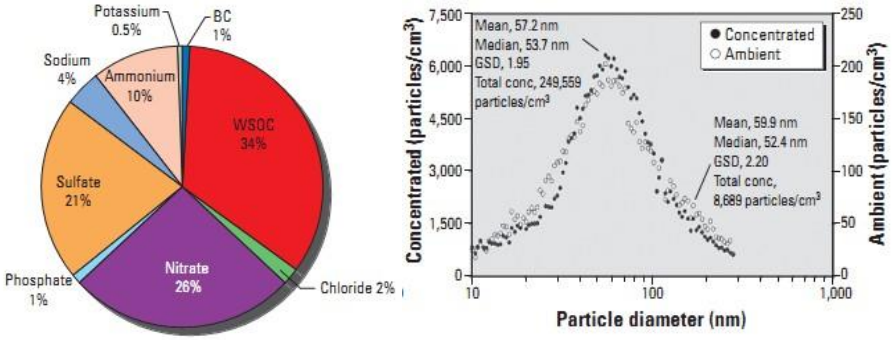

Fig. 3: Chemical composition and size distribution of re-aerosolized nPM Morgan et al.[37]

Size distribution measurements corresponding to ambient particles and re-aerosolized (i.e. concentrated) nPM in prior studies consistently show that re-aerosolization preserves the size characteristics of urban nPM (Fig. 3). Additional measurements of the aqueous-phase (i.e slurry) size distribution of nPM using Dynamic Light

Scattering (DLS) show that size distribution is well-preserved during water-extraction: with 95% of the nPM based on number, and 85% based on surface area similar to the airborne nPM phase.

(ii)nPM exposure of mice for Projects 3 &4, in participation with their personnel**.** Mice will be transferred to whole-body exposure chambers, see Morgan et al.[37] Temperature and airflow are controlled for adequate ventilation and to minimize buildup of animal-generated contaminants. Re-aerosolized nPM or HEPA-filtered air (for the control group) is delivered to the sealed exposure chambers for 5 hrs/day, 3 d/week. In all studies, mice did not lose weight or shown respiratory distress.[31, 37, 59] The exposure team consists of graduate PhD students supervised by C Sioutas and TE Morgan. All observations, exposures, surgeries (Project 4), and behavioral assessments are performed in the RRI Vivarium on the University Park Campus. After euthanasia by isoflurane, tissues are distributed to Projects 3 &4. For behavioral and cognitive tests, see Projects 3&4.

(iii) Comparability of nPM with other ultrafine PM in literature

We compared the cellular effects of ambient ultrafine nanoparticles (nPM) with manufactured titanium dioxide (TiO_2), carbon black, fullerol, and polystyrene (PS) nanoparticles (NPs)[60]. The study used an established phagocytic cell line (RAW 264.7) that is representative of a lung target for NPs. Physicochemical characterization of the tested nanoparticles showed a dramatic change in their state of aggregation, dispersibility, and charge during transfer from a buffered aqueous solution to cell culture medium. Nanoparticles differed with respect to cellular uptake, subcellular localization, and ability to stimulate the production of oxidants under biotic and abiotic conditions. Spontaneous oxidant production was compared by using an oxidizable probe (furfuryl alcohol) as well as an NADPH peroxidase bioelectrode platform. Among the particles tested, ambient nanoparticles (nPM) and cationic PS nanospheres robustly induced cellular oxidant production, GSH depletion, and toxic oxidative stress. This toxicity involves mitochondrial injury through increased calcium uptake and structural organellar damage. While increased TNF-r production could be seen to accompany nPM-induced oxidant injury, cationic PS nanospheres induced mitochondrial damage and cell death without inflammation. All considered, ambient nPM and NH2-PS nanoparticles showed the clearest evidence of toxicity and stand apart from the other particle types, even particles capable of spontaneous oxidant production (TiO_2 and fullerol). We therefore rank nPM and NH2-PS nanospheres as the most toxic, with ambient nPM additionally inducing proinflammatory responses.[60]

Several health studies involving inhalation exposures to PM have used “standardized” diesel exhaust PM (DEP) collected in chassis dynamometers, which can capture direct tailpipe emissions in controlled sets of experimental conditions. However, this type of PM collection does not take into account non-tailpipe emissions, which contribute to TRAP[61] particularly in light of the significant recent reductions achieved in direct exhaust emissions[62, 63]. Non-tailpipe emissions are significant sources of several potentially toxic metals including Ba, Cu, Fe, Mn and Zn[61] and are associated with PM-induced oxidative activity.[64-66] Moreover, dynamometer studies do not consider various urban atmospheric processes, such as dilution and photo-chemical transformation, which can alter the physicochemical and oxidative characteristics of the emitted particles.[30, 67]

Concentrated ambient particle systems[36, 40, 52] (CAPS) are very similar to our system, in that both types of aerosols are close to their “real-world” form; CAPS offer the advantage of providing the aerosol in real time and its airborne state, but with the disadvantage of substantial diurnal and seasonal changes in its chemical composition during the course of the exposures, which we cannot control, and which might have a significant effect on the progression of the observed health outcomes during the exposure

(iv) Anticipated problems. We do not anticipate any problems since we will involve aerosol sampling and characterization methodologies which we have extensively used and published in the past, as noted above.

SubCore C3. Brain Responses to TRAP-PM (HJ Forman, co-Director)

a. Significance: Goals (i) *test individual batches of collected nPM using THP-1 cells for cytokine expression*; and (ii) Western blot *assay of brain protein changes to in vivo nPM exposure for Projects 3 & 4. The Finch and Sioutas labs have been performing in vivo and in vitro studies on nPM since 2009. Each batch of collected nPM is characterized for chemical composition and biological activity. The uniformity of the nPM collections is illustrated by the replicable dose responses for TNFa induction in batches from 2010 and 2015 (Fig. 3 of Cheng et al 2016 [59]). For this P01, Core C3 will test individual nPM collections, using the differentiated THP-1 cell line, as described below. Table 2 lists the proteins for Western assay from Projects 3-4, chosen by their response in mouse brain to nPM or CCH. The new TLR4 pathway responses (TLR4, CD36, MyD88, TNFRI) were chosen for their involvement in TLR4-mediated neurodegenerative processes.*[70, 71]

Table 2	***nPM effect in vivo***	***chronic cerebral hypoperfusion (CCH) effect***
TLR4	*INCREASE, Proj 3, Fig. 6* [68]	*INCREASE* [69, 70]
CD36	*INCREASE, Proj 3, Fig. 6*	
MyD88	*INCREASE, Proj 3, Fig. 6* [68]	*INCREASE*[69, 70]
TNFa	*INCREASE* [37 59 71 68]	*INCREASE* [72 73 74 69]
IL1a	*INCREASE* [37]	*INCREASE* [74 69]
IL6	*INCREASE (Proj 3, unpublished)*	*INCREASE* [74 69 70]
IL10	*INCREASE (Proj 3, unpublished)*	*DECREASE* [74]
TNFRI	*INCREASE* [11]	
C5	*INCREASE* [31]	*INCREASE, Proj 4, Fig. 1* [31]
C5a	*INCREASE* [31]	*INCREASE* [31]
C5aR	*NO CHANGE* [31]	*INCREASE* [31]

b. Approach:

(i) THP-1 cell responses to compare nPM batches: THP-1 cells (human acute monocytic leukemia cell line) from American Type Culture Collection are cultured with standard conditions. Prior to measurements, cells (3 x 10^5 cells/cm^2) are differentiated into macrophages by 7.5 ng/ml phorbol 12-myristate-13-acetate (PMA) for 2 days, then normal media one day before treatment. The nPM from SubCore C2 is suspended in deionized water at 1 mg/ml and sonicated 2 min for dose-response of TNFα and IL-1a, assayed by RT-PCR.

(ii) Measurement of brain protein changes to in vivo exposure of mice from Projects 3 & 4. Western blot analysis using multiple infrared (IR)-labeled secondary antibodies for tissue samples from Projects 3 & 4. We will measure *TLR4, CD36, MyD88, TNFα, IL1α, IL6, IL10, TNFRI, C5, C5a, C5R* from 500 tissue samples per year. Frozen tissues are homogenized in RIPA lysis buffer (~5mg in 100-300 µL for optimal protein concentration of 1–5 mg/mL) with an electric homogenizer; 10-20 µg protein per well will be resolved on 12% SDS-PAGE acrylamide gel in Tris-Glycine-SDS Running buffer. Following electrophoresis, proteins are transferred to a PVDF membrane and probed with specific primary and secondary antibodies (Abcam, Cambridge, MA). Beta-tubulin[53] is used as loading control to normalize the signal intensity of each band. Averaged values from three independent experiments are used for the final statistical analysis. A SyngenePXi 6 Access Multi-application Gel Imaging System (Syngene, Frederick, MD) is used for Western blot imaging. This system offers IR fluorescence capturing quantification and is more sensitive and accurate with a broader dynamic range compared to the traditional X-ray film method. It enables multiplexed imaging of IR fluorophores and up to 5 different target proteins of different molecular weights can be detected simultaneously on the same blot using available different IR fluorophores dye-conjugated secondary antibodies. Fluorophores dye-conjugated secondary antibodies are from Abcam (Cambridge, MA) or LI-COR Biosciences (Lincoln, NE).

(iii) Data storage and access. Our ongoing collaboration has established a shared lab website with password protection. All Western blot images are digitized and saved. Initial data on specific bands are stored on Excel spread sheets, which will be accessed by Statistical consultant Prof Wendy Mack through our REDCap data base.

(iv) Anticipated problems. *Based on five years of in vivo exposures in ten studies, we do not anticipate significant variation in proinflammatory activities of different nPM batches, as measured by THP assay.* Western analysis is routine in the Forman lab.

VERTEBRATE ANIMALS

1. Provide a detailed description of the proposed use of the animals in the work outlined in the Research Strategy section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.
Core C will not handle mice. Core C2 will operate the air pollution exposure equipment to deliver nPM or filtered air through the exposure chambers. Personnel from Project 3 & 4 will be responsible for transferring the mice into the chambers, monitoring them during the exposure period, and returning them to their home cages. The exposure protocol is included in the Vertebrate Animal Sections of Project 3 & 4. Core C3 will obtain tissues from Project 3 & 4.

2. Justifications: Provide justification that the species are appropriate for the proposed research. Explain why the research goals cannot be accomplished using an alternative model (e.g. computational, human, invertebrate, in vitro).
In vitro studies or computer/ mathematical models cannot satisfactorily reproduce the white matter/ neuronal injury and functional deficits resulting from nPM/ CCH exposures. Due to cost, ethical concerns, and reproducibility, mice are most appropriate for these studies. We choose mice because of our experience in the models being tested, availability of applicable antibodies/ assays, and potential for future investigations. C57BL/6 mice are the standard murine strain used by most aging researchers and are available through the NIA Aging Mouse Colony. Further, all previous nPM data has been collected using C57BL/6 mice. Altering background strains for the study would likely introduce strain specific differences in exposure responses and outcomes. This variation would increase sample sizes. Male and female mice are selected to study sex influences and comply with recent NIH initiatives to balance sex in animal studies.

3. Minimization of Pain and Distress. Describe the interventions including analgesia, anesthesia, sedation, palliative care and humane endpoints to minimize discomfort, distress, pain and injury.
Discomfort, distress, pain, and injury will be minimized in all phases of the experiment. No pain or distress is expected based on our past nPM exposure studies using the same dose of nPM. Project 3 has detected subtle learning and memory deficits, mild systemic and neuroinflammation which do not appear to affect the well being of the mouse. Project 3 & 4 will monitor behavioral changes, e.g., loss of appetite, grooming and activity. Moribund criteria include one or a combination of the following: >20% weight loss, sustained hunched posture, respiratory difficulty, hypo/hyperthermia, inability of access food/water, inability to ambulate or make normal postural adjustments. Mice will be monitored for any signs of stress or pain as well as general criteria outlined by IACUC (Body Condition Score 2, BC2) and will be euthanized.

4. Euthanasia.
After completion of experimental procedures, all rodents will be euthanized by methods approved by the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

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SCOPE OF WORK: UW Subcontract for Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms

The University of Washington team, led by Principal Investigator Dr. Joel Kaufman, will be responsible for the development of the ambient air pollutant exposure models and the incorporation and merging of those models with Vietnam Era Twin Study of Aging (VETSA) datasets. Using a large suite of collected geographical covariates, and residential location information provided by the USC group, the UW team will develop, optimize, and test models including extensions of existing models for NO_2, $PM_{2.5}$, ozone, and component aspects of fine particulate matter. Additionally, the University of Washington will provide estimates of multipollutant exposures through clustered sources/predicted components of $PM_{2.5}$. The University of Washington team will then implement the model estimates into epidemiological analysis and participate in the development of manuscripts. The University of Washington subcontract will also be responsible for obtaining UW IRB approval to participate in these tasks, executing data use agreements as necessary, and maintaining approvals for the duration of the study.

1991 -	Diplomate in Occupational Medicine, American Board of Preventive Medicine
1991 - 1997	Clinical Assistant Professor, University of Washington, Departments of Medicine and Environmental Health, Seattle, WA
1997 - 2006	Associate Professor, University of Washington, Departments of Environmental & Occupational Health Sciences and Medicine, Seattle, WA
2002 -	Director, Occ and Env Medicine Program, University of WA, Seattle, WA
2003 - 2005	Member, Institute of Medicine Committee on the Gulf War and Health: Selected Environmental Agents, Pollutants, and Synthetic Chemical Compounds
2005 -	Fellow, American College of Physicians
2005 -	Fellow, American College of Occupational and Environmental Medicine
2006 -	Professor, Depts. of Environmental & Occupational Health Sciences, School of Public Health, Seattle, WA
2006 -	Professor, Department of Medicine, School of Medicine, Seattle, WA
2006 -	Professor, Department of Epidemiology, School of Public Health, Seattle, WA
2009 - 2010	Member, USEPA Clean Air Scientific Advisory Committee (CASAC), CO Panel
2013 - 2017	Member, USEPA Clean Air Scientific Advisory Committee (CASAC), NO Panel
2015 - 2018	Member, USEPA Clean Air Scientific Advisory Committee (CASAC), Particulate Matter Review Panel
2015 - 2019	Member, NIH Cancer, Heart, and Sleep Epi Study Section B (CHSB, previously CASE)

Other Experience and Professional Memberships

Member, Intl Society for Environmental Epidemiology; Chair 2014 International Conference
Member, American Public Health Association
Associate Editor, Environmental Health Perspectives
Member, American Thoracic Society
Editorial Board Member, American Journal of Respiratory and Critical Care Medicine

C. Contribution to Science

As a physician-epidemiologist studying environmental and occupational health factors in disease and disability, my work has focused on addressing problems at the nexus of epidemiology, environmental health sciences, toxicology, and clinical medicine. Leveraging advances in each of these disciplines, and conducting multi-investigator collaborations which include state-of-the-art quantitative methods, is common to each area.

1. Exposure to ambient air pollutants is associated with chronic diseases. Our epidemiological work has focused on well-conducted studies with good control of potential confounding factors and excellent outcome assessment. We approach our work with equipoise and have observed and published both null and positive findings. Our findings are most compelling and show more consistent effects for studies of long-term exposure than for short-term exposures.
   a. Miller KA, Siscovick DS, Sheppard K, Sullivan JH, Anderson G, **Kaufman JD**. Long-term exposure to fine particulate matter air pollution and cardiovascular events in women. New England J Med 2007; 356:447-58.
   b. Sullivan JH, Schreuder A, Sheppard L, Siscovick D, **Kaufman JD**. Short-term fine particulate matter exposure and onset of myocardial infarction in a community-based myocardial infarction treatment trial. Epidemiology 2005;16: 41-48.
   c. Young MD, Sandler DP, DeRoo LA, Vedal S, **Kaufman JD**, London SJ. Ambient air pollution exposure and incident adult asthma in a nationwide cohort of US women. Am J Respir Crit Care Med 2014. PMCID: PMC4299575
   d. Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC Jr, Whitsel L, **Kaufman JD**. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 2010;12:2331-2378. PMID: 20458016
2. Improved exposure assessment greatly improves the quality of research investigating the health effects of air pollutants. Sophisticated exposure monitoring campaigns combined with advanced statistical models can be built that accurately estimate exposures where people live.
   a. Keller JP, Olives C, Kim S-Y, Sheppard L, Sampson PD, Szpiro AA, Oron AP, Lindström J, Vedal S, **Kaufman JD**: A Unified Spatiotemporal Modeling Approach for Predicting Concentrations of

Multiple Air Pollutants in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. *Environmental health perspectives* 2014, 123(4):301-309. PMCID: PMC4384200

b. Wang M, Sampson PD, Hu J, Kleeman MJ, Keller JP, Olives C, Szpiro AA, Vedal S, **Kaufman JD**. Combining Land-Use Regression and Chemical Transport Modeling in a Spatio-temporal Geostatistical Model for Ozone and PM2.5. Environ Sci Technol. 2016; 50: 5111-8. PMID: 27074524 PMCID: PMC5096654 [Available on 2017-05-17]

c. Cohen MA, Adar SD, Allen RW, Avol E, Curl CL, Gould T, Hardie D, Ho A, Kinney P, Larson TV, Sampson P, Sheppard L, Stukovsky KD, Swan SS, Liu LJS, **Kaufman JD**. Approach to estimating participant pollutant exposures in the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). Environmental Science & Technology 2009;43:4687-93. PMCID: PMC2727607

d. Kioumourtzoglou MA, Spiegelman D, Szpiro AA, Sheppard L, **Kaufman JD**, Yanosky JD, Williams R, Laden F, Hong B, Suh HH. Exposure measurement error in PM2.5 health effects studies: A pooled analysis of eight personal exposure validation studies. Environ Health 2014;13:2. PMCID: PMC3922798

3. Experimental findings, including in human studies, that use realistic exposure systems, provide key corroborative evidence that environmental factors can affect the cardiovascular system. We have built and use a customized laboratory facility for generating exposures for translation of inhalation toxicology to enhance the clinical and mechanistic framework.

   a. Peretz A, Sullivan JH, Leotta DF, Trenga CA, Sands FN, Allen J, Carlsten C, Wilkinson CW, Gill EA, **Kaufman JD**. Diesel exhaust inhalation elicits acute vasoconstriction *in vivo.* Environmental Health Perspectives 2008;116(7):937-42. PMCID: PMC2453163

   b. Cosselman KE, Krishnan RM, Oron AP, Jansen K, Peretz A, Sullivan JH, Larson TV, **Kaufman JD**. Blood pressure response to controlled diesel exhaust exposure in human subjects. Hypertension 2012;59:943-8. PMID: 22431582 PMCID: PMC3654814

   c. Allen J, Trenga CA, Peretz A, Sullivan JH, Carlsten CC, **Kaufman JD**. Effect of diesel exhaust inhalation on antioxidant and oxidative stress responses in adults with metabolic syndrome. Inhalation Toxicology 2009;21:1061-1067. PMCID: PMC3075948

   d. Gould T, Stewart J, Slater D, McEwen N, **Kaufman JD**, Larson T. A controlled inhalation diesel exhaust exposure facility with dynamic feedback control of PM concentration. Inhalation Toxicology 2008;20:49-52 PMID: 18236222

4. Association of environmental factors with subclinical and intermediate disease markers can provide key insights into mechanisms of effects.

   a. Hajat A, Allison M, Diez-Roux AV, Jenny NS, Jorgensen N, Szpiro AA, Van Hee VC, Vedal S, **Kaufman JD**. Long-term exposure to air pollution and markers of inflammation, coagulation and endothelial activation: A repeat measures analysis in the Multi-Ethnic Study of Atherosclerosis (MESA). Epidemiology. 2015 May;26(3):310-20. PMCID: PMC4455899

   b. Adar SD, **Kaufman JD**, Diez-Roux AV, Hoffmann EA, D'Souza J, Stukovsky KDH, Rich SS, Rotter JI, Guo X, Raffel LJ, Sampson PD, Oron AP, Raghunathan T, Barr RG. Air pollution and percent emphysema identified by computed tomography in the Multi-Ethnic Study of Atherosclerosis. Environ Health Perspect. 2015 Feb;123(2):144-51. PMCID: PMC4314244

   c. Leary PJ, **Kaufman JD**, Barr RG, Bluemke DA, Curl CL, Hough CL, Lima JA, Szpiro AA, Van Hee VC, Kawut SM. Traffic related air pollution and the right ventricle: The Multi-Ethnic Study of Atherosclerosis. Am J Respir Crit Care Med. 2014 May 1;189(9):1093-100. PMCID: PMC4098110

   d. Adar SD, Klein R, Klein BE, Szpiro AA, Cotch MF, Wong TY, O'Neill MS, Shrager S, Barr RG, Siscovick DS, Daviglus ML, Sampson PD, **Kaufman JD**. Air Pollution and the Microvasculature: A cross-sectional assessment of in vivo retinal images in the population-based Multi-Ethnic Study of Atherosclerosis (MESA). PLoS Med. 2010 Nov 30;7(11):e1000372. PMCID: PMC2994677

5. Epidemiological research is enhanced by incorporating cutting-edge methodologies that span disciplines, including new statistical methods and potential confounding and complicating features.

   a. Szpiro AA, Sheppard L, Adar SD, **Kaufman JD**. Estimating acute air pollution health effects from cohort study data. Biometrics 2014 70:164-174. PMCID: PMC4080094

   b. Hajat A, Diez-Roux AV, Adar AD, Auchincloss AH, Lovasi GS, O'Neill MS, Sheppard L, **Kaufman JD**. Air Pollution and Individual and Neighborhood Socioeconomic Status: Evidence from the Multi-Ethnic Study of Atherosclerosis (MESA). Environmental Health Perspectives 2013;121:1325-1333. PMCID: PMC3855503

c. Hicken M, O'Neill MS, Auchincloss AH, Magzamen SL, **Kaufman JD**, Diez Roux AV. Do psychosocial stress and social disadvantage modify the association between air pollution and blood pressure? The Multi-Ethnic Study of Atherosclerosis. Am J Epidemiol 2013;178:1550-1562. PMCID: PMC3888274
d. Jones MR, Diez-Roux AV, Hajat A, Kershaw KN, O'Neill MS, Guallar E, Post W, **Kaufman JD**, Navas-Acien A. Race/ethnicity, residential segregation and exposure to ambient air pollution: the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Public Health 2014. PMCID: PMC4202969

Complete List of Published Work in My Bibliography (of >175 original articles):
www.ncbi.nlm.nih.gov/myncbi/joel.kaufman.1/bibliography/40707694/public/?sort=date&direction=descending

D. RESEARCH SUPPORT

Ongoing Research Support

R01 ES025888 (Kaufman, Chen, MPI) 7/1/2016-6/30/2021
Environmental Determinants of Pathological Brain Aging in WHI Memory Studies
This project will address the impact of long-term exposures on dementia/AD incidence, latent trajectories of internally validated neuropsychological biomarkers, evaluate the hypothesized mediation of these outcomes, and explore the associations between PM exposure sources/compositions and brain aging.

R01 ES023500 (Kaufman, Hansel, MPI) 02/24/14-10/31/18
SPIROMICS - Air Pollution Study
This multi-site, prospective cohort study is evaluating impact of air pollutants on progression and exacerbations of Chronic Obstructive Pulmonary Disease This project adds air pollution exposure monitoring and modeling to an NHLBI cohort study--the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS). Hansel and Kaufman are MPIs.

R56ES026528 (Kaufman, PI) 2/1/2017 – 1/30/2018 (estimate)
Air Pollution, Heart Failure and Atrial Fibrillation in MESA
This award funds air pollutant exposure assessment activities in the MESA cohort during the sixth clinical examination. This project is funded under the High Priority, Short-Term Project Award (R56) mechanism. NIH uses this one- or two-year mechanism to "underwrite highly meritorious applications which have fallen just beyond an established payline".

R01ES026187 (Li, Sheppard, MPI) 9/30/2016-7/31/2021
Title: Air Pollution, The Aging Brain and Alzheimer's Disease
The project examines the effects of air pollution on cognitive decline, all-cause dementia and AD incidence, and brain neuropathologies in the Adult Changes in Thought (ACT) study. We will collect new air pollution measurements to develop for ACT participants state-of-the-art long-term average air pollution predictions for ambient PM2.5, O3, NOx, and NO2. Our overarching goal is to identify air pollution risk factors and quantify their effects in order to promote healthy aging.
Role: Co-Investigator

1UG3OD023271-01 (Karr, Bush, LeWinn, Sathyanarayana, Tylavsky, MPIs) 09/21/2016 – 08/31/2018
Prenatal and Early Childhood Pathways To Health: An Integrated Model of Chemical and Social Exposures, Biological Mechanisms, and Sex-Specific Effects on Neurodevelopment and Respiratory Outcomes
This multi-PI cohort study involves over 3000 mother-child dyads as part of a new national program to understand the role of early life exposures in the development of core pediatric health outcomes. The Pathways study will evaluate exposures to air pollution, phthalates, and stress during pregnancy and impacts on early and later childhood development of asthma, atopy, and neurodevelopmental health.
Role: Co-Investigator

R01 HD078501, NIH/NICHD (Crowder, PI) 08/22/14-04/30/18
Title: Demographic Vulnerability, Neighborhood Pollution, and Racial Health Disparities
The project focuses on the ways in which social and economic conditions at the individual-, family-, and neighborhood-levels interact with neighborhood concentrations of environmental pollution to affect racial disparities in health and mortality. We assess neighborhood pollution, along with information on other neighborhood characteristics, to longitudinal data for a diverse group of individuals in the national Panel Study of Income Dynamics, to study long-term effects of pollution on mortality, self-rated health, physical limitations, and cancer, cardiovascular disease, and asthma.
Role: Co-Investigator

T42 OH008433, Centers for Disease Control and Prevention (Kalman, PI) 7/1/13-6/30/20

Northwest Center for Occupational Health and Safety
This is a multidisciplinary NIOSH Education and Research Center providing training for occupational health and safety professionals.
Role: Director of the occupational medicine component.

5P30ES007033, NIH/NIEHS (Kavanagh, PI) 4/1/16-3/31/21
PI: Kavanagh
Interdisciplinary Center for Exposures, Diseases, Genomics, and Environment (EDGE Center)
This is a renewal of a longstanding NIEHS Core Center. Dr. Kaufman is Deputy Center Director and the Director of the Integrated Environmental Health Sciences Facility Core
Role: Deputy Director, Core Director

RD-83479601, USEPA (Vedal, PI) 12/01/10-11/30/17
PI: Vedal
UW Center for Clean Air Research
The aim of the five project UW CCAR is to disentangle features of this complex mixture to provide insight into those that are especially toxic to the cardiovascular system. The ultimate aim is to identify the specific near-roadway emission sources and interactions that produce the greatest toxicity.
Role: Project lead for two projects

15PRE25680066, AHA (Cosselman, PI) 7/1/15-6/30/17
Mechanisms and Markers of the Cardiovascular Response to Diesel Exhaust in Humans
Doctoral award. This fellowship proposal integrates basic science with clinical measures to examine aspects of the vascular response in humans exposed to diesel exhaust, a model traffic-related pollutant.
Role: Mentor (unfunded).

Completed Research Support

R01ES020871 (Wellenius, PI) 7/15/12-3/31/17
Ambient Air Pollution and Incidental Stroke
The association between exposure to ambient particulate matter (PM) and PM chemical components and the risk of cerebrovascular events within the Women's Health Initiative (WHI) cohort is being investigated. Long-term concentrations of PM and PM components will be estimated using a national spatio-temporal model that makes use of national pollution monitoring data, geographic data and geostatistical estimation methods.
Role: Co-Investigator

R831697 (Kaufman, PI) 8/1/04-7/31/14
US Environmental Protection Agency
Prospective Study of Atherosclerosis, Clinical Cardiovascular Disease, and Long-Term Exposure to Ambient Particulate Matter and Other Air Pollutants in a Multi-Ethnic Cohort
This major, multi-site, prospective cohort study ("MESA Air") was conducted in the NIH/NHLBI Multi-Ethnic Study of Atherosclerosis (MESA) and examined the relationship between air pollutants, the progression of subclinical atherosclerosis, and incidence of cardiovascular events in several US communities.

P50 ES015915 (Kaufman, PI) 6/1/08 – 5/31/15
DISCOVER Center: Cardiovascular Disease and Traffic-Related Air Pollution
This specialized center examined the mechanisms by which traffic-related air pollution causes myocardial infarction and other cardiovascular diseases, by integrating a diverse set of research approaches from basic science to clinical research to population-based studies. There were five projects within this Center.
Role: Center Director, PI Project 1, Director of Administrative Core

R01 ES009411 (Spiegelman, PI; Kaufman, PI on UW sub) 05/01/09-02/28/15
Measurement Errors in Environmental Epidemiology
Primary aim of UW activity was to conduct a validation study relating personal exposure of individuals in epidemiological studies to routine air pollution exposure assessment methods, and advance methods to integrate measurement error correction into epidemiological analyses.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow thisformat for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Elizabeth A. (Lianne) Sheppard

POSITION TITLE: Professor and Assistant Chair

eRA COMMONS USER NAME (credential, e.g., agency login): LSHEPPARD

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Johns Hopkins University, Baltimore, MD	B.A.	06/1979	Psychology
Johns Hopkins University, Baltimore, MD	Sc.M.	01/1985	Biostatistics
University of Washington, Seattle, WA	Ph.D.	06/1992	Biostatistics

A. Personal Statement

My research interests focus on statistical methods for understanding the health effects of environmental and occupational exposures; they include study design, measurement error, exposure modeling and estimation, and estimation of environmental exposure effects with application to a wide range of health outcomes. The current proposal, which is focused on air pollution exposure and risk of Alzheimer's disease and dementia, is strongly related to several projects in my past and current portfolio. I am co-PI of the study Air Pollution, the Aging Brain and Alzheimer's Disease. Early in my career I worked on Alzheimer's disease research reflected in 3 citations below. As a biostatistician jointly appointed in two departments, I actively collaborate with many different principal investigators on multiple projects in the environmental and occupational health sciences. I have over 145 peer-reviewed publications; most pertain to statistics in the environmental and occupational health sciences. These include biostatistical methods papers (see Section C), numerous publications from the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air) study (including a May 2016 paper in *The Lancet* showing the relationship between air pollution exposure and increased progression of subclinical cardiovascular disease), and a publication in the *New England Journal of Medicine* estimating the risk in post-menopausal women of long-term air pollution exposure on cardiovascular disease incidence from the Women's Health Initiative cohort. In addition, I have extensive training and mentoring experience. In my role as Assistant Chair I actively support the development of our junior faculty. I direct a training grant to advance quantitative training in the environmental health sciences, BEBTEH (Biostatistics, Epidemiologic and Bioinformatic Training in Environmental Health) and an undergraduate research experience program to enhance diversity in the environmental health sciences, SURE-EH (Supporting Undergraduate Research Experience in Environmental Health). I am a Fellow of the American Statistical Association, a member of the HEI Review Committee, and a member of the Editorial Board of Epidemiology. I am one of seven statutory Clean Air Scientific Advisory Committee (CASAC) members, I have served on various CASAC Special Panels, I have served on two of EPA's Scientific Advisory Board Panels: Ethylene Oxide, and Toxicological Review of Libby Amphibole Asbestos, and I am currently serving on the EPA FIFRA Scientific Advisory Panel evaluating the carcinogenic potential of glyphosate.

1. Tsuang D, Kukull W, **Sheppard L**, Barnhart R, Peskind E, Edland S, Schellenberg G, Larsen E, Raskin M: Impact of sample selection on APOE E4 allele frequency: A comparison of two Alzheimer's disease samples. *J Am Geriatrics Soc*, 1996, 44:704-707. PMID: 8642164
2. Bowen J, Malter A, **Sheppard L**, Kukull W, McCormick W, Teri L, Larson E: Predictors of mortality in patients diagnosed with dementia of the Alzheimer's type. Neurology, 1996, 47:433-439. PMID: 8757016
3. O'Meara ES, Kukull WA, **Sheppard L**, Bowen JD, McCormick WC, Teri L, Pfanschmidt, Thompson JD, Schellenberg GD, Larson EB: Head injury and risk of Alzheimer's disease by apolipoprotein E genotype. American Journal of Epidemiology 1997, 146:373-384. PMID: 9290497
4. Adar SD, D'Souza J, Mendelsohn-Victor K, Jacobs DR, Cushman M, Thorne PS, **Sheppard L**, Thorne PS, Burke GL, Daviglus ML, Szpiro AA, Diez-Roux AV, Kaufman JD, Larson TV. Markers of inflammation and

coagulation after long-term exposure to coarse particulate matter: A cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis. *EHP*, 2015,123(6): 541–548. PMCID: PMC4455582

Positions and Honors

Positions and Employment

1993-2001 Research Assistant Professor, Department of Biostatistics & Department of Environmental and Occupational Health Sciences (DEOHS), University of Washington
2001-2006 Research Associate Professor, Department of Biostatistics & DEOHS, University of Washington,
2005-8,10,11 Member, EPA Clean Air Scientific Advisory Committee Special Review Panel for Ozone
2006-2009 Research Professor, Department of Biostatistics & DEOHS, University of Washington
2007-2009 Member, EPA Clean Air Scientific Advisory Committee Special Review Panels for NOx and SOx
2009-present Professor, Departments of Biostatistics & EOHS, University of Washington
2009-present Member, Health Effects Institute Review Committee
2011-2013 Member, EPA SAB Panel for Toxicological Review for Libby Amphibole Asbestos
2013-present Member, EPA Clean Air Scientific Advisory Committee Special Review Panel for NOx
2014-present Member, EPA Clean Air Scientific Advisory Committee Special Review Panel for SOx
2014-2015 Member, EPA SAB Chemical Assessment Advisory Committee for Ethylene Oxide
2014-present Assistant Chair for Research and Faculty Engagement, Dept. EOHS, University of Washington
2015-2017 Member, Statutory EPA Clean Air Scientific Advisory Committee

Other Experience and Professional Memberships

1995 Member, HEI Review Committee for RFA 94-2: Particulate Air Pollution and Daily Mortality
1996 Member EPA Review Panel for Intra-individual Variation in Human Susceptibility to Cancer
2001 Member, NIOSH Safety and Occupational Health Study Section
2006-2009 Consultant to the HEI Review Committee for the Public Health and Air Pollution in Asia (PAPA)
2007-2009 Member, NRC Committee on Contaminated Drinking Water at Camp Lejeune
2009 NIEHS Special Emphasis Panel for the Children's Env Health & Disease Prevention Centers
2009 Consultant, EPA Human Studies Review Board
2009-2012 Member: External Advisory Committee of the USC Children's Health Study
2010-present Member: Epidemiology Editorial Board
2010 External Reviewer: Emory University Graduate School's Proposed Doctoral Program in Environmental Health Sciences
2014-present Member, External Advisory Committee for the University of California, Berkeley-Stanford Children's Environmental Health Center

Honors

1991 Outstanding Student Award, University of Washington School of Public Health
2000 Nominee: Distinguished Graduate Mentor Award (UW)
2006 Elected Fellow, American Statistical Association
2009-2010 Genentech Endowed Professor of Biostatistics, University of Washington
2010 Distinguished Faculty Lecture, UW School of Public Health

B. Contributions to Science

Statistical Methods

1. Early in my career I collaborated with Ross Prentice to develop statistical methods for aggregate data studies. These studies are a substantial improvement upon ecological studies focused on the association between an exposure such as dietary intake and a health outcome such as breast cancer. They give better estimates of the health effect parameter targeted in individual-level studies while using a group-level study design and analysis coupled with some limited sampling of exposure data from individuals.

a. Prentice RL, **Sheppard L**: Aggregate data studies of disease risk factors. Biometrika 82:113-125, 1995.
b. **Sheppard L**, Prentice RL: On the reliability and precision of within and between population estimates of relative rate parameters. *Biometrics* 51:853-863, 1995. PMID: 7548704
c. **Sheppard L**, Prentice RL, Rossing MA: Design considerations for estimation of exposure effects on disease risk using aggregate data studies. *Stat in Med* 15:1849-1858, 1996. PMID: 8888477

d. **Sheppard L**: Insights on information and bias in group-level studies. Biostatistics 4:265-278, 2003. PMID: 12925521

2. Together with Adam Szpiro and other colleagues we have made important contributions on developing measurement error correction methods for inference about health effects for application to air pollution cohort studies. This work has fundamentally changed the perspective on measurement error. In these studies pollution is modeled using a statistical model. The measurement error induced has classical-like and Berkson-like components. These advances are critical to appropriate inference about air pollution health effects.

a. Szpiro AA, **Sheppard L**, Lumley T. Efficient measurement error correction with spatially misaligned data. *Biostatistics*, 2011, 12:610-23. PMCID: PMC3169665
b. **Sheppard L**, Burnett RT, Szpiro AA, Kim S-Y, Jerrett M, Pope CA III, Brunekreef B. Confounding and exposure measurement error in air pollution epidemiology, *Air Quality, Atmosphere & Health*, 2011, Jun;5(2):203-216. PMCID: PMC3353104
c. Szpiro AA, Paciorek C, **Sheppard L**. Does more accurate exposure prediction necessarily improve health effect estimates? *Epidemiology*, 2011, 22:680-685. PMCID: PMC3195520
d. Bergen S, **Sheppard L**, Sampson PD, Young-Kim S, Richards M, Vedal S, Kaufman JD, Szpiro AA. A national prediction model for components of PM2.5 and measurement error corrected health effect inference. *Environmental Health Perspectives*, 2013 Sep;121(9):1017-25. PMCID: PMC3764074

3. I have a strong interest in quantitative exposure modeling for application to epidemiological studies. This body of work spans both occupational and environmental epidemiology applications to include noise and air pollution exposures. Recently our team has significantly advanced spatial and spatio-temporal modeling methods for air pollution exposures.

a. Seixas N, **Sheppard L**: Maximizing accuracy and precision using individual and grouped exposure assessments. Scand J Work and Env 22:94-101, 1996. PMID: 8738886
b. Szpiro AS, Sampson PD, **Sheppard L**, Lumley T, Adar SD, Kaufman J. Predicting intra-urban variation in air pollution with complex spatio-temporal dependencies. *Environmetrics*, 2009, 21: 606–631. PMCID: PMC4029437
c. Lindström J, Szpiro AA, Sampson PD, Oron A, Richards M, Larson T, **Sheppard L**. A flexible spatio-temporal model for air pollution with spatial and spatio-temporal covariates. *Environmental and Ecological Statistics*, 2014, 21:411–433. PMCID: PMC4174563
d. Keller JP, Olives C, Kim SY, **Sheppard L**, Sampson PD, Szpiro AA, Oron A, Vedal S, Kaufman JD. A unified spatiotemporal modeling approach for predicting concentrations of multiple air pollutants in the Multi-Ethnic Study of Atherosclerosis and Air Pollution. *Environmental Health Perspectives*, 2015, Apr;123(4):301-9. PMCID: PMC4384200

4. We have solved the challenge of referent selection and proper analysis of the case-crossover study design for air pollution epidemiology. This is reflected in the accompanying editorial to our 2005 paper (Janes et al 2005b) which claimed “the issue of how to sample referent periods in case-crossover studies of air pollution is clearly answered and a standard approach is available that can easily be implemented using standard software tools. Now it is time for the field to move on to minimizing other, potentially much larger sources of bias in studies of the short-term effects of air pollution.”

a. Levy D, Lumley T, **Sheppard L**, Kaufman J, Checkoway H: Referent selection in case-crossover analyses of health effects of air pollution. *Epidemiology* 12:186-192, 2001. PMID: 11246579
b. Janes H, **Sheppard L**, Lumley T. Overlap bias in the case-crossover design, with application to air pollution exposures. *Statistics in Medicine*, 2005a, 24:285-300. PMID: 15546133
c. Janes H, **Sheppard L**, Lumley T. Case-crossover analyses of air pollution exposure data: Referent selection strategies and their implications for bias. *Epidemiology*, 2005b, 16:717-26.

Applications

5. I have made major contributions to studies of air pollution, noise exposure, manganese exposure, and other environmental and occupational exposures. Selected contributions include:

a. Miller KA, Siscovick DS, **Sheppard L**, Shepherd K, Sullivan JH, Anderson G, Kaufman JD. Long-term exposure to fine particulate matter air pollution and cardiovascular events in women, *New England Journal of Medicine*, 356:447-458, 2007. PMID: 17267905
b. Seixas NS, Neitzel RL, Stover B, **Sheppard L**, Feeney P, Mills D, Kujawa SG. 10-year prospective study of noise exposure and hearing damage among construction workers. *Occupational and Environmental Medicine*, 2012, 69:643-50. PMCID: PMC4570847
c. Adar SD, D’Souza J, **Sheppard L**, Kaufman JD, Hallstrand TS, Davey ME, Sullivan JR, Jahnke J, Koenig J, Larson TV, Liu LJS. Adopting clean fuels and technologies on school buses: Pollution and

health impacts in children. *Am J Respir Crit Care Med*. 2015 Jun 15;191(12):1413-21. [Epub ahead of print Apr 13]. PMCID: PMC4476560

d. Kaufman JD, Adar SD, Barr RG, Budoff M, Burke GL, Curl CL, Daviglus ML, Diez Roux AV, Gassett AJ, Jacobs, Jr DR, Kronmal R, Larson TV, Navas-Acien A, Sampson PD, **Sheppard L**, Siscovick DS, Stein JH, Szpiro AA, Watson KE. Air Pollution and Acceleration of Coronary Artery Calcification: The Multi-Ethnic Study of Atherosclerosis and Air Pollution. *The Lancet*, 2016, Aug 13;388(10045):696-704. [Epub 2016 May 24] PMCID: PMC5019949

Complete List of Published Work in MyBibliography (of >145 original articles published or accepted)**:**
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/44484767/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

1R01ES026187-01A1 REV Li (Multi-PI), Sheppard (Multi-PI) 09/30/2016-07/31/2021
Air Pollution, the Aging Brain and Alzheimer's Disease
This study provides an opportunity to develop comprehensive insights into the effects of air pollution on the aging brain, including the effects of air pollution on cognition, the risk of Alzheimer's disease, and various potential mechanisms of neurodegenerative disease and cerebrovascular disease. The proposed study seeks to advance fundamental research using state-of-the-art exposure science. A deeper understanding of these environmental exposures will contribute significantly to the identification of disease mechanisms and improvements in public health, particularly because these exposures can be modified by changes in regulations and individual behaviors.
Role: Multi Principal Investigator

1R25ES025503-01 Sheppard (PI) 07/01/2015-06/30/2020
Supporting Undergraduate Research Experience in Environmental Health (SURE-EH)
SURE-EH will provide environmental health science research experience and educational opportunities to at least four traditionally underrepresented undergraduate students per year from across the UW.
Role: Principal Investigator

T32ES015459 Sheppard (PI) 07/01/2009-06/30/2019
Biostatistics, Epidemiologic and Bioinformatic Training in Environmental Health (BEBTEH)
The purpose of this training program is to improve quantitative science expertise in the environmental health sciences (EHS) by producing quantitative science researchers with strong EHS skills and EHS researchers with strong quantitative science skills. The fundamental innovation of this program is its unified structure to bridge EHS with bioinformatics and biostatistics.
Role: Principal Investigator and Director

RD-83479601 Vedal (Center PI), Sheppard (Core PI) 12/01/2010-11/30/2017 (NCE)
UW Center for Clean Air Research
This center examines near-roadway pollution, a multi-pollutant atmosphere, consists of vapor and gas phase components that vary by vehicle emission source, road surface, extent of physical aging and the type and degree of atmospheric processing and photochemical reactions. The immediate aim of the UW CCAR is to disentangle features of this complex mixture to provide insight into those that are especially toxic to the cardiovascular system. The ultimate aim is to identify the specific near-roadway emission sources and interactions that produce the greatest toxicity.
Role: Project PI and Co-Investigator

1R01ES021488-01 Racette (PI) 12/1/2012-11/30/2017
Imaging Biomarkers of Neurotoxicity in Welders
The major goals of this project are to determine the relations between functional neuroimaging results and clinical signs and symptoms of parkinsonism among welders exposed to manganese. The project will also examine dose-response relations for neuroimaging results and neurobehavioral test findings.
Role: UW subcontract PI and Co-Investigator

R01ES025991 Racette (PI) 8/1/2015 – 4/30/2020
Motor and Cognitive Health Outcomes in a Mn-Exposed African Communities

The University of Washington team will be responsible for the biostatistical components of the proposed research on motor, cognitive and mood dysfunction in a manganese-exposed community in South Africa. Activities will include the design and implementation of statistical analyses related to the estimation and validation of the exposure data, the validation of health outcome data, and all statistical analyses directly addressing all three study aims.
Role: UW subcontract PI and Co-Investigator

Completed Research Support

R21OH010362 Simpson (PI) 9/1/2013-8/31/2015
Diesel Exposure in Mines: Biomarkers in Urine and Realtime Air Monitoring
This project will develop improved tools for measuring human exposure to diesel exhaust, including measurement of diesel specific chemicals in urine, and real-time measurements of several diesel exhaust components in air samples.
Role: Co-Investigator

P50 ES015915 Kaufman (Center PI) 4/1/2008-5/31/2015 (NCE)
DISCOVER Center: Cardiovascular Disease and Traffic-Related Air Pollution
The overall objective is to investigate the mechanisms by which traffic-related air pollution causes myocardial infarction and other cardiovascular diseases, by integrating a diverse set of research approaches.
Role: Project PI and Co-Investigator

K24 ES013195 Kaufman (PI) 08/01/2009-7/31/2015
Environmental Factors in Cardiovascular Disease
The goal of this midcareer investigator award is to expand training in multi-disciplinary research focused on the environmental health sciences at the University of Washington. Through collaborations with several ongoing training programs, the candidate will expand current research efforts and develop a mentoring program with trainees from several relevant disciplines. This program will integrate experimental, epidemiologic, and translational approaches to the role of environmental factors in cardiovascular and pulmonary disease.
Role: Co-Investigator

RD 831697010 Kaufman (PI) 08/01/2009-07/31/2014
Study of Atherosclerosis, Cardiovascular Disease & Exposure to Ambient Particulate Matter
The MESA Air Pollution Study is prospectively examining the relation between an individual level assessment of long-term ambient air pollution exposures and progression of subclinical cardiovascular disease in a multi-city, multi-ethnic cohort, by repeated assessment of coronary artery calcification, intima-media thickness of the common carotid artery, ankle-brachial index, and plasma markers of inflammation and endothelial activation.
Role: Co-Investigator

P42 ES04696 Gallagher (PI) 04/20/2009-03/31/2014
Effect-Related Biomarkers of Environmental Neurotoxic Exposures
The theme of this Superfund project is that biomarkers measured in accessible tissues are predictive of: a) toxicant exposures; b) early indicators of damage; and/or c) unusual susceptibility to toxic agents that commonly occur at hazardous waste sites.
Role: Co-Investigator

Subcontract with Harvard School of Public Health Spiegelman (PI) 05/01/2009-02/28/2014
Measurement Errors in Environmental Epidemiology
This project focuses on issues in air pollution epidemiology - in particular, the chronic effects of particulate exposure and elemental carbon on all-cause mortality, cardio-vascular mortality and lung cancer mortality.
Role: Co-Investigator

R01 ES017809 Seixas (PI) 07/01/2010-04/30/2014
Characterization of Manganese Exposure Biomarkers Among Welder Apprentices
Despite evidence of adverse health effects resulting from exposure to welding fume and, in particular, to manganese (Mn), biomarkers of exposure are poorly understood and have not been thoroughly evaluated in a longitudinal design. This study will evaluate the relationship between well-characterized inhaled MN exposure and a number of short- and long-term biomarkers of Mn exposure.
Role: Co-Investigator

Dear Proposed Subrecipient,

You are receiving this email because your organization was listed as a subrecipient on a proposal that the University of Southern California will be submitting for funding to one of the Public Health Service (PHS) agencies listed below.

- Agency for Healthcare Research and Quality (AHRQ)
- Agency for Toxic Substances and Disease Registry (ATSDR)
- Centers for Disease Control and Prevention (CDC)
- Food and Drug Administration (FDA)
- Health Resources and Services Administration (HRSA)
- Indian Health Service (IHS)
- National Institutes of Health (NIH)
- Substance Abuse and Mental Health Services Administration (SAMHSA)
-

The updated PHS Conflict of Interest regulations require us to verify your compliance with these regulations at time of proposal, and at various stages of the award. If your organization is compliant with the updated PHS Financial Conflict of Interest (FCOI) regulations, we encourage you to enroll your organization in the FDP Clearinghouse of institutions compliant with these regulations. Your organization does not have to be an FDP member to enroll in the Clearinghouse, and enrollment takes about two minutes. You may enroll at http://sites.nationalacademies.org/PGA/fdp/PGA_070596 . If your institution is a member of the Clearinghouse, simply indicate below.

If you are not enrolled in the FDP Clearinghouse, please complete and return the below certification at your earliest convenience. In the event you do not have an FCOI compliant policy, FDP has developed a model policy that you may adopt. For more information, please see: http://sites.nationalacademies.org/PGA/fdp/PGA_061001.

As a reminder, your institution may not be included in the proposal submission until the below certification is received. Should this proposal be awarded, no PHS funds may be expended by your organization related to this project until PHS FCOI requirements are met. Additional information regarding the new Public Health Service FCOI regulations may be found at: http://grants.nih.gov/grants/policy/coi/

SUBRECIPIENT FCOI CERTIFICATION

☒ Subrecipient Organization/Institution is enrolled in the FDP Institutional Clearinghouse of FCOI Compliant institutions.

☐ Subrecipient Organization/Institution certifies that it has an active and enforced conflict of interest policy that is consistent with the provision of 42 CFR Part 50, Subpart F "Responsibility of Applicants for Promoting Objectivity in Research." Subrecipient also certifies that, to the best of Institution's knowledge, (1) all financial disclosures have been made related to the activities that may be funded by or through a resulting agreement, a nd required by its conflict of interest policy; and, (2) all identified conflicts of interest have or will have been satisfactorily managed, reduced or eliminated in accordance with subrecipient's conflict of interest policy prior to the expenditures of any funds under any resultant agreement.

☐ Subrecipient does not have an active and/or enforced conflict of interest policy and agrees to abide by USC's policy, located online at http://ooc.usc.edu/Conflict-Interest-Research

APPROVED FOR SUBRECIPIENT

The information, certifications and representations above have been read, signed and made by an authorized official of the Subrecipient named herein. The appropriate programmatic and administrative personnel involved in this application are aware of agency policy in regard to subawards and are prepared to establish the necessary inter-institutional agreements consistent with those policies. **Any work begun and/or expenses incurred prior to execution of a subaward agreement are at the Subrecipient's own risk.**

INSTITUTION: University of Washington

By _____

Name Diane Wentz, Ph.D.

Title Grant & Contract Administrator/Authorized Signing Official

Resource Sharing Plan

Data Sharing Plan: We will share data in accordance with NIH policy. Processed data will be made public along with publications, and will be distributed through supplementary materials or our own websites.

Sharing Model Organisms: We will adhere to the NIH Grant Policy on Sharing of Unique Research Resources including the Sharing of Biomedical Research Resources Principles and Guidelines for Recipients of NIH Grants and Contracts. All 'model organisms' generated by this project will be distributed freely or deposited into a repository/stock center making them available to the broader research community, either before or immediately after publication. If we assume responsibility for distributing the newly generated model organisms, we will fill requests in a timely fashion. In addition, we will provide relevant protocols and published genetic and phenotypic data upon request. Material transfers will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements. Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with the NIH Principles and Guidelines document.

Genome Wide Association Studies: not applicable

All key resources for this proposal will be authenticated to enhance the reproducibility of our results, as appropriate and according to NIH policy. Key Biological Resources that will be utilized and validated in this proposal include:

Cell lines: None

Tissue from transgenic mouse strains (Project 3&4): EFAD and TLR4 knockout (TLR4-ko) mice.

EFAD (E3FAD and E4FAD) mice were generated by crossing 5XFAD to homozygous APOE3-, and APOE4-TR. 5xFAD mice are transgenic for 5 distinct FAD mutations (APP K670N/M671L+ I716V+ V717I and PS1 M146L+L286V). They are provided by University of Illinois at Chicago (Mary Jo LaDu, PhD).

TLR4 knockout (TLR4-ko), adult-inducible excision of TLR4 ($mCre^{+/-}$, $fl^{+/+}$) by tamoxifen, are CX_3CR1^{CreER} mice (JAX020940) crossed with $TRL4^{loxP/loxP}$ mice (JAX024872). The CX_3CR1^{CreER} mouse provides cell-specificity as Cre expression is under control of the CX_3CR1 promoter. CX_3CR1-expressing cells in adult mice are limited to microglia in brain and macrophages in gut, kidney (Jung et al 2000, PMID10805752; Yona et al 2013, PMID23273845). Cre is inducible by its linkage to an estrogen receptor mutant that is activated by treatment with tamoxifen. The $TRL4^{loxP/loxP}$ mouse has TLR4 flanked with two loxP sequences (TLR4 flox). The first generation (F1) mice are 100% heterozygous ($mCre^{+/-}$, $Fl^{+/-}$) and are currently breeding. F2 mice composed of 25% experimental animals ($mCre^{+/-}$, $fl^{+/+}$), 25% control group ($mCre^{-/-}$, $fl^{+/+}$) will be characterized for expression and functional efficacy of the TLR4 deletion, 3 months after tamoxifen treatment (75 mg/Kg, i.p. for 5 days or vehicle).

Transgenic mice will be validated by genotyping.

Antibodies: Only commercially available antibodies will be used.

Air Pollution particles: Collected, characterized and validated by Core C2 (Prof C. Sioutas, Director). These nPM characteristics are reported in recent publications by members of this P01 (Liu et al 2016; Morgan et al 2011) and are used in other studies supported by our NIA grants to CE Finch (AG040753; AG040683; AG051521).

Other resources used in this proposal will be standard laboratory reagents. Should we need to generate or obtain additional unique resources in the course of this proposal, they will be authenticated using methods similar to those described above, as appropriate.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0729333930000

Legal Name*: University of Southern California
Department: Contracts and Grants
Division: 95-1642394
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: CA
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Mr.	Steven		Misuraca	

Position/Title: Contracts and Grants Officer
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701
Phone Number*: 213-749-8207 Fax Number: 213-740-6070 Email: misuraca@research.usc.edu

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Traffic-Related Air Pollutants and Alzheimer's Disease: Risk, Susceptibility and Mechanisms in Women

12. PROPOSED PROJECT

Start Date*	Ending Date*
04/01/2018	03/31/2023

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Southern California
Duns Number:	0729333930000
Street1*:	3720 South Flower Street
Street2:	
City*:	Los Angeles
County:	CA
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90089-0701
Project/Performance Site Congressional District*:	CA-037

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Wake Forest University Health Sciences
DUNS Number:	9377379070000
Street1*:	Medical Center Blvd.
Street2:	
City*:	Winston-Salem
County:	
State*:	NC: North Carolina
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	27157-1023
Project/Performance Site Congressional District*:	NC-005

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ● No

If YES, check appropriate exemption number: 1 2 3 4 5 6 If

NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ○ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract_Project_1.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Project-1_A1_Bibliography_and_References_Cited.pdf
10.Facilities & Other Resources	Project_1_Facilities.pdf
11.Equipment	

Project-1 is designed to investigate the contribution of traffic-related air pollution (TRAP) to the risk, susceptibility and mechanisms of Alzheimer's disease (AD) and related dementias (ADRD) in a nationwide cohort of women from the Women's Health Initiative (WHI) Memory Studies (WHIMS). ADRD affects more women than men, and increasing evidence indicates different risk factors by sex, pointing to the need to identify modifiable environmental factors separately for women and men. In the last two decades, compelling data have shown that exposure to outdoor air pollutants including TRAP is a novel environmental determinant of brain aging. Despite the strong evidence for TRAP-induced neurotoxicity (e.g., increased Aβ deposit shown in Project-3), human data on whether and how TRAP exposure effects brain aging remain limited and inconsistent. Previous studies had notable limitations: (1) lacking prospective evidence for an increased ADRD risk associated with TRAP; (2) not based on our current understanding of early cognitive change, biomarkers, and neurobiological classification of AD; (3) not studying AD risk resulting from TRAP effects before late life (e.g., aged < 55); and (4) providing limited insight on the brain structure and neuropathology linking TRAP to early cognitive deficits or an increased risk of AD. To address these knowledge gaps cost-efficiently, Project-1 builds on two well-characterized and geographically-diverse cohorts of mid-aged and older women in the WHIMS of Younger Women (WHIMS-Y; n=1346, inception age 50-54) and WHIMS-MRI (n=1403, inception age 65-80) followed annually since 1996, both with comparable longitudinal assessments on episodic memory and executive function. We further capitalize an NIEHS-funded R01 in WHIMS which employs sophisticated air pollution models developed by the MESA-Air *("Multi-Ethnic Study of Atherosclerosis and Air Pollution"*) team to estimate TRAP (NO_2; $PM_{2.5}$; exposure sources/components). Supported by the Core B1, we take the novel population neuroimaging approach to studying pathological brain aging in communities, drawing on both clinical and neuropathological classification of AD defined in very well-characterized samples from the Alzheimer's Disease Neuroimaging Initiative with biomarkers of β-amyloid and neuronal injuries. State-of-the-art mediation and neurocomputational analyses will be conducted to examine the TRAP-affected brain structures and neuropathological pathways underlying the early cognitive deficits or MRI biomarkers of increased AD risk. **Aim 1** will determine the impact of TRAP on early biomarkers predictive of increased risks for AD, mild cognitive impairment, and preclinical AD in older women. **Aim 2** will examine the associations of cognitive decline reflecting early AD with TRAP exposure before/during late life. In **Aim 3**, we will take both targeted and agnostic approached with high-dimensional neurocomputation to elucidate the brain structure and neuropathology mediating the TRAP effects on pathological brain aging. **Aim 4** will evaluate whether APOE4 and vascular brain injuries increase the susceptibility to the neurotoxic effects of TRAP exposure.

FACILITIES AND OTHER RESOURCES

OVERVIEW:
Project-1 aims to: (1) determine the impact of traffic-related air pollutants (TRAP) on early biomarkers predictive of increased risks for AD, MCI, and preclinical AD in older women; (2) examine the associations of cognitive decline (episodic memory; executive functions) reflecting early AD with TRAP exposure before/during late life; (3) elucidate the brain structure and neuropathology mediating the TRAP effects on pathological brain aging, using both targeted and agnostic approached with high-dimensional neurocomputation; and (4) evaluate the contribution of APOE4 and vascular brain injuries to the individual susceptibility to neurotoxic effects of TRAP exposure from 1990-2016. To address these aims in a cost-efficient manner, Project-1 is designed as part of an ancillary study to Women's Health Initiative (WHI AS252, *"Environmental Determinants of Cognitive Aging in the WHI Memory Study"*), which had been approved by the WHI Ancillary Studies Committee and the NHLBI WHI Project Office (see the Letters of Support and Approval). The operation of AS252 was supported by a 4-year (2011-2015) NIA-funded study (R01AG033078; PI: Chen), which helped create a custom-made epidemiologic dataset combining comprehensive and longitudinal WHI clinical and longitudinal covariates database with the multi-dimensional/multi-sourced dementia and neurocognitive outcome data from WHIMS Suite of Studies before 2010. Specifically, Project-1 is built on a well-characterized, geographically-diverse cohort of mid-aged and older women in the WHIMS of Younger Women (WHIMS-Y; n=1326, inception age 50-54) and WHIMS-MRI (n=1403, inception age 65-80) followed annually since 1996, both with comparable longitudinal assessments of neuropsychological functions. Since 2010, two NIA-funded epidemiologic studies on cognitive aging in older and younger postmenopausal women in WHI have been funded. The proposed Project-1 will therefore draw additional longitudinal neuropsychological data on episodic memory and executive functions collected during the concurrent follow-up of WHIMS-ECHO and WHIMS-Y cohorts that were not available to the NIA R01. The scientific activities described in the subcontract at WFU will be coordinated within the WHI Southeast Regional Network, headed by the WHIMS PIs/senior investigators at WFU, which was the WHIMS Clinical Coordination Center (WHIMS) based at the Bowman Gray School of Medicine. Dr. Espeland is Co-PIs of the WHIMS, the WHI Study on Cognitive Aging, and the WHIMS-MRI. The subcontract at the Wake Forest University Health Sciences (which served as the WHIMS+WHIMS-Y Coordination Center) supports the continuing collaboration between USC team and Dr. Mark Espeland to ensure the research team will have the maximum benefit of the ongoing WHI/WHIMS data resources and information.

1. University of Southern California
A. Keck School of Medicine, Department of Preventive Medicine, Division of Environmental Health
The Department of Preventive Medicine has been one of the leading academic departments of preventive medicine and public health sciences for more than three decades. Ranked as the top academic department in preventive medicine with the largest NIH-sponsored researches, the department is home to worldwide authorities on chronic disease epidemiology (including cancer and cardiovascular disease), biostatistics, environmental medicine, health behavior, and prevention, detection, and treatment methods. The Division of Environmental Health (EH) is home to two federally funded regional research centers, including the Southern California Environmental Health Sciences Center (SCEHSC) and the Children's Environmental Health Center. The EH division is the operational house of the Children's Health Study, which is one of the most comprehensive cohort studies on the long-term health effects of air pollution among more than 15,000 school-aged children across 12 different communities in Southern California. Division faculty members have the scientific expertise in studying air pollution health effects in children. To support large-scale air pollution epidemiologic studies, the Division includes experienced staff scientists with the research capacities to conduct complex spatial epidemiologic analyses and monitor levels of the major components of smog—ozone gas, nitrogen dioxide, particulate matter and acid vapors—in population-based cohorts. The active research programs within the EH Division are almost entirely supported by extramural funds with approximately ten million dollars per year. Detailed description of the Dept. of Preventive Medicine and the EH Division can be found at:
https://pm.usc.edu/about.php?page_id=Mission
https://pm.usc.edu/research.php?page_id=Environmental_Health_Research
https://scehsc.usc.edu/

Intellectual Environment

The EH Division offers an excellent intellectual environment fostering innovative research in environmental epidemiology research and air pollution health effects sciences. EH Division faculty members study chronic health effects associated with environmental exposures, and many scientific products of their scholarly work have appeared in high-impact journals, such as the *New England Journal of Medicine*, the *Lancet*, and the *Proceedings of the National Academy of Sciences*. Of its 10 primary faculty members, two had served in the EPA's Clean Air Scientific Advisory Committee and one had served in the National Advisory Environmental Health Sciences Council.

Dr. JC Chen has been co-leading the USC AirPollBrain (APB) Network, a product of the program development project entitled ***"****Brain Health during Development and Aging in Urban Environments"* (co-PIs" Finch and Chen) funded by the USC Collaboration Fund (https://research.usc.edu/collaboration-fund-current-projects/) since 2010. This program brings together a network of USC faculty from Gerontology, the Viterbi School of Engineering, the College of Letters, Arts and Sciences, the Keck School of Medicine, School of Pharmacy, and Annenberg School for Communication and Journalism to better understand the scientific basis for the impacts of pollution on the brain, and to explore the health consequences. This program aims to promote the optimal neurodevelopment in humans and healthy brain aging by better understanding environmental effects of urban air pollution and gene environment interactions. Bimonthly meetings convene faculty and trainees on specific topics with a focus on developing new research projects and consortia. As of March 2016, the AirPollBrain has helped USC faculty obtained over $10 millions of NIH funding to support the continuing development of research and education programs in environmental neurosciences studying ambient air pollution and neurodevelopment/brain aging.

The research program described in this application draws upon the expertise of faculty and staff scientists in the Department of Preventive Medicine at the University of Southern California and collaborating researchers from the NIEHS-sponsored Southern California Environmental Health Sciences Center (SCEHSC). The Division of Environmental Health is home to two federally funded regional research centers, including the SCEHSC and the Children's Environmental Health Center. The NIH-funded SCEHSC is a multi-university consortium of over 60 leading researchers across the region. The SCEHSC has several research initiatives, including *"Air Pollution, Neurodevelopment and Neurological Diseases,"* led by Dr. JC Chen (the HSC PI). The major goal of the initiatives has been to incubate new programs of EHS research by providing infrastructure and support to grow new research into mature programs supported by investigator-initiated grants, program projects and new centers.

Laboratories and Facilities

The project PI is a core member of the SCEHSC, which includes the Integrative Health Sciences Facility Core (IHSFC) and the Biostatistics Facility Core (BFC). The research team will thus benefit from consultation and technical support in areas needed for exposure assessment of air pollution including aerosol science, environmental chemistry, and geospatial modeling. Facilities involved in the performance of the proposed research include offices and laboratories, in particular, the BFC and GIS Research Laboratory, which is part of the IHSFC resources of the SCEHSC.

(1) GIS Research Laboratory

The GIS Research Laboratory is a university- and project-supported laboratory with a number of impressive software and hardware resources. It has its own server room with an IBM Blade Center System H Chassis, HS20 Blade Servers, Xiotech Storage Area Network, Cisco MDS 9000 Fiber Channel Switches, and Quantum PX-502 Tape Library. The servers are located behind a Sonicwall Pro 5060 firewall with stateful and deep packet inspection, potentially analyzing packets all the way up to the application level. Applications requiring high-performance are attached to high-speed fiber-channel drives, 15k RPM, RAID 10. Second tier storage for static data that does not require high performance is provided on 7200 RPM SATA drives, RAID 5. Data is backed up daily incrementally and full backups are completed once a week and once a month. Tapes are rotated off site to Iron Mountain weekly as well.

Software resources that are supported from this hardware platform include Microsoft Windows 2007-2010 (64 bit), .NET v.2.0-3.5, PHP 5, Microsoft IIS 6-7, Apache Tomcat, Microsoft SQL Server 2005-2008R2, the CommVault Data backup software, the ESRI web tools (ArcGIS Server, ArcIMS, ArcSDE, GeoPortal Toolkit,

etc.), and Sentinel LM. The network resources include a Sonicwall Pro 5060 Firewall and Linksys 48-Port Gigabit Switches.

The lab administers the ESRI campus site license and provides the ESRI GIS, IDRISI GIS, and Trimble GPS software suites plus standard office, multimedia, and statistical analysis tools for the 60 or so faculty, graduate students, undergraduate research assistants, and staff that are affiliated with the GIS Research Laboratory and Geographic Information Science and Technology (GIST) graduate programs. All of the aforementioned computer facilities are supported by the College Information Technical Services Center and a dedicated computer services consultant housed in the GIS Research Lab itself.

(2) Biostatistics Facility Core of SCEHSC

The Biostatistics Facility Core (BFC) provides statistical support for planned and ongoing research projects by Center investigators. Core members assist in the development of research protocols from Center investigator who are requesting research grants or extramural support. This entails deciding on study design, calculating sample size or power, and reviewing proposed statistical methods. Core members also provide an interface between Center investigators and statistical resources by matching statisticians to investigators so that, to the maximum extent possible, Center projects will benefit from statisticians with particular expertise in the areas under investigation. Additionally, Core members stimulate collaboration between statisticians and other investigators by providing a focal point for statisticians to discuss issues with investigators from all the research cores. The Core also maintains a fast multi-processor computer that is accessible directly or through the campus network by all Center investigators. This resource provides a central repository for data, facilitates file sharing among Center investigators, and supports many commonly used software packages. The Core also assists Center member in the use of USC's High Performance Computing Cluster (HPCC), a university wide resource that provides thousands of processors for high-volume computing requirements. In addition to research support, the Core includes an educational component. This involves increasing the understanding of statistical issues among Center investigators through formal and informal consultations, broadening the awareness by statisticians of the many applications of statistical theory to environmental health problems, and enhancing graduate programs in biostatistics and epidemiology by providing a mechanism to assign graduate students to research problems in environmental health on which they can work under supervision. Core members are also very active in working with the Center Outreach and Education Core to make presentations and to disseminate research results.

(3) Other Facilities & Resources

Computing/Software Resources: The USC Information Services Division provides access to an array of high performance computing resources, including a Sun Fire 15K and HPC-Master Linux Cluster. The large siz e of this computer allows virtually unlimited user access and data storage needed by major data sets Key personnel in this study have Pentium or compatible machines with statistical and word processing software; all machines have Ethernet access to the mainframe and library information systems. PCs are integrated through a network that links machines and provides access to a library of local web-based software programs to access major datasets and other materials, or even to conduct data management and analysis. Information Technology Services includes the Keck School of Medicine's "Keck Tech" routine services to update software and link computers and databases. Services include: Online forum, Listserve, Online Calendar, Cyberspace file center, and other modules to assist in inter-institutional and international project management and coordination. Data entry, online survey, phone survey and tracking, coding, and other modules enhance the efficiency of project operations, especially during the phase of data collection, management, and analysis. The design and construction of the system comply with NIH's recommendations to develop and utilize programs with open ended source code and develop scalable programs that would be more cost effective when need to be ported to other research institutions or environments. The IT services and resources are available to all personnel on this study. Database, data analysis and methodological research in the Department of Preventive Medicine are largely accomplished via personal PCs/microcomputers that are linked to secured fileservers. All PCs are networked with the University system and directly to the Internet. Software includes all standard statistical packages; (SAS, SPSS, SPLUS, STAT, nQuery Advisor, StatXact, etc.); some in-house packages (POWER, EPILOC); database managers (SQL, ACESS, DBASE), word processors; all major programming languages (FORTRAN, C++, Visual Basic); spreadsheet (EXCEL); and file transfer programs and graphics.

Office Space for the USC Department of Preventive Medicine: The Department of Preventive Medicine at KSoM numbers more than 90 fulltime faculty members who successfully compete for over $50 million in extramural support annually. The PI and co-investigators on this application are Department faculty. The Department includes internationally recognized programs of research in biostatistics, epidemiology of cancer and other acute and chronic diseases, genetic epidemiology, health behavior and occupational and environmental health. The Department is known for its strong research, excellence in teaching and commitment to promoting community health through education. Undergraduate, medical and graduate students are trained in the areas of biostatistics, epidemiology, health behavior research, public health and occupational and preventive medicine.

The newest vanguard in the expansion of the USC Health Sciences Campus is a 120,000 square-foot facility that is home to the Department of Preventive. This three-story building houses offices, laboratories, classrooms, a student and faculty fitness center and a café. The space also houses the PhD, Master's and MPH programs, and other trainees.

Personnel Resources:
The Department of Preventive Medicine has a talented staff of statistical programmers who have been working as lead analysts/programmers for the Children's Health Study and a variety of research projects on spatial analyses, air pollution cohort studies and chronic disease/cancer epidemiologic studies dealing with multisource/ multidimensional exposure and outcome data, all based in the SCEHSC at the EH Division, in the Statistical Consultation and Research Center at the Divisions of Biostatistics, and in the USC Norris Comprehensive Cancer Center on the Health Sciences Campus. These outstanding programmers have acquired familiarity with spatial epidemiologic studies of large geographically diverse populations, developed a pertinent array of programming code, and acquired requisite attention to detail needed to create and combine the multidimensional data files for complicated spatial epidemiologic analyses.

Health Sciences Libraries/Other USC Libraries: The Health Sciences Libraries (HSL) includes the Norris Medical Library and the Wilson Dental Library. Together, the libraries support the teaching, research, and patient care missions of the USC health sciences schools. The HSL is an independent library system of the university, but cooperates extensively with the other university libraries, including the nearly twenty libraries of the Information Services Division, and the Law Library. Access to information is expanded through USC's membership in the Research Libraries Group, a national association of major universities and research institutions, and through membership in CALINET, a consortium of libraries at USC, UCLA, and Caltech. In addition, the USC libraries have developed reciprocal borrowing agreements with several local universities to improve faculty access to library resources.

The HSL, in cooperation with the Information Services Division, provides all USC users with access to a broad range of print and digital resources. The HSL licenses 14 databases including MEDLINE, EBM Reviews, and other health-related sources through Ovid; 160 electronic books and over 1600 electronic journals through Ovid and through individual publishers. The Norris Medical Library maintains a Web site that provides access to more than 1,700 biomedical information resources. The print collection includes 168,185 volumes and the Norris Medical Library receives 1,935 current periodicals. In addition, the Information Services Division licenses an additional 250 databases, approximately 2,500 electronic books, and nearly 2,200 electronic journals including over 250 in the biological sciences. The Norris Medical library has a Learning Resources Center which offers Internet, database searching, and software application workshops designed to meet the needs of USC health sciences students, faculty, and staff.

2. Wake Forest University Health Sciences

Facilities and Resources

Wake Forest University Health Sciences (WFUHS - academic entity) and Wake Forest Baptist Health (WFBH - clinical entity) together have over 900 faculty members, and over 500 adjunct faculty members in the community. Wake Forest School of Medicine has an enrollment of over 460 students. The control of WFBH is vested in the Board of Trustees of Wake Forest University. The Dean of WFUHS, Dr. Edward Abraham, is responsible for the operation of the School of Medicine through the President of the University (Dr. Nathan O. Hatch). The faculty, through the Faculty Executive Council, has general authority over long-range planning and all major changes in policy that affect the activities and welfare of the School (e.g. determining requirements for faculty promotion, student conduct, and the M.D. degree). The Board of Directors is responsible for the overall supervision of WFBH. In November 2008, John McConnell, M.D., was named the first CEO of the Medical Center.

The Medical Center has grown rapidly in recent years. Its catchment area is unique, drawing a substantial proportion of its clinical population from a 24-county region, particularly in western North Carolina and southwestern Virginia. The Medical Center has 1,004 licensed beds, a level 1 trauma center, and active services in cardiac and renal transplantation. The hospital is the largest tertiary care center for the Piedmont region of the Southeast. *US News and World Reports* ranks the Medical Center among the top 50 hospitals in the nation for cancer; cardiology and heart surgery; diabetes and endocrinology; ear, nose, and throat; gastroenterology; geriatrics; gynecology; nephrology; neurology and neurosurgery; orthopedics; pulmonology; and urology.

The active research program at WFUHS is reflected in the establishment of 9 Research Centers, which have the following research focuses: aging, arthritis, cancer, critical care medicine, human genomics and personalized medicine, hypertension and vascular disease, minority health and health disparities, nonhuman primate research, and public health genomics. There are three institutes, one in regenerative medicine, one in pediatric trauma, and one in translational science. Center and Institute Directors report directly to the Dean. The Wake Forest University School of Medicine received nearly $177.2 million in total costs for research from extramural grants in FY 2012-2013, of which 51% was federally funded. This ongoing success is a direct result of an active program by the administration to support, and for the faculty to embrace, new technologies and research opportunities as they emerge.

Office of the Dean. Edward Abraham, M.D., Dean, is responsible for several facilities and services, including: Office of Sponsored Programs: The Office of Sponsored Programs provides services related to research administration for Pre-award grant and contract administration includes: advice and guidance, identifying funding sources, institutional review and clearance of applications, and assistance with agency site visits. The Research Support Core provides pre-award grant editing, advice on funding sources and resubmissions, and statistical assistance to WFUHS faculty. Post-award grant and contract administration includes: advice and guidance, policy interpretation, agency liaison, and rebudgeting actions. The office also provides management and support of institutional committees: the Institutional Review Board, the Animal Care and Use Committee, the Intramural Research Support Committee, the University Patent Committee, and the Biosafety Committee.

The Department of Creative Communications provides audiovisual instructional design, production services and equipment for research activities. The services include photography, poster production, video and DVD production, medical illustration, typesetting, and graphic arts services. The Office of Printing Services has facilities for high-speed photocopying, offset printing, collating and binding. The charges for services are set to recoup costs and are generally less than those of outside vendors.

The Coy C. Carpenter Library contains nearly 30,000 books, over 118,000 serials, and 485 software resources in a 32,000 sq.ft. space. All catalog items are accessible via a computer-based catalog system. Computer searches, computer-assisted instruction, and document delivery services are also available.

The Division of Public Health Sciences (http://www.phs.wakehealth.edu)

The Department of Public Health Sciences was formed in 1989 from the Center for Prevention Research and Biometry, which had been founded in 1986 as evidence of the school's commitment to programs in prevention research. Thanks to the success and growth of the Department of Public Health Sciences, the Dean approved the creation of a Division of Public Health Sciences with three departments in January 2006.

The Division, currently directed by Gregory L. Burke, MD, MS, Professor and Chair, received more than $74 million in FY13 in extramural research funding from more than 120 collaborative research projects headed by Division faculty. Historically, the division has been ranked among the top two of similar groups nationally in NIH funding. The Division's overall goals are to further research programs in the areas of biostatistics, epidemiology, nutrition, and health promotion/disease prevention, and to strengthen research at the school by providing consultation in the development of research proposals, study design, analysis and other methodological issues. To achieve these goals, the Division is divided into three departments: a) Biostatistical Sciences, b) Epidemiology and Prevention, and c) Social Sciences and Health Policy. The Division, together with the Wake Forest University Translational Science Institute, offers a Master of Science degree in Clinical and Population Translational Science. This program is especially targeted to the training of physicians and post-doctoral fellows.

The Epidemiological Cardiology Research Center (EPICARE) within the Division offers competitively priced research laboratory technology for receipt and processing of electrocardiograms (ECGs). Currently, EPICARE is the ECG reading center for more than 20 national and international epidemiologic studies and clinical trials. Its investigators have been engaged in population electrocardiographic research for more than three decades. The Division has ongoing programs in aging, adolescent health research, cancer control, cardiovascular disease, community interventions, diabetes, health-related quality of life issues, nutrition, osteoporosis, renal disease, and women’s health. It also serves as the Coordinating Center for numerous multicenter studies. The Research Information Systems Unit (one of the sections within the Department of Biostatistical Sciences) specializes in state-of-the-art research computing methodology.

Outside the University and Medical School setting, the Division has formulated close collaborative relationships with a variety of other schools and programs across the state and country on numerous projects. Some examples of collaborative projects include ACAPS (The Asymptomatic Carotid Artery Plaque Study), ACCORD (Action to Control Cardiovascular Risk in Diabetes), AIED (Autoimmune Inner Ear Disease study), ARIC (The Atherosclerosis Risk in Community Study), CAFUS (Carotid Artery Follow-Up Study), CARDIA (The Coronary Artery Risk Development in Young Adults Study), CHS (The Cardiovascular Health Study), CHS-BA (Brachial Artery Substudy of CHS), CONTROL (Combination Oral and Nutritional Treatment of Late-Onset Diabetes Trial), C0-STAR (Effects of Selective Receptor Modulators on Cognitive Aging: The Study of Tamoxifen and Raloxifene), ERA (Estrogen Replacement and Atherosclerosis Trial), EUDL (Enforcing Underage Drinking Laws Program), EUDL-CT (Enforcing Underage Drinking Laws Randomized Community Trial), FIT (The Fracture Intervention Trial), FTG (Free to Grow: Head Start Partnerships to Promote Substance Free Communities), HEIRS (Hemochromatosis and Iron Overload Screening Study), ICPCG (International Consortium for Prostate Cancer Genetics), IRAS (Insulin Resistance and Atherosclerosis Study), IRAS Family (Insulin Resistance and Atherosclerosis Family Study), LIFE (Lifestyle Interventions for the Elderly), Look AHEAD (Action Health in Diabetes), MESA (Multi-Ethnic Study of Atherosclerosis), MIDAS (Multicenter Isradipine Diuretic Atherosclerosis Study), PEPI (Postmenopausal Estrogen/Progestin Interventions), PLAC-II (Pravastatin, Lipids and Atherosclerosis in the Carotids), PPT (Polyp Prevention Trial), PREVENT (Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial), SEA (Soy Estrogen Alternative Study), SEARCH (Diabetes in Youth Study), SECORDS (Southeastern Consortium on Racial Differences in Stroke), SPARC (The Study to Prevent Alcohol Related Consequences), SPRINT (Systolic Blood Pressure Intervention Elderly), T1DGC (Type 1 Diabetes Genetic Consortium-International), WHI (Women's Health Initiative), WHIMS (WHI Memory Study) and WHISCA (Women’s Health Initiative Study- Cognitive Aging).

The Division of Public Health Sciences is housed in several locations within Wake Forest Health Sciences. Faculty and staff are located at 525 Vine Street on the 4th floor, the ground floor of the Nutrition Research Center Building, and the first floor of Piedmont Plaza I. We maintain a library of software from all of our studies that enables us to provide timely materials. Our programmers convene regular meetings to discuss methods for improving our performance and for keeping up-to-date with all current technologies.

The Department of Biostatistical Sciences (Walter Ambrosius, PhD, Chair)
In 1986 the Center for Prevention Research and Biometry was established at Wake Forest University Health Sciences and in 1989, the Section of Biostatistics in the Department of Public Health Sciences emerged from this unit. In 2006 the Section became the Department of Biostatistical Sciences in the newly formed Division of Public Health Sciences. The Department has grown steadily to 24 faculty members and 93 staff including

biostatisticians, analyst/programmers, research information systems staff, project managers, data coordinators and support staff.

The faculty and staff in the Department conduct methodological research in survival analysis, sequential analyses, clinical trial design, categorical data analysis, analyses with missing data, measurement/ misclassification errors, multivariate/longitudinal analysis, re-sampling techniques, robust regression, metaanalysis, psychometrics, regression diagnostics, quantitative epidemiology, statistical genetics, image analysis, machine learning, and health service methods. Current institutionally oriented bioinformatics efforts include: genomic databases, quality control tools, novel analysis methods, analysis pipelines, and integration of statistical results from high-density SNP association studies with genomic databases for prioritization of genetic markers. Faculty and staff in DBS collaborate closely with the newly formed Wake Forest Biomedical Informatics Center.

The Department promotes basic, clinical, and epidemiological collaborative research of the highest methodological standards collaborating with investigators on study design, remote or centralized data entry systems, data management, quality assurance, data analysis, development of new statistical methods, sample size calculations, surveys and questionnaire development, publications, and manuscripts reviews. The Department provides scientific programming, data systems design and management, web-based data capture, study coordination systems and networked communications, integrating strict quality control specifications. Faculty within the Department direct the Center for Public Health Genomics and biostatistics cores within the Comprehensive Cancer Center and the J. Paul Sticht Center on Aging. Faculty also teach in the graduate program of the School of Medicine. This combination of experience and collaborative spirit places us as a national leader in the coordination of large multicenter studies.

DBS faculty and staff are currently funded by 159 different and very diverse research projects and collaborate with 20 departments and centers outside of the Division of Public Health Sciences. The total requested awards for pending grants that involve DBS members reached $136 million in 2012 while total FTEs on funded applications has risen to 90. The awards for grants involving DBS members total $55 million in 2011. DBS members co-authored 181 peer-reviewed journal articles and were first or senior authors on 46 publications in 2011.

Department of Epidemiology and Prevention (Alain Bertoni, MD, MPH, Chair)
The Department of Epidemiology and Prevention is dedicated to research and teaching in the etiology and prevention of chronic disease. The ultimate goal of these efforts is to reduce death and disability and improve quality of life. Areas of research by 11 faculty and 69 staff include cancer control, cancer epidemiology, cardiovascular disease epidemiology, diabetes epidemiology, epidemiology of aging, demography of aging, genetic epidemiology, health disparities, health services research and nutrition. There is also a focus on clinical trials methodology; the Department houses both coordinating centers and clinical centers. The faculty has extensive experience with large, multicenter studies and collaborates with clinicians and basic scientists from a variety of other departments in the Medical School, as well as institutions across the US and abroad. Faculty participate in teaching within the school and in the biomedical graduate program. The Department jointly sponsors, with other PHS Departments and the WFU Translational Science Institute, the Master of Science Graduate Program in Clinical and Population Translational Sciences.

Department of Social Sciences and Health Policy (Doug Easterling, PhD, Chair)
The Department of Social Sciences and Health Policy has expertise in health-related quality of life, prevention of substance abuse, medical effectiveness, health care outcomes research, psychosocial factors in health and disease, prevention of adolescent high-risk health behaviors, women's health issues, community interventions, and cost-effectiveness analysis of medical treatments. Areas of research interest of the 16 faculty members and 52 staff include adolescent health, alcohol, tobacco and drug use, community health, community interventions, health services research, health policy, psychometrics, women’s health, cognitive health and aging, medical outcomes and quality of life issues. Members of the Department have headed or participated in multicenter studies of college drinking, underage drinking, CVD, dementia, and hypertension.

Core Computational Informatics Resources:

RISU provides a variety of computational platforms for faculty and staff to perform their research activities. Ranging from interactive Linux systems used for primary statistical and genetic analysis to high performance compute clusters used for distributed parallel processing to Windows-based servers used for database and statistical processing, DPHS provides a host of resources to accomplish the research goals of its investigators.

Core Interactive Systems:

• (2) Linux Servers – IBM BladeCenter nodes with 8 Dual Core Xeon 2.40 GHz CPUs, and 98 GB RAM, each

High Performance Compute Clusters (HPCC)

The challenge of large scale data processing will be addressed by the high-performance computation facilities including DEMON-Isilon (1,358 2.6GHz Intel CPU cores, 4,992 706MHz Nvidia GPU cores, and 190 TB parallel storage space) at WFUHS, and DEAC (140+ Node / 482 core IBM Blade cluster & 60TB parallel storage space) at Wake Forest University. In addition to those large scale resources, we also have the following resources available for use:

• 23 Node / 92 core IBM Blade cluster, 828GB RAM

Central Storage

Each of the systems listed above connects to the centralized storage system. Providing over 130 TB of storage for researchers. Storage platforms include Isilon, EMC, and Compellent.

Software

PHS utilizes many specialized applications to perform statistical genetics research. This following list comprises a subset of these applications:

- SAS
- Splus
- R
- Matlab
- Gene Hunter
- Glogit
- Ghp
- Haploview
- HRT- Haplotype Runs Test

- Loki
- Mendel
- Merlin
- Non-Parametric Linkage Regression Analysis
- OSA- Ordered Subset Analysis
- PDT – Pedigree Disequilibrium Test
- Pedcheck
- PedSys
- PHASE
- Prest
- Solar
- Structure
- Unphased which includes haplotypic PDT and QPDT
- Zaplo/Profiler
- Dandelion- written at (Division of PHS) and performs case/control haplotype analysis using the EM algorithm
- Dprime- written at (Division of PHS) and calculates estimates of D, dprime, delta and r-square using independent individuals
- SNPGWA- written at (Division of PHS) for genome-wide association analysis, performs linkage disequilibrium measures, tests for Hardy-Weinberg Equilibrium, tests for general association, 3 genetic

models (dominant, additive and recessive), tests for lack of fit, performs an allelic test and does 2 and 3 marker haplotype analysis using EM algorithm.

The Division also manages over 40 Windows-based servers for uses including application servers, database servers and file servers. We have created our own clinical trials web platforms encompassing database servers, web hosting servers, and SAS servers for providing real-time access to clinical trial data to study staff.

All servers that are available to the Internet are secured in a DMZ zone, with two hardware firewalls. Local software firewalls are also used on each public server. Public server access is monitored at the hardware firewall location, by an outside monitoring company. The Division also monitors public servers, at the server software location, to help guarantee security. Operating system patches and hotfixes are implemented in a timely fashion, and can be managed remotely in an emergency.

All servers are physically located in one state-of-the-art 5,500 square foot data center in a new building designed for security and reliability. It is located at the new downtown campus of WFSM and provides a central presence for high-end computing (e.g.,computing clusters), data storage facilities, centralized backup, and disaster recovery.. The entire building's electrical needs are backed by a one Megawatt on-site generator. The data center provides a fully monitored environment for data processing equipment for the undergraduate campus of Wake Forest University (WFU), as well as a collocation facility for commercial and public service providers for the Innovations Quarter (downtown research park), ensuring multiple highbandwidth Internet paths and diverse state-of-the-art services.

The networking environment is a fully converged, high speed, multi-use network supporting video streaming, VoIP, video teleconferencing and high-bandwidth display signage in addition to the typical data traffic along with a fully integrated wireless environment across all campuses. WFU is one of the founding members of Internet II and utilizes the research and education network daily with our shared Virginia Tech-WFU School of Biomedical Engineering. WFU also provides the Piedmont region of North Carolina with a Regional Point of Presence (RPoP) called WinstonNet. This robust connection to Internet II and to the North Carolina Research and Education network (NCREN) is capable of 10 Gbps and is positioned to move to the Lambda Rail.

3. Scientific Resources from the Women's Health Initiative

Overview

The proposed application is an extension of the WHI ancillary study#252 (AS252), entitled "*Environmental Determinants of Cognitive Aging in the WHI Memory Study.*" (PI: Chen). Dr. Espeland at the Wake Forest University (WFU) School of Medicine is the Co-PI of WHI AS.252 which was designed to investigate the later-life environmental influence on cognitive aging. The research administration of WHI has been restructured, with its original 39 field centers reduced to several regional networks, each with specific Scientific Interest Groups (SIGs) to lead and coordinate the collective scientific efforts nationwide. One of the new scientific networks is the WHI Southeast Regional Network (WHI-SERN), headed by the WFU. The WHI-SERN is committed to supporting research projects related to the environment ("Environmental SIG") and cognition ("Aging SIG"). Functions of SIG include, stimulating scientific exchange, encouraging productive collaboration within specific topic areas, advancing science by developing innovative scientific papers and ancillary studies, providing opportunities to engage current and new scientists to WHI, and coordinating activities in an area of mutual scientific interest. The proposed research is aligned with the SIGs of the WHI-SERN. As the co-PI of the WHI SERN, Dr. Espeland will continue his close collaboration with Dr. Chen and his USC research team to access the data from the WHIMS Suite of Studies and WHIMS-Y, as well as other elated data resources available to this application.

Multi-disciplinary Research Management

Coordination of all the proposed scientific activities requires multi-center management, data analyses, and multi-disciplinary communication. Drs. Chen, Espeland & He have had extensive experiences in collaborating with multi-disciplinary investigators from institutes based in different geographic regions. Several successful telecommunication tools used in both previous and ongoing projects, such as teleconferencing, videoconferencing, email correspondence, and web-based blogs, will be adapted in the proposed project. Verbal communication between the project PIs with other investigators, either by teleconference or videoconference, will take place on a bi-weekly basis. The investigators from the USC will post pertinent information, presentations, and data analysis to an internal project blog, and coordinate with the subcontract PIs to update the respective progress in the bi-weekly meetings and monthly work-in-progress meetings.

WHI Biological and Other Extant Data Resources

The WHI Biological Data and other (dietary/nutrition/psychosocial factors) extant data resources offer this application the great potential to pursue mechanistic studies/interactions. It is noteworthy that WHIMS participants were well represented in the WHI Genetics and Biomarkers Studies (https://cleo.whi.org/data/Pages/GWAS-Data.aspx)

WHIMS GWAS Data

GWAS data from minorities (African Americans and Hispanics) are already available online through SHARe (SNP Health Association Resource cohort). GWAS data (Illumina Omni Express, estimated completion date: ~8/31/12) from WHIMS participants with EA (European American) ancestries (~n=4660) will be available through the database of Genotypes and Phenotypes (dbGaP), a shared data system to archive and distribute the results of studies that have investigated the interaction of genotype and phenotype. The WHIMS GWAS data are expected to be available soon after QC and imputation is complete.

WHIMS Candidate Genes

SNP and haplotype variations in several candidate genes (including APOE, BDNF, COMT, KIBRA, and SORL1) in WHIMS participants have been characterized in an NIH-funded ancillary study (R00AG032361 AS250 PI: Driscol). Dr. Driscol (University of Wisconsin – Milwaukee) has agreed to allow the USC-based NIEHS T32 (T32ES013678 "*Environmental Genomics*") trainees to access theses data. Manuscript proposals are in progress to combine the multiple air pollutants and other environmental data from WHI AS252 (the parent study of the proposed R01) and the AS250 genetic data to investigate G*E contributions to cognitive aging (global/domain specific), risk of MCI/dementia, and MRI-measured brain ischemia and structural changes.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Jiu-Chiuan Middle Name Last Name*: Chen Suffix: M.D.

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Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: MD,SCD,MPH Degree Year: 1992

Attach Biographical Sketch*: File Name:

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Mark Middle Name A. Last Name*: Espeland Suffix: Ph.D

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County:
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Phone Number*: 336-716-2826 Fax Number: 336-716-6427

E-Mail*: mespelan@wakehealth.edu

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1981

Attach Biographical Sketch*: File Name: Espeland_Biosketch_Chen_final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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State*: CA: California
Province:
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Zip / Postal Code*: 900330000

Phone Number*: (213) 740-1038 Fax Number:

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 2014

Attach Biographical Sketch*: File Name: petkus_biosketch_23may2017.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Collins Middle Name Yuchen Last Name*: Liu Suffix: M.D.

Position/Title*: Assistant Professor
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Zip / Postal Code*: 90089-9405

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: M.D. Degree Year: 2004

Attach Biographical Sketch*: File Name: Liu_NIH_bios_2016_(JC).pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD,MS,BA Degree Year: 2009

Attach Biographical Sketch*: File Name: Nation_Daniel_bio_4.14.17.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 1972

Attach Biographical Sketch*: File Name: Gatz_biosketch_P01_2017.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

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Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Ph.D. Degree Year: 2005

Attach Biographical Sketch*: File Name: biosketch_Millstein_April2017_Neuroimaging.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Steven Middle Name Yong Last Name*: Cen Suffix: Ph.D

Position/Title*: Assistant Professor
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Zip / Postal Code*: 90089-9238

Phone Number*: 323-442-1570 Fax Number:

E-Mail*: cen@usc.edu

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Project Role*: Other (Specify) Other Project Role Category: Biostatistician

Degree Type: Ph.D. Degree Year: 2005

Attach Biographical Sketch*: File Name: STEVEN.CEN_BIOSKETCH_5.23.2017_R01_JC.pdf

Attach Current & Pending Support: File Name:

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OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ❍ Yes ● No

*Agency-Defined Phase III Clinical Trial? ❍ Yes ❍ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ❍ Yes ❍ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

❍ Yes ❍ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

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4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	Introduction_to_Project-1_A1.pdf
Research Plan Section	
2. Specific Aims	Project-1_SAs_(WHIMS)_A1.pdf
3. Research Strategy*	Research_Strategies_P01_Project-1_A1.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	P1_Human_Subjects_0520.pdf
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	P1_Inclusion_of_Women_and_Minorities_0520.pdf
8. Inclusion of Children	P1_Inclusion_of_Children_0520.pdf
Other Research Plan Section	
9. Vertebrate Animals	
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	Wake_Forest_Sub-Contract_Package.pdf
13. Letters of Support	All_Support_Letters_v0301_(1).pdf
14. Resource Sharing Plan(s)	P1_Resource_Sharing_0520.pdf
15. Authentication of Key Biological and/or Chemical Resources	
Appendix	
16. Appendix	

INTRODUCTION TO PROJECT-1

To investigate the contribution of TRAP to the risk, susceptibility and mechanisms of Alzheimer's disease (AD) and related dementias (ADRD) in women, Project-1 builds on two well-characterized, geographically-diverse, prospective cohorts of mid-aged and older women in the WHIMS of Younger Women and WHIMS-MRI, both followed for 20 years. These resources were regarded as "*a great strength*" of the overall PPG. A wide appreciation was given to our outstanding research team (e.g., PL recognized as "*a leader in this area*" plus "*other renowned investigators and collaborators*") and excellent institutional environments. The panel highlighted "*many important strengths*", such as the *"outcome data very comprehensive…includes longitudinal...MRIs,"* using "*spatiotemporal modeling will reduce exposure misclassification*," "*sophisticated analyses, including causal inference testing methods*," and "*excellent integration between study components*." Reviewers also noted some minor weaknesses and possible points of confusions, as parsed below in *italic* and followed by brief summaries of our responses (detailed in Section#). Accordingly, the Sections with submitted changes in *texts (Georgia font)* are labeled as **[***revised***], [***new***]** or otherwise left unchanged in the A1 proposal.

Significance: Reviewers' critiques included **(Rev#1)** "*air pollution steadily declining across US cities*" and **(Rev#2)** "*public health relevance of evaluating historical exposures; …a challenge to detect significant effects (of low levels)*"

➢Retrospective studies like Project-1 provide the critical human data needed for rigorous *ex post* analyses to reaffirm the effectiveness of existing regulatory policies or justify stronger programs for future actions.[1] Despite the overall trend[2] of decreasing $PM_{2.5}$ (which substantially increased the dementia risk in older women[3]), on-road vehicles have contributed to an increasing proportion of PM emissions,[4] and yet whether/how TRAP exposures affect the brain health of urban-dwelling aging Americans remain unclear (section A1).

➢Using the lower levels of exposure estimates (3y average $PM_{2.5}$ before 2005-6), our preliminary analyses (C9.3) showed the promise of high-dimensional neurocomputational approach. The adverse $PM_{2.5}$ effect on WM remained with $PM_{2.5}$ <12μg/m^3 (*C9.3*) We also estimated that the statistical powers are sufficient (C10).

(Rev#1) *on cohort representativeness:* The biracial distribution (Blacks and Whites) in WHIMS was comparable to the US older women in 1990s (*C12.1*)

Outcome Data: *"shifts in some measurement approaches"* **"***power issues related to the analysis of AD and MCI data"*

➢Reviewers likely referred to the shift from in-person to telephone-based cognitive testing across subcohorts in 2008-9 (e.g., WHISCA → WHIMS-ECHO; Table C4.2). These phone-based assessments had great reliability and test validity,[5] and such approaches substantially increased enrollment/retention/data completeness and reduced potential biases.[6] To account for the variation in outcome measure, our statistical analyses will include an additional covariate indicating the different testing approach and subcohort (C8.2).

➢We delete the WHIMS AD/MCI data that were included in A0 proposal (Aim-1b) to test predictive validity of ADNI-derived risk scores. To avoid further confusion, this validation substudy is only described in Core B1.

TRAP Exposures: Rev(#1;#2;#3) asked **"***how time-varying exposure(s)…be developed"* for subjects who moved; **(Rev#1)** was unclear on *"unknown exposure contrasts and trends"* and *"how…exposure…windows be selected"*

➢The prospective design (C5) allowed us to account for time-varying exposure levels due to relocation since enrollment, and we could estimate the residence-specific exposures (weighted by the duration at each location) already used in the published work.[3, 7, 8] See examples of exposure contrasts and trends in Core C1.

➢Our analyses plans will include both long-term (cumulative) and intermediate-term (e.g., 1- to 3-year moving averages) exposures preceding the outcome measures (C9.1; C9.2).

(Rev#1)*"heterogeneity of exposures…not described"* **"***limited discussion on…ultrafine PM fraction of PM… other microenvironments…* [and] *TRAP in 1990s or before" "IMPROVE and other… networks do not emphasize traffic sites."*

➢Standard MESA-AIR models follow a "regionalized" framework combining temporal basis functions with spatially-varying coefficients and spatiotemporal residuals[9] (see Core C1 which the PL directs). This approach captures both regional- and local-scale exposure variability. For this application, we will use the downscaled spatiotemporal model to estimate monthly NO_2 (gaseous surrogate of TRAP) concentrations in 1990-2016. We further noted the limitations to characterize nPM and estimate pre-1999 $PM_{2.5}$ from traffic (*C12.1*).

➢The P01 is focused on ambient exposure from traffic. Exposures from other "microenvironments" are unlikely to confound the observed associations (*C12.1).*

(Rev#2) raised other questions about *"time-varying confounding" "15% addresses geocoded at the zip-code level"* and *"statistical power and interpretations in the event of null findings."*

➢Time-varying confounding was considered in the listed covariates (C8.2) and statistical analyses (C9). We will conduct sensitivity analyses excluding addresses geocoded at the zip-code levels (C5). The estimated statistical powers are sufficient (C10). Since Project-1 is built on a population context found with neurotoxic effects of $PM_{2.5}$,[3, 7, 8] even null findings with TRAP may point to the importance of other PM sources.

(Rev#3)*"mixed results of some prior and similar investigations"* The same conclusion was given in our review on PM and vascular brain injuries, and we also identified two major limitations to be addressed by Project-1 (A2).

Scope of Work/Budget: Need to clarify *"Roles of team members" "potential overlap with ongoing projects at USC"*

➢ Beyond the Personal Statement, the expected role/contribution was noted in the Budget Justification, which also tabulated the comparison of Project-1 (2017-22) vs. other R01s (completed 2011-15; new 2016-2021).

2. Specific Aims [*revised*]

Growing evidence supports that outdoor air pollution is a novel environmental determinant of brain aging.[10-12] In the last two decades, compelling experimental data have documented significant neurotoxicity of ambient air pollutants,[13-16] including traffic-related air pollution (TRAP)[17-23] in urban environments. Although lagging behind neurotoxicological data, recent epidemiologic studies began to show that individuals living in places with higher levels of regional air pollutants (including $PM_{2.5}$: particulate matter <2.5μm;[24-26] and O_3[26, 27]) had cognitive deficits or accelerated cognitive decline.[28-30] Since 2014, *six studies[3, 31-35] reported an increased risk for dementia associated with exposures to regional air pollutants. For instance, in our NIA-funded study (R01AG033078), high regional $PM_{2.5}$ increased the ADRD risk in a nationwide cohort of older women (aged >65) from the Women's Health Initiative Memory Study (WHIMS).*[3] Despite the strong evidence for TRAP-induced neurotoxicity in animal models[18-23] (e.g., Project-3 showed Aβ deposit ↑ with TRAP), human data on **whether and how TRAP exposure affects the aging brain remain limited and inconsistent.** Project-1 will extend the NIA-funded R01 to further address several notable knowledge gaps in the current literature on air pollution-neuroepidemiology of pathological brain aging. (1) We lacked convincing *prospective* cohort data showing ADRD risks increase with individual-level TRAP exposure. *The extant evidence from three published studies was inconclusive, because of the limitations to their exposure characterization that was based on measuring the proximity to major roadways,[35] relying on aggregated exposure estimates[31] prone to ecological biases, or modeling the TRAP exposure after or shortly before dementia ascertainment.[36] (2)* We did not know whether such neurotoxic effects start before late-life (e.g., aged <60). *(3)* No studies examined the putative exposure effects on early biomarkers or neurobiological classification of AD.[37-39] All published studies relied on claims data[31-33] (which have questionable validity [40]) or used clinical criteria[41] to define dementia.[34, 36] *(4)* No studies assessed TRAP effects on early cognitive changes predictive of AD. *(5)* Very few neuroimaging studies elucidated the neuropathology underlying the neurotoxic effects of TRAP on aging brain.

Project-1 builds on two well-characterized and geographically-diverse cohorts of mid-aged and older women in the WHIMS of Younger Women (WHIMS-Y; n=1346, inception age 50-54) and WHIMS-MRI (n=1403, inception age 65-80) followed annually since 1996, both with comparable longitudinal assessments on episodic memory and executive function. We further capitalize one NIEHS-funded R01 which employs sophisticated models developed by the MESA-Air *("*Multi-Ethnic Study of Atherosclerosis and Air Pollution") team to estimate residential TRAP exposure (NO_2; $PM_{2.5}$; sources/components). We take the novel population neuroimaging approach to studying pathological brain aging in communities, drawing on both clinical and neuropathological classification of AD defined in well-characterized samples from the Alzheimer's Disease Neuroimaging Initiative (ADNI) with biomarkers of Aβ and neuronal injuries, plus the Core B-developed tools to predict early risks for AD (overall and neuropathological categories), mild cognitive impairment (MCI), and preclinical AD, based on structural MRI (sMRI) matrices of harmonized measures of grey matter (GM), white matter (WM), and WM hyperintensities (WMH). State-of-the-art mediation and computational analyses will be conducted to examine the TRAP-affected brain structures/neuropathological pathways underlying the early cognitive deficits.

(AIM 1) To assess the TRAP effects on early biomarkers of AD/MCI /preclinical AD risk in older women
The associations between TRAP and sMRI biomarkers of predicted risks for AD/MCI/preclinical AD in WHIMS-MRI will be investigated in multicovariate-adjusted regression models. In WHIMS-MRI cohort, we hypothesize that TRAP exposures increase the risk scores *for clinically- and neuropathological biomarkers-defined MCI/AD*.

(AIM 2) To examine the associations of cognitive decline of early AD with TRAP before/during late life
We will employ the latent curve modeling to test the hypothesis that TRAP adversely influences the decline in episodic memory and executive function, starting with exposures estimated during middle age (aged 50-54 in WHIMS-Y) and continuing into later life (in WHIMS-Y+ WHIMS-MRI).

(AIM 3) To elucidate the brain structure and neuropathology mediating the TRAP effects on brain aging
(Aim 3a) Using structural equation models, we will examine whether the putative adverse effects (Aims 1&2) are mediated through reduced brain surfaces/volumes of GM/WM in vulnerable areas (medial temporal lobe; hippocampal-amygdala complex) or by increasing WM hyperintensities. (Aim 3b) We will perform agnostic computations in high-dimensional space to identify neuroanatomic structures mediating TRAP neurotoxicity.

(AIM 4) To assess the biological susceptibility to neurotoxicity of TRAP exposure
We will examine whether the adverse TRAP effects are stronger in women with APOE4 or greater WMH.

Bringing together experienced USC investigators and collaborating scientists with complementary expertise, Project-1 will greatly advance our understanding of the role of TRAP in contributing to early AD risk, biological susceptibility and mechanisms of AD in late life.

3. Research Strategy

A. Significance

Project-1 is designed to investigate the contribution of traffic-related air pollution (TRAP) to the risk, susceptibility and mechanisms of Alzheimer's disease (AD) and related dementias (ADRD) in a nationwide cohort of women from the Women's Health Initiative (WHI) Memory Studies (WHIMS). AD is the most common form of pathological brain aging affecting elderly. In 2017, one in 10 Americans over age 65 is living with AD. Of these more than 5.5 million people with AD in the US, two-thirds are women. Epidemiologic data indicate that women are 2-3 times as likely as men to be affected by AD over the lifetime,[42-44] and neurobiological experiments also support this differential risk by sex.[45] There is increasing evidence for different risk factors by sex and gender[46-48] not simply attributed to the higher longevity of women versus men.[45] Concerning the increasing ADRD burden as an important women's health issue, many have called for research on identifying environmental risk factors.[42, 49] However, scientific evidence linking environments with ADRD risks remains elusive,[50] and there is a very short list of candidates that are modifiable during late life.

A1. Ambient Air Pollution – a Novel Environmental Determinant of Brain Aging [*revised*]

Growing evidence supports the emerging concept that exposures to ambient air pollutants are a novel environmental determinant of brain aging.[12] In animals with *in vivo* exposures to ozone or particulate matter (PM), numerous neurotoxicological experiments have shown oxidative stress and neuroinflammation and other neurotoxic reactions in multiple brain regions.[51-53] Post-mortem data suggest that poor air quality might accelerate brain aging (e.g., β-amyloid accumulation) in mice[18, 54, 55] and humans,[56] and result in possible neurodegeneration (e.g., neuro-fibrillary tangles) in canines.[57, 58] As a result, there is an increasing concern about the potential impact of ambient air pollution on human brain aging.[59] In the last few years, cross-sectional studies reported relatively low performance in various tests of cognitive functions among adults and older people residing in places with higher levels of ambient air pollutants (including fine PM [$PM_{2.5}$] [24-26] and ozone[26, 27]) or in proximity to major roadways.[60, 61] Adverse PM effects on cognitive declines were also shown in most longitudinal studies.[28-30, 62] Seven studies [3, 31-36] had examined neurodegenerative effects of ambient air pollution, all pointing to a possible increase in ADRD risk. However, there were great uncertainties with these reported associations, due to their methodological limitations. Four studies[31-33, 35] used claims data to determine incident dementia/AD, an approach with questionable validity.[40] Four studies[31-34] were of retrospectiv e design, which was subject to selection biases.[63] *Of the three studies examining TRAP, methodological limitations to their exposure characterization were evident. One study only measured the proximity to major roadways,[35] the second study relied on aggregated exposure estimates[31] prone to ecological biases, or the third study using a spatial model towards the end of study follow-up to estimate the NO_x exposure in earlier years,[36] which obscured the temporality of the reported association.*

Supported by the NIA R01 (AG033078), **we conducted the first US-based prospective cohort study linking individual-level air pollution exposure estimates with increased ADRD risk ascertained by validated protocols.** We found that regional $PM_{2.5}$ exposure increased the risk for all-cause dementia a nationwide cohort of older women from WHIMS. [3] *Our preliminary data (section **C9.1**) also showed that high $PM_{2.5}$ exposure (exceeding the US EPA standard of 3-year average >12 μg/m³) in 1999-2010 increased the clinical AD risk by 71%.*

Despite the growing epidemiologic evidence for the association between ambient air pollution and pathological brain aging, we identified several notable limitations to these published studies. First, there were no convincing prospective cohort data showing ADRD risks increase with individual-level TRAP exposure during the biologically-relevant period, despite the strong evidence for neurotoxicity of TRAP in animal models [18-23] (e.g., increased Aβ deposit shown in Project-3). Second, previous studied failed to provide any insights for air pollution exposure effects on earlier biomarkers or neurobiological classification of AD.[37-39] All published studies relied on claims data[31-33] (which have questionable validity [40]) or used clinical criteria (e.g., the DSM-IV[41]) to define dementia.[34, 36] Third, no studies had examined TRAP effects on early cognitive changes predictive of AD. Of the five published longitudinal studies on ambient air pollution and cognitive decline,[3, 28-30, 62, 64] only one included TRAP exposure data with non-significant associations.[29] Fourth, previously studies included mostly older adults, with no data on whether/how TRAP before late life (e.g., aged < 55) affects AD risk. Project -1 will address these limitations, and the expected results will elucidate whether and how TRAP exposure ***affects the early biomarkers of AD and*** **neuropsychological processes predisposing the affected individuals to increased risks for preclinical AD, MCI and AD,** according to our current knowledge of the pathogenesis and clinical/neurobiological classification of AD.

A2. Mechanistic Mediators Linking Air Pollution Exposures with Pathological Brain Aging [*revised*]
Only limited epidemiologic data are available to inform the mechanistic mediators underlying the neurotoxic effects of ambient air pollutants on brain aging. **No previous studies had conducted mediation analyses linking brain atrophy or vascular brain injuries (VBI) with cognitive deficits predictive of AD.** For this application (Aim 3a), we hypothesize that the adverse effects of TRAP on cognitive impairment are mediated through multiple pathways, including structural neurotoxicity to the medial temporal lobe and frontostriatal system and increased VBI. The medial temporal lobe-memory system [65] and the frontostriatal-executive system [66] are two vulnerable neural networks affected in both brain aging and neurodegenerative disease.[67] **The neurotoxic effects of air pollutants on these vulnerable neural systems had not been examined using longitudinal MRI data.** Cortical thickness represents a better morphometric phenotype,[68] which so far had not been examined in the limited literature on air pollution and brain aging. The existing WHIMS-MRI protocols were based on volumetric measures, and did not allow fine-scale GM and WM segmentation in the medial temporal lobe and frontostriatal systems, which will be provided to Project-1 through the Neuroimaging Data Support Core B1. We and others had conducted ROI-based analyses,[7, 8, 69] but found little evidence that $PM_{2.5}$ in late life exposure was associated with hippocampal volume in the elderly. These null findings should be better interpreted with the understanding of exposure characteristics and population context of the studied cohorts.[7, 8] Because both studies relied on air pollution exposure models for $PM_{2.5}$ estimation after 1999, we cannot exclude the possibility that reduced hippocampal volume might be associated with higher exposures in 1990s or in older adults exposed TRAP, which will be investigated in Project-1. In the WHIMS-MRI cohort, we also discovered the novel association between $PM_{2.5}$ and small brain volume specifically in normal-appearing WM (of frontal and temporal lobes).[7, 8] *This novel neurotoxicity on WM was recently substantiated in experimental models exposed to small particles from regional background[70] or TRAP.[71]* Using the voxel-based morphometry method,[72] we further showed the associations between $PM_{2.5}$ exposure and patterns of smaller GM volumes primarily in the dorsolateral and medial prefrontal cortex, regions associated with higher cognitive function such as working memory, episodic memory retrieval, and executive function. *This observed adverse effect of $PM_{2.5}$ on smaller GM volumes was consistent with particle-induced synaptic neurotoxicity with decreased neurites in both wildtype[23] or EFAD mice [3]exposed to TRAP.*

The hypothesized VBI underlying air pollution-brain aging is receiving great attention,[59] as extensive literature has documented that ambient air pollutants increase the risks of coronary heart disease and stroke [73-75] and elevate blood pressure,[76-78] all contributing to cognitive decline and increased dementia risk.[79-85] However, previous studies that examined VBI associated with PM exposures had yielded mixed results.[8, 69, 86-89] One earlier small study in Boston showed that $PM_{2.5}$ was associated with diminished cerebral blood flow of the elderly.[86] Two studies reported inconsistent associations between PM exposure and white matter hyperintensities (WMH) vs. silent cerebral infarcts.[90, 91] In the WHIMS-MRI cohort, we also found regional $PM_{2.5}$ exposure was not associated with the volume of small-vessel-ischemic-disease in older women.[8] Although several large cohort studies pointed to the role of TRAP in increased stroke risk and mortality,[75, 92, 93] the effect of TRAP on VBI remained unclear. *Importantly, none of these previous studies examined the putative adverse TRAP effects on VBI using longitudinal MRI data and their assessment was limited to the global measures of VBI disregarding the possibly different neuropathological correlates of WMHs present in periventricular space and deep subcortical area. These limitations will be addressed in Project-1.*

A3. Biological Susceptibility to Neurotoxicity of TRAP Exposure [*revised*]
APOE is the most important known genetic risk factor for late-onset AD, with the APOE4 allele conferring dramatically increased risk in a dose-dependent manner and more profound impact on women.[94] Although it was suggested that studying environmental factors should account for APOE,[95] recent studies (including our new publication[3]) suggest air pollution on brain aging was not confounded by APOE.[34, 36, 96] APOE4 status imparts an increased AD risk by both Aβ-dependent[97-99] and Aβ-independent mechanisms (e.g., altered cholesterol metabolism; mitochondrial dysfunction; blood-brain barrier breakdown)[100-102] that are all relevant to the reported toxic effects of exposure to airborne particles, supporting the hypothesized interaction with TRAP. *For regional $PM_{2.5}$, we also observed much stronger adverse effects on clinically significant cognitive decline and increased dementia risk among older women with APOE4.*[3]
Cerebrovascular injuries may promote the exposure-induced neurodegeneration (Overall Program; Fig.1). Under this framework, we posit that adverse TRAP effects stronger in women with greater WM hyperintensities. This hypothesis was supported by our previous observation of greater WM loss associated with $PM_{2.5}$ among older women with cardiovascular disease (including stroke).[8]

B. Innovation

The following features make this an innovative application for studying environmental risk for dementia/AD.

(1) The proposed Aim 1 likely represents the first major attempt to assess the impact of TRAP on AD risk, susceptibility and mechanism, by drawing on state-of-the-sciences to better understand the pathogenesis and clinical/neurobiological categories of AD associated with air pollution exposure in women.
(2) The design of Project-1 is highly cost-efficient, as it expands the use of several NIA-funded cohorts with comprehensive outcome data (annual neuropsychological assessments; longitudinal sMRI data; well-validated clinical ascertainment of MCI and AD) and further leverages one NIEHS-funded R01 which employs state-of-the-art spatiotemporal modeling to assess TRAP exposure in WHIMS.
(3) Project-1 benefits from the population neuroimaging approach taken by the Neuroimaging Data Support subcore (Core B1). This approach integrates the new knowledge from clinical neurosciences to better characterize the AD phenotype classification in community-based epidemiologic studies. Using the ADNI database provides us a sensitive, empirically derived, continuous measures of early biomarker for AD risk based on a well-characterized brain imaging database of cognitively normal subjects and AD patients.
(4) Because of increased vulnerability during the postmenopausal period,[103, 104] the WHIMS-Y cohort (Aim 2) offers a unique opportunity to investigate/compare the exposure effects before and during late life.
(5) The proposed Aim 3 analyses for mechanistic mediation involving complex brain structures are innovative. The novel application (Aim 3a) of structural equation models (SEMs) offers several analytical advantages (e.g., accounting for measurement errors in characterizing latent biological intermediates/phenotypes; accommodating parsimonious models for multivariate outcome variables) to draw causal inference in ways not readily attainable by using conventional regression models.[105] The high-dimensional mediation analyses (Aim 3b) coupled with causal inference testing methods offer a powerful approach to systematically and agnostically examining the specific brain structures mediating the TRAP neurotoxicity on brain aging.

C. Approaches

C1. Overview: This application aims to: (1) determine the impact of TRAP on early biomarkers predictive of increased risks for AD, MCI, and preclinical AD in older women; (2) examine the associations of cognitive decline reflecting early AD with TRAP exposure before/during late life; (3) elucidate the brain structure and neuropathology mediating the TRAP effects on pathological brain aging, using both targeted and agnostic approached with high-dimensional neurocomputation; and (4) evaluate the contribution of APOE4 and vascular brain injuries to the individual susceptibility to neurotoxic effects of TRAP exposure from 1990-2016. To address these aims in a cost-efficient manner, Project-1 is built on a well-characterized, geographically-diverse cohort of mid-aged and older women in the WHIMS of Younger Women (WHIMS-Y; n=1346, inception age 50-54) and WHIMS-MRI (n=1403, inception age 65-80) followed annually since 1996, both with comparable longitudinal assessments of neuropsychological functions. This project further leverages the NIEHS-funded ancillary study to WHI (R01ES025888; PIs: Chen & Kaufman) which employ the sophisticated spatiotemporal models developed by the MESA-Air *("Multi-Ethnic Study of Atherosclerosis and Air Pollution"*) team to estimate residential exposure to TRAP (NO_2; $PM_{2.5}$; sources/components). We capitalize the data support from Neuroimaging Core B, which takes the novel population neuroimaging approach to studying pathological brain aging in communities, drawing on the clinical and neuropathological classification of AD defined in well-characterized samples from the Alzheimer's Disease Neuroimaging Initiative (ADNI) with biomarkers of Aβ and neuronal injuries. Given the structural MRI (sMRI) matrices developed by Core B, we will examine whethwe TRAP exposure is associated with these biomarkers for increased early risks of AD (overall and neuropathological categories), MCI (overall and neuropathological categories), and preclinical AD in WHIMS-MRI, using multivariable-adjusted linear regression models (Aim 1), accounting for differential drop-out. Project-1 will provide the WHIMS data on incident MCI/ADRD (>180 cases in WHIMS-MRI by 2016, centrally-adjudicated) for the Core B1 team to prospectively evaluate the performance of ADNI-derived risk scores in predicting the clinical phenotype. Based on annual assessment of episodic memory and executive function, the putative adverse effects on the individual trajectories of early cognitive endophenotypes of AD (Aim 2) will be examined in latent curve models with TRAP exposures estimated starting at age of 50-54 (in WHIMS-Y) and continuing into later life (in both WHIMS and WHIMS-Y). Using a targeted approach with the Core B-harmonized measures of GM, normal-appearing WM, and WMH, we will employ structural equation models to test the hypothesized mediation involving the latent construct of medial temporal lobe and frontostriatal systems (Aim 3a). We will also conduct agnostic high-dimensional whole-brain mediation analyses (Aim 3b) coupled with causal inference testing methods to systematically examine the specific brain structures mediatin g the TRAP neurotoxic effects on brain aging. Multivariable-adjusted analyses with statistical

interactions using linear regression and Cox models and latent curve models will be performed to assess whether the susceptibility to neurotoxicity of TRAP increases in subpopulations with APOE4 alleles and greater VBI (Aim 4).

C2. Selection of Study Population

The following facts support Project-1's rationale for selecting WHIMS: (1) WHIMS-MRI is one of the largest community-based, prospective cohorts with both longitudinal sMRI and well-validated clinical data on ADRD; (2) longitudinal data with annual assessment of episodic memory and executive function are available in both WHIMS-MRI and WHIMS-Y (Table C4.2), thus enabling a very cost-efficient design to examine whether the TRAP effects on the early cognitive endophenotypes of AD start during late mid-life (aged 50-54 in WHIMS-Y) and continue into later life (aged ≥65 in WHIMS+WHIMS-Y); (3) women are more susceptible to the APOE4-associated brain aging including elevated AD risk and older women appear to be more sensitive to the adverse vascular effects of particulate air pollutants;[106-110] and (4) the geographic diversity of WHI provides the optimal environmental context with a wide range of exposures for better statistical power and boarder generalizability.

C3. Study Design and Population

We propose a prospective cohort design that includes all participants in the WHIMS-MRI (n=1403, inception age 65-80) and the WHIMS of Younger Women (WHIMS-Y; n=1346, inception age 50-54), followed annually since 1996, both with comparable longitudinal assessments on episodic memory and executive function (see C4.2). The WHIMS-MRI, including MRI-1 (2005-6)[111, 112] and MRI-2 (2010-11),[113] is a structural neuroimaging follow-up study of older women participating in the WHIMS main trials (1996-2005). [114-117] Both WHIMS (N=7479, aged 65-80 years) and WHIMS-Y (N=1346, aged 50-54 years)[118, 119] were ancillary studies to the WHI trial in 40 clinical centers, which recruited community-dwelling post-menopausal and older women from 48 states (plus Washington, DC) [120] in 1996-9 and all free of dementia, according to standardized study protocols involving neuropsychological testing, clinical evaluation, and central adjudication. Detailed information about the WHIMS Suite of Studies, including study design, population characteristics, recruitment methods, measurement protocols, and follow-up schedule has been published.[111-114, 117, 121-125] Briefly, the WHIMS main trials and WHIMS-Y were conducted in 38 of the 40 WHI clinical centers and enrolled 4,532 (in WHIMS-MRI) +814 women from the WHI Estrogen plus Progestin (E+P) trial and 2,947 (in WHIMS-MRI)+512 women from the WHI Estrogen-Alone (E-alone) trial. The WHIMS-MRI-1&2 was conducted in 14 of 38 active WHIMS sites selected to maximize both geographic and racial/ethnic diversities. As of March 2016, we had 1403 women's MRI-1 data certified for analyses, and both MRI-1 and MRI-2 data obtained by the same imaging protocols were also certified in 730 women. Approximately 4.7 years following the WHIMS MRI-1, MRI-2 will allow us to assess the brain structural change and progression of neuropathology. Table C3 presented the sociodemographic features and common clinical attributes of this joint sample included in Project-1.

Table C3: WHIMS-MRI + WHIMSY Population Characteristics

Race/ Ethnicity	African-American	Hispanic	NH-White[a]	Other or Unknown	Total
	231	79	2349	80	2739
	(8.4%)	(2.9%)	(85.8%)	(2.9%)	(100%)
Education	**≤High school**		**>High school**	**Missing**	**Total**
	601		2125	18	2744
	(21.9%)		(77.4%)	(<1%)	(100%)
Clinical Attributes with Prior Medical Histories	**Depression**	**HTN[b]**	**DM[c]**	**CVD[d]**	
	91	784	97	287	
	(6.1%)	(52.9%)	(6.5%)	(19.4%)	
	BMI: <25	**BMI: 25-29**	**BMI: ≥30**	**Missing**	**Total**
	832	938	958	21	2749
	(30.3%)	(34.1%)	(34.8%)	(<1%)	(100%)

[a]NH: Non-Hispanic; [b]HTN: hypertension; [c]DM: diabetes; CVD: cardiovascular disease

C4. Outcome Data in the WHIMS-MRI and WHIMS-Y

The ongoing follow-up of WHIMS-MRI+WHIMS-Y cohort provides comprehensive data on clinical phenotypes (*C4.1*), neurocognitive functions (*C4.2*), and structural brain MRI (*C4.3*).

***C4.1 Data on Incident Dementia and Dementia Subtypes* [*revised*]**

Project-1 will provide to Core B1 with the WHIMS data on incident MCI/ADRD (>180 cases in WHIMS-MRI by 2016, centrally-adjudicated) to prospectively evaluate the performance of ADNI-derived risk scores in predicting the clinical phenotype. A detailed description and validity of the standardized outcome measurements used to ascertain incident MCI/ADRD for WHIMS+WHIMS-Y has been published.[116, 117] In brief, the standardized protocols consisted of annual screening of global cognitive function, neuropsychological and functional assessment, and collection of clinical data to rule out possible reversible causes of cognitive impairment, all concluded with central adjudication for final classification of normal, mild cognitive impairment, or dementia (vs. non-demented) with clinical phenotypes of possible etiologies (AD; vascular; and other subtypes). For annual screening, centrally-trained/-certified and masked interviewers administered the Modified Mini-Mental State (3MS) Examination[126] during each clinic visit. Women who screened positive

according to age-/education-adjusted 3MS cut-points proceeded to extensive neuropsychological testing (including the Consortium to Establish a Registry for AD battery) [127] and behavioral symptoms/function assessment. These participants subsequently received a detailed neurological examination and neuropsychiatric evaluation by board-certified physicians with experience in diagnosing dementia. Each woman then suspected of dementia underwent cranial CAT scan and a series of laboratory tests to rule out possible reversible causes of cognitive decline and dementia. Beginning in 2008-9 for both WHIMS-ECHO and WHIMS-Y, annual screening was conducted by telephone using the Telephone Interview for Cognitive Status-modified (TICSm),[128] which was highly correlated (0.89) with 3MS in previous studies. For women screened positive (i.e., TICSm<31), a reliable and pre-identified informant was interviewed by telephone using the standardized Dementia Questionnaire (DQ)[129] to assess the history of cognitive and behavioral changes, functional impairments, and health events that could have affected cognitive functioning (e.g., stroke). For participants no longer able to attend the clinic- or telephone-based assessments (e.g., becoming dependent or death), the WHIMS investigators developed Supplemental Case Ascertainment Protocol (SCAP), which consisted of DQ and was shown to reduce undercounting of clinic-based cases and better characterize the risk factor relationships.[130] Data from all longitudinal assessments (screening; behavioral/functional; clinical; SCAP; DQ) and relevant supplemental information from the WHI (e.g., cardiovascular events) were then submitted to the central adjudication committee (of 3 board-certified neurologists/ geriatric psychiatrists with extensive experience in diagnosing dementias) for final outcome classification, based on the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*), which had been found to have substantial inter-rater reliability and good validity for AD (compared with post-mortem confirmation).[131] Following the accepted criteria [117] at WHIMS baseline, MCI was defined as poor performance (≤10th percentile in CERAD norms) on at least one CERAD test, evidence of functional impairment (but not severe enough to interfere with activities of daily living), and absence of psychiatric or other medical disorders (including probable dementia) that could explain the cognitive impairment. The DQ interview of proxies was validated against the 'gold standard' of a clinical evaluation with sensitivities and specificities >90% and inter-rater (face to face vs. phone) agreement of >94%.[129, 132, 133] Others using telephone-based assessment and DQ plus prior clinical/neuropsychological data also report high diagnostic validity to identify dementia cases in ethnically-diverse populations.[134] Among the WHIMS-MRI participants, we expect >180 cases of MCI/ADRD cases will be identified by December 2016 (including 152 with MCI not yet progressed to ADRD, as of December 2015).

C4.2 Longitudinal Data on Cognitive Endophenotypes

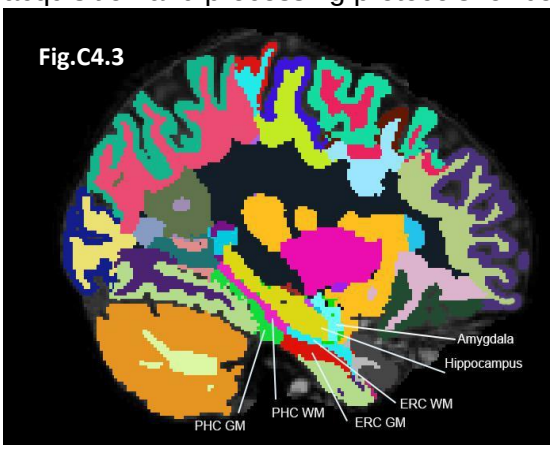
Aim 2 focused on episodic memory and executive functions, because functional declines in these domains represent the hallmark of early AD.[135-139] In the WHIMS Suite of Studies, these domains were assessed by well-validated instruments. The *episodic verbal memory* was assessed by the California Verbal Learning Test (CVLT)[140] and East Boston Memory Test (EBMT),[141] whereas the *executive functions* (including attention/working memory) were assessed by the Digit Span (DigSP) [142] and Oral Trail Making Test (OTMT)[5]–*attention (Part A) and executive function (Part B).* For the WHIMS-MRI cohort (n=1403), the CVLT+DigSP data (average#~6 repeated measures) were available in 930 subjects enrolled in the WHI Study on Cognitive Aging (WHISCA, 1999-2010). These WHISCA data (N=2302, aged 66 to 84 years) were collected by an *annual battery* during clinic visit. Detailed testing procedures of the WHISCA assessment,[143-147] scoring scheme, and quality assurance had been reported elsewhere.[124, 125, 148-151] After the extension of WHIMS ended in 2008, 896 WHIMS-MRI subjects participated in the WHIMS Epidemiology of Cognitive Health Outcomes (WHIMS-ECHO; n=2917, 2008-*onward*), which was initiated concurrently with WHIMS-Y to continue the annual neuropsychological assessment (including CVLT, DigSP, EBMT, and OTMT), based on the telephone-based cognitive batteries. We had reported the great reliability and test validity of these assessments,[5] and also showed that using these batteries substantially increased enrollment/retention/data completeness, thereby improving study validity.[6] These unique data will allow us to examine the putative adverse effects on these early neuropsychological processes associated with air pollution exposures estimated at age of 50-54 (in WHIMS-Y) and whether such exposure effects continue into later life in WHIMS-MRI (aged 66-84 years with WHISCA data and aged 75-93 years with WHIMS-ECHO data) and WHIMS-Y (aged 62-74).

Table C4.2

Cohorts/Subcohorts	Episodic Memory	Executive Functions
WHIMS (N=7479), 1996-2016 (aged 65-80 at Y0)		
WHISCA (n=2302), 1999-2010	CVLT	DigSP
n=930		
WHIMS-MRI (n=1403)		
n=896 WHIMS-ECHO (n=2917), 2008-2016	CVLT+EBMT (2008-2016)	DigSP+OTMT (2008-2016)
WHIMS-Y (N=1346), 1996-2016 (aged 50-54 at Y0)	CVLT+EBMT (2008-2016)	DigSP+OTMT (2008-2016)

C4.3 Brain Structure Data from WHIMS-MRI

The WHIMS MRI QC Center[152-154] (Dept. of Radiology, University of Pennsylvania) developed 1.5T scan acquisition and processing protocols for both WHIMS MRI-1&-2. Standard T1-, T2-, proton density-weighted, and FLAIR scans were acquired. Briefly, the scans were obtained with a field of view=22 cm and a matrix of 256×256. Included were oblique axial spin density/T2-weighted spin echo (TR:3200 ms, TE=30/120 ms, slice thickness=3 mm), fluid-attenuated inversion recovery (FLAIR) T2-weighted spin echo (TR=8000 ms, TI=2000 ms, TE=100 ms, slice thickness=3 mm), and oblique axial three-dimensional T1-weighted gradient echo (flip angle=30 degrees, TR=21 ms, TE=8 ms, slice thickness=1.5 mm) images from the vertex to the skull base parallel to the anterior commissure–posterior commissure (AC-PC) plane. Following the optimized sMRI protocols developed in ANDI to reduce variability across scanners produced by different manufacturers, the Core B1 Data Support will perform post-acquisition corrections to minimize noise in the images, as well as monitoring protocols based on a standardized phantom to detect system errors.[155, 156] To harmonize the sMRI measures between WHIMS and ANDI, these imaging data will be automatically processed with *FreeSurfer* (including the longitudinal stream[157]). In addition to the standard GM segmentation, automated WM parcellation[158] in FreeSurfer will be performed to derive the subcortical WM volumes in MTL. Dr. Thompson (Core B Director) and other USC colleagues have recently developed the EPIC (Evolving Partitions to Improve Connectomics)[159] method and apply this novel analytic framework to evaluate the brain connectivity represented by a cortical segmentation for an in a support vector machine (SVM)-based classifier of AD vs. controls. In brief, given the reprocessed sMRI data, the EPIC analyses combined the dilated cortical segmentation intersecting with the WM fibers to generate the whole-brain structural connectivity matrices. The representation of these complex sMRI networks was partitioned and mathematically refined to estimate the optimal parcellation differentiating AD vs. controls. Dimensionality of the resulting brain structural networks was reduced by principal components analysis (PCA). A support vector machine (SVM)-based supervised learning method was employed to optimize the classier.



For this application, as noted in the Program Summary (Table C), Project-1 will contribute to testing the several hypothesized mechanistic mediation pathways (e.g., MTL-memory systems; front-striatal executive systems) by conducting the targeted region of interest (ROI) analyses (see section ***C9.3***; Aim 3a), with each defined brain area assessed for GM+WM, and separately for GM and WM alone. For MTL-WM, we will focus on the entorhinal cortex (ERC WM) and parahippocampal gyri (PHC WM) (Fig.C4.3). We will use the ADNI-defined signatures of cortical thinning that best differentiating AD from cognitive normal controls, to examine the hypothesized GM thinning associated with TRAP. The extant ADNI protocols[160] for automated WMH detection will be used to derive the WMH volume, which represents the vascular brain injuries for the hypothesized mediation effect on declining episodic memory and executive functions. The harmonized whole-brain measures, including vertex-wise sMRI measures of normal-appearing structures include cortical thickness, surface area, GM volume, and WM volumes, will be submitted for the high-dimensional analyses (see section ***C9.3***; Aim 3b).

C5. Geocoded Residential Information

Geocoded information of participants' addresses will be used to define the residential locations where air pollution exposures were estimated. Because address data were collected prospectively with annual updates starting approximately three years before WHI inception and continuing during follow-up, we will be able to account for the varying exposure levels due to relocation. The geocoded location data (longitudes and latitudes) in 1993–2016 will be made available through (R01AG033078; R01ES025888) at no additional cost. Project-1 benefits from continuing efforts by WHI investigators to provide high-quality geocoded data, following the same standardized procedures,[161-163] with very high matching reported (e.g., 99.8% in the 2012 update of WHI address records; 85.8% by street+14% by zipcode centroid). Detailed results of reliability and validation study with high level of accuracy in geocoding of WHI addresses have been published.[161, 164] *For the statistical analyses (C9), we will conduct sensitivity analyses excluding addresses geocoded at the zip-code levels.*

C6. Exposure Assessment

Given the geocoded data (section C5) of all home addresses in 1993-2016, we will conduct the individual-level, residence-specific estimation for ambient NO_2 (a gaseous surrogate), elemental carbon (EC) component of

$PM_{2.5}$ (a marker of diesel exhaust particle),[165-168] and predicted source profiles of $PM_{2.5}$, based on the methodological approaches to be harmonized across Projects 1& 2, through the Environmental Data Support Core C1. Dr. Chen (Project-1 PI) will lead this effort, through a subcontract with U. Washington where Dr. Joel Kaufman (subcontract PI) leads the MESA-AIR (Multi-Ethnic Study of Atherosclerosis and Air Pollution) team. Core C1 will support the proposed extension/implementation of a suite of MESA-AIR spatiotemporal modeling tools (see Core C1 for details). In brief, the MESA-AIR approach represents state-of-the-art in modeling air pollution exposures for large cohort studies, as it incorporates the methodological advances and environmental data resources developed from the EPA's long-term investment in MESA AIR (2006-onward).[169] The MES-AIR modeling framework integrates measurements from the U.S. EPA Air Quality System[170] with comprehensive (n>*800*) spatiotemporal covariates assembled in the MESA Air Exposure Assessment Core Database.[171] The MESA-AIR approach models pollutant concentrations as a linear combination of temporal basis functions with spatially varying coefficients and spatiotemporal residuals. The resulting exposure estimates therefore capture both regional- and local-scale variability.[172] In the context of WHI, Chen and Kaufman have been working on the R01ES025888 (2016-21) which supports the downscaling of yearly MESA-AIR models to estimate monthly outdoor concentrations between 1990 and 2016 for NO_2 and between 1999 and 2016 for $PM_{2.5}$ on the national-scale. Although downscaling of spatiotemporal models is very computationally intensive, we have previously shown that exposure estimation processes starting with shorter time-scales followed by temporal aggregation is more preferable than directly modeling yearly exposure.[173] Figure C6 depicted the preliminary results of a downscaled **MESA-AIR model of monthly $PM_{2.5}$ in one climatic zone (the Southeastern US) in 1999-2013, with the model performance via 10-fold cross validation showing a high degree of estimation accuracy (R_{CV}^2 = 0.73).** Also available to this PO1 is a new spatiotemporal model of **historical annual $PM_{2.5}$ concentrations in the continental US from 1980-1998**. These prediction model performed well when validated using IMPROVE Network and USC-based Children's Health Study data (**R^2=0.84–0.91**).[174] Validated national spatial exposure models[175] will also be implemented to estimate annual average concentrations of major $PM_{2.5}$ components, focused on EC (a marker of diesel exhaust particle). Recent advancement includes novel approaches to characterizing PM composition (and hence sources). For instance, MESA-AIR team recently developed new statistical methods (the "predictive k-means and predictive sparse principal component analyses") to identify profiles of $PM_{2.5}$ composition from the EPA's Chemical Speciation Network (see details in Core C1). This novel approach, already successfully implemented to predict profile labels at the residential locations of the nationwide cohort of Sister Study,[176] will be available to this P01 program to separate the TRAP-$PM_{2.5}$ from other identified sources.

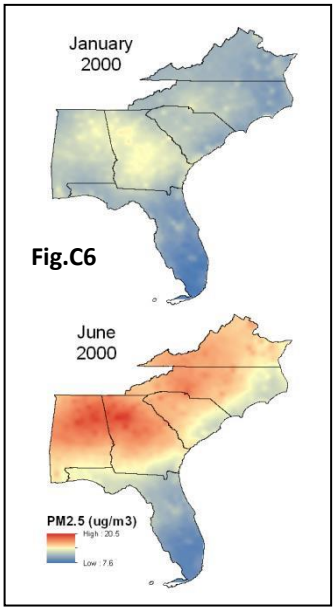


Fig.C6

C7. APOE Alleles

The proposed Aim 4 analyses will be conducted on WHIMS-MRI subjects (n=952) with APOE genotype data (ε4 carriers=~20%, comparable to the prevalence in a national representative sample[177, 178]). These data came from a WHI core study (W63) resource derived from the genome-wide association study sample, which included WHI participants with European ancestry (primarily non-Hispanic whites). APOE genotypes of 4504 WHIMS participants were assigned, based on rs429358 and rs7412 genotype results from imputation and harmonization. The imputation was conducted using the 1000 Genomes Project reference panel and the MaCH algorithm as implemented in Minimac (R^2 = 0.98 for each SNP in the study population).[179] Although these data were missing in a subset of WHIMS-MRI, we did not find evidence that APOE4 alleles confound the adverse $PM_{2.5}$ effects on brain volumes[8, 72] and cognitive impairment[3] (see section *C9.1*).

C8. Other Pertinent Covariate Data [*revised*]

The comprehensive WHI covariates data offer the unique opportunity to assess the potential confounding and other sources of biases. To adjust for/assess the potential confounding, we took a more conservative approach which accounts for both prior knowledge and empirical data to select two sets of covariates.[180] First, we will use the directed acyclic graph (DAG) [181] to identify a list of important ***confounders*** *a priori* (section *C.8.1*) for the putative associations. These covariates have established causal relationships with cognitive impairments, *and* they often determine where the people live (and thus the estimated exposure levels). These DAG-identified confounders will be included in the fully adjusted analyses. Second, we used the change-in-estimate (≥10%) as the analytic criteria for evaluating the ***potential confounding*** introduced by other covariates (section *C.8.2*), i.e., those personal factors or environmental characteristics that are likely predictive

of cognitive decline and dementia/AD risk in WHIMS+WHIMS-Y participants, but they may not confound the associations unless these covariates also empirically correlate with the estimated exposures.

***C8.1 Covariate Data for Confounders*:** These variables include: (1) sociodemographics: age and race/ethnicity; (2) individual socioeconomic position (SES): education, family income, employment status, occupation (Managerial/Professional, Technical/Sales/ Administrative, Service, Operators/Fabricators/ Laborers, Homemaker only); and (3) lifestyle factors: smoking, physical activities, and alcohol consumption.

***C8.2 Other Covariates*:** These include: (1) assigned intervention (E+P or E-Alone; Placebos); (2) prior medications: hormone replacement (never vs. past vs. current hormone user), statin, aspirin, hypnotics/ sedatives and anti-depressants; (3) physical attributes: body mass index (BMI) and blood pressure; (4) medical conditions: histories of CVD, stroke, DM, hypertension, hypercholesterolemia and depression; (5) other comorbidities (e.g., Parkinson's; lung, liver and kidney diseases; cancers); (6) psychosocial factors: stressful life events (as measures in the Alameda County Study); [182] and social support (9-item measure from Medical Outcomes Study social support questionnaire);[183] (7) urban/rural categorization for the residential neighborhoods (per U.S. census 1990 and 2000 Urban/Rural Classification); (8) census tract-level contextual socioeconomic characteristics of residential neighborhoods defined for the WHI cohort;[184-186] (9) meteorological variables (including averages of dew point and temperature), using distance-weighted k-nearest-neighbor average within 50 km buffers based on daily data from the National Climatic Data Center Network;[187] (10) censoring status during follow-up (mortality; incident CVD); *and (11) indicators for subcohort membership and cognitive testing methods (clinics- vs. phone-based). Possibility of time-varying confounding by these covariates (except for those in [1] and [2]) will be evaluated in the statistical analyses (section C9).*

C9. Specific Analytic Approaches to the Proposed Aims [*revised*]

***C9.1 (Aim 1) To assess the TRAP effects on early biomarkers of AD/MCI /preclinical AD risk* [*revised*]**

We will construct hierarchical/multilevel models[188] for continuous outcome data (sMRI-derived risk scores), adjusting for relevant covariates (section C8). The exposure variables *will include both* long-term cumulative estimates *determined at baseline* preceding *the outcome measures (e.g., NO_2 exposure in 1993-2005/2006 before MRI-1)* and time-varying exposures estimated at each follow-up time (e.g.,3-y moving average *preceding MRI-1 and -2*), adjusting for potential confounding by time-varying or time-independent covariates (section C8).

***Sensitivity Analyses* [*new*]**: *Study participation and/or longitudinal dropout may depend on cognitive function at WHIMS baseline[189] or brain structure at WHIMS MRI-1. Air pollution exposures increase overall mortalities and risks of chronic diseases including CVD[74]and dementia.[3] These together raise the legitimate concern with the possibility of selection biases, which may result from selective enrollment or differential loss to follow-up. We will plan to use pattern-mixture and propensity scores approaches [190, 191] to examine the sensitivity of our results to missing data or differential dropout. It was interesting to note that our previous analyses in WHIMS using propensity scores showed very little impact of missing data on the HT results.[154] Our team members [192]are also familiar with the inverse probability-weighted estimation [193, 194] estimation which could be applied to correct such biases.*

***Preliminary results* [*revised*]**: *Supported by the completed R01AG033078, we recently* showed that elevated $PM_{2.5}$ (3-year average >12 μg/m^3) was associated with 1.65-fold higher risk of accelerated cognitive decline and 1.92-fold increased risk of dementia, with potentially greater impact in ε4 carriers in older women (Fig.C9.1). We employed a conventional spatiotemporal modeling approach[173, 195] to estimating $PM_{2.5}$ and examined its adverse effects using Cox models to estimate the hazard ratios (HRs; 95% confidence intervals) for *clinically-significant* global cognitive decline (>8-point loss in 3MS) and dementia risk among non-Hispanic white women (N=3647 with ε3 or ε4; aged 70.3±3.7) in 1999-2010. Our results showed that residing in high $PM_{2.5}$ locations (exceeding the US EPA standard of 3-year average >12 μg/m^3) increased the incidence of global cognitive decline and all-cause dementia, and these observed adverse $PM_{2.5}$ effects were exacerbated among women of ε4/4. The overall incidence rate of accelerated global cognitive decline increased by 81% (hazard ratio or HR=1.81; 1.42-2.32), comparing older women residing at locations with high versus low $PM_{2.5}$ (Fig.C9.1A). The incidence of all-cause dementia in older women with high $PM_{2.5}$ exposure was 92% higher (HR=1.92; 1.32-2.80) than the incidence with low exposure (Fig.C9.1B). Incidence rate for all-cause dementia associated with high $PM_{2.5}$ exposure increased by 68% (HR=1.68; 0.97-2.92), 91% (HR=1.91; 1.17-3.14), and 295% (HR=3.95; 1.18-13.19) respectively in ε3/3, ε3/4, and ε4/4 carriers. High $PM_{2.5}$ exposure was also associated with greater incidence of global cognitive decline by 65% (HR=1.65; 1.23-2.23), 93% (HR=1.93; 1.29-2.90), and 264%

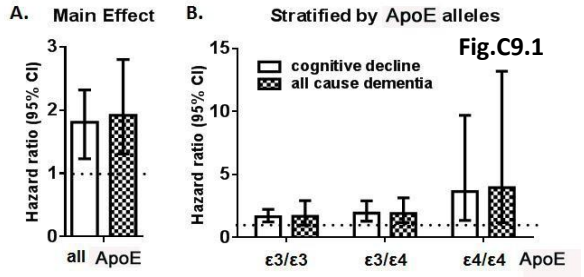


Fig.C9.1

(HR=3.64; 1.36-9.69) in women of ε3/3, ε3/4, and ε4/4 alleles. In addition to the covariates in 3.3.8.1, all these estimates had adjusted for geographic region, spatial random effect, and clinical characteristics (use of hormone treatment; depression; BMI; hypercholesterolemia; hypertension, DM and CVD histories). *We found $PM_{2.5}$ accelerated cognitive decline even before progressing to dementia. For both neurocognitive endpoints (clinically-significant global cognitive decline; all-cause dementia), very similar pattern of gene-environment interactions involving $PM_{2.5}$ and APOE, and the neurotoxic effects were present even in women with only background genetic risk women (of APOE ε3/3). These important observations implied that the underlying neurodegenerative processes affected by $PM_{2.5}$ likely have started at the preclinical stage.* The additional exposure with $PM_{2.5}$ clustering analyses and source estimates will allow us to more specifically examine the effect of TRAP-$PM_{2.5}$ on *early biomarkers predictive of increased risks for AD, MCI, and preclinical AD in older women.* We had recently expanded the analyses to examining the association of regional $PM_{2.5}$ and clinical AD. Although the relatively small number of AD cases prohibited the test of interaction with APOE alleles, incidence rate of AD increased by 71% (HR=1.71; 1.18-2.48) associated with residing in location with high $PM_{2.5}$ exposure in the full sample (n=134 AD in the final adjusted model; N=6128).

C9.2 (Aim 2) The associations of cognitive decline of early AD with TRAP before/during late life

For Aim 2, latent curve models (LCMs)[196] will be constructed to test the hypothesis that TRAP adversely influences the decline in episodic memory and executive function, starting with exposures estimated during middle age and continuing into later life. We will start with the standard *non-linear* mixed-effects models using the latent curve technique (Equations 1-3).[197-200] Let $X_{r,Si,0}$ be the estimated long-term exposures at baseline and $X_{r,\,Si,j-t}$ be the estimated level of TRAP aggregated over 1-year *to 3-year* period for the j^{th} year at the residential location s_i. To illustrate the latent structural analyses linking exposure with the trajectories of cognitive function, we start by defining the first-order latent curve model:

$$\boldsymbol{Y[T]_i = y_{0,i} + B[T]*y_{1,i} + e[T]_i} \quad \text{(Equation 1)},$$

where $y_{0,\,i}$ (in lower case) represents unobserved or latent scores for the initial level of each subject *i*; *B*[T] denotes a set of coefficients describing the average group change as a fixed-effect non-linear function of the timing of observations (e.g., *B*[T] = 0 or 1 or *B*[T] =*Age*[T]); $y_{1,i}$ (in lower case) is the *latent slope*, representing unobserved individual-specific latent score change over time (e.g., per year); and *e*[T] represents unobserved but independent errors of measurement at each time T. We then proceed with the second-order model:

$$\boldsymbol{y_{0,i} = \mu_0 + \beta._o X_{r,Si,0} + f(Z_{r,ij},\theta.) + e_{0,i}} \text{ and } \boldsymbol{y_{1,i} = \mu_1 + \beta._1 X_{r,Si,\,j-t} + f(Z_{r,ij},\theta.) + e_{1,i}} \quad \text{(Equations 2\&3)},$$

where both the latent initial level (with the expected initial score, μ_0, e.g., at the centered age 70) and latent slope (with the expected average change per year, μ_1) are influenced by long- and intermediate-term exposures ($X_{r,Si,0}$ and $X_{r,Si,j-t}$), with corresponding effect estimates $\boldsymbol{\beta._o}$ and $\boldsymbol{\beta._1}$, adjusting for other covariates and random residuals ($e_{0,i}$, $e_{1,i}$) assumed to have variances (σ_0^2, σ_1^2) with a correlation=ρ_{01}.

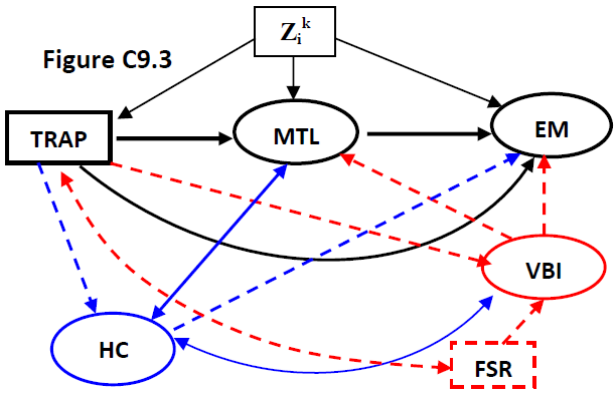
In addition to the *non-linear* mixed-effects models, the LCMs encompass other variants of latent structural analyses that we will explore. For instance, the growth mixture models[201, 202] can be used to identify subgroups with different cognitive trajectories that may be linked to increased dementia risk and/or exposure to air pollution. We may also construct LCMs with changepoints,[203, 204] explicitly modeling the time of accelerated decline as the random changepoint that may be affected by TRAP.

C9.3 (Aim 3) **To elucidate the brain structure and neuropathology mediating the TRAP effects**

Conceptually speaking, Aim-3a employed ROI-based approach to mediation analyses involving the hypothesized target brain regions, whereas Aim-3b took an agnostic approach to examining the topologies of the hypothesized mediators (e.g., cortical thinning) in high-dimensional space. The proposed Aim-3a analyses will be performed by structural equation models (SEMs)[205] in Mplus, [206] whereas the Aim-3b high-dimensional analyses conducted within the EPIC framework.[159] SEMs[205] will be used as primary analytic tool for Aim-3a, because of several desirable features noted in Section B. In the conceptual diagram below (Figure C9.3), we used MTL and episodic memory (EM) to illustrate their structural relationships in mediating the putative TRAP→ early cognitive decline predictive of AD, although the other pathways (blue- and red-colored) will be also examined separately in the full analyses. We follow the conventional symbols of path diagrams,[105, 207, 208] using ovals to represent latent variables and boxes to specify the observed variables. The structural relationships between variables are described by directed (single-direction) arrows for linear effects regressing dependent variables on independent variables. Although double-headed arrows were given to illustrate the correlation of AP with covariate vector (Z_i^k), certain covariates will be treated as common causes of TRAP and AD (Section *C8.1*). For clarity of presentation, we omitted residual errors in the variables and also avoided the other possibly complex correlation structures involving TRAP, WM, GM, EM, and vascular brain injuries (VBI), which will be explored in the final SEMs. In analytic terms, these variables of mediators could be defined either as a "latent construct" (with its assumed errors in measurement) or as "classifiable measures" (e.g., the

Framingham stroke risk score[209, 210] to represent VBI[211]) for the pertinent hypotheses to be tested. The use of these classifiable measures may reduce the dimensionality of SEM parameters in case of computational difficulties. Let TRAP and the path to early AD share a vector of common causes or correlates specified as a fixed covariate matrix $\mathbf{Z_i^k}=(\mathbf{Z_{i1}}, \mathbf{Z_{i2}}, \mathbf{Z_{i3}}, \ldots, \mathbf{Z_{ik}})^{\mathbf{T}}$. Following the notations of the Bentler-Weeks model, [212] all the observable variables (TRAP) and latent factors (e.g., MTL or HC) in a structural equation model can be specified as either dependent variables, η, (e.g., MTL; EM) or independent variables, ξ, (e.g., TRAP; $\mathbf{Z_i^k}$). The putative mediators are considered as dependent variables in the Bentler-Weeks model. The structural relationships can be expressed with a matrix equation: $\eta = \beta\eta + \gamma\xi$, where η and ξ are vectors of dependent and independent variables, respectively; β and γ are matrices of unknown regression weights; and Φ is the covariance matrix representing the associations among the independent variables in ξ. Evaluation of the SEMs will be carried out using Mplus.[206]

Figure C9.3



In Aim 3b, we will employ mediation analysis in an agnostic strategy to identify regions of the brain that mediate effects of TRAP on brain aging. The causal inference test (CIT),[213, 214] developed by Dr. Millstein, has played an important role in 'post GWAS' analyses that seek to identify biological mediators of high-dimensional genetic influence disease phenotypes. It is the unique approach of the CIT, based on component statistical tests of evidence in support of measurable conditions consistent with causality that confers advantages over other causal inference approaches, such as the ability to distinguish causality from reverse causality. A growing number of groups have used the CIT to identify mediators such as methylation[215-222] and gene expression[215, 217, 223-227] but the CIT is also applicable to the problem of identifying molecular mediators of environmental exposures, such as air pollution. A recent modification of the CIT[213] accommodates a computationally efficient non-parametric permutation-based FDR estimator, resulting in a statistically powerful approach when multiple null hypotheses are false. Suppose WM in a particular region of the brain represented by multiple voxels is affected by TRAP, resulting in cognitive decline. Then WM would mediate the effect of TRAP on cognitive decline in this region. Each voxel in the region would correspond to a false null hypothesis, reducing the overall proportion of true null hypothesis, thereby increasing statistical power[228] when the FDR approach is applied. We will identify localized regions of the brain that correspond to clusters of low values of FDR. Dr. Millstein's FDR approach provides confidence intervals that quantify uncertainty in the estimate, even in the presence of weak effects, i.e., when FDR is greater than 0.05. Clusters will be identified using a convex clustering approach that employs the proximal distance algorithm.[229] By adjusting penalty parameters such that there are no clusters under a uniform distribution, we can identify clusters that significantly depart from that condition. If multiple regions are found with suggestive evidence of mediating TRAP effects, we will increase our power by jointly testing those regions. To reduce the search space of mediators, we will incorporate prior knowledge, for example, using the ADNI-defined signatures of cortical thinning that best differentiates AD from cognitive normal controls (section C4.3). The CIT is currently designed to accommodate a single candidate mediator, but we will extend the software to simultaneously handle a set of mediators by leveraging such approaches as partial sparse canonical correlation analysis[230, 231], which will be used to generate CIT component tests. This software extension will be freely provided to the research community through the CRAN project website (https://cran.r-project.org/web/packages/). Two published studies [232, 233] on the mediation analyses of environmental neurotoxicity followed approaches closely related to the 30-year old Sobel test [234] that was not designed to distinguish causality from reverse causality. The CIT is uniquely suited to address this challenge, as has been demonstrated in simulation studies.[213, 214]

Preliminary results **[***revised***]**: We conducted voxel-based morphometry (VBM) analyses to examine the local-scale variation in cortical GM and WM volumes associated with $PM_{2.5}$ exposure.[7] Our results demonstrated that agnostic approach may be more powerful to detect spatial patterns not readily seen in ROI-based analyses. We found statistically significant associations of smaller GM volumes with increased $PM_{2.5}$. The identified local areas were primarily in the dorsolateral and medial prefrontal cortex, regions associated with higher cognitive function such as working memory, episodic memory retrieval, and executive functions. In our previous ROI analyses, regionally-defined GM volumes did not vary by $PM_{2.5}$ exposure.[8] In the VBM analyses, *we used the lower levels of exposure estimates in the middle of WHIMS follow-up (2005-6), but the observed effect of 3-y average* $PM_{2.5}$ on smaller WM volumes involves the same regions (frontal, parietal, and temporal lobes) as found the ROI analyses *using the long-term cumulative exposure since 1999.*[8] *In repeating the ROI*

analyses with 3-y $PM_{2.5}$ <12μg/m³, we found the adverse $PM_{2.5}$ effect on MW volumes remained (data not shown).

C9.4 (Aim 4) To test the genetic and cerebrovascular susceptibility to neurotoxicity of TRAP

We will use the multi-level mixed-models and LCMs to examine whether the adverse TRAP effects are stronger in women with APOE4 or greater WMH.

For instance, let $Y_{r,ij}$ denotes the continuous response variable (e.g., Aβ(+)MCI risk score) for subject *i* measured at j^{th} year and living in residential area *r*; and $Y_{r,ij}$ is assumed to have a normal distribution after log- or logit-transformation if needed. To evaluate the potential effect modification by APOE4 for the hypothesized TRAP effect on sMRI biomarkers, we will construct the following model:

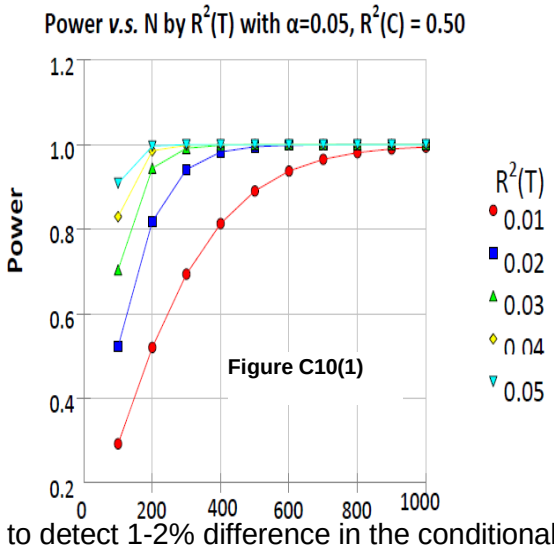
$$Y_{r,ij} = \alpha + U_r + V_i + \beta_{2i}X_{r,Si,j-t} + f(Z_{r,ij},\theta) + \varepsilon_{rij}$$

$$= \alpha + U_r + V_i + (\beta_{2,0} + \delta_{APOE4}I_{APOE,\,i})X_{r,Si,j-t} + f(Z_{r,ij},\theta) + \varepsilon_{rij} \quad \text{.....(Equations 4\& 5),}$$

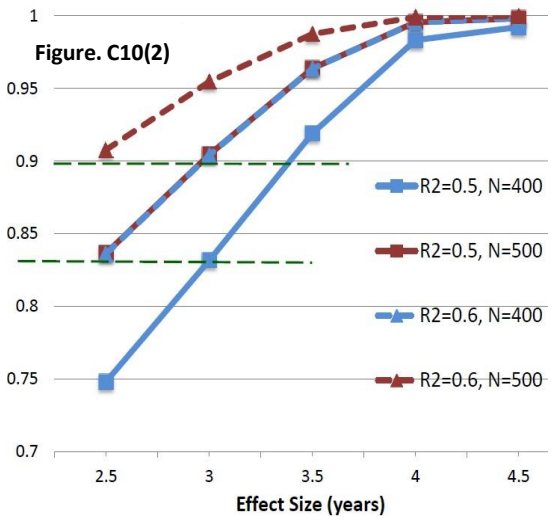
where U_r is i.i.d. N(0, σ_u^2) and ε_{rij} is i.i.d. N(0, σ_ε^2) with "i.i.d." standing for 'independent and identically distributed, α for the overall intercept, U_r for the random-area effect (e.g., MRI clinic-specific intercept), V_i for the random-subject effect (to account for within-subject correlation), $Z_{r,ij}$ for all relevant covariates with corresponding effects specified by a vector of coefficients θ, and $I_{APOE,\,i}$ is the indicator variable for APOE4 status (=1 if carriers; 0=otherwise). The overall TRAP effect estimate of interest is given by β_{2i} and $\boldsymbol{\delta}_{APOE4}$ quantifies how much the effect of TRAP on sMRI biomarkers is changed in carriers vs. non-carriers ($\boldsymbol{\beta_{2,0}}$).

C10. Statistical Power Analyses

In this section, we estimate the statistical power for our main analyses. In **Figure C10(1)**, we depict our sample size and statistical power analyses for the proposed aims. For Aim-1 relating various predicted risk scores to TRAP in multiple regression models, [235] we used the empirical distribution derived from the AD-PS (AD Pattern Similarity) score defined in an ADNI study contributed by Dr. Espeland (subcontract PI at WFU). The AD-PS computation was based a high-dimensional machine learning algorithm of high-dimensional volumetric analyses, with higher scores indicating increasing AD risks. We assume our AD risk scores calculated for WHIMS MRI participants (aged 71-89) using the EPIC matrices will follow a similar distribution as shown for the observed AD-PS (mean ± SD: 0.26±0.21) among cognitively normal controls (aged 75.9±5.0) in ADNI.[236] These power analyses were conditioned on a set of 20 covariates that jointly accounted for 50% of TRAP variability (i.e., R^2(C)=0.50). These results were presented with varying statistical power as a function of sample size (N) for a range of effect size (i.e., R^2(T)=0.01–0.05) reflecting the % of outcome variance attributable to TRAP. Our analyses showed that sample sizes of 1000 and 700 will provide >95% and >99% power to detect 1-2% difference in the conditional probability for AD, with better power for stronger effects (3-5% difference). We consider the assumed 1% difference as clinically significant, because in ADNI it was equivalent to 1-2 years of pathological brain aging.[236]



Because our targeted approach (Aim 3a) to defining mechanistic mediation was based on well-established pathways (e.g., MTL→EM), the success of proposed SEM analyses involving brain MRI data will be largely determined by the effects regressing TRAP on brain structures in the subpopulations with cognitive endophenotypes (CEP, e.g., EM), including 930 for the mediation involving the differences in MRI-1 and 582 in the mediation analyses involving the changes across MRI-1 and-2. As shown In **Fig. C10(1)**, our proposed analyses with a study size of N>900 (N>550) may have >97% (>90%) power to detect 1% difference (change) in sMRI measures linked to CEP.



Power calculations are not straightforward for SEMs with interactions. To illustrate the statistical power of Aim 4 analyses (**Fig. C10(2)**) for joint effects on sMRI biomarkers (Aim 1) or CEP (e.g., EM in Aim 2), we adapted the method by Borm et al.[237] using ANCOVA with various sizes of subsamples (N=400-500), model-adjusted R^2 (0.5-0.6), and a range of standardized effect estimates (equivalent to several years of brain aging) comparing different groups of the highest vs. lowest levels/categories. For comparison

of two groups defined by median of the putative "moderator" (e.g., WMH), a sample with N>1000 in WHIMS-MRI will have 83% power to detect 20% differences in effect sizes (2.5 vs. 3.0 years of brain aging by TRAP) affecting CEP with assumed model-R^2=0.5. Such differences could separately be measurable in both WHIMS-Y (N>1200), with better power expected for larger model-adjusted R^2=0.6. These analyses also illustrated that Aim 2 analyses will have sufficient statistical power (all > 90%) to detect early cognitive decline (>2.5 years of brain aging) associated with TRAP during late life (n=896-930 with an average of 6 repeated measures in WHIMS MRI) or before late life (n=1346 in WHIMS-Y with annual assessment 2008-2016).

C.11: Study Organization and Research Management: This multi-center project is built on the PI's existing collaboration with WHI/WHIMS investigators. We leverage the research team and infrastructure/resources already developed by an NIA-funded R01 (see the comparison table in Budget Justification) based in the WHIMS cohort. It further draws on faculty scientists from the Alzheimer's Disease Research Center and Department of Psychology with expertise in neuropsychology (Gatz; Nation; Petkus), clinical neurology (Liu) and neuroimaging (Liu; Nation) epidemiology of AD (Gatz), latent structure modeling (Petkus) and high-dimensional data analyses (Millstein). The proposed research timeline is summarized in Table C11.

Table C11. Proposed timeline of major research activities by fiscal year

	FY1				FY2				FY3				FY4				FY5			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
WHIMS MRI+WHIM-Y Updates / QA	X	X	X	X		X		X		X		X		X		X	X	X	X	X
Environ Dataset Assembling/ QA	X	X	X	X																
TRAP → early biomarkers; MCI		X	X	X	X	X	X	X												
TRAP → CEP of early AD	X	X	X	X	X	X	X	X												
Mediation SEMs/High-dimensional							X	X	X	X	X	X	X	X	X	X	X	X		
Moderation Analyses							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Presentation/ Publication /Reports		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

C.12. Study Limitations and Strengths

C.12.1 Limitations [*revised*]

(1) Exposure Estimation Errors: Although the proposed exposure models represent state-of-the-art approaches with dramatically reduced exposure misclassification compared to prior research, the estimated exposures are still prone to measurement error. We will not have estimates of all participant micro-environments, which are nearly impossible in a large population-based study, nor time-activity information in the cohort. Collecting time-activity information is not feasible in a retrospective study in which cognitive impairment has occurred. Several novel approaches to account for classical and Berkson-like measurement error in exposures, are being refined for R01ES025888, and we will apply these statistical tools as appropriate in our analyses. [238-240]

(2) Other *Exposure Sources and Characterization*: We will not characterize other sources of air pollutants (e.g., indoor; occupational exposure; *commuting*), because our P01 focuses on ubiquitous ambient environment, and there is very little or no correlation between ambient air pollution and exposure from indoor sources. [241-244] Previous studies have also found very little or no confounding by other sources of environmental pollutants.[27, 245-249] *Because AQS did not monitor particles smaller than $PM_{2.5}$, we could not characterise nPM or ultrafine fraction of PM. Our pre-1999 TRAP exposure estimation primarily replies on the spatiotemporal NO_2 model, because there was very limited information available regarding size distribution of PM and TRAP in the 1990s or before.*

(3) Generalizability: We could not generalize our findings to younger women or men of any age. However, selecting the post-menopausal and older women is well justified to address the Project-1 aims (Section C3). Studying early-life/adulthood exposures to ambient air pollution and the dementia risks in contemporary cohorts remains a great challenge, because good-quality air exposure data and models only became available after the 1990s. An ideal population will be a nationwide sample of middle-aged women and men with gender-specific analyses, but no such cohorts exist with comprehensive data available as in WHIMS-MRI+WHIMS-Y. *It is noteworthy that WHIMS+WHIMS-Y was a multi-ethnic population (see enrollment table) with its biracial (Black and White) distribution representative of US older women in 1990s.*[250]

C.12.2 Strengths

This application has the following major strengths: (1) the study design is very cost-efficient, as it builds on two large, unusually well-characterized, geographically diverse cohorts with unique clinical, neuropsychological, and structural brain MRI data plus comprehensive covariates; (2) this collaborative project brings the novel population neuroimaging approaches to marry with state-of-the-art air pollution neuroepidemiology to advance our knowledge in population neurosciences of TRAP and brain aging; (3) this application is the translational product of an outstanding collaboration by expert investigators representing diverse disciplines and taking a true "team science" approach; (4) there is great potential to further leverage WHI biological and other data resources (e.g., *GWAS and Biomarker Studies* https://cleo.whi.org/data/Pages/GWAS-Data.aspx; dietary/nutrition/ psychosocial) to pursue future studies on biological mechanisms/interactions; and (5) the strong preliminary data indicate a high probability that new knowledge gained from this Project-1 will greatly improve our understanding of the role of TRAP in contributing to the risk, heterogeneity, and mechanisms of AD in late life.

HUMAN SUBJECTS PROTECTION

Risks to Subjects

The proposed research project is part of a WHI/WHIMS ancillary study nested within the completed Women's Health Initiative Memory Study (WHIMS) and WHIMS in Younger Women (WHIMS-Y). Specifically, Project-1 sample was drawn from two well-characterized and geographically-diverse cohorts of mid-aged and older women in the WHIMS-Y (n=1351, inception age 50-54) and a subset of WHIMS participants who were enrolled in WHIMS-MRI (n=1403, inception age 65-80), followed annually since 1996. The proposed research activities involve no direct contact with the original participants and no use of biological samples from participants enrolled in WHIMS+WHIMS-Y. Since there is no direct interaction with subjects, the resulting risk is minimal and limited to breach of confidentiality.

Human Subjects Involvement and Characteristics

The proposed research involves no primary data collection from the study participants. There is no proposed contact with the original participants. The project has been reviewed and approved by the WHI Ancillary Studies Committee and the NHLBI-WHI Project Office. The data are not publicly available. Confidentiality of the WHI data will be ensured. No personal identifiers will be used. All WHI data will be kept on password-protected computers in offices restricted to authorized personnel on keypad-protected floors.

All investigators and personnel with access to WHI data will sign a Data Distribution Agreement (DDA) and will abide by WHI rules for publications and presentations. Key personnel with access to geocoded data will follow the existing procedures for special Data and Materials Distribution Agreement (DMDA), including the use of legally binding DDA data securoty provisions for data containing personal identifiers, that conforms to the specifications by the USC IRB& HIPAA regulations, the UW IRB& HIPAA regulations and UNC IRB and submitted for clearance by the WHI CCC (Clinical Coordination Center).

Adequacy of Protection Against Risks

Recruitment and Informed Consent

WHI participants provided informed consent at all examinations using a form approved by the Institutional Review Board at participating academic centers and by the Office of Management and Budget (OMB 0925-0414).

Protection Against Risk

To ensure the confidentiality of the WHI data, all derived data will be linked to the main WHI/WHIMS database by the unique WHI identification number for each participant and no other personal identifiers will be used. All WHI data will be kept on password-protected computers in offices restricted to authorized personnel on keypad-protected floors. All investigators with access to WHI data will sign a DDA, and will abide by WHI and the USC, University of Washington, and Wake Forest University IRBs and protocols related to human subject protection. This risk of breaching confidentiality will be minimized, as the processing of geocoded information has been incorporated into the management and analysis of data, with tested procedures designed to safequard confidentiality. These procedures are based on our experience accrued in several prior studies of this kind that also involve access geocoded information and apply the data linkage to derive air pollution exposure estimates for large-scale epidemiologic studies. In the process of extensive spatiotemporal modeling of exposure to ambient air pollution, the UW investigators will use two server systems to separate the environmental data from geocoded data. This approach will further strengthen the security of confidential residential information and allow all researchers to access and evaluate environmental data until it is suitable for data fusion with geocoded data. The fully evaluated and processed environmental data would then be transferred to the second data server, which will follow all confidentiality protocols. Also, the purposeful compartmentalization of data management and analysis, as set in place by the WHI CCC, only provides data without personal identifiers for parent and ancillary study analyses at the performance site, as in the case for the proposed project. Other important elements in this process are the use of legally binding data use agreements that stipulate how our academic collaborators and commercial geocoders must operate. The required education on human subject protection of human subjects has been documented by each collaborating institution for all involved investigators in this application. The data with personal identifiers will be contained exclusively within a secure data management and computing environment established for

confidential data and not accessible to other programmers, investigators, or data managers. All ancillary study data will be provided to the WHI CCC when the final data set is compiled.

Potential Benefits of the Proposed Research to the Subjects and Others
The proposed ancillary study offers no directly tangible benefits to individual WHIMS or WHIMS-Y participants.

Importance of the Knowledge to be Gained
The long-term goal of this project is to understand the influence of ambient air pollution exposures on the neuropsychological processes and mechanistic pathways leading to dementia including Alzheimer's disease (AD). In 2015, one in 9 Americans over age 65 is living with AD at a cost of $226 billion annually. Of these more than 5 million people with AD in the US, two-thirds are women. However, scientific evidence remains elusive for the modifiable factors associated with Alzheimer's risk. The proposed hypotheses, if substantiated, will greatly strengthen the potential causal role that traffic-related air pollution plays in the development of Alzheimer's disease. This project holds the promise to improve our understanding of novel and modifiable environmental risk factors for dementia/AD that is relevant to millions of aging Americans and their families.

Inclusion of Women and Minorities

Dementia, including Alzheimer's disease (AD) imposes a greater burden on women, because two-thirds of the 5 million affected by AD in the US are women, and women are two to three times as likely as men to have AD across the lifetime. This Project-1 study is designed to investigate the contribution of traffic-related air pollution (TRAP) to the risk, susceptibility and mechanisms of AD and related dementias (ADRD) in older women.

To this end, the sample for the proposed analysis is 100% female. The women sample came from the WHIMS & WHIMS-Y cohorts, both based in the larger WHI Clinical Trials which were designed to test the hypothesis that postmenopausal hormone therapy (HT) reduces the incidence of all-cause dementia and also to assess the efficacy of HT on age-related changes in specific cognitive functions in postmenopausal women including older (aged 65-80 years in WHIMS) and younger (aged 50-54 in WHIMS-Y) women.

The WHIMS & WHIMS-Y is a multi-ethnic cohort including both older and younger postmenopausal women of racial/ethnic minorities and its biracial (Black and White) distribution was well representative of US older women in mid-1990s (See Table 2-2: http://www.census.gov/prod/1/pop/p23-190/p23-190.pdf).

PHS Inclusion Enrollment Report

This report format should NOT be used for collecting data from study participants.

OMB Number:0925-0001 and 0925-0002
Expiration Date: 10/31/2018

***Study Title:** Traffic-Related Air Pollutants and Alzheimer's Disease: Risk, Susceptibility and Mechanisms in Women

***Delayed Onset Study?** ❑ Yes ☑ No

If study is not delayed onset, the following selections are required:

Enrollment Type ☑ Planned ❑ Cumulative (Actual)

Using an Existing Dataset or Resource ☑ Yes ❑ No

Enrollment Location ☑ Domestic ❑ Foreign

Clinical Trial ❑ Yes ☑ No

NIH-Defined Phase III Clinical Trial ❑ Yes ☑ No

Comments:

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	5	0		0	0					5
Asian	14	0		0	0					14
Native Hawaiian or Other Pacific Islander	0	0		0	0					0
Black or African American	231	0		0	0					231
White	2349	0		79	0					2428
More than One Race	19	0		0	0					19
Unknown or Not Reported										
Total	2618	0		79	0					2697

Inclusion of Children

Data on children are not included in this research project. The data set is limited to women who were enrolled in the WHIMS trials and WHIM-Y which included only women aged 65–80 years and 50-54 respectively.

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University of Southern California Department of Contracts and Grants

SUBRECIPIENT CERTIFICATION FORM

Our records indicate that your organization is currently being considered for receipt of a subaward under research funds awarded to the University of Southern California. Please ensure that all Documents in Section A are competed, accurate and attached, check all applicable Certifications In Sections B through D and attach requested Information. Additionally, if applicable, the Uniform Guidance (2 CFR 200) requires the University of Southern California to ensure that your organization is in compliance with the requirements of 2 CFR 200. As such, please check all applicable Audit Questions In Section C and attach requested Information.

SUBRECIPIENTS'S LEGAL NAME: Wake Forest University Health Sciences

SUBRECIPIENT'S PI: Mark A. Espeland

USC's PI: JC Chen, Ph.D. PRIME SPONSOR: NIA

use PROPOSAL TITLE: Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms

SUBRECIPIENT'S TOTAL FUNDS AWARDED: 166,163

SU8REIP1ENT'S PERFORAMANCE PERIOD: BEGIN: 04/01/2018 END: 03/31/2023

SECTION A - Proposal Documents

The following documents will be included in the subaward and are covered by the certifications below:

STATEMENT OF WORK *(required)*

BUDGET *(required)*

BUDGET JUSTIFICATION *(required)*

SECTION B - Certifications

1. Facilities and Administrative Rates included in this subaward have been calculated based on:

   [j] Our federally-negotiated F&A rates for this type of work, or a reduced F&A rate that we hereby agree to accept.
   (If this box is *checked, please attach* a *copy of your F&A rate agreement must be furnished to* USC *via hard copy, website, or email before a subawarrJ will be issued.)*

   0 Other rates (please specify the basis on which the rate has been calculated in Section E *Comments* below)

   D Not applicable (no indirect cost request for subrecipient)

2. Fringe Benefit Rates included in this subaward have been calculated based on:

   [j] Rates consistent with or lower than our federally-negotiated rates
   (If this box is *checked, a copy of your FB rate agreement must be furnished to USC before* a *subaward will be issued.)*

   D Other rates (please specify the basis on which the rate has been calculated in Section C *Comments* below).

3. Human Subjects [j] Yes 0 No Approval Date: _____

   If "Yes": Copies of *the /RB approval and approved HInformed Consenr form must be issued. Please attach or provide these documents* to *USC's PI as* soon *as they beco ovided before any subaward will be available. Thisis required before any subaward will be issued.*

   If "YesH and NIH funding is involved:

   Have all key personnel involved completed Human Subjects Training? [j] Yes 0 No

   Note: All key personnel engaged in human subject research must take the NIH human subjects training or human subject research training (http://grants1.nih.govlgrantsfpolicy/hs educ fag.htm)

4. Animal Subjects 0 Yes [j] No Approval Date: _____

   If "Yes": A copy of the IACUC approval must be provided before any subaward will be issued. Please attach or provide these documents to USC's *PI as soon as they become available.*

University of Southern California Department of Contracts and Grants

SUBRECIPIENT CERTIFICATION FORM

5. Stem Cells D Yes Ii] No

{If "Yes": A copy of the Stem Cell approval must be provided before any subaward will be issued. Please attach or provide these documents to USC's PI as soon as they become available.

6. Conflict of Interest (applicable to NIH, NSF, or other program requiring federal financial disclosure)

D Not applicable. This project is not being funded by NIH, NSF, or any other program requiring federal financial disclosure.

If funded by NSF:

0 Subrecipient Organization/Institution certifies that it has an active and enforced conflict of interest policy that is consistent with the provision of 42 CFR Part 50, Subpart F "Responsibility of Applicants for Promoting Objectivity in Research." Subrecipient also certifies that, to the best of Institution's knowledge, (1) all financial disclosures have been made related to the activities that may be funded by or though a resulting agreement, and required by its conflict of interest policy; and, (2) all identified conflicts of interest have or will have been satisfactorily managed, reduced or eliminated in accordance with subrecipient's conflict of interest policy prior to the expenditures of any funds under any resultant agreement.

0 Subrecipient does not have an active and/or enforced conflict of interest policy and agrees to abide by USC's policy and related procedures. See: http J www.usc.edu/admin/oprs/oolicies/coi.html for the text of USC's policy.

If funded by PHS/NIH:

Ii] My organization DOES HAVE a PHS-compliant Financial Conflict of Interest (FCOI) policy and my organization will rely on this policy and associated procedures to comply with the PHS Conflict of Interest regulation.

D Yes, we are registered as an organization with a PHS-compliant FCOI policy with the FOP Clearinghouse. (http://sites.nationa1academies.orgIPGA/fdo/PGA_070596)

0 My organization DOES NOT HAVE a PHS-compliant policy in place but will have one at the time of award. (A sample FOP FCOI policy can be found at http:/lsites.nationalacademies.orq/PGA/fdp/PGA 061001)

list the names of individuals working on this project who are responsible for the design, conduct, or reporting of this research in Section E *Comments* below.)

D Subrecipient does not have an active and/or enforced conflict of interest policy and agrees to abide by USC's policy, located online at http/looc.usc.edu/Conflict-Interest-Research

7. Cost Sharing/Matching/In-Kind 0 Yes Ii] No Amount:_____
Cost sharing, Matching, and/or In-Kind amounts and justification should be included in the subrecipient's budget

8. Certification Regarding Debarment and Suspension

Is the entity, PI or any other employee or student participating in this project debarred, suspended or otherwise excluded from or ineligible for participation in federal department, agency, assistance programs or activities?

0 Yes Ii] No (If "Yes", explain in Section E *Comments* below)

Subawards to any entity or individual Included in tho Federal excluded Parties are prohibited.

9. Ethics In Research Training (applicable to projects funded by NSF)

Ii] Not applicable because this project is *not* being funded by NSF.

D Subrecipient Organization/Institution hereby certifies that it will ensure that all undergraduates, graduate students, and postdoctoral researchers who will be supported by this NSF proposal will be trained on the oversight in the responsible and ethical conduct of research.

10. Is subrecipient a for-profit entity? 0 Yes !!ii No

If "Yes", USC PI must complete the attached Fair and Reasonable Cost Analysis Form.

University of Southern California Department of Contracts and Grants

SUBRECIPIENT CERTIFICATION FORM

SECTION C - Audit Status

1. Audit Status

III We have completed our A-133 audit for the most recent fiscal year. There were no material weaknesses, material instances of noncompliance, reportable conditions; or findings related to any subaward(s) from USC. A complete copy of the audit report is enclosed or a URL links provided in Section E.

O We have not yet completed our A-133 audit for the most recent fiscal year. We will advise you of the results and provide a complete copy of the audit report when it is completed on (date):_____

Note: In the Interim a complete copy of subrec/p/ent's most recent audit report or the Internet URL fink to a complete copy, must be furnished to University of Southern California before a subaward wf/I be Issued.

0 We have completed our A-133 audit for the most recent fiscal year. There were material weaknesses, material instances of noncompliance, reportable conditions, or findings related to subaward(s) from USC University. A complete copy of the audit report is enclosed, including our corrective action plan. The specific audit finding(s) noted in the audit report relating to subaward(s) from USC are discussed in Section E.

D We are not subject to OMB Circular A-133 because (check all that apply):

0 Non-profit entity (under federal funding threshold)
0 Government entity
O For profit entity
D Foreign entity (not formed under U.S. laws), or another exception applies (explain in Section E):

Note: For organizations that are not subject to OMB Circular A-133 or 2 CFR 200, University of Southern California will require entity to complete the attached Audit Certification and Financial Status Questionnaire and provide audit reports or financial statements.

SECTION D - Federal Funding Accountabilit and Transparenc Act

1. Location of Subrecipienl (Name, Address, City, Stale, Zip + 4, Congressional District, and Country):

Wake Forest University Heolth Sciences. Medical Center Blvd.Winston Stem. NC 27157-0001 NC-005

Note: If primary place of performance Is different than Location of Subreclplent, provide local/on of where the project will be performed (Name, Address, City, State, Zip +4, Congressional District, and Country)

2. DUNS Number (+4) of Subrecipient receiving award: 937727907

3. Is Subrecipient owned or controlled by a parent entity? O Yes No

NOTE: If yes, please provide the Name, DUNS Number (+4), and Location (Address, City, State, Zip + 4, Congressional District, and Country) of parent entity:

4. Does Subrecipient currently have an active registration in the System for Award Management (www.sam.gov)?
III Yes 0 No

NOTE: Organ zations that have not registered with SAM wlll need to obtain a DUNS number first and then access the SAM onllne registration through SAM home page at https://www.sam.gov/. Subreclplent must maintain their currenthformationh SAM.

5. Exempt from reporting compensation III Yes D No If "No", proceed with filling out the top 5 paid officers below.
Executive compensation information for the Subrecipient must be reported if: More than 80% of annual gross revenues are from the Federal Government, and those revenues are greater than $25M annually; compensation information is not already available through reporting to the Security and Exchange Commission (SEC).

Officer 1	Name		Compensation	
Officer 2	Name		Compensation	
Officer 3	Name		Compensation	
Officer 4	Name		Compensation	
Officer 5	Name		Compensation	

University of Southern California Department of Contracts and Grants

SUBRECIPIENT CERTIFICATION FORM

SECTION E - Comments

http://finance. wfu.edu/audited-financial-statements

APPROVED FOR SUBRECIPIENT

The information, certifications and representations above have been read, signed and made by an authorized official of the Subrecipient named herein. The appropriate programmatic and administrative personnel invdved in this application are aware of agency policy in regard to subawards and are prepared to establish the necessary inter-institutional agreements consistent with those policies. Any work begun and/or expenses Incurred prior to execution of a subaward agreement are at the Subreciplent's own risk.

Rentz. M;[Vake Forest University Health Sciences i

Signature or Subrecipient s Authorized Official	Legal Name of Subrecipient's Organizabn/Institution
Meredith rentz, MS,Director, Office of Sponsored Programs	Medical Center Blvd
Name and Title of Authorized Official (Print)	Address
nihawards@wakehealth. edu	Winston Salem, NC 27157
Email	City. State,Zip
336-716-2382	223849199
Phone Fax	FederalEmployer Identificat ion Number (EIN)
4/27/17	93772907
Date	DUNSorOUNS+4 number

Workscope

Mark A. Espeland, PhD, will collaborate with Dr. Chen and the rest of the study group throughout this proposal on analyses and publications. He will help to develop analysis plans, propose and collaborate on publications, and assist in the overall dissemination of study findings. He will also provide an interface between the WHI and this proposal and is well-familiar with WHI policy relating to publications and data access. Julia Robertson will provide Dr. Chens' group with the WHIMS data necessary for the conduct of the study (in Year 1) and will assemble final study bases for the WHI data archive in Year 5. Ms. Robertson will also develop reports, address quality control issues, and collaborate on study publications. She will develop data interfaces that permit the secure transfer of study data, provide documentation on constructed variables, and provide guidance throughout the study on the interpretation of analytical findings. She will work closely with Dr. Espeland to develop summary measures from imaging and cognitive data for analytical databases.

O"ARTM•Nf O• H£ALTH •HUMAN SDVKES

Public Health Service

National Institutes of Health
National Heart, Lung, and Blood Institute
Bethesda, Maryland 20892

· January 16, 2008

TO: JC Chen
Gerardo Heiss

FROM: Jacques E. Rossouw, M.D.
Branch Chief, NHLBIIDPPS/WHIB

SUBJECT: Approval of Ancillary Study 252
Environmental Determinants of Cognitive Aging in the WHI Memory Study

This ancillary study has recei ved the approval of the WHJ Anci llary Study Committee and the NHLBVDPPS/WHIB Project Office and the Executive Committee. However, you are encouraged to work with Dr. Whitsel, who developed the geocoding on which your study depends. You are also encouraged to consider the Ancillary Study reviewer comments as you prepare for funding submission.

Continued adherence to all the WHI Ancillary Studies and Publications and Presentations policies and proced ures are required. The ancillary study must not interfere with the conduct of the main study, and must not make use of WHI staff beyond that portion of salaries covered by the ancillar y study funding.

Any proposed changes to the design of the study must be approved by the Ancillary Study Committee prior to implen1entation. Please provide annual and final progress reports to the Ancillary Study Committee Coordinator at the *CCC.*

cc: Nancy Morris, NHLBI/WHI
Helen Penor, CCC
Robert Brunner, ASC
Marcia Stefanick, ESEC Chair

FRED HUTCHINSON
CANCER RESEARCH CENTER
A LIFE OF SCIENCE

WOMEN'S
HEALTH
INITI

WHI Clinical Coordinating Center

January 31, 2008

Jiu-Chiuan Chen,
University of North Carolina at Chapel Hill
School of Public Health
2104G, McGavran-Greenberg, CB#7435
Chapel Hill, NC 27599

Dear Dr. Chen:

After thorough review of your proposed WHI Ancillary Study 'Environmental Determinants of Cognitive Aging in the Women's Health Initiative Memory Study," the WHI Clinical Coordinating Center (CCC) at Fred Hutchinson Cancer Research Center (FHCRC) agrees to provide the work outlined on the following pageat no cost to you if this study is funded.

For planning purposes, please keep in mind that the CCC requires a60-day response time. As much advance notice as possible with regard to data to be provided will help keep the response time to a minimum.

We understand that this proposal will be submitted to the NIH. Fred Hutchinson Cancer Research Center will abide by all NIH rules.

Current OHRP regulations require that any research activity obtaiJ1ing data from a repository for which FHCRC is responsible, have FHCRC IRB approval. Therefore, if you receive funding, wewill need from you your local IRB approval documentation, as well as the final protocol. We will also require a copy of your local IRB Annual Reports to assist in the preparation of our Annual Reports. The WHI CCC will not be allowed to release WHI data without FHCRC IRB approval of the study using the data.

Please address any correspondence regarding the WHI CCC's involvement in this ancillary study to:

Jenny Schoenberg
Women's Health Initiative - Clinical Coordinating Center
Division of Public Health Sciences
Fred Hutchinson Cancer Research Center
PO Box 10924
1100 Fairview - M3-A410
Seattle, Washington 98109-1024

Phone: 206-667-6365
FAX: 206-667-4142

If I can be of further assistance, please feel free to contact me.

Best wish

Gamet Anderson,
Principal Investigator

cc: J. Schoenberg, J. Shupe, M.Pettinger R:\Studies\AncillaryStudies\250-299\AS252 JCChen\OtherAdmin\AS252 Memo.doc

January 31, 2008

WHI Ancillary Study #252
Environmental Determinants of Cognitive Aging in the Women's Health Initiative Memory Study
(P.I. JC Chen)

Federal Contract #NOl-WH-2-2110 funds will cover the following personnel for this study at no cost to the University of North Carolina:

A scientific liaison to coordinate CCC activities, and maintain communications between ancillary study investigators and the CCC.

A Systems Analyst/Programmer to prepare data file(s) consisting of data items necessary for the conduct of the study, including: location data (longitudes/latitudes) 1993-2004; selected SES indicators (developed by AS# 140); address data 2004-2007.

A Project Coordinator to provide administrative assistance, IRB application and renewal, and to assist with resolving study-related problems for this ancilla11 study.

Resource Sharing

We will comply with the extant NIH policies to make the results of proposed Project-1 and accomplishments of related activities available to the research community and to the public at large.

Those data on estimated exposures to ambient air pollutants generated for the WHIMS+WHIMS-Y participants could potentially become valuable resources for further environmental health research in this unique study population. Since the proposed Project-1 research is part of a WHI ancillary study, access to the environmental data collected in this study and other pertinent data resources for developing either manuscript proposals or ancillary study proposals have to follow the established WHI policies, including, but not limited to, "Publications and Presentations Policy" and "Ancillary Study Policy." Detailed of these policies can be found at the following link:
https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/PP%20policy.pdf
https://www.whi.org/researchers/Document%20%20Ancillary%20Studies/AS%20policy.pdf

We do not expect any inventions nor application for patents resulting from the proposed research activities of this Project-1.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 8043557900000

Legal Name*: The Regents of University of California UC San Diego
Department:
Division: University of California San D
Street1*: 9500 Gilman Drive
Street2:
City*: La Jolla
County: California
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92093-0041

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
	Evelyn	V.	Olaes	

Position/Title: Senior Grant Analyst
Street1*: 9500 Gilman Drive
Street2:
City*: La Jolla
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92093-0742
Phone Number*: 858-822-1406 Fax Number: 858-822-0834 Email: eolaes@ucsd.edu

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Urban Air Pollution and Pathological Brain Aging: A Nationwide Twin Study in Men

12. PROPOSED PROJECT

Start Date*	Ending Date*
04/01/2018	03/31/2023

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of California San Diego
Duns Number:	8043557900000
Street1*:	9500 Gilman Drive
Street2:	
City*:	La Jolla
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	92093-0674
Project/Performance Site Congressional District*:	CA-052

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of California Riverside
DUNS Number:	6277974260000
Street1*:	245 University Office Bldg.
Street2:	
City*:	Riverside
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	92521-0217
Project/Performance Site Congressional District*:	CA-041

Project/Performance Site Location 2

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	VA Puget Sound Health Care system
DUNS Number:	0202329710000
Street1*:	1600 S. Columbian Way
Street2:	
City*:	Seattle
County:	King
State*:	WA: Washington
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	98101-1532
Project/Performance Site Congressional District*:	WA-009

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: 1 2 3 4 5 6 If

NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_2_7_Abstract.pdf
8. Project Narrative*	Project_2_8_ProjectNarrative.pdf
9. Bibliography & References Cited	Project_2_9_References.pdf
10.Facilities & Other Resources	UCSD_FacilitiesOtherResources.pdf
11.Equipment	UCSD_EquipmentR.pdf

Substantial evidence indicates that exposure to outdoor air pollutants may accelerate cognitive aging. Emerging data from animal models also point to a possible increase in the risk of Alzheimer's disease (AD) and related dementias with exposure to traffic-related air pollutants (**TRAP**). Animal models of TRAP show strong evidence of neurotoxicity, but existing studies of neurotoxic effects of TRAP on human brain aging have important knowledge gaps: 1) little long-term address history data; 2) little data on exposure effects before late life; 3) limited data on cognition, particularly mild cognitive impairment (MCI) or AD; 4) limited neuroimaging measures; 5) no examination of potential confounding early-life factors: 6) studies of older adults mostly on women; and 7) need for better understanding of gene-environment interactions. Project 2, built on the NIA-funded longitudinal Vietnam Era Twin Study of Aging (**VETSA**; R01 AG018386 & AG022381), is ideal for addressing these gaps. It includes male twins ages 51-60 at VETSA 1 (n=1291) and 55-66 at VETSA 2 (n=1205). VETSA is a geographically-diverse cohort from 49 states, offering great variability in TRAP exposure. Subjects have 10-12 hours of neuropsychological, psychosocial, and health/medical data at each timepoint; 545 and 447 have structural magnetic resonance imaging (MRI) at VETSA 1 and 2, respectively. We will address gap #1 by collecting and geocoding residential history data back to 1993, and in conjunction with Environmental Exposures Core C, create cumulative indices of TRAP. We will then be able to directly address gaps 2-7. TRAP will be characterized by the estimated ambient levels of NO2 (a gaseous surrogate), elemental carbon (EC) component of $PM_{2.5}$ (a marker of diesel exhaust particle), and predicted source profiles of $PM_{2.5}$. The age of VETSA subjects is ideal for examining TRAP effects *before* late life. We examine the following aims: **Aim 1. Assess TRAP effects on brain structure/function: Main effects.** We predict TRAP exposure will be associated with higher AD-related brain signature scores, cerebral hypoperfusion (paralleling Mouse Project 4), and white matter hyperintensities. TRAP exposure will be associated with poorer cognitive function and greater cognitive decline over time. **Aim 2. Assess the impact of TRAP on cognitive and brain aging: Mediation.** We will examine associations between TRAP and specific measures of cognition with a focus on episodic memory, executive function, and processing speed, as well its mediation by specific brain measures. Diffusion tensor imaging (**DTI**) indices of brain microstructure have not been examined in prior studies. We will examine mean diffusivity in both grey and white matter in medial temporal and frontal regions. **Aim 3. Examine gene-environment (GE) interaction. 3a**) We hypothesize that adverse TRAP effects on brain and cognitive decline will differ as a function of *APOE*-ε4, and polygenic risk scores for AD, inflammatory processing, and tau metabolism/processing. **3b**) We will use MZ within-pair difference analysis as another approach to shed light on how genetic and environmental influences work in tandem.

Chronic traffic-related air pollution (TRAP) exposure effects are an important public health issue, but they have been understudied in midlife, particularly as they relate to risk for MCI and AD. Better understanding the effects of TRAP exposure on brain structure/function in middle-aged adults is a first step in understanding the mechanisms by which TRAP may lead to cognitive decline. This may provide a focus for future interventional research in at-risk individuals.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ❍ Yes ● No

*Agency-Defined Phase III Clinical Trial? ❍ Yes ❍ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ❍ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

❍ Yes ❍ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	Project_2_1_Introduction.pdf
Research Plan Section	
2. Specific Aims	Project_2_2_SpecificAims.pdf
3. Research Strategy*	Project_2_3_ResearchStrategy.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	UCSD_Twins_Human_Subjects_Protection_Plan.pdf
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	UCSD_InclusionWomenMinoritiesR.pdf
8. Inclusion of Children	UCSD_InclusionChildrenR.pdf
Other Research Plan Section	
9. Vertebrate Animals	
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	UCSD_MultiplePD_PI_LeadershipPlan.pdf
12. Consortium/Contractual Arrangements	VETR_Consortium_Package.pdf
13. Letters of Support	Franz_Kremen_LetterOfIntent_P01_2017.pdf
14. Resource Sharing Plan(s)	UCSD_ResourceSharingPlans.pdf
15. Authentication of Key Biological and/or Chemical Resources	
Appendix	
16. Appendix	

INTRODUCTION

We thank the reviewers for helpful critiques that substantially strengthened the project. Changes are in *italics*.

R1 Lack history of air pollution interests; consideration for air pollution largely off-loaded to Exposure Core. We added Co-I, Dr. Jennifer Vanos (see biosketch)[1-3]. She brings an extensive background in effects of air pollution to the study; she will be an effective liaison between Project 2 and Core C (Exposures Core). We will hire a postdoc with a background in pollution effects. We added considerable detail on TRAP to the text.

R1/R2 Not all subjects will have same degree of TRAP data quality. How will exposure uncertainty be addressed? R2 Time for primary hypotheses about exposure periods not clear how will time-varying exposures be dealt with? Exposure data will be available in contiguous 48 states; time period is 1993 to 2013 (see **4.2.1**). State-of-the art spatiotemporal historical prediction model techniques developed by MESA-Air (part of Core C) provide finely resolved TRAP exposure estimates with national coverage. Although the long-term models (1993+) do not have the same effectiveness as the post-1999 models due to lack of monitoring data, to an unprecedented extent the long-term models allow for meaningful assessment of long-term concentrations for epidemiological analyses[4-7]. The majority of VETSA participants live near major cities where monitoring was continuous and intensive; <0.1% live outside the contiguous states. Measurement error will be higher in subjects from less monitored areas and will be accounted for in data analyses[5,8]. Detailed address histories combined with yearly temporal TRAP resolutions will allow for analysis of time-varying exposures. Core C members have statistically validated algorithms to examine time-varying exposures and error estimation[4,6-13].

R2 If they believe that long-term exposure affects baseline cognition function, adjustment is not appropriate. Exposure affects brain and cognition, but cognitive ability is also positively correlated with brain volume. There is no simple solution, so we will compare analyses with and without adjusting for early adult cognitive ability.

R1 Details for the AD risk score based on allelic variants is a bit hazy; use of two forms of AD risk scores (genetic and pupil/tau-derived) is confusing; genetic risk scores are a potential modifier of TRAP effects, while ADNI-derived risk scores may be impacted by TRAP, but this language detracts from the description. To avoid confusion we now use "risk score" only for polygenic risk scores (**4.2.2.e**); these are always risk factors. Other measures (e.g., ADNI-derived brain signatures) may be risk/predictive factors (i.e., relative to cognitive decline), but it is also a major idea of the proposal that they can be outcomes (i.e., relative to TRAP exposure).

R2 Success hinges on willingness/ability of subjects to supply residential history. How long a history? R1.It is unknown how many participants will return address history questionnaires, and how complete this information will be. Address histories will be from 1993 to 2013. We and the twin registry already have some address history info. Addresses were most recently updated in 2016-2017. We will also use proven techniques developed by Core C based on the MESA-Air study (details in **4.2.1**). At ages 61-71, most will be cognitively competent; for others we have informants. We use a multi-method approach (e.g., letters, phone follow-ups, informants, computer searches for non-responders) and a participation incentive. We anticipate a 90% response rate given our prior return rate of 82% and residential history being a small burden relative to the 10-12 hours of assessments our twins completed at each study wave.

R1 Range of data for various cognitive domains and health measures would be good to consider power calculations; power section could a more thorough for all endpoints. R2 Power may be an issue; stable MCI cases with imaging data is <50. Do not know the range of exposures, so not clear if power is sufficient. We added Co-I, Xin Tu, a senior biostatistician to enhance analysis and power sections[14-17]. Analyses have adequate power for continuous cognitive measures with MRI, and for TRAP-cognition associations for the full sample. Due to the smaller N the MCI analyses in the MRI analyses will be exploratory. Health and cognitive data were collected on all subjects. Rates of health conditions and general cognitive ability are similar to American men in their age range[18,19]. Many studies focus only on urban centers. VETSA subjects live across the US with a full range of rural, suburban, and urban exposures.

R2 Lack of new data collection specifically for the Project. Address history and geocoding are new data.

R2 No discussion of potential selection bias. Agreeing to participate results in some selection bias in all epidemiologic studies; bias appears to both under- and over-estimate relationships[20]. At baseline, VETSA subjects were unselected except for age (51–60), with only 18% attrition at follow-up. The sample is similar to American men in their age range on health and lifestyle factors based on CDC data[18,21]. We have an "attrition replacement" subsample at VETSA 2, so we can compare attrition and practice effects. Health and survivor bias are less than if we began when subjects were older, as in many studies of aging (see 4.1 & 4.3.8).

R3 Pupillary hypothesis is plainly exploratory and not well-integrated. A midlife sample enables us to identify early risk indicators. Pupillometry can detect risk for decline very early, providing an objective measure of the effort needed on a task *before* performance declines[22-25]. Greater effort is consistent with increased need for compensation, hence, increased risk for decline. Biological indications of its links to early risk for MCI and AD are that the pupil response being largely driven by the locus coeruleus (LC), and Braak et al.'s finding that the LC is the earliest site of tau deposition. Pupil dilation responses do tend to be greater with age as cognitive efficiency declines, but we have only a 10-year age range and our analyses controlled for age (see **4.2.2.d**).

1. SPECIFIC AIMS —PROJECT 2 (Franz, Kremen)

Substantial evidence indicates that exposure to outdoor air pollutants may accelerate cognitive aging[26-28]. Emerging data also point to increased risk for Alzheimer's disease (**AD**) and related dementias associated with ambient air pollutants[29-46]. *The overarching theme of this P01 is to examine neural pathways in cognitive responses to air pollution.* Animal models of traffic-related air pollutants (**TRAP**) show strong evidence of neurotoxicity, but there are still important knowledge gaps in prior studies of neurotoxic effects of TRAP on human cognitive and brain aging: 1) little long-term address history data; 2) little data on exposure effects before late life; 3) limited data on cognitive effects, particularly mild cognitive impairment (MCI) or AD; 4) limited neuroimaging measures; 5) limited examination of potential confounding early-life factors; 6) studies of older adults mostly on women; and 7) need for better understanding of gene-environment interactions.

Project 2's use of the longitudinal Vietnam Era Twin Study of Aging (**VETSA**; R01s AG018386, AG018384, AG022381) is ideal for addressing these gaps[21,47,48]. It includes male twins ages 51-60 at VETSA 1 (n=1291) and 55-66 at VETSA 2 (n=1205). VETSA is a geographically-diverse cohort from 49 states, offering great variability in TRAP exposure. Subjects have 10-12 hours of neuropsychological, psychosocial, and health/ medical data at each time point; 545 and 447 have structural magnetic resonance imaging (MRI) at VETSA 1 and 2, respectively. We will address knowledge gap #1 by geocoding residential history data back to 1993. Geocodes will be used by the **Environmental Exposures and Neurotoxicology Core C** to derive individual long-term TRAP exposure indices with state-of-the art methods developed by MESA-Air (Multi-Ethnic Study of Atherosclerosis & Air Pollution). *These TRAP indices, in conjunction with innovative AD-related brain signature scores developed by **Neuroimaging Core B** based on Alzheimer's Disease Neuroimaging Initiative (ADNI) data, will then allow us to examine the neurotoxic effects of TRAP on brain and cognitive aging in midlife,* directly addressing knowledge gaps 2-7[49]. NIH[50] and the NIA-Alzheimer's Association[51,52] consensus statements stress the urgency of *early identification beginning in midlife* to predict AD and cognitive decline. As with cardiovascular disease, early detection and intervention is key. The age and detailed characterization of VETSA subjects is ideal for examining effects of long-term TRAP exposure *before* late life in people who are cognitively normal or have early mild cognitive impairment (MCI). Using monozygotic (MZ) twin within-pair analyses, we will also examine effects of exposure controlling for genetic background and rearing environment.

Our **Overarching Hypothesis** is that TRAP exposure promotes the convergence of vascular and inflammation-based pathology and neurodegenerative processes resulting in reduced structural brain integrity, which, in turn, accelerates cognitive decline and risk for MCI and AD. In VETSA, we showed that we can identify MCI in late midlife[53]. In addition, we showed that we can objectively measure compensatory effort required to perform a cognitive task even before cognitive deficits emerge using task-evoked pupil dilation, an innovative biomarker of early risk for MCI and AD[23]. Our basic model focuses on mediation effects; their omission in prior studies is likely to have missed key aspects of TRAP-related effects. Complementing and expanding Project 1's study of women, Project 2 contributes to the overall P01's scientific theme of risk, heterogeneity and mechanisms of long-term TRAP exposure on brain and cognitive aging.

Aim 1. Assess TRAP effects on brain structure/function: Main effects. Hypotheses: **1a**) TRAP exposure will be associated with higher AD-related brain signature scores. **1b**) TRAP exposure will be associated with cerebral hypoperfusion measured via arterial spin labeling (paralleling Mouse Project 4) and increased WM hyperintensities. These are likely to be associated with vascular-related medical conditions. **1c**) Higher TRAP exposure will be associated with *poorer cognitive function and greater cognitive decline over time*.

Aim 2. Assess the impact of TRAP on cognitive and brain aging: Mediation. Hypotheses: **2a**) TRAP exposure will negatively impact episodic memory; this association will be mediated by medial temporal system abnormalities including perforant path abnormalities, i.e., volume reductions in white matter (**WM)** associated with entorhinal cortex (**ERC)** and parahippocampal gyrus (**PHG**) (paralleling Mouse Project 3), and gray matter (**GM**) abnormalities in ERC, PHG, and hippocampus. **2b**) TRAP exposure will be inversely associated with executive function and processing speed; this association will be mediated by mean prefrontal thickness and frontal WM volume. We will also examine GM and WM mean diffusivity (**MD**) in these regions. **2c**) Diffusion tensor imaging (**DTI**), which provides indices of brain microstructure, may be more sensitive than volume or thickness measures, but has not been examined in prior studies. We will examine MD in both WM and GM mediators of memory in the medial temporal regions, and executive function and processing speed in the frontal regions. We will also examine TRAP effects on WM tracts, predicting increased axial or radial diffusivity in tracts connecting frontal regions, thereby indicating axonal loss or demyelination, respectively, as underlying mechanisms. **2d**) Task-evoked pupil dilation indicates need for compensatory effort and is likely associated with tau deposition[23,54-61]. We hypothesize that TRAP exposure will be associated with increased task-evoked pupil dilation, which will be mediated by the AD-related brain signature score linked to elevated tau.

Aim 3. Examine gene-environment (GE) interaction. 3a) We hypothesize that adverse TRAP effects on brain and cognitive decline will differ as a function of *APOE*-ε4 as well as polygenic risk scores for AD, inflammatory processing, and tau metabolism/processing. **3b**) We will use MZ within-pair difference analysis as another approach to shed light on how genetic and environmental influences work in tandem.

2. SIGNIFICANCE

2.1 Ambient Air Pollution: An Environmental Risk Factor for Cognitive and Brain Aging

Alzheimer's disease (AD) and related disorders are extremely costly at personal, social, and institutional levels. Few successful individual-level interventions for modifiable risk factors (e.g., cardiovascular disease, physical activity, diabetes) have emerged. Existing studies suggest that exposure to ambient air pollutants, in particular TRAP, contributes to accelerated cognitive decline and brain aging, as well as increased risk for MCI and AD[42]. Improved knowledge of these associations could enhance efforts to curb fine particulate matter emissions, thereby affecting potential outcomes at a population level. With widespread recognition that neuropathologic changes take place long before the onset of clinical dementia, there is also recognition that early identification of risk for MCI and AD is a most important research goal[52,62,63]. AD has jumped from the 32nd ranked disease for years of life lost in 1990 to 9th in 2010 (largest increase of any disease), and from 17th to 12th for years lived with disability[64]. Expenditures for Americans 65 and older for AD and other dementias were estimated at $214 billion in 2014 alone[65]. Taken together, these factors argue for the need to study the impact of pollutant exposure on cognition and brain longitudinally in middle-aged adults.

Long-term exposure to air pollutants, especially fine particulate matter with aerodynamic diameters <2.5 ($PM_{2.5}$), increase risks for cardiovascular disease and stroke[27]. Animal and human studies show that ozone or PM exposure leads to neurotoxic reactions, including oxidative stress and neuroinflammation, in multiple brain regions[34,38,66-74]. Animal and human studies suggest that poor air quality may also accelerate brain aging indicative of increased risk for AD (accumulation of Aβ[34,69,70,75] and tau/neurofibrillary tangles[34,75-77]), but many of the human studies were conducted in children and young adults. Recent studies by the Project 1 team and others report associations between increased $PM_{2.5}$ exposure and smaller total brain and white matter volumes[78-80], and more silent brain infarcts[79] in older adults. $PM_{2.5}$ exposures have also been associated with poorer cognitive function, although results of pollutant exposure have been inconsistent with respect to which cognitive functions are affected[40-46,81,82]. A study of older women also found that pollutant exposure increased risk for MCI[42]. Traffic-related air pollution (TRAP) is a major source of $PM_{2.5}$ and other pollutants. Thus, these findings suggest a key role of TRAP exposure in brain aging and cognitive function, including risk for MCI/AD.

Braak stages of neurofibrillary tangles show the neuroanatomical progression of AD including neurodegeneration from entorhinal cortex to the hippocampus via the myelinated perforant path[83]. Parahippocampal white matter (**PWM**), includes the perforant path, and recent MRI studies have shown PWM reductions in AD and more moderately in amnestic MCI[84-86], and accelerated memory decline and increased AD risk in cognitively normal older adults[85,87]. TRAP exposure has neurotoxic inflammation and oxidative stress effects. Vascular and inflammatory processes increase the risk of neurodegeneration and AD. And the hippocampal complex and AD are vulnerable to cerebral ischemia. Thus, we hypothesize that TRAP exposure promotes the convergence of vascular- and inflammatory-based neurodegenerative processes. We will focus on this overarching hypothesis in multiple ways: examination of entorhrinal and parahippocampal white matter (paralleling Mouse Project 3); extended examination of white matter with DTI; extending DTI to include axial and radial diffusion as indices of possible mechanisms; extending the study of gray matter volumes to DTI-based mean diffusivity to examine microstructural cytoarchitecture disruptions; and examining cerebral perfusion via arterial spin labeling (paralleling Project 4). In addition, we will conduct cognitive analyses focusing on episodic memory, executive function, and processing speed.

After briefly describing the Project 2 sample, we address 6 significant knowledge gaps (**KG**) below.

2.2 The Sample: Vietnam Era Twin Study of Aging (VETSA; AG018386, AG018384, AG022381)

In Project 2, we will study participants in VETSA (Kremen PI; Franz Co-Director), a set of longitudinal projects on cognitive and brain aging ongoing since 2002. VETSA has 3 key design features: 1) a midlife baseline before substantial age- and AD-related declines have taken place; 2) a large sample with a narrow age range to enhance power to examine within individual differences in change; and 3) a twin design to elucidate genetic and environmental influences. Our baseline assessment extensively characterized a twin cohort of men at ages 51-60 (mean=56 at VETSA 1) and followed them longitudinally six years later (see **Table 1** in Approach section). We administered an extensive neuropsychological test battery to avoid ceiling effects in midlife adults and address the heterogeneity of cognitive abilities and trajectories[21,53,88-93]. We identified subjects with MCI and will compare the association of TRAP and AD-related brain signatures in cognitively normal versus MCI subjects. *We also collect detailed health, medical, and psychosocial data on all participants.* We genotyped *APOE* and now have newly acquired genome-wide genotyping data, allowing for the identification of polygenic risk scores for AD and other phenotypes. VETSA subjects reside in 49 of 50 states. We build on this rich, stable study for the present proposal by also capitalizing on its geographically diverse sample that is perfect for a study of the impact of TRAP on risk factors for MCI and AD. **These features make VETSA uniquely positioned to address the knowledge gaps and the aims of this proposal.**

2.3 Addressing Significant Knowledge Gaps (KGs)

KG1: Limited Data on Long-Term Address Histories

It is important to account for cumulative exposure over time, but studies of dementia risk have used aggregated exposure[94] or exposure shortly before or after dementia[95]. *Using addresses updated in 2016-2017, we will contact VETSA participants to obtain detailed residential histories from 1993 to current time covering a period of approximately 20 years. These data are particularly important to be able to examine exposure variability for individuals who have resided at more than one address (see section 4.2.1) and create measures of long-term chronic TRAP exposure.*

KG2: Little is Known About Exposure Effects on Brain Before Late Life

Most neuroimaging studies on air pollutants have included older adults, but studies of middle-aged adults and the transition from middle to early old age are crucial for advancing knowledge about the impact on risk factors and biomarkers for MCI or AD. It is exactly this transitional period that the VETSA sample covers.

KG3: Limited Data on Cognitive Effects, Particularly Cognitive Functions Associated with MCI or AD

Almost all extant studies of pollution and cognition employed screening instruments or just 2-3 brief individual cognitive tests, which creates problems regarding possible ceiling effects, low reliability, and inadequate coverage of cognitive domains. Coverage is also too limited to draw firm conclusions regarding specificity of effects for MCI- or AD-relevant cognitive domains. *Our work in VETSA has demonstrated the value of extensive neurocognitive batteries*[90,96,97]. Episodic memory was weakly covered in prior studies. Assessment of executive function was barely present and often confounded with processing speed. These are important considerations as several studies show that there are both dysexecutive and amnestic subgroups of adults with MCI or mild AD; indeed, a meaningful proportion (20-25%) has executive deficits greater than their memory deficits[98-105]. These studies have also shown evidence suggesting that the dysexecutive subgroup has more prefrontal abnormalities and is less likely to carry an *APOE*-ε4 allele. Interestingly, the Project 1 team has recently shown negative TRAP exposure-prefrontal volume associations based on voxel-based morphometry[106]. The VETSA neurocognitive battery was specifically designed to be extensive and taxing enough to have the necessary breadth and depth of coverage needed for a middle-aged sample.

KG4: Measures Examined in Neuroimaging Studies of Air Pollutant Exposure Have Been Very Limited

Studies to date (in 2.1) have primarily examined total cerebral volume, gray and white matter volumes of major cortical lobes, and one study of white matter hyperintensities (**WMHs**). However, the first voxel-based morphometry study by the Project 1 team showed smaller cortical gray and subcortical white matter volumes associated with $PM_{2.5}$ in older women[106]. Neuroimaging findings are still too limited to draw firm conclusions. Complementing Project 1, we will examine men and extend neuroimaging modalities (See Approach & **Fig. 6**).

Structural MRI. In Project 2, we will expand on prior studies of structural MRI to include cortical surface area and thickness. We have conducted many analyses of thickness and surface area in both region-of-interest-based and map-based vertex-wise studies, as well as white matter, subcortical gray matter and ventricular volumes[47,107-123] (e.g., **Figs. 1 & 2**). VETSA was the first to show that cortical thickness and surface area are phenotypically and genetically independent at the global level[47,108]. We expect TRAP exposure effects on cortical volumes to be primarily accounted for by effects on thickness, not surface area.

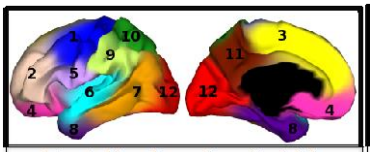


Fig. 1. Map-based cortical SA parcellations (*Science*, 2012)[96]

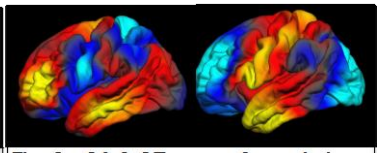


Fig. 2. SA & CT maps of correlations with cognition (*Neuroimage.*, 2016)[104]

WMHs. WMHs in VETSA subjects are predominantly in periventricular and deep parietal and frontal regions, and associated with age and hypertension[124]. WMHs were greater in those with uncontrolled hypertension[124]. To improve understanding of WMH-TRAP exposure associations, we will examine whether those associations differ as a function of key factors such as genetic risks.

DTI. VETSA subjects with longstanding hypertension had reduced fractional anisotropy and increased mean diffusivity—indices of white matter integrity—in most of 9 white matter tracts measured[125]. Longstanding hypertension was defined as having hypertension at both VETSA 1 and 2; shorter duration was defined as hypertension at VETSA 2 but not VETSA 1. Unlike WMHs, DTI effects were stronger in ε4 carriers for 2 tracts connecting frontal regions with other brain areas (uncinate & inferior fronto-occipital fasciculi). Results held up after control for lifestyle and cardio-vascular risk factors. DTI results were unaffected by shorter duration of hypertension or better blood pressure control, indicating differences from WMHs. Thus, microstructural white matter alterations appear early in the course of hypertension and may persist despite adequate treatment.

TRAP exposure seems like a likely additional contributing factor. The Project 1 team found reduced white matter volume associated with $PM_{2.5}$[78]. In preadolescent children, there is evidence of a TRAP exposure association with frontal connectivity, but not with cortical thickness or fractional anisotropy[126]. Their DTI protocol was conducted on a 1.5T scanner with 25 directions. With 3T scanners and 51 directions, our VETSA

2 DTI may be more sensitive. Also, effects on aging and developing brains may differ. With DTI, we can also make stronger inferences about possible underlying mechanisms affecting white matter (e.g., demyelination indexed by radial diffusivity[127-129] or axonal loss or injury indexed by axial diffusivity[127,130,131]). Based on our results, and the voxel-based morphometry results of the Project 1 team, we hypothesize an interaction such that TRAP exposure-diffusivity associations will be stronger in ε4 carriers in tracts linked to frontal regions.

We also recently published 2 papers on cortical and subcortical **gray matter mean diffusivity** showing that they are phenotypically and genetically different from volume[132,133]. Hippocampal mean diffusivity has been found to differentiate cognitively normal and MCI adults[134-136] and to predict memory decline[137] and progression to AD, often with greater accuracy than gray matter volume[137-141]. In addition to white matter DTI, we will examine the relationship of pollutant exposure and gray matter mean diffusivity. Hippocampal volume was not associated with exposure in the Project 1 team's study[78], but there could still be disruption at the microstructural level as indexed by hippocampal mean diffusivity. We have these DTI data in VETSA.

Arterial Spin Labeling/Resting Cerebral Perfusion. Animal models and human studies strongly suggest reduced cerebral perfusion as a biomarker for progression to MCI and AD[142-146]. Hypoperfusion has been observed in MCI compared with cognitively normal adults in several AD-related brain regions (e.g., inferior temporal and parietal, precuneus, posterior cingulate, entorhinal cortex)[142-144,146], and it may be somewhat dissociated with structural differences (e.g., hippocampal volume[146]). Given the expected association between pollution exposure and vascular risk factors, we expect pollutant exposure to be associated with cerebral hypoperfusion in these AD-related regions. In VETSA 2, we collected arterial spin labeling data on 123 individuals, allowing us to conduct the first examination of the association between TRAP exposure and cerebral perfusion. Moreover, this exploratory substudy provides a synergy with Mouse Project 4's hypothesis about chronic hypoperfusion, and is consistent with the overarching hypothesis that TRAP exposure promotes vascular- and inflammatory-based neurodegenerative processes.

KG5: More Studies on Air Pollutant Exposure and Risk for MCI or AD Were Conducted on Women

Our Project 2 study of men complements the Project 1 study of women.

KG6: Need for Genetically-Informative Designs to Understand Genetic *and* Environmental Influences.

Because the *APOE*-ε4 allele is the major risk allele for AD, we will examine *APOE* status as a moderator. In VETSA, we found, for example, no main effect of *APOE* status on hippocampal volume, but as predicted from animal models, there were *APOE* x testosterone interactions; only men with *both* an ε4 allele and low testosterone had smaller hippocampal volumes and poorer episodic memory[110,119,147].

Polygenic risk scores are of interest for cognitive and brain aging and MCI/AD, but also related phenotypes such as inflammation that affect relevant MRI measures (See Approach for details). These have not been examined in studies of TRAP exposure. We will examine moderator effects of polygenic risk scores based on externally-validated SNP-based polygenic scores from large-scale genome-wide association studies.

We will also capitalize on our twin data to implement **MZ within-pair analyses**, a powerful design because each pair is perfectly matched for genetic background, age, and rearing environment. MZ pair differences reflect nonshared environment. Fisher's test of heterogeneity[148,149] (see Approach) indicates the presence of GxE interaction, i.e., differential sensitivity of particular genotypes to particular environments (in this case, TRAP). We have summarized multiple studies using this approach, including our own, that show such GxE interactions[47,150]. Measured genes (e.g., *APOE* or polygenic scores) can also be included in MZ within-pair models, which can be more powerful than studies of unrelated individuals[151]. This tests for "variability genes" that are associated with trait variation rather than just trait mean[152,153]. In particular, evidence by our group and others shows that effects of environmental risk differs as a function of *APOE* genoytpe[110,119,147,150,154-158]. Finally, a postmortem study based on samples of convenience from Mexican cities found that the effects of pollutant exposure (including $PM_{2.5}$ & O_3) were much more strongly associated with hyperphosphorylated tau and Aβ even in children and young adults[75]. We will examine whether effects consistent with elevated tau are present in VETSA subjects and if they differ as a function of *APOE* genotype.

We are uniquely positioned to address these knowledge gaps.

2.4 Early Identification of Risk for Cognitive Decline in VETSA: A Key Component of Project 2

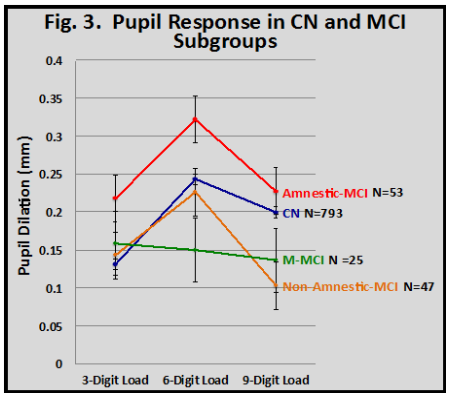
There is now a consensus on the urgent need for early identification of AD because the pathological process begins decades before onset of dementia[52]. Toward that end, early identification of MCI—a transitional phase in the progression to AD—and even identification of individuals at risk before onset of MCI are both critically important. The VETSA project (Project 2) will enable us to examine whether TRAP exposure is associated with risk of cognitive decline prior to MCI and/or with MCI itself.

Early Identification of MCI. We showed that we could identify MCI in VETSA subjects who were only in their 50s—to our knowledge, the youngest demonstration of MCI to date and the first evidence of MCI

heritability[53,159]. *Details of our approach to diagnosing MCI, including our unique use of general cognitive ability (**GCA**) scores from age 20[160], as well as the number of MCI cases are provided in Approach section 4.2.2.b. We hypothesize that greater TRAP exposure will be associated with poorer cognitive performance and MCI.*

Earlier Identification of Risk for Decline with Task-Evoked Pupil Dilation (TEPD). *In VETSA 2 we introduced pupillometry to measure TEPD, a validated psychophysiological biomarker of cognitive effort[23]. Even in the absence of deficit, if 2 people have the same cognitive score, the one requiring more effort to attain that score is likely to be at higher risk for cognitive decline or MCI because they are closer to the point at which they can no longer compensate with increased effort[161]. Two well-known TEPD phenomena highlight its usefulness in assessing risk for decline[23,56-61]: 1) increased cognitive load requires greater effortful resource allocation, indicated by increased pupil dilation; 2) when beyond cognitive capacity or ability to compensate, system breakdown is reflected by drop-off in pupil dilation.*

In VETSA 2, we assessed TEPD by measuring pupil size increase from baseline during digit span tasks at low, moderate, and high cognitive loads (3, 6 & 9 digits)[23]. We compared MCI and cognitively normal (**CN**) groups (**Fig. 3**). Normal performance (max span) but increased TEPD in amnestic single-domain MCI indicated compensation capacity remaining. *The use of digit span is not to focus on that specific cognitive ability. Indeed, digit span differentiated amnestic single-domain MCI and CN groups even though it was not impaired in amnestic MCI.* Consistent with their apparent inadequate compensatory capacity to generate enough effort, non-amnestic single-domain-MCI subjects had worse performance than the CN group, but they had lower cognitive reserve (age 20 general cognitive ability). Lack of adaptation to cognitive load in the multi-domain MCI group suggests compensatory functions becoming exhausted with advancing disease. *The CN group has a normal distribution of TEPD. Thus,* we predict that even in the CN group, greater TRAP exposure will be associated with greater TEPD, indicating need for greater compensatory effort even before cognitive deficits become manifest.



Pupillary responses reflect activation in the locus coeruleus (**LC**) and related brain systems that modulate cognitive effort allocation[56-60,162,163]. Degenerative LC changes take place in the earliest stages of AD[54,83]. In Braak et al.'s study of >2300 brains, LC was the earliest site of tau deposition with tau misfolding appearing before tau spread to the cortex in successive Braak stages (predating Aß)[164,165]. *The ventral attention network is strongly linked to the LC[166], and we recently found that ventral attention network activations during resting state fMRI were associated with TEPD patterns (submitted ms.).*

In sum, key to the goal of early identification of risk, TEPD objectively indexes cognitive effort, providing an innovative means to detect risk for decline, even before observable performance deficits[167-169]. We hypothesize that greater TRAP exposure will be associated with greater TEPD, after accounting for differences in general cognitive ability. Because TEPD also reflects the function of the AD-tau-related LC attention network[162], we also predict that TEPD will be associated with the brain signature associated with tau pathology derived from ADNI data by Neuroimaging Core B.

Significance Summary. After accounting for different brain pathologies, Boyle et al concluded: "Identification of the mechanisms that contribute to the large unexplained proportion of cognitive decline is urgently needed to prevent late life cognitive decline"[170]. Here, we address the infrequently studied contribution of TRAP exposure's *neurotoxic effects on brain and cognitive aging in midlife. The transition from midlife to early old age has still received insufficient attention, even though early identification is now recognized as critical for addressing modifiable risk factors. Our richly characterized, large longitudinal community-based cohort allows for consideration of how TRAP may drive neurodegeneration, addresses an important transitional period with respect to exposure in men, and complements Project 1's study of women, as well as Project 3 and 4 mouse studies' examination of DTI and perfusion. Our wealth of high quality cognitive, neuroimaging, medical and psychosocial data at 2 timepoints is a strength and allows for multilevel modeling. In addition, the use of GWAS and twin data has the capacity to yield new findings about biological effects of exposure to air pollution.*

3. INNOVATION

There are multiple features that make this proposal innovative: **1.** To our knowledge, this is the first study of the impact of TRAP on early indicators of risk for MCI and AD *before* late life, including risk for cognitive decline. ***2.*** *We will utilize state-of-the-art indices of cumulative long- and short term TRAP exposure indices created by Environmental Exposures Core C.* **3.** We will examine mediator/moderator models—something that has not been done in prior studies of TRAP. **4.** We will utilize newly derived AD/MC/tau brain signatures created via state-of the art methods developed by the Neuroimaging Core B. In particular, we focus

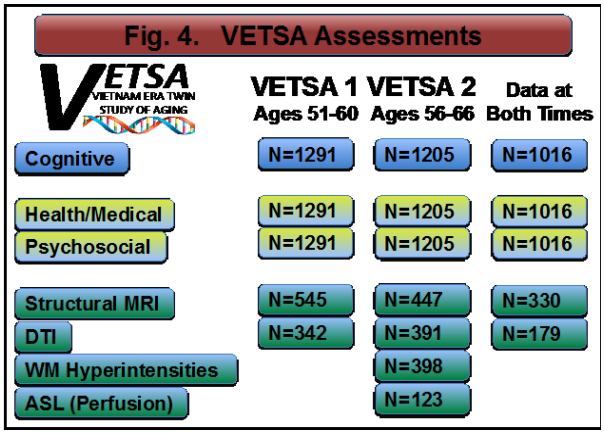
on GM and WM DTI, indices of brain microstructure that are not captured by standard thickness or volume measures, and may be more sensitive than the latter measures. We will also examine cerebral perfusion in a subset of subjects (n=123). No other studies of the impact of TRAP have examined DTI or perfusion. **5.** We have more extensive neuropsychological assessments than prior studies. This is important for obtaining robust measures that are not based on single tests, and it is particularly important for adults who are only in middle-age in order to have good sensitivity and avoid ceiling effects. Uniquely, we also have a general cognitive ability score from age 20, which provides additional insight into cognitive change and the impact of cognitive reserve. ***6.*** Our all-male, national sample will extend prior studies of the impact of TRAP in older adults because most have most have been based on female samples. **7.** The twin nature of the sample means that we can perform MZ within-pair analyses that are uniquely able to provide information about gene-environment interplay. To our knowledge, this is the first twin study of effects of TRAP exposure on cognitive and brain aging in adults. **8.** Polygenic risk scores and *APOE* genotype will be assessed as moderators. Genome-wide genotyping data on VETSA subjects provides an additional source of innovation. *This has never been examined. Genetic moderators will be incorporated into state-of-the-art mediation models using structural equation modeling and latent variable analysis.*

4. APPROACH

This will be a very cost-efficient project because of all of the cognitive, neuroimaging, demographic, clinical, and genotyping data that is already collected. Prior assessments in the VETSA Parent Projects provide the majority of data for Project 2. The major time and expense is related to collection of residential histories, geocoding, and data analyses by Project 2. The Project 2 team will work closely with the Environmental Exposures Core C which will create individual time-varying long-term TRAP indices and with Neuroimaging Core B for re-processing and harmonizing MRI data to create brain signature measures.

4.1 Participants: Ascertainment and Representativeness

VETSA 1 subjects were *randomly recruited* from a large, prior Vietnam Era Twin Registry (VETR) study conducted in 1992[171]. *Subjects were age 51-60 (mean age 56) in VETSA 1 and 56-67 (mean age 62) in the VETSA 2 follow-up (**Fig. 4**).* VETSA is a geographically diverse, national sample with subjects residing in 49 of 50 states. Indeed, it is a reasonably representative epidemiological sample with strong demographic and health similarities to census/CDC data for similarly aged men (e.g., education, income, diabetes, obesity, hypertension)[48,172-176]. Mean GCA is near average (IQ=104-5)[177-180]. Most of the major AD studies (e.g., ADNI) have highly educated samples; VETSA is more representative with mean education of 13.8 years (SD=2.1). This community-dwelling sample comprises twins who were in the military sometime between 1965-1975; *nearly 80% were not in combat or in Vietnam*. Actually, 20% of men over 60 in the U.S. are Vietnam era veterans[181], but that is ignored in almost all studies. Although VETSA is an all-male sample, it complements the all-female Project 1. We had an 82% retention rate in VETSA 2; *the mean time to follow-up was 5.6 years. An additional 243 attrition replacement subjects were enrolled into the study at VETSA 2[21,182]. Thus, there is a total of 1480 individuals in either or both waves of the study (see **Fig. 4**).* Sample sizes corresponding to different VETSA assessments are shown in **Fig. 4**. At VETSA 2, 391 individuals have structural MRI, DTI, and WMH data, 55 of whom have MCI (Ns for MCI are detailed in 4.2.2.b). *All participants have cognitive, health/medical, genetic, and psychosocial data.* For MZ within-pair analyses, there are 330 MZ pairs at VETSA 2, and 108 pairs with all 3 of these imaging modalities.



4.2 Measures

4.2.1 New Data Collection

Residential Histories. *The VETR maintains updated, but not historic, address and contact information for the twins; addresses were last updated in 2016-2017. This information will be used for re-contacting VETSA subjects by VETR staff who are in regular contact with twins in order to maintain enthusiasm for research activities. The VETR does not allow investigators to make initial contact with potential subjects for new studies. Using procedures validated by members of Environmental Exposures Core C to obtain as accurate histories as possible, the VETR will mail letters that explain the present study and ask twins to complete and return residential history questionnaires. Additionally, we will use multiple telephone follow-ups, informants, and electronic database searches for non-responders. Address histories will be from 1993 to current date. Existing VETSA address data will serve as a reliability check.* Subjects will receive $25 for participation. We expect a high rate of participation because this request requires little time or burden. *Subjects are committed to*

VETSA and have already participated in multiple waves with full-day assessments involving higher burden. Subjects will be approximately 66 (range 61-71) years old when contacted for address histories and will have multiple modalities (i.e., verbal, written) in which to provide addresses, increasingly the likelihood of participation and reliability of reports. With national distribution comparable to Project 1 and the MESA projects[38,183], TRAP exposure measures will be obtainable from the 48 contiguous U.S. and will represent a wide range of exposures to air pollutants. Fewer than 0.1% of participants live outside the contiguous states.

Geocoding Residential Data. Residential history data will be entered into a database by the Project 2 team. Advised by Environmental Exposures Core C, we will use standardized geocoding procedures[184-186]. Geocoding can be conducted at high levels of reliability, validity and confidentiality[186]. We focus on residential history because even middle-aged adults are likely to spend a higher proportion of their time near their residences. *Residential instability will be tracked and examined in data analyses.*

Traffic-Related Air Pollution (TRAP) Indices.

Recent state-of-the art spatiotemporal historical prediction model techniques developed by members of Environmental Exposures Core C (e.g., MESA-Air) provide precise and finely resolved exposure estimates with national coverage in 48 contiguous states[4-6,183]. TRAP will be characterized by estimated ambient levels of NO_2 (a gaseous surrogate), elemental carbon component of $PM_{2.5}$ (a marker of diesel exhaust particles)[187-190], and predicted source profiles of $PM_{2.5}$. Using geocoded VETSA data, Core C will create individual-level, residence-specific time-varying exposure estimations of these TRAP exposures from 1993 to current. The innovative approaches to estimate TRAP exposures developed by Core C's team integrate measurements from the EPA Air Quality environmental data resources with spatiotemporal covariates assembled in the MESA-Air Exposure Assessment Core Database (http://depts.washington.edu/mesaair).This approach models pollutant concentrations as a linear combination of temporal basis functions with spatially time-varying coefficients and spatiotemporal residuals with a universal Kriging framework produced across the contiguous U.S. at fine resolution[6,10-13,191-194]. The resulting exposure estimates therefore capture both regional- and local-scale variability[5,7,9,13]. Yearly temporal resolutions will be provided. The MESA-Air database includes over 150 geographical/spatiotemporal variables that may be of use as covariates in the current study[192,193]. The same TRAP indices will be developed for Projects 1 and 2 (details in Core C). The historical MESA-Air model for 1999-2013 has high levels of cross-validated accuracy of prediction (R^2 values >= 0.85) and well-calibrated prediction intervals[4,183].

Due to fewer air monitoring sites prior to 1999, the models starting with 1993 data cannot perform with the same effectiveness as the 1999+ models. However, to an unprecedented extent the new models allow for meaningful assessment of long-term concentrations for epidemiological analyses[4-6,183]. The majority of VETSA participants live near major population areas where monitoring was continuous and intensive. Measurement error will be higher in participants who live in less monitored areas or outside of the contiguous US[8]. Data analyses will be conducted with and without participants in less monitored areas. Detailed address histories combined with yearly temporal TRAP resolutions will allow for analysis of time-varying exposures. The MESA-Air team has developed and statistically validated algorithms for examining time-varying exposures and estimation error which will be included in data analyses[5-7](see also Core C). We will also include covariates reflecting participants' residential history (e.g., moving), regional characteristics, and exposure uncertainty.

Our collaborators in Environmental Exposures Core C have made significant contributions to understanding the sources and impacts of measurement error on estimation of health effects of air pollution in epidemiologic cohort studies; to designing exposure models to minimize the impact of this error and correct for it using post hoc bootstrap and asymptotic calculations as well as methods for selecting and combining data from multiple sources, exposure estimation, choice of exposure prediction models, and reduced-rank spatiotemporal modeling, and adjusting for unmeasured temporal confounding in estimating acute air pollution health effects as well as actively compared the reliability and validity of different models for TRAP indicators and developed efficient pre-adjustment methods that use all available exposure data[5-10,195]. These resources will be available to VETSA investigators throughout data cleaning/preparation and data analysis. Long-term (1993 to current) and shorter-term (3 years prior to each VETSA data collection) air pollution concentration measures will be created and used in data analysis.

ADNI-Derived AD-Related Brain Signatures. Based on ADNI data, Neuroimaging Core B will create *6* AD-related "signatures" based on cortical patterns that best characterize cases versus controls[196,197]. As detailed in Core B, "Evolving Partitions to Improve Connectomics" (EPIC)[198] is a data-driven approach that clusters or subdivides regions to partition the cortex so as to optimize the classification of disease based on brain connectivity or cortical thickness[199]. Beginning with vertex-wise cortical thickness measurements derived from FreeSurfer, it will use ADNI data to arrive at thickness patterns that best separate high- and low-risk subjects. EPIC uses logistic regression as a classifier and leave-one-out validation within ADNI. Predicted scores will indicate the probability of being in the high-risk category based on the patterns for each analysis. *Thus, higher scores mean the person is more "case-like."* Mean thickness values within the AD-related

signatures will be included in analyses. We will also use TRAP exposure cortical thickness signatures created by Neuroimaging Core B, based on the highest and lowest quartiles of TRAP exposure for training sets. EPIC will provide the mean cortical thickness within that signature pattern for every subject. This mean value can then be included in structural equation models to evaluate a mediation effect between TRAP and cognition. The 6 AD-related brain signature measures are: **1)** AD; **2)** incident MCI, i.e., cognitively normal who progress to MCI within 2 years; **3)** pre-clinical AD, i.e., cognitively normal Aβ positivity (Aβ+); **4)** tau, i.e., signature associated tau positivity; **5)** MCI; ***6) Aβ+ vs. Aβ-.*** *These will allow for early identification of brain disease patterns; we hypothesize that TRAP exposure and MCI will be associated with higher, more case-like, scores*.

4.2.2 Previously Collected Measures (from the VETSA Parent Project)

4.2.2.a Neuroimaging (MRI) Measures

The Neuroimaging Core B will harmonize MRI measures across Projects 1 and 2, accounting for scanner differences, and will assist in extending Project 2 measures to include FreeSurfer automated white matter parcellations[200-202] and voxel-wise DTI measures within those white matter parcellations. Resting cerebral perfusion is in VETSA only, and will not be handled by Neuroimaging Core B. The imaging methods provided here delineate the acquisition and post-processing that has already been completed.

VETSA Original Image Acquisition. Imaging data were acquired on Siemens 1.5T scanners at VETSA 1 and on 3T GE and Siemens scanners at VETSA 2 with 8-channel phased array head coils. Three sequences were acquired, including a 3D T1-weighted volume and 2D proton density(PD)- and T2- weighted images. The full multi-channel dataset was employed in a complementary processing path for precise measurement of cerebral white matter and abnormalities within the white matter (i.e., hyperintense regions on T2) along with definition of CSF in subarachnoid spaces and measurement of intracranial volume (ICV). T1 was a sagittal IR-FSPGR with TI=600ms, TE=minimum full, flip angle=8, slice thickness=1.2mm. The 2D sequences were coronals with 2mm slice thickness and include a T2: TE=94ms, TR=4.6s, ETL=16, 2 NEX; and a PD: TE=17ms, TR=3s, ETL=4. Sequences had FOV=24cm and frequency encoding in the S/I direction.

Diffusion data were acquired using single-shot EPI with isotropic 2.5 mm voxels and 51 directions with a b-value of 1000 s/mm2 (matrix size=96x96, FOV=24 cm, 47 axial slices, slice thickness=2.5 mm), covering the entire cerebrum and brainstem without gaps. Two brief EPI scans were also acquired to correct distortion caused by magnetic field inhomogeneities. T1 images were rigidly resampled into alignment with an atlas brain for a common, standard orientation across participants. Diffusion-weighted images were corrected for eddy current distortion[203], head motion[204], B0-susceptibility distortions[205], and gradient nonlinearity distortions[206]. These images were registered to T1 images and rigidly resampled into the standard T1-based orientation at a 2x2x2mm resolution[207]. Cubic interpolation was used for all resampling steps. Conventional DTI methods modeled the tensor as an ellipsoid where eigenvalues |1, |2, and |3 defined the 3 primary axes[208-212].

T1-Weighted Volumetric Segmentation, Cortical Reconstruction and Parcellation[200,201,206,213-221]. T1 images were corrected for gradient nonlinearities[220] and image intensity non-uniformity[222] that significantly improve detection of subtle changes[206]. The semi-automated 3D segmentation procedure delineates 11 "subcortical" structures including hippocampus, cerebellum, and ventricles using a probabilistic atlas and Bayesian classification rules[223,224]. Cortical surface reconstruction allows whole neocortex surface-based analyses for cortical thickness (CT) and surface area (SA), and regional parcellations[213-219]. The Desikan parcellation atlas is particularly relevant to aging and AD, and has high reliability with manual parcellations[221]. FreeSurfer also parcellates white matter regions underlying specific cortical regions[200,201]. Segmentation and CT measures[213-219] have high correlations with manual measures in vivo and ex vivo[215,225], and many reports shows their sensitivity to subtle brain changes in aging, AD and other conditions[223,225-237].

Structural White Matter Abnormalities. We used validated methods for measuring white matter hyperintensities, which also necessitated defining CSF in subarachnoid spaces and measurement of ICV[238-242]. This included re-slicing to a standard space; intra-subject mutual information registration[243]; bias-correction with N3[222]; skull-stripping; gray matter, white matter, and CSF segmentation; and semi-automated white matter hyperintensity designation based on location and geometric rules.

DTI: Indices of White and Gray Matter Microstructure. DTI measures may reflect greater axonal coherence, density or myelination (fractional anisotropy)[210,244,245] and myelin changes and variations in intra/extra cellular spaces (mean diffusivity; MD)[246]. We also obtain axial and radial diffusivity, which are thought to reflect axonal loss or injury[127,130,131] and demyelination[127-129], respectively. Measures of cortical and subcortical MD add additional useful information. For example, our new findings indicate that gray matter mean diffusivity is more strongly related to age than gray matter volume[132]. Atlas-based tract analysis[247] of white matter DTI was based on a manually-constructed probabilistic diffusion tensor atlas with information about locations and orientations of 23 fiber tracts[247]. T1-weighted images are used to map into a common space. Based on the T1 FreeSurfer-based anatomical stream, cortical, basal ganglia, and thalamic gray matter and all CSF are excluded during the fiber tract atlas application to ensure that tracts course only

through white matter regions. Average fractional anisotropy and mean diffusivity are calculated for each tract, weighted by the fiber probability at each voxel. Fibers include: cingulum, fornix, parahippocampal projections, thalamic radiations, corticospinal tract, uncinate, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, superior longitudinal fasciculus (dorsal components and arcuate fasciculus). The protocol also allows estimation of fiber orientation density to capture more complex fiber architecture[248].

Arterial Spin Labeling/Resting Cerebral Perfusion. By magnetically labeling water, an endogenous contrast agent is formed that can trace the flow of blood into an imaging plane, and a quantitative measure of perfusion can be calculated by region. ASL was conducted at VETSA 2 with a FAIR QUIPSS II method (TI1/TI2=600/1600ms; 1 cm tag-slice gap; 10 cm tag width; TR 4000 ms) covering 24 slices (220mm FOV; 64x64 matrix; 4mm thick with 1mm gap)[249,250]. Arterial spin labeling raw image data are converted into calibrated cerebral blood flow maps, segmenting high-resolution anatomical images to identify voxels primarily of CSF. Image inhomogeneities are corrected for with a smoothed version of the minimum contrast calibration image[251]. CSF voxel selection is applied to the CSF calibration image to obtain an estimate of the average CSF signal intensity, which is in turn used to estimate the equilibrium magnetization of arterial blood[250,252]. Use of this estimate, along with literature values for blood magnetic relaxation times, provides a scaling term that is applied to the average difference of the control and tag images to form a cerebral blood flow image in units of ml/(100g-min). FreeSurfer regions of interest can create masks at the resolution of the quantitative cerebral blood flow maps in order to calculate mean cerebral blood flow in each region.

4.2.2.b Neurocognitive Battery (Table 1)

General Description. We use an extensive and taxing cognitive battery to avoid floor and ceiling effects in middle-aged adults, and because *change is neither monotonic nor uniform*[96]. Our battery, with the same measures at VETSA 1 and 2, covers a broad range of cognitive functions with emphasis on executive functions, processing speed and episodic memory. **Cognitive reserve** is also an important construct in cognitive aging and risk for MCI or AD[285-287] . We are in the rare situation of having a direct reserve measure—Armed Forces Qualification Test (AFQT)[160]—from age 20 that is more sensitive than a proxy measure such as education[288]. In 35- and 42-year follow-ups we found that the AFQT had high reliability and test-retest correlations of .73-.85[19,160].

Table 1. Basic In-Lab and At-Home Assessments

***In-Lab*—Neurocognitive Domains and Tests**	
Handedness	Oldfield Handedness Inventory[253]
Gen'l. Cogn. Ability	AFQT[254];
Verbal	WASI Vocabulary[255]; Wide Range Achievement Test-3 Reading subtest[256]
Abstract Reasoning	WASI Matrix Reasoning[255]
Working Memory	WMS-3 Digit & Spatial Span[257]; Letter-Number Sequencing[257]; Reading Span[258]; AX-CPT[259]
Inhibitory Control	AX-CPT[259]; Stroop Interference[260] (adjusted for word & color)
Other Executive Functions	Verbal Fluency (letter, category); Trail Making Test (switching adjusted for other conditions); Category switching adjusted for fluency[261]. All from D-KEFS[261]
Processing Speed	D-KEFS Trails scanning, number sequencing, letter sequencing[261]; Simple/Choice RT; Stroop Word, Stroop Color[260]
Episodic Memory	WMS-3 Visual Reproductions & Log. Memories[257]; CVLT-2[262]
Visual-Spatial	Mental Rotation[263]; Hidden Figures[264], AFQT box folding[254]
Context Processing	AX-CPT (signal detection perceptual sensitivity measure [d^1])[259]
***In-Lab*—Objective Health Measures/Medical History Interview:** Chronic Diseases, Psychological disorders, Cancer History, Pain & Stiffness, Tinnitus, Smoking, Alcohol Use, Head Injury, Sleep Habits, Incontinence, Sexual Function, Family History of Dementia, Cause/Dates of Parental Death; Height, Weight, Girth, Aud. & Vis. Acuity, Vis. Contrast Sensitivity, Walk Test, Rise from Chair, Pulse Oximetry, Grip Strength, BP (4 x), Ankle-Arm Index, Pulmonary Function Tests, Fasting Blood Chemistries (Glucose, Insulin, LDL, HDL, TGs, CRP), DNA, Meds ('Brown Bag')[177,265-269]	
***At-Home*—Psychosocial Questionnaire:** Demographics, Exercise, Activities, Life Complexity, Life Events, Subjective Health (SF-36), Memory Complaints, Health Habits & Utilization, Depression, Anxiety, Well-Being, Life-Satisfaction, Perceived Stress, PTSD Checklist, Life Events, Attachment Style, Marital Adjustment, Social Support, Personality, Self-Esteem, Impulsivity, Control[270-284]	
***Telephone*—MRI Screening**	
***Remote-Lab*—**Genome-Wide Genotyping; APOE	
AFQT=Armed Forces Qualification Test; WASI=Wechsler Abbreviated Scale of Intelligence[255]; WMS=Wechsler Memory Scale[257]; CPT=Continuous Performance Test[259]; RT=Reaction Time; D-KEFS=Delis-Kaplan Executive Function System[261].	

MCI. VETSA MCI criteria are based on the Jak/Bondi actuarial-neuropsychological approach, *which has been validated in multiple independent samples*[289-294]. In ADNI subjects, the Jak/Bondi definition outperformed the traditional clinical ADNI diagnosis on all validators: more converted to AD; fewer reverted to normal; higher proportion had *APOE*-ε4 alleles; and more abnormal levels of CSF Aß$_{42}$, tau, and p-tau compared to cognitively normal subjects[289]. Also prevalence based on Jak/Bondi criteria was lower, consistent with increased false positives with a "one-test" approach.

Clinical diagnostic criteria for MCI or preclinical AD by the NIA-Alzheimer's Association[51,52] require limited "one-test" cognitive assessment or clinical judgment. ADNI took this approach, using only a single Wechsler Memory story. This approach overlooks the possibility of impairment in other memory tests or other cognitive domains, reduces reliability compared with multiple tests, and increases false positives[289,292,294,295]. For example, over 20% of healthy older adults have one impaired score in 2 different cognitive domains; less than 5% have 2 or more impaired *within* a domain[296]. The Jak/Bondi approach emphasizes more thorough neuropsychological assessment, which is important for a disorder marked by cognitive impairment.

In our approach, the diagnosis requires 2 tests within a domain that are below the impairment cutoff as this has been shown to have the best balance between sensitivity and specificity[289]. MCI definitions can then

be subdivided by amnestic/non-amnestic, single/multiple-domain, or specific domains. We employ 18 neuropsychological measures covering 6 cognitive domains[53,297]. Uniquely, we adjust for age 20 AFQT, ensuring that MCI reflects decline rather than just low ability. We determine consensus diagnoses based on all current and prior test, interview, and questionnaire data, including concerns about or changes in function[298]. Some researchers might consider our VETSA 1 classifications to be pre-MCI, but pre-MCI adults are still at increased risk for progression to MCI and dementia; cognitive deficits can predict progression in those cases even in the absence of observed clinical impairment[299]. *Within VETSA, our MCI diagnosis has been validated in subjects who were only in their 50s by: 1) showing greater hippocampal atrophy amnestic MCI compared with CN subjects[159]; and 2) new data (Logue et al. under review) showing that higher AD polygenic risk scores, based on the International Genomics of Alzheimer's Project (IGAP) data, were associated with significantly greater odds of having amnestic MCI than being CN even after excluding APOE-related SNPs (best differentiation: odds ratio [OR]=1.43; OR=3.22 for upper vs. lower quartile of polygenic risk score distribution).*

MCI Stability. *In VETSA 2, a total of 162 people had MCI. Of 967 with data at both timepoints, 107 (11%) had MCI at VETSA 1; 148 (15%) had MCI at VETSA 2. Additional cases are from replacement subjects. For those with MRI data, 66/518 (12.7%) had MCI in VETSA 1; 69/422 (16.4%) in VETSA 2.* Given the age of the sample, there are few incident cases. However, it is well known that any diagnosis of MCI may change over time. The optimal approach in VETSA is to focus on stable cases (diagnosis at both timepoints). These are most likely to be valid MCI cases: *120/148 (86%) were stable MCI; 61 of those had MRIs at both times.*

4.2.2.c Health/Medical and Psychosocial Measures (Table 1)

VETSA has detailed psychosocial and lifestyle questionnaires *completed by all subjects at VETSA 1 and 2* that assess demographics, marital history, types of activities, religious involvement, living arrangements, exercise frequency[270,271], subjective health[272], memory complaints, health utilization, depression[273], anxiety[274], well-being and life satisfaction[275,300,301], stress and posttraumatic stress symptoms[276,277,302], social engagement, attachment and social support[279-281], and personality[281-284]. Medical history interviews, functional tests at both times, and blood chemistries at VETSA 2 provide information on vascular-related conditions, pulmonary function, *APOE* load, fitness, hormonal levels, physical health, head injury, and impaired motor and sensory capacities that may affect brain and cognitive aging[88,303-314]. Blood pressure and pulmonary function are associated with white matter abnormalities and/or cerebral volume decreases[315-318]. Some data also indicate that controlling for vision and hearing can be as effective as controlling for processing speed in explaining age-related variance in several cognitive abilities[319]. These measures, *collected from all subjects*, reflect major moderating/mediating influences on cognitive aging outcomes.

4.2.2.d Pupillometry

Pupillometry is conducted with a NeurOptics PLR-2000 portable pupillometer that records pupil responses for up to 15 sec. at 13 samples hand-held per sec. Spans of 3, 6, and, 9 digits (low, moderate, high loads) are presented aurally at 1 digit per 1.5 sec. while subjects view a gray dot on a white background (~200 lux) in a viewing cone. The cone covers 1 eye; the subject covers the other with his hand, avoiding confounds of external light or head movement. Resolution is superb: mean error=.052mm (99% CI=.048-.056; NeurOptics data, n=655). Pupil responses are screened for artifacts; a MATLAB algorithm also removes blinks and minor artifacts. A smoothing filter is used, and average pupil areas are calculated. We measure: 1) digit recall; 2) baseline pupil size; and 3) effortful resource allocation (change in pupil diameter relative to baseline pupil size). *Pupillometry was collected on all subjects at VETSA 2.*

4.2.2.e Genotyping and Polygenic Risk Scores

Genome-wide genotyping on all VETSA twins was done with the Illumina OmniExpress-24 chip by deCODE. Imputation based on 1000 genomes *is completed*. *APOE has been genotyped separately[320]. Using all SNPs in our genotyping data, we have also calculated an AD polygenic risk score based on the largest meta-analysis of AD risk genes and the full International Genomics of Alzheimer's Project (IGAP) data[321-324]. The meta-analysis[322] also denoted subsets of AD risk genes that were related to different functions. There are 2 of these subsets that are clearly related to the present proposal, so we will focus on them as additional relevant polygenic risk scores. The 2 functions and the genes that will comprise the respective polygenic risk scores are: inflammation and immune response (APOE,BIN1,CR1,CD33,MS4A,CLU,ABCA7, EPHA1,MEF2C,HLA-DRB5-DBR1,INPP5D,TREM2); and tau metabolism/processing (BIN1,CASS4,FERMT2, MAPT).*

To boost the power of polygenic risk score in VETSA, we will utilize recently developed gene enrichment and genetic-pleiotropy-informed methods[325-335]. Details of the fitting algorithm are in *Bioinformatics*[335]. By incorporating auxiliary information (functional annotations of the genome and pleiotropic relationships), information from test statistics are up- or down-weighted depending on level of 'enrichment'. The estimation methods are empirical, i.e., the resulting conditional false discovery rate (FDR) is allowed to depend on the auxiliary information, but if the information is not helpful the resulting estimates are not biased toward

any given solution[329]. This novel conditional FDR incorporates prior information about gene element-based functional annotations of SNPs, so SNPs from categories enriched for non-null associations have a lower FDR for a given test statistic than SNPs in unenriched categories. This readjustment of FDR is based on functional annotations by fitting a covariate-modulated parametric 2-group mixture model. In a test with Crohn's disease, the result was dramatically increased power; the number of loci declared significant at an FDR of .05 increased by a factor of 5.4[335]. Also, SNPs declared significant replicated in much higher numbers than with usual FDR, yet there were similar replication rates for a given FDR cutoff in *de novo* samples[335].

4.2.2.f Data Collected Prior to VETSA

We also have data *on all twins* extending back to age 20[48]: a) age 20 AFQT, height, weight, BMI, blood pressure; b) 1986, 1992 height, weight, BMI, health, health history, social support, combat history[172,174], posttraumatic stress symptoms[336] and c) 1992 lifetime DSM-III-R diagnoses/symptoms[171].

4.3 Data Analysis

4.3.1 General Analysis Approach. *The proposed study involves two time-points of data for the primary outcomes. To effectively address missing data under the general missing at random (MAR) mechanism, longitudinal regression and mediation models will be employed for examining the proposed hypotheses[337,338]. These models also readily address correlated outcomes within each twin pair. Unless indicated otherwise, data from both VETSA 1 and 2 will be included in all analyses in which data at time 1 for attrition replacement subjects will be treated as missing completely at random (MCAR)[338-340]. Also, the correction for correlated observations is not needed for MZ within-pair analyses.*

For regression analysis (main and moderation analyses), both the (parametric) generalized linear mixed-effects models (GLMM) and (semi-parametric) augmented weighted generalized estimating equations (AWGEE) will be used[16,339]. Unlike GLMM, which posits distribution models, AWGEE requires no such assumption, yielding valid inference for a broader class of data distributions under the most popular MAR mechanism in real studies. Thus, if discrepancies arise between the two, AWGEE results will be reported. Mechanistic hypotheses concerning mediation (main and moderation analyses) will be examined using both parametric and semi-parametric structural equation models (SEM)[15,341]. As in regression analysis, the semi-parametric SEM-based mediation models require no distribution assumption, providing valid inference for a broader class of data distributions. Thus, results from the semi-parametric models will be reported if discrepancy arises between the two approaches. Model fit will be assessed by chi-square test, the comparative fit index (CFI), the index of Tucker and Lewis (TLI), and Root Mean Square Error of Approximation (RMSEA)[341-343].

We set α=0.05 for hypothesis testing, and use Holm-Bonferroni adjustments for <10 tests[344,345], Westfall-Young resampling-based maxT procedure for 10-100 tests, and FDR methods (an inherently exploratory hypothesis-testing context) for >100 tests.

4.3.2 Covariates. Other covariates may include*: AFQT score at age 20 , demographics (age, race/ethnicity, education, occupation); health/medical variables (body mass index, blood pressure, metabolic syndrome components, cardiovascular diseases, depression, alcohol, PTSD, neurologic conditions, head injury, cancers, medications); and psychosocial factors (SF-36, stressful life events, including combat exposure), residential instability. These measures are listed and referenced in Table 1.*

TRAP exposure-related covariates derived by Environmental Exposures Core C include: exposure uncertainty, urban/rural categorization for residential neighborhoods based on 1990 and 2000 U.S. census classification; census tract-level contextual SES characteristics of residential neighborhoods; and meteorological variables (e.g., dew point and temperature averages) based on distance-weighted k-nearest-neighbor average within 50 km buffers based on daily data from the National Climatic Data Center Network[346].

4.3.3 TRAP Exposure. *As described in section 4.2.1 and in Environmental Exposures Core C, time-varying, exposure-related TRAP indices will be created by Core C for Project 2. They will include ambient-predicted spatiotemporal concentration for ambient-source PM2.5 and gaseous pollutants from 1993 to 2013 for each subject's address (which may change over time due to relocation) modeled in universal Kriging frameworks. For analyses, long-term (1993 to 2013) and shorter-term (3 years prior to VETSA 1 and 2) exposures, variables will be created by averaging the time-varying TRAP measure(s) over time. Pollutant concentration may vary due to a number of characteristics (e.g., time, distance to monitoring station, availability of different types of air quality indicators, mobility, changes in geography) which will be evaluated, operationalized and included in data analyses. A number of these issues have already been addressed by members of Core C[5-10].*

4.3.4 Aim 1. Assess TRAP effects on brain structure/function: Main effects. Hypotheses: **1a**) TRAP exposure will be associated with higher AD-related brain signature scores. **1b**) TRAP exposure will be associated with cerebral hypoperfusion measured via arterial spin labeling (paralleling Mouse Project 4) and

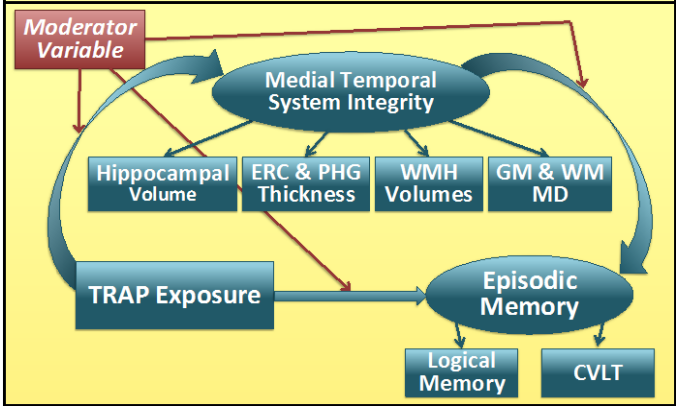
increased WM hyperintensities. These are likely to be associated with vascular-related medical conditions. **1c**) Higher TRAP exposure will be associated with *poorer cognitive function and greater cognitive decline over time*.

Analytic Strategy for Aim 1a-c*) The longitudinal methods discussed in* ***4.3.1*** *will be applied to examine both the hypothesized long-and short-term effects of TRAP on brain signature scores, where the AD-related brain signature score or cognitive measures will be the response (dependent variable) and TRAP exposure will be the predictor, controlling for covariates (both TRAP and non-TRAP related). The hypotheses will be supported by a significant predictor main effect.*

4.3.5 Aim 2. Assess the impact of TRAP on cognitive and brain aging: Mediation models. Hypotheses: **2a**) TRAP exposure will negatively impact episodic memory; this association will be mediated by medial temporal system abnormalities including perforant path abnormalities, i.e., volume reductions in white matter (**WM)** associated with entorhinal cortex (**ERC)** and parahippocampal gyrus (**PHG**) (paralleling Mouse Project 3), and gray matter (**GM**) abnormalities in ERC, PHG, and hippocampus. **2b**) TRAP exposure will be inversely associated with executive function and processing speed; this association will be mediated by mean prefrontal thickness and frontal WM volume. We will also examine GM and WM mean diffusivity (**MD**) in these regions. **2c**) Diffusion tensor imaging (**DTI**), which provides indices of brain microstructure, may be more sensitive than volume or thickness measures, but has not been examined in prior studies. We will examine MD in both WM and GM mediators of memory in the medial temporal regions, and executive function and processing speed in the frontal regions. We will also examine TRAP effects on WM tracts, predicting increased axial or radial diffusivity in tracts connecting frontal regions, thereby indicating axonal loss or demyelination, respectively, as underlying mechanisms. **2d**) Task-evoked pupil dilation indicates need for compensatory effort and is likely associated with tau deposition[23,54-61]. We hypothesize that TRAP exposure will be associated with increased task-evoked pupil dilation, which will be mediated by the AD-related brain signature score linked to elevated tau.

Analytic Strategy for Aim 2a-d*) Shown in* ***Fig. 5*** *is an example of a path diagram for mediation analysis (excluding moderators [in red]) with TRAP exposure's influence on cognition taking place through its effect on brain structure. Ellipses represent latent variables and rectangles represent measured variables; errors of measurement are not shown but will be estimated. The mediation approach discussed in* ***4.3.1*** *will be applied, with each measure of medial temporal system abnormalities as the mediator and episodic memory as the outcome. If the null hypothesis of full mediation is rejected, direct and indirect intervention effects, and percent of mediation will be reported. Both long- and short-term TRAP effects will be examined. The same basic model is applicable to Aim 2b-d. The mediators may be assessed at time 1 (or, if not available, at time 2) for causal interpretation.* Additionally, we can test models with AD-related brain signature scores as mediators; these could be measured variables or latent brain signature variables created using the regions of interest comprising those scores.

Fig. 5. Sample SEM Model



4.3.6 Aim 3. Examine gene-environment (GE) interaction. 3a) We hypothesize that adverse TRAP effects on brain and cognitive decline will differ as a function of APOE genotype (ε4+ vs. ε4-) as well as polygenic risk scores for AD, inflammatory processing, and tau metabolism/processing. **3b**) We will use MZ within-pair difference analysis as another approach to shed light on how genetic and environmental influences work in tandem.

Analytic strategy for Aim 3a & b*. 3a) The same longitudinal methods in Aim 2 will be applied to test a series of moderation hypotheses (see Fig 5, red rectangle). For each outcome measure of brain or cognition, TRAP exposure and a moderator variable under consideration and their interactions will serve as predictors of the model, controlling for covariates. Depending on the analysis, moderators (see* ***Fig. 5****) will be APOE genotype or a particular polygenic risk score. The hypothesis will be supported by a significant TRAP-by-moderator interaction.* ***3b****) We will use MZ within-pair difference analysis as another approach to shed light on how genetic and environmental influences work in tandem. We can evaluate the extent to which individual differences in change are accounted for directly and indirectly from TRAP exposure.* Regarding Aim 3b, examinations of within-twin pair differences among MZ twins are potent tools to consider environmental factors and G-E interplay. We will implement within-pair MZ difference approaches to evaluate the presence of GxE[148,149,347-349]. These approaches are variants of the co-twin control method[350] but have increased power because continuous measures can be used. In the within-pair difference approach, differences within MZ pairs

indicate unique person-specific environmental influences, as genes contribute to between-pair but not within-pair MZ differences. There is, of course, some measurement error contained in within-pair differences as well. If, for example, *APOE* genotype or polygenic risk scores contribute to heterogeneity in within-pair differences, it suggests that genetic factors contribute to differential sensitivity to environments (GxE). Our strategy will include: 1) testing for mixture distributions of MZ within-pair differences for continuous traits (e.g., cognitive domains, MRI measures); 2) evaluating whether particular genotypes or polygenic risk scores explain heterogeneity in MZ within-pair differences; 3) evaluating whether MZ within-pair differences in brain and cognitive decline correlate with differences in TRAP exposures dependent on genotype or polygenic risk scores. We are mindful of work suggesting the importance of considering and adjusting for measurement error, non-shared confounders, and non-normality in the application of the described within-twin pair methods[351,352].

4.3.7 Power Analysis. The various MRI-based, cognitive, and polygenic risk score measures are all continuous variables. The only dichotomous variables that are the focus of the analyses are MCI and *APOE* genotype. With respect to aim 1, *phenotypic power estimates are based on sample sizes n=125 for ASL* and from n=255 (highly conservative adjustment for correlated observations) to n=1200 for other outcomes for a two-sided α=.05. For main effects in Aim 1, w*e have >80% power to detect small effect sizes (Cohen's d=0.19 [n=255] or d=0.04 [n=1200]) in mean level change in continuous measures from time 1 to time 2 (assuming a within-subject correlation 0.5) for 1a and 1c, and medium effect size d=0.43 for 1b. For mediation hypotheses in Aim 2, we have >80% power to detect small indirect mediation (r^2=0.02-0.04) for n=1200 and medium indirect mediation (r^2 = 0.09) for n=255. For moderated mediation via APOE or polygenic risk scores in Aim 3a, we have >80% power to detect differential mediation comparing small to medium indirect effects (e.g., APOE ε4+ vs ε4 in contrast to cognitive measures).* For MZ within-pair differences in Aim 3b, we have >80% power to test for mixture distributions, with within-pair variance ratios ranging from 1.2 to 2.6 across genotypes (e.g., *APOE*). *Ns are smaller for MCI comparisons with respect to a few MRI-based measures; therefore, we consider those particular analyses to be exploratory. We have power >80% to detect small mean, d=0.27 (n=1200) or medium to large, d=0.68 (n=255), effect size differences between MCI vs non-MC assuming a 13% rate of MCI. For testing moderation of bivariate associations by MCI status, e.g., TRAP and brain risk signatures, we have >80% power to detect medium to large effects assuming a 13% MCI rate.*

4.3.8 Limitations and Strengths

Limitations. *Measurement error will be higher in participants who live in less monitored areas or outside of the contiguous US[8]. The majority of VETSA participants, however, live near cities with continuous and intensive monitoring. Analyses will be conducted with and without participants in less monitored areas. We will also include covariates reflecting participants' residential history and will be able to analyze whether mobility is due to cognitive status (or other measures).* Further, some measurement error due to the inability to estimate time-activity information or micro-environments is inevitable. However, members of Environmental Exposures Core C have developed innovative methods of accounting for such error *and residential mobility affects PM2.5 less than NO2*[8].[353-355]. Other pollutants will not be estimated, but prior studies find little or no confounding due to other pollution sources[41,356-360]. It is not possible for us to examine conversion to MCI prospectively in this proposal because there are too few subjects in the VETSA sample who convert from cognitively normal at VETSA 1 to MCI at VETSA 2.

Strengths. Air pollution exposure effects are an important public health issue, but they have been understudied in midlife, particularly as they relate to risk for MCI and AD, and particularly in men. The well-characterized, geographically diverse VETSA sample is ideal for addressing these gaps. Starting data collection from a younger age (age 51-60) also helps reduce selection bias. To our knowledge, no human study has examined effects of air pollutants on brain microstructure or cerebral perfusion, but VETSA DTI and ASL data make these advances possible. Although we cannot examine conversion to MCI, we do have a large proportion of MCI subjects who were stable, i.e., they met criteria for MCI on both occasions. This is a strength that is different from the ability to examine conversion. A fair amount of people diagnosed with MCI revert to normal on follow-up, but those diagnosed with MCI at 2 timepoints are more likely to be "true" MCI cases. The enormous amount of already collected VETSA data also make this project extremely cost-efficient. *As part of a thematic program project, the proposed study capitalizes on collaboration among multiple teams that provide state-of-the-art methods for measuring TRAP exposures, early identification of cognitive decline, and analysis of neuroimaging data.* It will also contribute to translational science with aims that are synergistic with the two animal model proposals within the proposed program project.

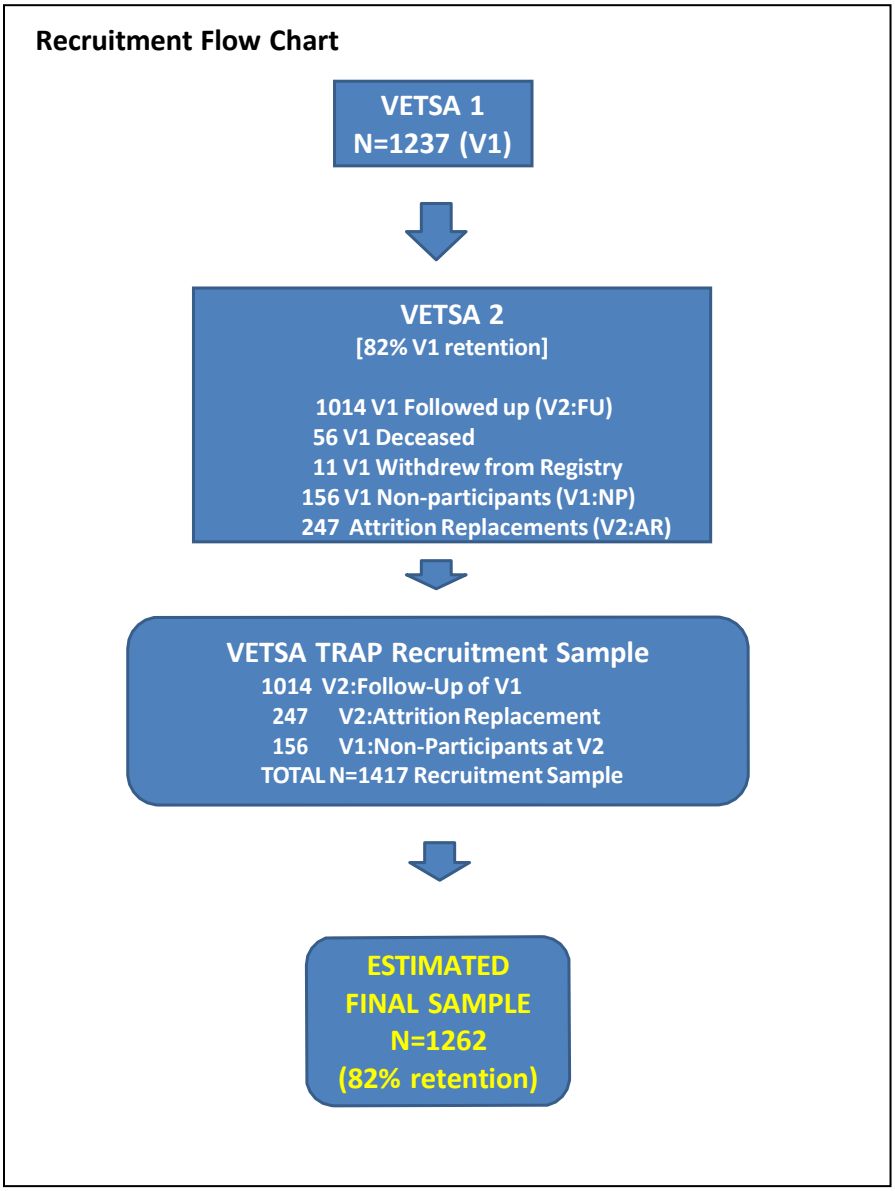
PROTECTION OF HUMAN PARTICIPANTS

1. Risks to the Subjects

A. Human Subjects Involvement, Characteristics, and Design.

a) Selection of Participants and Eligibility:

All twins in this study (VETSA-TRAP) were participants in either the baseline VETSA study (2002-2008; R01 AG018384; AG018386 PIs Kremen & Lyons), participants in the VETSA 2 follow-up and/or are attrition replacement subjects added in VETSA 2 (2008—2014): N=1417. The two separate grants,VETSA MRI and VETSA Cortisol (R01 AG022381; AG022982; PI: Kremen), collected additional data from eligible VETSA participants. VETSA 2 followed up the 1237 VETSA 1 and VETSA 1 MRI participants with an 82% retention rate and an additional 243 attrition replacement twins were added. Attrition replacement subjects were randomly recruited from remaining VETR twin pairs in the same age range (51-66 years old). Of the men who did not participate in VETSA 2, 56 (4.5%) had died.



Twins are all members of the Vietnam Era Twin Registry (VETR) at the Puget Sound VA Healthcare System in Seattle, WA, and signed agreements with the VETR to be contacted to participate voluntarily in research studies. The VETR, created in 1986, is a registry of same sex male twin pairs who both were in the U.S. military between 1965 and 1975 (the "Vietnam Era").

Original ascertainment. In brief, the VETSA 1 participants were *randomly recruited* from 3322 male-male twin pairs that were assessed in the VETR 1992 Harvard Drug Study. The Harvard study, *which did not select on the basis of any participant characteristics*, recruited from 7500+ twin pairs in the VETR. It attempted to enroll as many twins from the VETR as possible. The majority of twins did not serve in Vietnam or Southeast Asia (78%).

The VETR maintains regular contact with all of the twins in the Registry through newsletters and the twins' participation in research studies; thus their database has current addresses (so as to expedite mailings). The VETR also has authority to use Lexus Nexus to assess status of non-responding twins.

Eligibility. Our proposal will collect detailed address information from all participants from VETSA 1 and/or 2. Average age was 56 (range 51-60) at VETSA 1 and age 62 (range 56-66) at VETSA 2. Addresses were most recently updated in 2016—2017 when participants were on average 66 years old.

Characteristics. The sample comprises a relatively representative epidemiological sample of community-based men in regards to marital, work, income, and health characteristics of American men in their age range based on the United States (U.S.) Census and Center for Disease Control data (CDC). In VETSA 2, for instance, 79% of participants were married; 4.6% never married; 2010 U.S. census figures show 72% of men age 50-59 currently married and 10% never married. Median income for households 55-59 years old in the U.S. was $65,571 while for the VETSA 2 men household median income was $60,000-$69,000. Many health characteristics were very parallel for men in this age group.

Because this is a study of the long term effects of air pollution on brain and cognitive aging building on previously collected data, children are not included in our proposal. No other vulnerable populations (i.e.

fetuses, neonates, prisoners, institutionalized individuals) are part of the targeted sample.
b) Overview of Measurement:
Participants will be mailed a consent and a form requesting information on address history since 1994; up to 3 mailings will be sent, with reminder calls following the second mailing.
c) Collaborating sites and roles of collaborating investigators.

1. VET Registry (Smith, site PI): Mail questionnaires and consent to collect address history.

   Nicholas Smith, PhD (Director VET Registry and PI, VETR subaward) is responsible for overseeing address collection and providing historic data to VETSA investigators.

d) Data protection. The VETR protects the identity of participants in the twin registry by creating identification numbers that are only accessible to the registry. Dr. Franz and Kremen, and VETR staff will have restricted access to the name-to-number lists. All faculty and staff are required to have training in protections of human subjects and follow internal guidelines (e.g., data cannot be left unattended on desks; computers need to be locked when not in active use, data cannot leave premises, etc.).

Only de-identified data sets are provided to co-investigators, students, or post-doctoral fellows conducting data analysis; these are given to the user as encrypted password protected files and—if mailed—are mailed via trackable mail. All investigators, staff, and students associated with the VETSA must be compliant at all times with university and VA human subjects' protections certifications. All personnel sign "access agreement forms" on which they acknowledge the need for confidentiality as well as the highest standards of ethical and professional behavior.

B. Sources of Research Material. New data to be collected are residential address histories from 1994 to present which will then be geocoded. Other VETSA data includes interview responses, results of neuropsychological tasks, questionnaires, interviews and sequencing data from previous data collections. Participants will be mailed a consent form, and a letter describing the study along with a standardized residential address history form.
a) Sources
1) Address history (new)—collected by mailed questionnaire.
Data analyses will include previously collected data/phenotypes from VETSA participants:
2) Previously collected data from VETSA 1 & 2 (cognitive, psychosocial, psychological, blood chemistry); VETSA 1 & 2 MRI studies: raw images plus cortical and subcortical brain structures, diffusion tensor imaging. VETSA 1 Neuroendocrine:cortisol, testosterone, and DHEAS. For all subjects: APOE and GWAS data.
3) Previously collected archived data from the VETR includes data gathered at induction (~age 20), health surveys administered at ages 37 (Survey of Health) and 40 (NHLBI study), and diagnostic psychiatric interviews ("Harvard Drug Study") in 1992 (~age 42).
b. *How specimens, records, and/or data are collected, managed, and protected*.
VETSA TRAP participants will be informed about the nature of the study and invited to participate by letter. They will be provided with an address history form, which they will complete and return to the VET Registry. The VETR will then provide that form to the VETSA staff for geocoding.

Data are protected by assigning new IDs, limiting access to data on a need-to-know basis, and keeping entered data in password protected files. Only de-identified data sets are shared.
c. Who will have access to individually identifiable private information and how is it protected?

The staff at the VETR, Drs. Franz and Kremen are the only persons who will have access to individually identifiable private information. Geocoded location data will not be linked with any PHI. There is a very remote possibility someone could be identified by their genetic information, this is addressed in the section on adequacy of protection against risks.

Electronic data are protected by password-protected computers and electronic files that are additionally protected by university firewalls and virus protection. All UCSD servers and network access are encrypted. File cabinets and rooms housing computers are locked when not in use by project staff. Codes that link project IDs and personal information necessary to contact and schedule twins are kept in locked databases on separate protected computers from the ones with data collected as part of the VETSA.

C. Potential Risks.

a. Potential risks, likelihood, and seriousness to human subjects.

The main risks are risks due to breach of privacy and confidentiality. To circumvent this, confidentiality is protected by using ID numbers, rather than names, on study documents and in any analyses. Hard copies and computer-based data are identified only by code. Hard copies of data are and will continue to be maintained in locked file cabinets, and computer-based data are and will be stored in password-protected files. The code-book that links ID numbers to participants is and will be maintained in a locked cabinet accessible on

a "need-to-know" basis to the UCSD Principal Investigators only. Geocoded data provided to Core C for creation of TRAP indices will be blind to any other identifying information. Any MRI data provided to Core B will be de-identified.

b. Alternative procedures. The alternative procedure(s) are to not provide cumulative address histories (addresses at VETSA 1 and VETSA 2 were already provided, so some address data is present) or to not participate.

2. Adequacy of Protection against Risks

A. Recruitment and Informed Consent. The participation of every subject in this research is voluntary. All participants will be asked to sign a consent form; previous consents allow for use of their previously collected data in ongoing research.

a. Recruitment. Recruitment will take place through letters mailed by the VETR.

b. *Informed Consent*. An informed consent is part of the mailed recruitment letter packet which also includes the address history form.

During the consent process, per UCSD policies, the participant is informed of the following information about the test protocol. He will be:

a) asked to provide as much information about his address history from 1994 to present;
b) informed that the data will be rendered anonymous and distributed only to qualified investigators;
c) informed that all efforts will be made to maximize his confidentiality;
d) informed that there will be no cost to participants;
e) informed that there are no treatments associated with the study;
f) informed that there are generally low risks associated with tests being conducted;
g) informed that all information we collect will remain confidential, and names will not be attached to any data at any time;
h) reminded that their previously collected data will be used in the course of data analysis, at no cost to them;
i) informed that the de-identified data collected in the course of this study may be made available to other researchers who have access to the (unidentifiable) data collected in this study, and may have commercial interests;
j) informed that under circumstances of unique genotypes (if previously collected DNA is examined), there is a very remote possibility that enough information might be developed to allow individuals to be identified and consequently become subject to any risk(s) that might arise as a result of that identification;
k) assured that genetic material and the genetic information will not be shared with any health maintenance or health insurance companies;
l) informed that the researchers will not give participants specific information about what is found out about their genes;
m) informed that non-participation is an alternative to participating in this study, and if the subject decides not to participate, there will be no penalty or loss of any benefits to which the participant is entitled.

B. Protection against Risk.

a. Procedures to protect from risks:

The other risks to human participants relate to the privacy and confidentiality of names and addresses, interview, genotype, and test data. The names of participants who are unwilling to participate in the proposed study will not be released to investigators. Although the names and addresses of Registry members who do participate are released to specified investigators, the unique Registry identification number that links individual Registry data currently in the possession of the investigators with specific names will not be included. Only a unique project ID is included.

To protect identity, geocoded location will be kept separate from other data and will never appear in any master data set; geocodes will only be used to create cumulative air pollution indices and will not be available to personnel outside of an internal core group of investigators. Only measures of cumulative TRAP (this measure is non-identifying) will be given to other researchers or will be included in the master database. Staff who work on linking the geocoded data with air pollution data will not have access to any other information/data about the participant.

To reinforce the absolute requirement for strict confidentiality, all project investigators and staff will sign a confidentiality agreement. Violation of confidentiality will be sufficient grounds for immediate suspension from employment. Risks of participation are minimal.

b. Privacy/Confidentiality of study reports:

Using procedures already in place as part of currently funded projects, staff and investigators will be trained extensively on the importance of confidentiality. In terms of data management and data sharing, the sample participant data is given a unique identification number that is separated from personally identifying information. This ID number is used not only for data entry, but also for communication between sites.

Any data provided by VETSA to collaborators will be stripped of identifiers, and will be transmitted via access to password protected firewalled servers at each site. These data will then be merged with other data at UCSD. After merging, these data will be collated and posted on a secure server at UCSD for examination and analysis by study investigators. All data will be reported as aggregates and never identified by individuals' names. These procedures have been shown to be effective in reducing potential risks to confidentiality. We constantly monitor best practices to maximize confidentiality/privacy.

3. Potential Benefits of the Proposed Research to the Subjects and Others

Participants do not receive any direct benefit. The importance of the results to the fields of neuropsychology, and cognitive impairment is due to the potential for prediction and early intervention for those affected by MCI. If risk and protective factors as well as genetic and environmental influences on cognitive aging can be identified early, the information may be useful for developing interventions for modifiable risk factors. Prevention of loss of cognitive function by even a few years can have a major impact on the personal, financial, social, and societal burden of cognitive decline. Thus we believe the risks to the participants are reasonable in relation to the anticipated benefits to research participants and others.

4. Importance of the Knowledge to be Gained

The development of mild cognitive impairment is an important indicator of Alzheimer's and has potentially severe consequences for the individual and their family. Further, the economic cost of Alzheimer's far surpasses other disorders of similar prevalence. The findings from this proposed study will contribute to the improved identification of individuals at risk for MCI and AD, and could potentially improve outcomes. The procedures in the proposed study all involve minimal risk, and are therefore reasonable in relation to the importance of the knowledge that may be expected from this study.

An improved understanding of genetic and environmental (and interactive) causes of MCI and cognitive decline will make significant contributions to the potential for developing effective interventions to mitigate or slow cognitive decline. Alzheimer's disease and other dementias are extremely costly diseases in terms of years of life-lost, years of disability, as well as their social burden on families (see US Burden of Disease Collaborators. The state of US health, 1990-2010: Burden of diseases, injuries, and risk factors. *JAMA.* Aug 14 2013;310(6):591-608[12]). The project is a unique and valuable resource for studying aging in the US population as well as in veterans. It is unique among other twin studies in its focus on middle-aged adults and use of cutting edge detailed biomarkers, cognitive assessments, brain measures, environmental measures, and its broad array of important psychosocial and health phenotypes collected at multiple time points.

5. Data and Safety Monitoring Plan (DSMP)

The procedures in this study involve minimal risk and we therefore anticipate a very low level of adverse events. If, however, an adverse event does occur, we will immediately notify the UCSD University Office of Responsible Research Practices and the NIH.

The risks of participation in this study are minimal and we believe we have developed protocols that successfully minimize existing risks. Thus, the benefits of conducting this research program far outweigh the risks. The project provides information with great public health relevance regarding traffic related air pollution as risk factors for AD, MCI, cognitive aging or brain age-related declines. Thus the risks to participants are reasonable in relation to the importance of the knowledge that may be expected to result.

INCLUSION OF WOMEN AND MINORITIES

No new participants will be added to Project 2. Project 2 new data includes collection of address histories from previous VETSA participants so as to create longitudinal TRAP exposure scores for the sample thus the characteristics are those of the parent study. However, within the P01, Project 1 studies only women so the all-male Project 2 counterbalances that.

A. *GENDER*

Only male twins will participate. Women are not included for several reasons—first, this study is built primarily on use of previously collected data from the VETSA studies which only included men from the VET Registry. The VET Registry includes only male-male pairs who both served in the military between 1965--1975. The absence of women in the Registry is not the result of an arbitrary decision during the construction of the Registry; rather, it reflects the sex differences in the likelihood of serving in the military during the Vietnam era. Finally, this is a longitudinal study so the participants will focus on those people previously enrolled in the VETSA studies (all men).

With regard to the original construction of the VET twin registry in 1986 and ascertainment of women. During the Vietnam era approximately 5,500,000 men served in the US military. During the same period, 187,381 women served in the military and very few of these women were twins (this indicates that approximately 3.4% of veterans from this era are women). Given that we are able to obtain data from approximately 3,200 male twin pairs from that era, one interpolation would suggest that we might have been able to include 112 female pairs maximum. However, this number is predicated on a number of assumptions that are probably overly favorable. For example, if the percentage of men from the entire population who served in the military was 40%, then the joint probability of both members of a male pair serving in the military if the service of each twin is independent of his co-twin's service would be the joint probability of two men both serving together (0.4 x 0.4 = .16). If the percentage of women from the population who served in the military was 1%, then the probability of both members of a female twin pair serving in the military would be significantly lower than for men (0.01 x 0.01 = .0001). Thus, for every 1,600 pairs of male-male twins there would be one female-female pair. This analysis suggests that the expected number of female pairs would be two. The actual number probably lies somewhere between these most and least favorable estimates, but closer to the latter. Another concern in including female pairs in the Registry was the bias against locating married women when the registry was constructed. Because the identification of twins in the Registry depended upon both twins having the same last name, any pair in which either or both twins changed her last name could not have been identified. We do not have information about the number of female veterans who were married or the proportion of those who changed their last name, but this factor would have systematically eliminated a proportion of female twins.

B. *MINORITY GROUPS*

In regard to inclusion of minority individuals in our study, based on VETSA data collection from previous years, the expected distribution of ethnicity is 88% non-Hispanic white, 4.3% African-American, 2.9% Hispanic, 0.6% Native American/Alaskan. The remaining participants describe themselves as more than one race or declined to answer. This distribution is very close to the ethnic and racial distribution of the VET Registry.

PHS Inclusion Enrollment Report

This report format should NOT be used for collecting data from study participants.

OMB Number:0925-0001 and 0925-0002
Expiration Date: 10/31/2018

***Study Title:** Urban Air Pollution and Pathological Brain Aging: a Nationwide Twin Study in Men

***Delayed Onset Study?** ❑ Yes ☑ No

If study is not delayed onset, the following selections are required:

Enrollment Type ☑ Planned ❑ Cumulative (Actual)

Using an Existing Dataset or Resource ☑ Yes ❑ No

Enrollment Location ☑ Domestic ❑ Foreign

Clinical Trial ❑ Yes ☑ No

NIH-Defined Phase III Clinical Trial ❑ Yes ☑ No

Comments: This new data collection for the air pollution study is based on a longitudinal study of men (VETSA); there were no women in the sample and new data only will be collected from research participants who participated previously. Thus the number and gender of participants in the categories for planned enrollment reflect that of previous VETSA participants.

Racial Categories	Ethnic Categories: Not Hispanic or Latino			Ethnic Categories: Hispanic or Latino			Ethnic Categories: Unknown/Not Reported Ethnicity			Total
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	5		0	4					9
Asian	0	0		0	0					0
Native Hawaiian or Other Pacific Islander	0	4		0	0					4
Black or African American	0	48		0	6					54
White	0	1124		0	19					1143
More than One Race	0	44		0	8					52
Unknown or Not Reported										
Total	0	1225		0	37					1262

INCLUSION OF CHILDREN

Children and adolescents will not be included as research participants. First this is a longitudinal study of cognitive aging, beginning at mid-life, thus by definition the study is of older adults. This proposal focuses on early identification of mild cognitive impairment as well as mid- to late-middle age change in cognitive and other functioning. Therefore, although a lifelong study of children and/or adolescents would be immensely valuable, it is not feasible at this time.

MULTIPLE PD/PI LEADERSHIP PLAN

Rationale for choosing a multiple-PD/PI approach

Drs. Kremen and Franz have collaborated as co-Directors of the joint VETSA projects (2002--ongoing), starting with co-writing the first VETSA grant. Given that Project 2 relies on VETSA data, it makes sense to continue this as a collaboration. The long history of collaboration between these two PIs has been very productive and free of problems.

Roles and administrative, technical, and scientific responsibilities for the project for each of the PDs/PIs and other collaborators.

Dr. Franz at UCSD will provide oversight of the entire research project and implementation of all policies, procedures and processes. Dr. Franz and Kremen will be responsible for the implementation of the Scientific Agenda and the specific aims and ensure that systems are in place to guarantee institutional compliance with US laws, DHHS and NIH policies including biosafety, human research, data and facilities.

Dr. Franz will serve as contact PI and will assume overall fiscal and administrative management including maintaining communication among investigators and key personnel through monthly meetings. She will be responsible for communication with NIH and submission of annual reports. Dr. Franz will supervise the data collection activities of the address histories. The PIs will share responsibility for overseeing each of the specific aims. There are some differences in the expertise of each PI. Dr. Kremen has a long history conducting cognitive neuroscience-oriented research, initially with schizophrenia and for well over a decade with cognitive aging research. His expertise has allowed him to integrate experimental neuroscience methods with imaging data and the twin method to address critical issues in the domain of cognitive and brain aging in novel ways. Dr. Franz brings an extensive background in risk and protective factors (environmental, medical, health, and psychosocial) for cognitive and brain aging.

Fiscal and management coordination

Dr. Franz will be responsible for fiscal and research administration at UCSD.

Process for making decisions on scientific direction and allocation of resources

The two PIs will have equal responsibility for making decisions on scientific direction and the allocation of resources. As mentioned above, they have been close collaborators for over 15 years. During that entire period there has never been an issue that resulted in conflict or an important decision that was not reached quickly with complete agreement.

Data sharing and communication among investigators

The PIs and investigators will meet weekly to discuss experimental design, data analysis, and all administrative responsibilities. The PIs and co-investigators share their respective research results with each other. They discuss any changes in the direction of the research projects and the reprogramming of funds, if necessary.

A very important function for the leadership structure of Project 2 is facilitating the efficient attainment of the project's goals. To this end, we have multiple meetings where priorities for papers and analyses are discussed. Priorities are set out according to the central aims of the grant and the core issues. We use people with different expertise to weigh in on the papers and analyses in their areas. Anyone wishing to undertake data analyses and prepare a paper or conference presentation submits an abstract explaining the research question to be addressed along with the main variables, required resources, etc. A key consideration is always determining the fit of any proposed analysis with the major study goals.

Publication and intellectual property (if needed) policies

Publication authorship will be based on the relative scientific contributions of the PIs and key personnel. Proposals for data analysis and manuscript preparation will be submitted to the EC to prevent duplication of effort. We do not foresee any issues regarding intellectual property resulting from the proposed study.

Procedures for resolving conflicts

If a potential conflict develops, the PIs will meet and attempt to resolve the dispute. If they fail to resolve the dispute, the disagreement will be referred to the VETSA Executive Committee.

If either PI moves to a new institution, attempts will be made to transfer the relevant portion of the grant to the new institution. In the event that a PI cannot carry out his duties, a new PI will be recruited as a replacement at one of the participating institutions.

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CONSORTIUM ARRANGEMENTS

The following institution and Principal Investigator is collaborating on Project 2 (VETSA Air Pollution study) and will have a consortium arrangement with UCSD. The institutional agreements is attached.

INSTITUTION	PRINCIPAL INVESTIGATOR
Vietnam Era Twin Registry (Seattle VA)	Nicholas Smith, PhD

SCOPE OF WORK

VETR16C02: Urban Air Pollution and Increased Risk for Pathological Brain Aging: A Nationwide Twin Study in Men.

Vietnam-Era Twin (VET) Registry staff, under the direction of Registry Director, will work with Dr. Kremen from the University of California, San Diego (UCSD) to create study-specific data sets for the project entitled “Urban Air Pollution and Increased Risk for Pathological Brain Aging: A Nationwide Twin Study in Men.”

VET Registry staff, in accordance with the VET Registry policies and procedures, will coordinate initial recruitment efforts for this study; this includes mailing written study questionnaires and telephone follow up for non-responders to this effort.

Over the specified recruitment period, the VET Registry will obtain and gather the names and most recent addresses and telephone numbers of about 1,500 twins. VET Registry staff will attempt to locate and recruit every twin to complete the questionnaire from the sample requested by UCSD. The location efforts for both current mailing addresses and telephone numbers will include: National Change of Address searches; Post Office address correction requested on all correspondence; directory assistance; manual searches of electronic directories; Lexis Nexis searches; and telephone contact location efforts.

VET Registry staff will print and mail introductory letters, written questionnaires, consent forms, addendums, and provide business reply envelopes (BRE).

In an effort to achieve a high response rate among twins, VET Registry staff will send each twin as many as three mailings (more, if requested by a twin). In addition, twins who do not respond either positively or negatively to the mailings will be called to determine whether we have located the right individual and whether they would be willing to participate in the study. Additional questionnaires will be mailed with BRE as required.

VET Registry staff will forward all completed questionnaires from twins to VETSA on a bi- monthly basis along with a count of all cases in final disposition: deceased, incapacitated; refused; and study (survey) completion.

VET Registry staff will also mail compensation to twins who return a completed questionnaire.

VET Registry staff will send progress reports to the investigators on a weekly or bi-weekly basis throughout the course of their involvement in the study.

VET Registry staff will update its database with members’ updated contact information obtained during data collection.

Throughout the duration of the study, the VET Registry manager, research coordinators, and research assistants will track and monitor the use of Registry data, Registry members, and maintain institutional approvals. When the study is complete, Dr. Kremen will transfer any new study data to the VET Registry Data Manager who will prepare data collected and generated as part of this study for archiving into the VET Registry data repository.

Nicholas L. Smith, PhD
Director, VET Registry

Personnel

Nicholas L. Smith, PhD, VET Registry Director, will devote 0.24 calendar months to this project in Project Years (PYs) 1-5. Dr. Smith is the Director of the Seattle Epidemiologic Research and Information Center and the VET Registry. He is also a Professor of Epidemiology at the University of Washington and works primarily in the area of cardiovascular epidemiology with a focus on pharmaceuticals and genetics. Dr. Smith has overall administrative and scientific responsibility for the VET Registry. He will work in concert with Dr. Kremen to implement the VET Registry portion of the study. Dr. Smith's time is donated.

Cindy Liu, **Program Manager** will devote 0.60 calendar months to this project in PYs 1-5. The Manager will manage all the activities of the VET Registry. She is responsible for supervising all VET Registry staff including the Research Coordinator and Research Assistants. The Manager will be the primary liaison for the study, and will work with VET Registry and University of California, San Diego study staff to launch this study. The Manager will dynamically monitor the release of VET Registry data to the project and its return or destruction at the study site during the course of the study. Using email and telephone communication with study staff, the Manager will keep track of the progress of the study. The Manager will be responsible for managing the mortality search and will coordinate the newsletter and website production activities. The Manager will design several tracking data systems for subjects. The Manager will be supervised by Dr. Smith; they will have regular meetings about study progress.

Emily Wing, **Research Coordinator,** will devote 1.20 calendar months in PY 1, and 0.84 calendar months in PYs 2-5. The Research Coordinator will be responsible for daily operations of the VET Registry as it involves the proposed study. This includes maintaining ongoing communications with and providing updates to the research staff throughout the duration of the study. This also includes answering and fielding any inquiries from VET Registry members about the study to appropriate research staff. In PY 1 the Research Coordinator will develop the R&D and Human Subjects protection applications for submission to the VA Puget Sound R&D and IRB committees. The Research Coordinator will also oversee and track VET Registry IRB and other VA Puget Sound reviews and approvals for this study, and will work with the study site to ensure the Registry has copies of study site IRB approvals. Additional responsibilities include updating, managing, and tracking Data Use Agreements (DUAs), and other data security plans. The Research Coordinator will track and monitor VET Registry member participation in the study including updating VET Registry dispositions. The Research Coordinator will also receive and track mailing materials as it involves the proposed study. The Research Coordinator will also be responsible for tracking training at the level of the study site, and tracking any publications produced by the study site. The Research Coordinator will also conduct core Registry activities such as mortality searches, newsletter production, and website support.

Bailey Clopp, **Research Assistant,** will devote 5.40 calendar months in PYs 1-2.The Research Assistant will be responsible for record keeping maintenance for the VET Registry as it involves the proposed study. The Research Assistant will coordinate the tracking of written questionnaires, and perform data entry for completed and returned surveys. Tracking activities include hardcopy storage, and quarterly audits of study questionnaires. The Research Assistant will oversee the storage of all hardcopy documents in the VET Registry member files.

Kate Moore, PhD, **Data Manager,** will devote 0.60 calendar months in PYs 1, 2 and 5, and 0.36 calendar months in PYs 3 and 4 to this study. The VET Registry Data Manager and will work with the University of California, San Diego data managers to prepare requested data variables, perform data extractions, provide consultation, and prepare data files for transmission, and to prepare data collected as part of this study to be archived into the VET Registry data repository.

Scientific and Ethics Oversight Committee (SEOC)

Costs for the annual review of the status of this project will be used to pay costs incurred by the VET Registry Scientific and Ethics Oversight Committee (SEOC). The SEOC meets either by telephone conference or in-person to review all VET Registry studies and any significant modifications and/or amendments. The SEOC provides external oversight and advice to the VET Registry Director. The SEOC is composed of 5-6 senior scientists in biomedical research. SEOC costs are estimated at $1,259 in PY 1, increasing by 2% each PY.

Site Visit
To assure compliance with regulatory and Registry requirements at least one site visit to University of California, San Diego will be conducted by Registry staff during the duration of the investigator's study. This cost has been budgeted at $2,000 in PY 3.

Supplies
Standard office supplies include mailing supplies and express shipping. This has been estimated at $975 in PYs 1 and 2.

Other Expenses
Mortality search- the VET Registry conducts on-going location efforts for all Registry members. One aspect of this process is the submission of the VET Registry members to the National Death Index maintained by the National Center for Health Statistics. For the members in this study the mortality search cost will be $162 in year PY 1, increasing by 2% each PY.

Address updates- the VET Registry conducts on-going location efforts for all Registry members. One aspect of this process is the submission of the VET Registry members to a commercial location service provider before sending materials to VET Registry members. This cost has been estimated at $263 in PYs 1 and 2.

Newsletter- the VET Registry maintains contact with all Registry members using a newsletter the Registry develops and mails to all members. This newsletter allows us to stay in touch with Registry members as well as to update addresses. For this study the newsletter costs will be $1,096 in year PY 1, increasing by 2% each PY.

UNIVERSITY OF CALIFORNIA, SAN DIEGO UCSD

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SANTA BARBARA • SANTA CRUZ

DEPARTMENT OF PSYCHIATRY
9500 GILMAN DRIVE (MC 0603)
LA JOLLA, CALIFORNIA 92093-0603

Dr. Caleb Finch and Dr. Jiu-Chiuan Chen
Davis School of Gerontology
University of Southern California
Los Angeles, CA 90089-1061

Re: UCSD R01 as part of P01 “Urban Air Pollution and Alzheimer’s Disease: Risk, Heterogeneity, and Mechanisms”

May 15, 2017

Dear Tuck and JC,

Bill Kremen and I are enthusiastic about our participation in your P01 “Urban Air Pollution and Alzheimer’s Disease: Risk, Heterogeneity, and Mechanisms” (4/01/2018-3/31/2023) through our R01/Project 2 proposal. We believe that this collaborative multidisciplinary examination of the effects of urban air pollution on cognitive and brain outcomes in both humans and rodents has potential to make major contributions to our understanding of environmental risk factors for Alzheimer’s disease and their mechanisms of action.

We look forward to continuing to work with you and the rest of the P01 team over the next five years. We will participate in scientific planning, data collection of the historic geolocation data for the VETSA twins, data analyses, and manuscript preparation. With our all-male VETSA sample, we will provide a valuable addition to age-related research the effects of air pollution, which has been conducted on primarily older women.

This collaborative project is truly unique and the extensive data from these different studies will doubtless yield many valuable insights; the work you are proposing to do is very important and is poised to address critical public health issues in our rapidly growing aged population. We and our colleagues look forward to working with you on this exciting project.

Sincerely yours,

Carol E. Franz, PhD
Associate Professor
University of California San Diego

William S. Kremen, PhD
Professor
University of California, San Diego

OTHER RESEARCH PLAN SECTION 13. RESOURCE SHARING PLAN(S)

A. Resource and data sharing plan: General. Intellectual property and data generated under this project will be administered in accordance with both University and NIH policies, including the NIH Data Sharing Policy and Implementation Guidance of March 5, 2003.

Ownership of sole or joint inventions developed under the project will be owned by the institution(s) employing the inventor(s). Inventors shall be determined by U.S. Patent law, Title 35 SC. University and Participating investigators/institutions will disclose any inventions developed under the project and such inventions will be reported and managed as provided by NIH policies. Sole inventions will be administered by the institution employing the inventor. Joint inventions shall be administered based on mutual consultation between the parties. Similar procedures will be followed for copyrights.

Materials generated under the project will be disseminated in accordance with University/Participating institutional and NIH policies. Depending on such policies, materials may be transferred to others under the terms of a material transfer agreement.

Access to databases and associated software tools generated under the project will be available for educational, research and non-profit purposes. Such access will be provided using web-based applications, as appropriate.

Publication of data shall occur during the project, if appropriate, or at the end of the project, consistent with normal scientific practices. Research data which documents, supports and validates research findings will be made available after the main findings from the final research data set have been accepted for publication. Such research data will be redacted to prevent the disclosure of personal identifiers.

B. Resource and data sharing plan: Specific. Sharing of data generated by this project is an essential part of our proposed activities and will be carried out in several different ways. We would wish to make our results available to the community of scientists interested in genetic and environmental influences on successful aging or age-related decline in the following domains--cognition; brain; personality-psychosocial; and health-medical. We also wish to communicate our results to a general (lay) audience of persons interested in or concerned about aging. Conversely, we would welcome collaboration with others who could make use of the assessment protocols developed in the project.

Our data sharing plan includes the following activities:

Presentations at National Scientific Meetings. Presentations of our findings at national and international meetings is an ongoing activity (e.g., such as the Gerontological Society of American, Cognitive Aging Association, World Congress on Psychiatric Genetics, International Twin Studies meeting, Behavior Genetics Association, Brain Imaging meetings; American Psychological Association; American Psychiatric Association, to name a few). These meetings are key sources of new information for other researchers on a variety of topics related to genetic and environmental influences on aging. Dissemination at meetings will also include providing information about how to access the data. All VETSA personnel are asked to put access information on their business cards and on their university faculty websites and acknowledgements. Information on public access will be disseminated at meetings, at ICPSR/NACDA, at the VETSA and VET Registry websites, and on list servers such as those for Twin Research, Behavior Genetics Association (BGA), Geriatric Society of America (GSA) and American Psychological Association.

Newsletter. The Vietnam Era Twin (VET) Registry publishes an annual newsletter for registry twins. The VET Registry is within the Department of Veterans Affairs, and it controls all access to registry participants and to data derived from them. The newsletter's intent is to disseminate new information regarding twin research. The activities and discoveries of the VETSA research project will be provided regularly to the Registry for dissemination. The VET Registry newsletter will be asked to advertise information on access to the archived data in the newsletter. This newsletter is also distributed widely throughout the VA.

Web Site. A description of the VETSA project has already been submitted to the NIA website ("Publicly Available Databases for Aging-Related Secondary Analyses in the Behavioral and Social Sciences"). http://www.nia.nih.gov/bsr, under the heading of "Databases Under Development." We have a university supported website where summaries of the scientific presentations and a bibliography of publications is posted, written primarily for a general audience as well as information about access to the VETSA data.

Collaborations with Consortia. A number of consortia have come into existence that seek to harmonize data across studies in order to increase sample sizes and power. VETSA investigators currently belong to the Consortium on Interplay of Genes and Environment across Multiple Studies (IGEMS), the goal of which is to examine gene and environmental interplay of social contexts and aging through combining data from multiple studies. We have been invited to work with the International Network of Twin Registries Harmonization Consortium. All of these interdisciplinary collaborative efforts disseminate and distribute the VETSA data to other researchers and enlarge its impact far beyond its original goals.

Access to Data. We will release data to other investigators through multiple mechanisms in accordance with UCSD, NIH, and VET Registry policies (after the main findings from the final research data set have been accepted for publication). We avoid publication of partial data because of the risk of misleading findings; therefore, it is our view that data should be made publicly available only for the completed datasets for each wave of the study.

We actively pursued the goal of creating a state-of-the-art system for maximizing public access and availability of all VETSA data. Data will be released through contact with the principal investigators, and through requests to the VET Registry at the Puget Sound VA. Dissemination will also occur through the PIs and/or the VET Registry. It should be noted here that previously collected data on these participants from 1986, induction to the military, 1990, 1992 are already housed at the VET Registry. Researchers who wish to use these older data need to work with the VET Registry. The cross-walk between the Registry ID numbers and the VETSA ID numbers is controlled by the VET Registry. VETSA biospecimen data (DNA; frozen plasma) are stored at the VET Registry in keeping with Registry/VA rules to protect the privacy of the participants. Access to biospecimen data is through the VET Registry. One of the tasks of the data management team will be to distribute data to eligible PIs requesting data; data can only be used for research purposes and not identify any individual research participant and, per UCSD policy, PIs will need to provide evidence of IRB approval for their project.

We acknowledge the intellectual property and scientific publication elements of the NIH data-sharing policies. We will ask that all researchers using the data for publications or presentations credit NIA/NIH by adding acknowledgements of the NIH grants that generated the data as well as the VET Registy.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0729333930000

Legal Name*: University of Southern California
Department: Contracts and Grants
Division: 95-1642394
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: CA
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Mr.	Steven		Misuraca	

Position/Title: Contracts and Grants Officer
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: CA
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701
Phone Number*: 2137408207 Fax Number: Email: misuraca@research.usc.edu

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Age-sex-ApoE allele interactions in neuronal and white matter vulnerability to air pollution

12. PROPOSED PROJECT

Start Date*	Ending Date*
04/01/2018	03/31/2023

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Southern California
Duns Number:	0729333930000
Street1*:	3720 South Flower Street
Street2:	
City*:	Los Angeles
County:	CA
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90089-0701
Project/Performance Site Congressional District*:	CA-037

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: 1 2 3 4 5 6 If

NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Prj3_Abstr_2017_Final_TM.pdf
8. Project Narrative*	
9. Bibliography & References Cited	Prj3_Bibliography_2017_Final.pdf
10.Facilities & Other Resources	Proj3_FACILITIES_AND_OTHER_RESOURCES.pdf
11.Equipment	Proj3_EQUIPMENT.pdf

Project 3 Abstract: Caleb Finch, with Todd Morgan and Christian Pike
Age-Sex-ApoE Allele Interactions in White Matter Vulnerability to Air Pollution.
To reviewers: *new text is italicized*. Accelerated cognitive aging is strongly associated with air pollution fine-sized particulate matter ($PM_{2.5u}$) in two *recent reports of community-living elderly* [1, 2]). Besides cognitive deficits and loss of white matter, the WHIMS cohort incurred higher risk of dementia (Project 1).

In mouse models, nano-sized PM (nPM) from traffic-related air pollution (TRAP-nPM) is pro-amyloidogenic. In our experimental model, mice receive whole body exposure to TRAP-nPM for 150 hours intermittently during 10 weeks (Core C2). *Besides brain-wide inflammatory responses*, TRAP-nPM causes selective damage to hippocampal CA1 myelinated neurons (Fig. 4) that are most vulnerable in AD and to cerebrovascular ischemia. Because AD risk is elevated in women, particularly in apoE4 carriers, we propose to study age-sex-ApoE allele interactions in the vulnerability of myelinated pathways to nPM using EFAD mice. We also examine the blood-brain barrier (BBB), which shows greater age-related leakiness in human ApoE4 carriers. New data show that nPM increases activity in a TLR4 inflammatory pathway that mediates post-ischaemic neurodegeneration. *We hypothesize that TRAP increases AD risk by TLR4-related inflammatory processes that synergize with CA1-specific hippocampal neurodegenerative mechanisms.*
Aim 1 *(refocused)*: Age and sex in brain susceptibility of C57BL/6 (B6) mice to nPM. Both sexes of B6 mice will be exposed to nPM at ages 2 mo, 10, and 18 mo, spanning reproductive senescence. We will assay hippocampus mediated learning and hippocampal subregional analysis of myelin degeneration and neuron atrophy. Microglial and TLR4-TNFα pathway responses will be evaluated by immunohistochemistry and Western blots. Cerebrovascular and white matter tract responses to nPM exposure is assessed in Core B2 by in vivo multiphoton imaging for regional CBF and BBB permeability and angiography. DTI-MRI at 80µm isotropic spatial resolution will provide fractional anisotropy maps for white matter connectivity. BBB cellular integrity is assessed by confocal microscopy. Collaboration with Project 4 examines B6 brains for synergies of nPM with chronic cerebral hypoperfusion (CCH).
Aim 2: Age-Sex-ApoE allele interactions in EFAD mouse responses to nPM. The Aim 1 parameters of age and sex for hippocampal-mediated learning and neurodegeneration in hippocampal subfields are examined in EFAD mice. Cerebrovascular amyloid and microbleeds will be evaluated in EFAD mice. We hypothesize that nPM will show female bias in accelerating AD changes.
Aim 3 *(major revisions): Role of TLR4. A new mouse model with inducible macrophage/microglial-specificTLR4 knockout (i-mTLR4-ko) will evaluate TLR4 contributions to nPM-induced myelin degeneration and neurite atrophy. This new model will identify targets of TLR4 pathway components in the effects of TRAP on myelinated pathways.*

FACILITIES AND OTHER RESOURCES

The **University of Southern California** provides a rich scientific environment that offers both an intellectual community and infrastructure that will contribute to the success of the proposed research project. The work will be completed in the Leonard Davis School of Gerontology at the University of Southern California.

The **Leonard Davis School of Gerontology** at **USC** is a multi-disciplinary research center with ongoing research conducted by demographers, biologists, psychologists, sociologists, physicians, and public policy analysts who are in the same home department. The School is headquartered in the Andrus Gerontology Center, an 88,000 square foot building, housing several wet laboratories, a 250-seat auditorium, the gerontology library, offices, and meeting rooms. The endowment of the Leonard Davis School is over 70 million dollars, including nine fully funded chairs.

The AirPollBrain Group at USC is an organized research group of scholars who do research on ways to promote optimal neurodevelopment in humans and healthy brain aging by better understanding environmental effects of urban air pollution and gene-environment interactions. The APB group developed from a core of USC's leadership in urban environmental health research in the Los Angeles Basin, with collaborating institutions. Taking a multi-disciplinary research, APB addresses effects of environmental pollution on the brain across the lifespan. APB is pursuing five main goals: I, Identify environmental risk factors impacting brain health in human populations with different environmental settings and genetic backgrounds; II, Develop experimental paradigms relevant to human studies and public health interventions; III, Integrate the existing environmental, neurosciences, and public health sciences programs at USC; IV, Provide the best sciences to support evidence-based regulations to protect public's health?; V, Create resources for training and education in interdisciplinary environmental health sciences from basic environmental biology to societal levels of analysis. Bimonthly meetings convene faculty and trainees on specific topics with a focus on developing new research projects and consortia.

USC has a number of research centers that provide a resource rich intellectual environment for the work proposed in this application:

NIA Center on Biodemography and Population Health: The USC/UCLA Center on Biodemography and Population Health (CBPH) aims to enhance understanding of biological and other processes that contribute to population health at older ages and thereby contribute to more effective program and policy efforts to improve health and reduce health disparities. The CBPH promotes theory-based integration of biological measurement into population-based studies and on-going development and validation of biological measurement protocols. This center is directed by investigator Crimmins.

The USC Alzheimer's Disease Research Center (USC ADRC): The USC ADRC focuses on mild cognitive changes related to aging, Alzheimer disease (AD) and cerebrovascular disease (CVD) in multi-ethnic communities.. The ADRC integrates USC strengths in epidemiologic and longitudinal studies; in the molecular biology of aging and its diseases; and in genomics to determine basic and clinical and psychosocial approaches to the detection, cause, prevention, and treatment of early-stage cognitive impairments in human and in animal models. Research is also focused on normal brain aging processes and the transition to clinical stage dementias.

The Southern California Environmental Health Sciences Center: SCEHSC was established in 1996 to promote environmental health research in Southern California. The Center aims to more fully characterize environmental health hazards, understand the basis for personal vulnerability, and translate research into preventive action to reduce the burden of environmentally-related diseases. This Center has played an important role in the development of the air pollution and cognition initiative which is a focus of a significant program of research at USC.

The Statistical Consultation and Research Center (SCRC): The SCRC is an organized research unit within the Department of Preventive Medicine at the University of Southern California which integrates statistical, epidemiological and computing resources for researchers. There is also an organized Statistical Consulting Group across the campus consisting largely of social scientists. In addition, faculty in the Molecular and

Computational Biology group provide collegial consulting in the approaches of molecular biology, computational biology and genetics.
Spatial Analysis Services: The GIS Research Laboratory has a permanent professional staff that administers campus-wide GIS infrastructure, collects and disseminates geospatial datasets, and provides training and consultation in the use of geospatial technologies. The GIS Research Laboratory supports research projects and courses needing geographic information science and spatial analysis inputs. In addition, the GIS Research Laboratory offers a large number of geospatial software solutions to the USC community and has developed a variety of web-based tools and extensions to help the USC community with their geoprocessing and spatial analysis needs.

Computer Facilities: The Leonard Davis School employs four full-time IT personnel to maintain the computing system in the building and access to computers and networks for researchers and graduate students. The Gerontology IT/Computing Services provides guidance, resources, and instruction to Gerontology faculty, staff, teaching assistants, research assistants, and students to enhance learning and teaching at the Andrus Gerontology Center through the effective use of digital and communications technology. It provides the faculty, staff and the students with the computing resources, hardware, and software. File servers, document servers and web servers which are securely hosted in the building to provide file sharing and web hosting services for the Leonard Davis School. The project personnel use IBM compatible computers with Microsoft Office, SAS, STATA, and internet productivity tools (software for e-mail and internet browsing). The computer lab of the Andrus Center, used in teaching classes, includes network desktop computers, wireless laptops, scanners, LCD projector and network printers. There are file servers, document servers and web servers which are securely hosted in the building to provide file sharing and web hosting services for the Center. Secure networks have been developed for users of large-scale population datasets such as the Health and Retirement Survey (HRS). There is also a secure room in the Leonard Davis School for both analysis of restricted data sets such as those linked to geographic indicators, medical records, Social Security records, or death records. This room has several computers, a large data storage capability but no internet access. Security is assured with special cabinets, door locks and alarm system. The office suite of the two investigators working with the HRS data contains two dedicated non internet connected computers for use with secure data.

Office Facilities: All project personnel in the USC Leonard Davis School of Gerontology have individual offices in the Leonard Davis School that are equipped with computers, hard lines, and wireless internet. The Leonard Davis School is housed in the Andrus Center, an entire building specifically for gerontology research and instruction. The 88,000 square foot structure houses several wet laboratories, a 250-seat auditorium, the gerontology library, offices, and multiple meeting rooms with video capabilities.

Libraries: The University of Southern California libraries, with the support of other parts of the Information Services Division, support many digital initiatives aimed at providing seamless access to information of every kind. The ultimate goal of developing and expanding digital access, and the ability of users to manage it, is to provide "one stop shopping," regardless of information format, for students, faculty and other researchers. The libraries maintain a large and ever-growing body of electronic resources, which include very large aggregator databases, reference tools, and discipline-specific resources for every area of study. The Integrated Document Delivery (IDD) team works with a global network of institutions to borrow, lend, and otherwise make available materials that support scholarly research for USC faculty, staff, and students. Interlibrary loan borrows books, dissertations, government documents, microforms and other loanable materials that are not owned by USC or are unavailable from USC's collection. Once received, articles are delivered online and physical items are made available at the Doheny Library Information Services desk. In addition, IDD services provides articles and documents owned by USC Libraries. The requested articles are scanned, converted to PDF format, posted on our server, and then delivered to the patron via a direct link in an email.

In addition to the substantial University Library, the Andrus Gerontology Center Library maintains one of the largest collections of scholarly, professional reference materials related to aging in the country, with a

retrospective collection including over 15,000 monographs and more than 300 gerontology journals. Access to these materials is facilitated by a professional staff including a full-time librarian specializing in gerontology.

Resources For Web Casting, Webinars Etc: As part of the campus-wide Technology Enhanced Learning (TEL) and Distance Learning (DL) initiatives, the School of Gerontology has added two state-of-the-art Smart Classrooms and increased technological integration in the Leonard Davis Auditorium. The renovations provide top-of-the-line equipment including:

- Dual screen DTP projection
- Digital surround sound system
- Wireless handheld & lavaliere microphones
- Assistive hearing device system
- Document camera system & Virtual whiteboard
- Multiple cameras for webcasting complete with TV style control room
- Video teleconference unit

These facilities are staffed by two full time employees and two part time employees; both of whom are skilled at the production of video and web-based material.

USC Animal Facility: This is maintained by the Department of Animal Resources under the supervision of Don Casebolt, DVM. The Department employs 2 additional veterinarians, a manager of animal husbandry, 2 facility supervisors and a staff of 12 trained animal care workers (certified by the American Association of Laboratory Animal Science). USC has been accredited under the American Association for Accreditation for Laboratory Animal Care, since 1966. An Institutional Animal Care and Use Committee (IACUC) reviews all applications to ensure ethical and humane treatment of animals. Dr. Morgan is an active member of IACUC. The Ray R Irani Hall Animal Facility was completed in 2007. The facility has a total capacity of 10,000 rodent cages within the 14,770 net square feet consisting of 20 animal holding rooms, 10 procedure rooms, 2 quarantine rooms, additional rooms for cage processing, bulk autoclave & storage, and office, locker & break rooms. The facility features a “suite” design concept of grouping 4 animal holding rooms with 2 procedure rooms into an individual security-controlled suite. Our group has exclusive use of 1 of these suites. The individually-ventilated rodent cages ensures consistent air exchange rates of 50-80 air chages per hour within each cage to reduce ammonia and carbon dioxide levels. Incoming air is high-efficiency particulate arresting (HEPA) filtered for control of airborne particles. All components of the cages and racks are autoclaved and changed weekly. Wall-mounted supply air blowers and central exhaust plenums reduce noise and vibration. Automatic watering system is supplied to each cage.

Laboratory Facilities and Resources

Finch & Morgan:

Labs: 3 labs (including image analysis room with fluorescent and bright field microscopes, computers and software), a cold room and a kitchen for preparation of solutions.

Computer: 3 Macs; 3 PC; including printers, scanners

Office: Drs. Finch and Morgan have separate offices next to their lab in the Gerontology Center.

Pike:

Labs: 2 labs (including cell culture room with incubators and sterile hoods),

Computers: 5 Macs

Office: Dr. Pike has an office next to his lab.

EQUIPMENT RELEVANT TO THIS PROPOSAL

Finch & Morgan:

Major laboratory equipment: Real-time PCR (Opticon2); PCRExpress (Hybaid); GeneAmp PCR System 9600 (PerkinElmer), Odyssey, dual infrared detection system (LI-COR), Image Analysis System (digital cameras, digital video camera, slide scanner, Olympic binocular microscope with flourescence capability; 1 compound microscope (Leica) with computer controlled motorized stage and digital video capture); Spectrophotometer (NanoDrop), Ultracentrifuge, 3 Ultralow freezers; 2 floor temperature-controlled shakers; cryotome; microplate spectrophotometer & fluorimeter.

CO2 waterjacket cell culture incubator (2); Hybaid hybridization ovens (2); UV Stratalinker 1800; SpeedGel SG200 vacuum gel dryer; Speedvac™ concentrator, centrifuge and mechanical vacuum pump; 2 tissue culture laminar hoods BSL 2; inverted microscope for tissue culture; X-ray developer; Eppendorf refrigerated centrifuge with rotors; fluorescence microscope w/camera; BioRad Trans-Blot system; BioRad Protean II Multicell Electrophoresis system.

Custom-made exposure cages for sealed delivery of specified air (re-aerosolized nPM or HEPA-filtered room air) for specified doses and duration of mice.

Pike:

Equipment:

1. Tissue culture facility (Andrus Gerontology Center room 304A): 2 Nuaire incubators outfitted with dual auto-changing CO_2 tanks, Olympus IX-70 inverted microscope with fluorescence capability, Olympus SZ6045 stereo dissecting microscope, biological safety hood, two laminar flow hoods, water bath, incubated rotary shaker, clinical centrifuge, 4°C refrigerator, and -20°C freezer.
2. Quantitative microscopy set-up: Olympus BX-50 microscope with CAST stereology options including linked computer, motor-driven stage, and microcator.
3. Main lab space (Andrus Gerontology Center room 304): Olympus BX40 microscope, Olympus BX-50 microscope with Nomarski and fluorescence capabilities, Eppendorf refrigerated benchtop centrifuge, microplate reader, two microfuges, refrigerator, 4°C refrigerator, -20°C freezer, two -80°C freezers, benchtop rotary shaker, water bath, electrophoresis equipment, FluorEChem gel documentation system, PCR cycler.

SHARED:

Much of the major equipment used by the eight biology laboratories within the Andrus Gerontology Center are maintained as shared resources. In addition to the equipment within the PI's lab, equipment and resources available to lab members that will be useful to the proposed work include NanoDrop spectrophotometer, Beckman tabletop ultracentrifuge, confocal microscope, phosphoimager, liquid scintillation counter, spectrophotometer, refrigerated centrifuge, cryotome, Zeiss Axiophot fluorescence microscope with computer assisted digital photography, Dumas/Dresal Optical Density and analysis system, floor temperature-controlled shakers and access to a cold room.

Included within the shared resources are access to cores:

Behavioral Core is located at the USC Ray R Irani Animal Facility in a dedicated room adjacent to the animal housing rooms in a separate suite to eliminate unnecessary disturbances. All equipment for performing the following behavioral tasks is available:

1) Spontaneous Alternation Behavior (measuring exploratory behavior and short-term spatial memory);
2) Novel Object Recognition and Placement (measuring episodic and recognition memory);
3) Barnes maze (measuring spatial learning and memory).
4) Contextual Fear Conditioning (measures hippocampus-dependent memory).

Data is collected, analyzed and archived using the Nodlus EthoVision XT Tracking System with computer-directed videocamera capture and image analysis software. Details are provided in facilities section.

Aging Biomarker Core Facility. This core provides services to measure various aging-related biomarkers to support research projects and grant applications in the gerontology research community. The goal of the core is to provide high-quality assay services to measure various established and/or develop and validate novel aging-related biomarkers to meet the specific needs of investigators in a timely, cost effective, and integrated

manner. Major platforms used by the core includes: 1) Meso Scale Discovery system (MSD), which runs ultra-sensitive multiplex assays that can simultaneously detect up to 10 analytes from very small sample volume (10-25 µL), utilizing the next generation electro-chemiluminescence detection that offers a unique combination of sensitivity, dynamic range and convenience. 2) Enzyme linked Immunosorbent Assays (ELISAs) that are available commercially or developed/validated in-house for unique and/or novel age-related analytes, using the SpectraMax M3 multi-mode microplate reader providing three modes of detection (UV-visible absorbance, fluorescence intensity and glow luminescence) in one platform so as to allow the widest range in bioresearch applications. We currently provide a wide range of age-related biomarkers that can be detected in body fluids, tissues, cell lysates of various species including inflammatory markers (TNFα, IL-6, CRP, etc.), Alzheimer-related markers (Aβ, phospho-tau, humanin, etc.), DNA damage and repair markers, oxidative stress markers, hormones (insulin, GH, IGF-I, IGFBPs, etc.), telomere length, etc. The core is located within the Davis School of Gerontology in a designated space (~300 sq ft) within a recently renovated research facility. The core is driven largely using automated systems operated by a dedicated staff member.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: CALEB Middle Name E Last Name*: FINCH Suffix: Ph.D

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Organization Name*: University of Southern California
Department: Davis School of Gerontology
Division:
Street1*: 3715 McClintock Avenue
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Phone Number*: (213) 740-1758 Fax Number: (213) 740-0853

E-Mail*: cefinch@usc.edu

Credential, e.g., agency login: cefinch

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: PHD,BS Degree Year: 1969

Attach Biographical Sketch*: File Name:

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: TODD Middle Name E Last Name*: MORGAN Suffix: Ph.D

Position/Title*: Research Professor
Organization Name*: University of Southern California
Department: Davis School of Gerontology
Division:
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Street2:
City*: Los Angeles
County:
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Credential, e.g., agency login: temorgan

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD,BS Degree Year: 1990

Attach Biographical Sketch*: File Name: Morgan_NIH_bio_AirPoll_P01_2017.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: CHRISTIAN Middle Name J Last Name*: PIKE Suffix: Ph.D

Position/Title*: Professor
Organization Name*: University of Southern California
Department: Davis School of Gerontology
Division:
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County: Los Angeles
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Province:
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Credential, e.g., agency login: cjpike

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD,BS Degree Year: 1994

Attach Biographical Sketch*: File Name: Pike_biosketch_Finch_Chen_P01_2017.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: WENDY Middle Name JEAN Last Name*: MACK Suffix: Ph.D

Position/Title*: Professor
Organization Name*: University of Southern California
Department: Preventive Medicine
Division: Keck School of Medicine
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Street2:
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County:
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E-Mail*: WMACK@USC.EDU

Credential, e.g., agency login: WJMACKPI

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1989

Attach Biographical Sketch*: File Name:

Attach Current & Pending Support: File Name:

Tracking Number: Funding Opportunity Number: PAR-16-359 . Received Date:

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

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4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	Prj3_Intro_2017_Final.pdf
Research Plan Section	
2. Specific Aims	Prj3_SA_2017_Final_TM.pdf
3. Research Strategy*	Prj3_Res_Plan_2017_Final_TM.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	Proj3_Vertebrate_Animal_Section.pdf
10. Select Agent Research	Proj3_Select_Agent_Research.pdf
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	
13. Letters of Support	Wendy_mack._letter_FINAL.pdf
14. Resource Sharing Plan(s)	Proj_3_Resource_Sharing_Plan.pdf
15. Authentication of Key Biological and/or Chemical Resources	Proj3_Authentication_of_Key_Resources_Plan.pdf
Appendix	
16. Appendix	

P01 Project 3 A1

New publications most relevant to this P01:

(1) Cacciottolo M et al 2016, The APOE4 allele shows opposite sex bias in microbleeds and Alzheimer's Disease of humans and mice. Neurobiol Aging PMID: 26686669;
(2) Cacciottolo M et al 2017, Particulate air pollutants, ApoE alleles, and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. Transl Psych 7. PMID: 28140404;
(3) Woodward N et al 2017, Traffic-related air pollution accelerates myelin and neuritic aging changes in a mouse model, with specificity for CA1 neurons. Neurobiol Aging. PMID: 28212893;
(4) Woodward N et al 2017, Toll-like receptor 4 in glial inflammatory responses to air pollution in vitro and in vivo. J Neuroinflamm. PMID: 28410596.
(5) Liu Q et al 2016, Stroke damage is exacerbated by nano-size particulate matter in a mouse model. PLoS One. PMID: 27071057. Collaboration with Project 4.

Response to Critique (Crtq): Major text changes are *italicized* in Project 3 A1.

1. High in vivo dose (Crtq p4). Response; *Similar exposure doses (300 ug/m^3) are used by other labs and represent human exposure in proportion to the 30-fold shorter mouse lifespan, cited in Project 3 Methods and Core C2.*
2. In vitro TNFa, uncertain meaning. (Crtq p16): Response: *Aim 3, in vitro TNFa studies are deleted.*
3. B6 mice as models for human age-by-sex interaction (Crtq p5). Response: *C3 Methods discusses middle-aged mice as models for the human peri-menopause for which Finch, Morgan, and Pike have been major contributors.*
4. TNFR1-ko Uncertain meaning. (Crtq p33,35): Response: The TNFR1-ko is replaced, see 6&7 below.
5. Lack of 'dose-response' (Crtq p39). Response: *In vivo dose response is studied in R01 AG051521 (Finch PI).*
6. C1. Approach, Major revisions with new data

Fig 1. Sex and ApoE allele differences in brain TNFa and IL6 of control mice (not nPM exposed)
Fig 2. Hippocampus-mediated behavioral deficits are greatest in E4FAD female mice (not nPM exposed)
Fig 3. Female and ApoE4 bias in response to nPM exposure
Fig 4. nPM targets hippocampal CA1 subfields for neurite atrophy, myelin, and microglia
Fig 5. nPM causes behavioral deficits in the 'novel object in context' (NOIC) test.
Fig 6. TLR4 inflammatory pathway genes respond to nPM in the brain. TLR4, CD33, MyD88, TNFa. New data are shown for response of brain TLR4 inflammatory pathways to air pollution, in addition to those we recently reported (Ref 4 Woodward et al 2017 above).

C2. Experimental design

7. Preliminary data (Fig 6) shows in vivo TLR4 pathway responses to nPM (including TNFa) and cites our new bioinformatics analysis of TLF4 pathways relevant to neurodegeneration (Ref 4 above, Woodward 2017). The TLR4 pathways mediate pulmonary edema responses to air pollution and show effects on Abeta levels in a whole genome TLR4-ko (references cited). Moreover, TLR4 is involved in post-ischemic neural damage (references cited). Aim 1 is refocused on TLR4 inflammatory pathways and includes other TLR4 components: TLR4, CD36, MyD88, and TNFa.
8. Aim 1 includes the complement system C5, C5a, C5 receptor, which are also studied in Proj 4, e.g. C5a is increased by in vivo exposure to nPM (Project 4, Fig. 1).
9. Expected Results has an expanded discussion of sex differences.
10. Aim 3 is completely revised. We replaced the TNFR1-ko with an inducible microglial-targeted TLR4-ko (i-mTLR4-ko) from CJ Pike, our co-I. The new i-mTLR4-ko will be available to Project 4 to study the hypothesis that adult deletion of microglial TLR4 will lessen post-ischemic neurodegeneration, as suggested by TLR4 targeted drugs. We anticipate that i-mTLR4-ko will be of value to other labs studying air pollution and neurodegeneration. We added TLR4 to Sect B Innovation.

Relation of Project 3 to other funded projects.

1. R01 AG051521 (10/2015; 09/2020; Finch PI; Pike, co-I) Amyloid and inflammation: modulation by ApoE, gender, air pollution, and drugs. Studies of sex-ApoE allele interactions in cerebral microbleeds and cerebral amyloid angiopathy of nPM exposed mice. nPM exposure dose responses. No overlap.
3. R21 AG05020 (04/2016-03/2018; Finch PI). Air pollution nano-particulate matter, APP processing, and glutamate receptors. Studies of a new inhibitor of APP processing (gamma secretase modulator). No overlap.
4. U.S. Army Medical Research Acquisition Activity, PD160021P1 (10/2017-09/2121). Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System. Studies of a-synuclein proteinopathy spread in neuronal pathways during exposure to nPM. No overlap.

Specific Aims

Accelerated cognitive aging is associated with traffic-related air pollution (TRAP), particularly for the $PM_{2.5u}$ which is monitored by the EPA for a primary standard of 12 ug/m^3 annual average. Recent studies of community-living elderly from the WHIMS cohort show deficits of cognition [3] and white matter [4] in association with for residence in PM2.5 above the EPA standard elevated residential levels of PM2.5 (Project 1). Moreover, the WHIMS cohort showed a 70% higher risk of dementia which was enhanced further in ApoE4/4 homozygotes (P01 Overview, Section A2 and Project 1). In the same report, we showed that air pollution nano-sized particles increased brain amyloid deposits preferentially in AD mice carrying human ApoE4.

For experimental studies of brain responses to TRAP, mice are given whole body exposure to a neurotoxic nano-sized subfraction of TRAP, designated as nPM, in distinction from the broader ultrafine class of $PM_{0.2u}$. The TRAP-nPM is pro-amyloidogenic in vivo and in vitro [1]. The TRAP-nPM represents a continuous sample of urban air pollution and are extensively characterized for chemical composition and cytotoxicity (Cores C2, C3). After exposure to nPM for defined dose-duration, pilot data show inflammatory responses of TNFα and damage to neurites and myelin with selectivity for hippocampal CA1 subfields (Supporting Data*). Given the CA1 vulnerability in AD and cerebral ischemia, we hypothesize that nPM and other components of PM.2.5 synergizes with basic neurodegenerative mechanisms in AD that impact CA1 neurons though mechanisms involving the TLR4 pathway, which also elevates TNFα* (Fig. 6). Because AD risk is elevated in women, particularly in ApoE4 carriers, we propose studies of age-sex interactions (Aim 1) and interactions with ApoE4 (Aim 2). Aims 1 and 2 also examine the blood-brain barrier (BBB), which shows greater age-related leakiness in ApoE4 carriers. With Project 4 (Wm Mack), we will analyze interactions of nPM with chronic cerebral hypoperfusion (CCH) for effects on myelinated pathways in hippocampus.

Aim 1 (*refocused*): Age and sex in brain susceptibility of C57BL/6N (B6) mice to nPM. Both sexes of B6 mice will be exposed to nPM for 3 months, starting at age 2 mo, 10, and 18 mo; the later ages encompass female reproductive senescence for synergies with Project 1 (WHIMS). Ovarian cycling status during nPM exposure will be monitored by vaginal cytology to identify nPM impact on the peri-menopausal transitions, which begin by 6 mo and are complete by 20 mo in B6 mice [5, 6]. Hippocampal subregions are examined by histochemistry for neurite atrophy; for myelin degeneration (myelin basic protein, myelin-associated glycoprotein), microglial activation (Iba-1, CD68), and *TLR4 and TNFα pathway inflammatory factors (TLR4, CD36, MyD88; IL6, TNFα; TNFRI*). Hippocampal-mediated learning will also be assayed.

Cerebrovascular and white matter tract responses to nPM exposure will be assessed by Core B2 by in vivo multiphoton imaging for regional CBF and BBB permeability and angiography. DTI-MRI, at 80μm isotropic spatial resolution, will assess white matter connectivity in fractional anisotropy maps. BBB cellular integrity will be assessed by confocal microscopy.

Collaboration with Project 4 examines nPM-CCH interactions in B6 mice for hippocampal myelinated pathways.

Aim 2: Age-Sex-ApoE allele interactions in EFAD mouse responses to nPM. EFAD transgenic mice carrying apoE alleles and familial AD genes (EFAD) will examine the Aim 1 parameters of age and sex for neurodegenerative changes in hippocampal subfields and for hippocampus-mediated learning. These studies will also define the peri-menopause in EFAD mice, a gap in knowledge, which is relevant to older ages in the expanding use of EFAD mice*. Our prior studies with B6 aging females showed strong correspondence to human peri-menopausal stages by the clinical STRAW classification [5].* Because EFAD mice show female excess of cerebral arterial amyloidosis (CAA) and microbleeds [7], nPM exposure effects on the BBB will be examined by Imaging Core B2 (Aim 1). We hypothesize that E4FAD females will be most vulnerable to BBB disruption with ensuing microbleeds, and CA1 neuron and myelin damage. It is premature to anticipate age-sex interactions.

Collaboration with Project 4 examines nPM-CCH interactions in EFAD mice for hippocampal myelinated pathways.

Aim 3 *(revised): Role of TLR4. A new mouse model with inducible microglial-specific TLR4 knockout (i-mTLR4-ko) will evaluate TLR4 contributions to nPM-induced myelin degeneration and neurite atrophy. This new model will identify new mechanistic targets of TLR4 pathway components in the effects of TRAP on myelinated pathways. After careful characterization, the new i-mTLR4-ko will be provided to Project 4 for study of TLR4 pathway responses to CCH, followed by CCH-nPM interactions.*

Research Design

A. Significance:

The search for environmental neurotoxic factors in AD and related disorders (ADRD) has recently considered traffic-related air pollution (TRAP) and the fine-size class of air pollution particles, PM2.5. Two recent human population studies showed strong associations of ambient PM2.5 with increased dementia: *the Womens' Health Initiative Mental Study (WHIMS) in our recent publication with JC Chen, Cacciottolo et al [1]and in a major population study from Canada[2] (P01 Overview). ApoE4 carriers showed the greatest cognitive deficits in WHIMS and the most amyloid deposits in E4FAD mice Cacciottolo et al[1]. This is the first evidence for gene-environment interactions in a widely distributed AD-risk gene.*

Nano-sized particulate matter from TRAP (nPM) is pro-amyloidogenic *Cacciottolo et al*[1], confirming other rodent models [8-10]. Moreover, TRAP-nPM has neuronal specificity for the hippocampal CA1 myelinated neurons (Fig. 4) that are most vulnerable in AD. ***We hypothesize that TRAP increases AD risk by brain wide inflammatory processes that synergize with AD-pathway specific neurodegenerative mechanisms.*** This hypothesis adds specificity to analysis of environmental factors in AD by linking TRAP exposure to amyloidogenic mechanisms and hippocampal targets in AD memory loss. Specifically, we propose that TRAP components accelerate cognitive decline and increase AD risk by exacerbating neurodegenerative pathways from the entorhinal cortex to hippocampus via the myelinated perforant path (the Braak progression of neurofibrillary tangles). These classic findings were extended by MR of the posterior parahippocampal gyrus white matter (PWM) which includes the perforant path. Clinical AD had smaller PWM volumes, while anamnestic MCI had intermediate PWM loss [11, 12]. Moreover, cognitively normal elderly with initially smaller PWM volumes incurred accelerated memory decline and increased AD risk [11, 13].

Vascular targets are included in this hypothesis because hippocampal CA1 neurons are also vulnerable to cerebral ischemia. Moreover, the rate of carotid artery thickening is strongly associated with ambient PM2.5 in several human populations [14, 15]. The blood-brain barrier (BBB) may also be compromised. For example, mice exposed to mixed vehicle exhaust had increased BBB permeability, with altered tight junction proteins (occludin and claudins) and increased metalloproteinases MMP2 and MMP9, which degrade tight junctions of the BBB [16]. In vitro, macrophages exposed to PM2.5 impaired tight junctions of endothelia, with increased permeability and monocyte transmigration[15]. These findings concur with Calderon-Garciduenas' postmortem findings from a highly polluted Mexican mega-city, which showed extravascular lipids in frontal cortex; nano-sized PM in vascular endothelia; and, serum antibodies to occludin and zonulin-1, consistent with impaired BBB [17]. Although ApoE alleles remain to be studied for TRAP-interactions with BBB, the ApoE4 allele itself increases BBB leakage in studies from Zlokovic[18]. Moreover, ApoE4 controls without nPM exposure show activation of proinflammatory CypA-NFkB-MMP9 pathway in vascular pericytes. Thus TRAP promotes at least two of the major pathological processes associated with ADRD. Vascular changes also involve the ApoE4 allele risk factor of AD, which also increases BBB leakage [18]. Pilot data suggest gene-environment interactions of ApoE4 in WHIMS and in EFAD mice with human ApoE genes (P01 overview, Fig. 1&2). These findings are consistent with postmortem samples from highly polluted Mexican megacities which show myelin damage, diffuse amyloid deposits, and inflammatory changes in young brains[19], further exacerbated in ApoE4 carriers[20].

Human imaging findings further show regionally selective loss of white matter volume in association with high PM2.5 exposure for the WHIMS cohort[4]; recent MR analysis shows grey matter volume loss in frontal cortex gyri[21] (Project 1). On the cell level, chronic nPM exposure decreased levels of glutamate receptor subunit GluR1 in the hippocampus of wildtype B6[22] and EFAD mice, which is relevant to the hippocampal-mediated spatial learning impairments of aging and AD. In B6 mice exposed for 8 months exposure to concentrated TRAP PM2.5, Fonken[23] showed loss of dendritic spines in CA1 neurons, and impaired spatial learning in the Barnes maze. *We also found CA1 vulnerability in B6 or EFAD mice after a much shorter nPM exposure [1, 24]: Fig. 4B, neurite atrophy in CA1 neurons but not in the adjacent dentate gyrus; Fig 4C, CA1 selectivity for loss of myelin basic protein; 4D, microglial activation in CA1.* This regional specificity parallels the CA1 neuronal vulnerability during AD and post-ischemia.

We recently reported [24] a possible ceiling effect of response in middle-aged mice. While y*oung male B6 mice (3 mo) responded to nPM with CA1 neurite atrophy (Fig. 4B) and reduced CA1 myelination (Fig. 4C), middle-aged female B6 mice (18-20 mo) had minimal responses to nPM, suggesting ceiling effects (not shown).* These blunted responses match the lack of nPM induction at 18 mo of Nrf2 and HOX-1, basic xenobiotic detoxification responses [25] in collaboration with Forman's lab. The generalizability of this ceiling

effect will be examined with the additional endpoints in aging male and female B6 and EFAD. The proposed studies with Project 4 for nPM plus cerebral hypoperfusion will further explore this finding.

Rodent brains show *complex inflammatory responses to TRAP components that are remarkably consistent across multiple genotypes and exposure models*[26],[27]. Microglia are robustly activated*, with induction of TNFa throughout the brain. Further studies on TNFa led us to consider the TLR4 (Toll-like Receptor 4), which controls several pathways in AD and post-ischemic neurodegeneration*[28]*. Whole genome TLR4-ko in ADtg mice increased fibrillary and soluble amyloid levels* [29-31]*, with varying effects on microglial activation* [29, 30]*. In vitro, microglial TLR4 is activated by the Aβ peptide via the scavenger receptor CD36* [32]*. In vivo and in vitro, nPM induced TLR4 and MyD88 on the NFkB pathway (Fig 6), consistent with known TLR4 pathways* [32, 33]*. We hypothesize thatTLR4-dependent pathways in microglia mediate the synergies of cerebral ischemic damage with air pollution that are observed clinically, and in a mouse model in collaboration with W Mack of Proj 4* [34]*. This hypothesis will be tested by i-mTLR4-ko targeted to microglia (revised Aim 3). Because whole genomeTLR4-ko protected mice from airway inflammation*[35]*, we anticipate that a microglial targeted i-mTLR4-ko will have less myelin damage and CA1 neurite atrophy, which in wildtype mice are associated with hippocampal CA1 subfield microglial activity (Fig. 4).*

B. Innovation These studies innovate in developing mouse models for neurodegenerative responses to TRAP.

1. Our exposure model uses continuously collected nanosized/ultrafinePM from an urban near-freeway site; the nPM subfraction is well characterized for chemistry, particle size, and neurotoxicity [22, 36-39]

2. This is the first comparison of response to air pollution of ages across phases of reproductive senescence, in both sexes of wildtype mice, and in EFAD mice carrying human FAD genes and ApoE alleles.

3. The first in vivo studies of air pollution effects on cerebral blood flow.

4. *The first mechanistic studies of TLR4 in TRAP neuroinflammatory responses*.

5. The first research in animal models of air pollution to combine neurotoxicological and vascular approaches.

C.Approach

C1. Supporting data *(completely revised).*

a. Sex differences in E4FAD levels of TNFa and IL6 (unpublished).

We extended published results in EFAD mice to show female and apoE4 bias in brain TNFa and IL6. Prior studies showed similar E4 female excess of cerebral amyloid angiopathy (CAA) and cerebral microbleeds[7]*.*

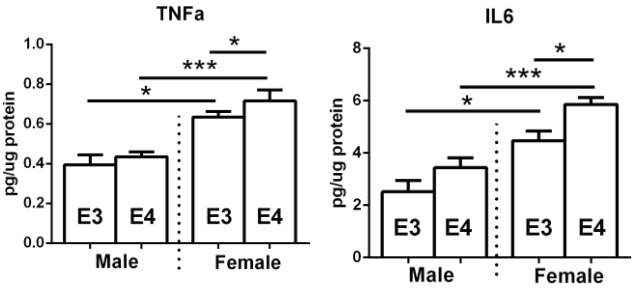


Fig. 1. *Sex and ApoE differences in brain TNFa and IL6. Female E4FAD mice have highest levels of TNFa and IL6. MSD multiplex cytokine ELISA of cerebral cortex. Females have higher TNFa (left panel) and IL6 (right panel) than males. E4FAD females have the highest levels of both cytokines, while males did not differ. N=5-6, Mean+/-SEM, * p<0.05; ** p <0.01, *** p<0.005, **** p<0.001*

b. Hippocampus mediated behavioral deficits are greatest in E4FAD female mice (unpublished).

Our primary behavioral measure is contextual fear conditioning, which is hippocampus mediated and involves the CA1 subregion during retrieval [40, 41]*. In female EFAD mice, the freezing behavior during retrieval testing for context was weaker in E4FAD vs E3FAD (left panel). Similarly, novel object recognition was weaker in E4FAD vs E3FAD females (right panel) in a task mediated by hippocampal-parahippocampal regions, including entorhinal cortex*[42]*.*

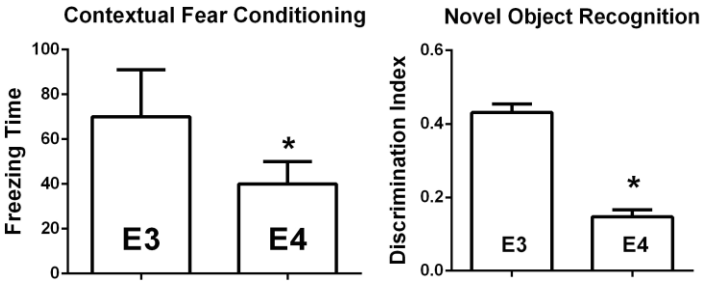


Fig 2. *ApoE allele effects on learning behaviors. Female E4FAD mice age 7 mo had weaker performance than E3FAD in contextual fear conditioning (left panel) and novel object recognition (right panel). N=5-6, Mean+/-SEM, *, p<0.05 relative to E3FAD.*

c.*Female and apoE4 bias in response to nPM exposure: Aβ load (female published, REF; male unpublished).*

EFAD female and male mice were chronically exposed to nPM in our standard model [22]: Exposure in vivo to nPM or Filter air. Cerebral cortex total Aβ was increased by nPM in female E4FAD. The higher level of baseline Aβ load in E4FAD versus E3FAD parallels human AD [7].

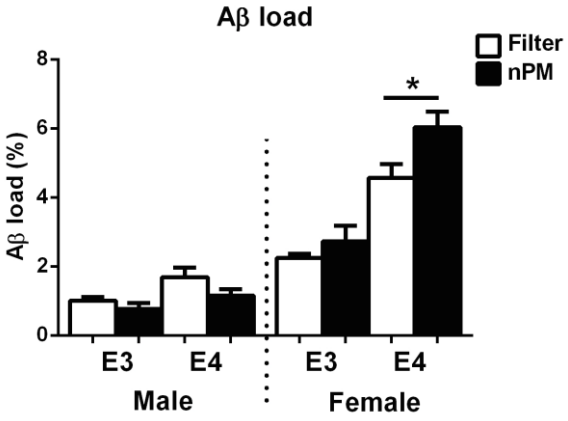
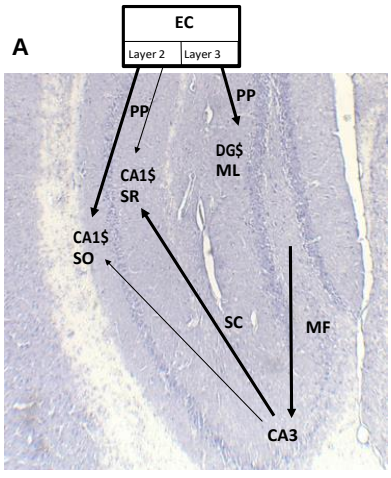


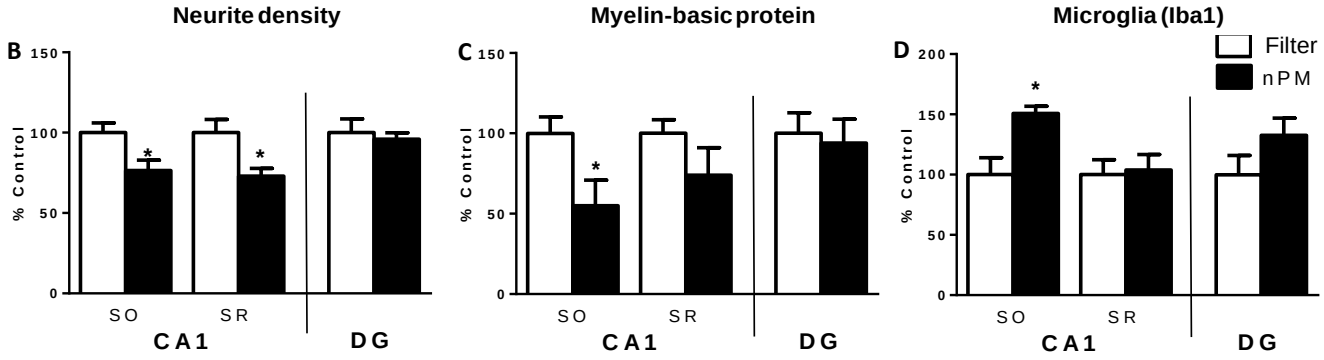
Fig. 3*. Sex and ApoE differences in response to TRAP nPM exposure increased Aβ load to highest levels in female E4FAD mice. Mice were exposed to nPM or filtered air. Cerebral cortex sagittal sections were analyzed for Aβ plaque load (4G8 antibody). Female E4FAD mice had 2.8-fold greater increased total plaque load after nPM than E3FAD mice [1]*

d.*nPM selectively targets hippocampal CA1 subfields causing decreased neurite density and myelinated pathways with increased microglial activation. (recently published, [24])*

Female B6 mice (3 mo) were exposed to 150 hr nPM or filter air. Neurite atrophy (-25%, Fig. 4B), reduced myelin-basic protein (-50% MBP, Fig. 4C) and increased microglial activation (+50%, Fig. 4D) were restricted to the CA1 region, without change in DG (dentate gyrus). MBP loss and increased microglial Iba1 immunostaining was further restricted to the stratum oriens (SO). The nPM selectivity for myelinated CA1 neurons parallels CA1 vulnerability in human AD, supporting the nPM exposure model for TRAP-acceleration of AD pathogenesis.

Fig. 4. Hippocampal CA1 selective vulnerability to TRAP.
In young B6 females, the CA1 subfield was damaged by nPM exposure/150 hours, while the dentate gyrus was not altered. A) Schematic of entorhinal cortex - hippocampal sub-region connectivity. B) Neurite density as total silver-stained area per field; C) Myelin basic protein (MBP) immunohistochemistry; D) Microglial activation, Iba1 immunohistochemistry.
*N=9, % of respective control, Mean +/- SEM, *p<0.05,*
Abbreviations: CA, *Cornu Ammonis*; DG, dentate gyrus; EC, entorhinal cortex; MF, mossy fibers; MO, molecular layer; SO, stratum oriens; PP, perforant path; RD, stratum radiatum, SC, Schaffer collaterals.





e.*nPM causes deficits in hippocampal-mediated learning. The 'novel object in context' test (NOIC) assesses hippocampal mediated object and context recognition[43] and showed significant deficits from nPM exposure of B6 male mice.*

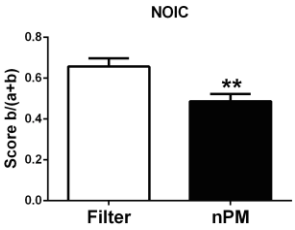


Fig 5: *Novel Object in Context Recognition (NOIC): Male B6 mice were tested for hippocampal mediated object and context recognition after exposure to nPM or filtered air. Discrimination index was analyzed on day 5 and reported as time of exploration of novel object divided by total time of exploration. nPM exposed mice showed a decreased score (-25% , p=0.01). Mean +/- SEM; N: ctrl=9, nPM=10*

f. TLR4 pathway responds to nPM toxicity in the brain

The TLR4 pathway (Fig. 6) was identified by RNAseq analysis of corpus callosum from nPM exposed mice (Project 4, unpublished). TLR4 was further implicated by PCR of hippocampal tissue from nPM exposed young male B6 mice showing nPM-induction of TLR4 (Fig. 6B), MyD88 (Fig. 6D) and TNFa (Fig. 6E), recently published[24]. Additionally, CD36 was elevated in an independent cohort nPM exposure (Fig. 6C, unpublished), This cohort also verified the TLR4, MyD88 and TNFa increases shown here. TNFR1 was also increased (not shown). Notably, the nPM had no detectible endotoxin activity (LPS), confirmed by lack of response to nPM in the MyD88-independent endocytosis pathway, which is LPS-mediated.

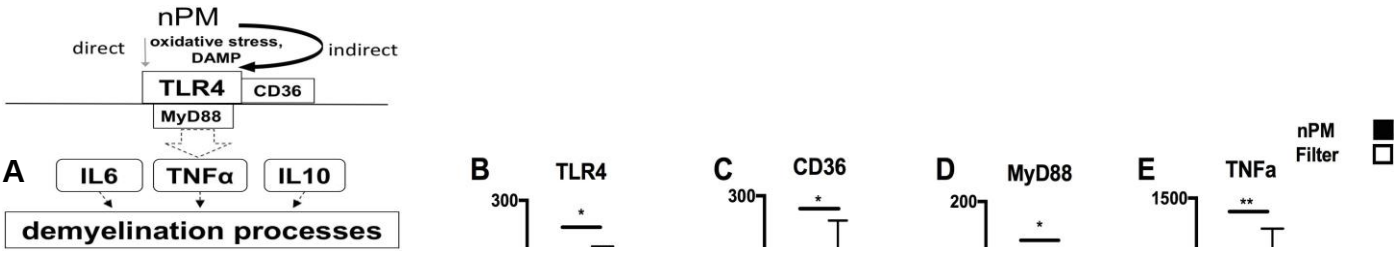


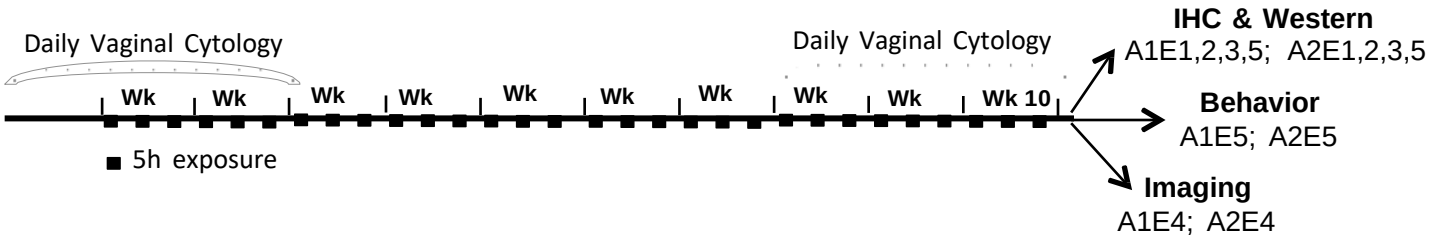
Fig. 6. *Chronic nPM exposure induced components of the TLR4 pathway. Young male B6 exposed to nPM exposure/150 hrs. A: Schematic of nPM induction of the TLR4 pathway. B,C,D,and E: TLR4 pathway mRNA responses in hippocampal response to nPM vs filtered air by q-PCR. N=7, Mean+/-SEM, *p<0.05; **p < 0.01.*

C2. Experimental Design Mouse exposures (Core C2) use a nanoscale fraction of TRAP which is sampled continuously by state-of-the-art aerosol samplers [22, 44]. The continuous sampling of TRAP in our paradigm is important because air pollution components that vary widely between sites, season, and by time of day. Thus, PM emitted in the morning by traffic are transformed during the day by photo-chemical reactions involving ozone and hydroxyl radicals, with further seasonal variations across the PM size classes [45]. Three distinct PM size classes are recognized, with different chemistry and bioactivity: coarse PM, 2.5-10μ in diameter ($PM_{2.5-10}$); fine PM, <2.5μ (PM2.5); ultrafine PM, <0.20μ. The bioactivity in vivo and in vitro varies differs by PM size and chemical composition. Coarse PM are mostly trapped in the upper airways, whereas smaller PM penetrate deeply into the bronchi and the alveolar region of the lung [46]. The smaller, and currently unregulated ultrafine

PM are more bioactive than PM2.5 in promoting atherosclerosis in mice [47] and in macrophage responses in vitro [48]. Core C2 collects ultrafine PM on Teflon filters, for elution by sonication into an aqueous suspension. This fraction was designated as nanoscale PM (nPM) to distinguish it from the total ultrafine TRAP-PM0.2. The nPM are enriched in water-soluble organic carbon, as well as transition elements and metals (e.g. Fe, Cu, V, Ni), but is depleted in black carbon [22] (CoreC2, Fig. 3). At low levels in vitro (1-10 µg/ml), nPM increases production of free radicals and oxidants (NO, $O_2^{\cdot-}$, H_2O_2) in glia and neurons [36]. For mouse exposures, suspended nPM are re-aerosolized, retaining the same size distribution as their airborne state [22].

Aim 1 (*refocused)*: Age and sex in brain susceptibility of C57BL/6 mice ('B6', wildtype) to nPM. We propose to analyze sex differences in response to chronic nPM exposure for ages that span reproductive senescence: young (2-4 mo), middle-age (10-12 mo) and older (18-20 mo) B6 mice and that are relevant to peri-and postmenopausal women (Project 1). *Young male B6 mice (3 mo) responded to nPM with CA1 neurite atrophy (Fig. 4B) and reduced CA1 myelination (Fig. 4C). We recently reported that aging female B6 mice (18-20 mo) had minimal responses to nPM, suggesting ceiling effects*[24], which match the lack of nPM induction at 18 mo of Nrf2 and HOX-1 [25] (Section A).

Because of logistic issues (workload and restricted availability of mice from the NIA Aging Colony), we will first compare sexes at each age in separate exposures (Expt. 1: 3 mo; Expt. 2: 10 mo; Expt. 3: 18 mo). We will examine hippocampal subregional selectivity by histochemistry and Westerns (details below). Ovarian activity will be documented with daily vaginal cytology for two 3-week periods: 1 week prior and 2 weeks during initial nPM exposure and the final 3-weeks of nPM exposure. Initial age comparisons will not examine behavioral differences. See Chart at end of Methods for the scheduling of experiments.



Once we determine the earliest age of vulnerability for nPM effects on myelinated pathways, we will expose that age/sex group to nPM (Expt. 4) for imaging by Core B2 (Y2).: Expt. 4 examines nPM effects on cerebral blood flow and BBB integrity, and on white matter tracts using advanced techniques. Measurement of cerebral blood flow responses to chronic nPM is of high interest for two reasons: (1) the age-decreases in basal blood flow in CA1, CA3 and dentate gyrus of rat hippocampus [49] and (2) the strong association of carotid thickening with PM2.5 in human populations [14]. Core B2 will receive each mouse from Exp 4 to examine in sequence on the same brain:
i) in vivo BBB permeability using dynamic contrast enhanced (DCE)-MRI;
ii) in vivo cerebral blood flow, BBB permeability and angiography measurements by 2-photon microscopy;
iii) postmortem ultra-highfield diffusion tensor imaging (DTI)-MRI to detect connectivity changes through various diffusivity and tractography (fiber tracking) maps and immunohistochemistry of white matter tracks and BBB integrity using confocal microscopy. The (DTI)-MRI technologies will give expected resolution of white matter tracts at 80u, e.g. Fig. 2 of Core B2 [50, 51].

Logistics and costs limit us to examining 2 groups of N=10 per year for these complex and labor-intensive analyses. Thus, we must alternate imaging resources between Proj 3 (Y2&5) and Proj 4 (Y3&4).

Tissue Preparation and Processing: After whole body saline perfusion, brains are rapidly removed from the skull and hemisected at bregma for frontal and posterior portions. The entire frontal portion is post-fixed in 4%-paraformaldehye and prepared for Klüver-Barrera myelin histochemistry (Project 4). The remaining posterior portion is hemisected: one hemisphere for immunohistochemistry (IHC) and the other hemisphere microdissected for hippocampal subregions (CA1-enriched and DG-enriched) and the entorhinal cortex (Project 3). The hemisphere side is alternated by individual within experimental groups to minimize contributions from hemisphere asymmetries. nPM effects on AD-relevant regions will be characterized by IHC.
i) neurite atrophy by silver staining that shows AD-like CA1 neuronal selectivity as we recently reported[24];
ii) white matter changes by IHC for myelin degeneration in perforant path and CA1 by myelin basic protein (MBH) & myelin-associated glycoprotein (MAG); *complement C5, C5a, C5 receptor (Proj 4)*

iii) microglial activation by IHC (Iba-1and CD68). While we appreciate the importance of microglial-astrocyte interactions, our focus we will not include astrocytes.
iv) microbleeds in cerebral cortex by hemosiderin;
v) plasma protein leakage in cerebral cortex and hippocampus by IgG and albumin.

Microdissected entorhinal cortex and hippocampus CA1 & DG subregions, will be evaluated for
i) Project 3: APP (sAPPb:sAPPa, Western) and Aß pepides (Aß38,40,42, MSD multiplex ELISA).
ii) Core C3: *TLR4 pathway (TLR4, CD36, MyD88), cytokines (TNFα and TNFRI; IL1α, IL6, IL10), and complement system (C5, C5a, C5R): Western blots. Core C3 Table 2 lists responses of these proteins to nPM and chronic cerebral hypoperfusion.* We include complement C5 components, which was increased in nPM exposed mice used for controls in experimental cerebral ischemia (Project 4, Fig.1 and[34]) The 300 µg total soluble protein (RIPA buffer extract) for each microdissected region per brain suffices for multiple Western blots.

Expt. 5 will compare the three ages for the sex showing greatest vulnerability; this direct comparison (Expt. 5) is not redundant because it controls for intangible seasonal and other variations between exposures. The Pike Lab (coPI) will examine hippocampal-mediated learning and memory in mice of Expt. 5 (Y3). Because behavioral tests cause stress, which could alter TNFα and other stress-sensitive proteins, we prefer to use this group exclusively for cognitive studies.

Collaboration with Project 4: Mice with chronic cerebral hypoperfusion (CCH; sub-ischemic arterial wall constriction by spiral collar) will be simultaneously exposed to nPM in parallel with our mice. Since many of our endpoints are shared across both projects (e.g., TNFα, C5a, IBA-1), we will directly compare our age-matched mice ('intact', no surgery) with Project 4 sham constricted (neck opened but no arterial collar) and CCH- mice. Brain sections are shared by both projects to match aims of each project: Project 3, CA1 neurons, entorhinal perforant path projections; Project 4: white matter, corpus callosum is examined by both Projects 3 & 4.

Expected Results: *We must confront an apparent contradiction from the presence of FAD genes. The new EFAD data show more amyloid increase by nPM in female than male E4FAD (Fig 3). This finding is consistent with the greater amyloid load in female ADtg mice and postmortem AD brains[52, 53]. However, estrogen is neuroprotective in FAD mice by reducing Aβ production [54]. In wildtype rodents show clear female advantage in experimental stroke, which cause smaller infarcts in young female rats, with diminished sex differences by 10-12 mo [55]. Moreover, in wildtypes without FAD genes, estrogen is neuroprotective by reducing Aβ production [54] ; by attenuating neuron damage in CA1 from seizures [55]; and by decreasing post-ischemic white matter damage and neurocognitive deficits (Project 4, Fig. 4). Thus, we expect young female vs male B6 mice to show less CA1 neurite atrophy, less loss of myelin basic protein, and less microglial activation from nPM exposure, with subregional specificities. Further, we predict the possible loss of female advantage with age with estrogen deficits in acyclic mice. Studies on middle-aged mice at 10 and 18 mo will define this transition (Section C, Ovarian activity).*

Despite the rarity of microbleeds in wildtype B6 mice [7], their low baseline of ≤5 microbleeds/cortex might favor detection of response to nPM in association with the BBB leakage, as observed in rats with a different TRAP model (see above).

For Project 4 collaboration on CCH interaction with nPM, we anticipate synergy of nPM and CCH with greater attrition of CA1 neurites and CA1 myelin, because of the CA1 vulnerability to ischemia. From above, we anticipate the 'young female advantage' because of estrogen neuroprotection to ischemia. *Based on previous results showing an age-ceiling effect on the nPM response (discussed above), we do not expect to observe greater effects of nPM at advanced age; however, this may not translate to nPM/CCH-exposed mice as a result of diminished vascular reserve and microvascular failure secondary to CCH (discussed in Project 4, A4).* We anticipate that mice showing behavioral impairments will also show more neuronal damage, e.g. in CA1 neurite atrophy or myelin degeneration.

Caveats: Aging females have complex individual patterns of estrogen loss that may increase experimental variance. We do not propose expensive assays for estradiol of individual mice, but serum will be stored. If we do not see effects of nPM exposure on the novel object *in context* and fear conditioning tests *(Fig. 5),* we may consider the Barnes maze and other behavioral tests for hippocampus mediated cognition.

Future Directions: Our focus on brain regional effects of nPM exposure in association with age, sex and genotype will guide our analysis of potential drug interventions, e.g. by sex, age, ApoE allele, and brain region specific vulnerability. Ongoing studies (R01AG051521, Finch PI) and R21 AG05020, Finch PI) examine potential protection from nPM according to the hypothesis that TRAP is proamyloidogenic. Several drugs are being tested for neuroprotection to TRAP in our nPM model: a novel soluble GSM (gamma secretase modulator) which lowers brain Aβ42 in B6 males, without altering Notch proteolysis [56, 57]; and memantine, which shows cognitive benefits to AD in humans and mice, in association with lowering Aβ oligomers [58-60].

Aim 2: Sex-ApoE allele interactions in EFAD mouse responses to nPM. Studies on EFAD mice employ Aim 1 parameters for neurodegenerative changes in hippocampal subfields and for hippocampal-mediated learning. Based on the female excess of Aβ in EFAD mice from our recent report [18], we hypothesize that nPM will show female bias in accelerating AD changes. These studies in Y2-Y5 will define the peri-menopause in EFAD mice by vaginal smears at older ages relevant to those examined in the expanding use of EFAD mice.

As in Aim 1, we first examine E3FAD and E4FAD male and female mice at each age in separate exposures: Expt. 1: 2-4 mo; Expt. 2: 10-12 mo; Expt. 3: 18-20 mo. Expt. 5 will compare the three ages for the sex and genotype showing the most vulnerability. See above for schematic of experiment timeline. As in Aim 1 we will first determine the most critical age of vulnerability for nPM effects on the BBB (CAA, microbleeds, plasma protein leakage). Then, we will expose that age/sex group to nPM (Expt. 4) for imaging (Y5).

Collaboration with Project 4: Brain sections from nPM exposed EFAD mice will be provided to Project 4 for analyses of hippocampal myelinated pathways, *complement C5, C5a, C5R IHC* and neuronal endpoints. These baseline results will guide future Project 4 experiments on examining the synergies of chronic cerebral hypoperfusion (CCH) and nPM in EFAD mice. Our nPM exposed EFAD mice will serve as controls for CCH mice in Project 4. Our collaboration with the William Mack Lab is well established, see co-authored[34].
Expected Results: Because female EFAD vs male EFAD have higher TNFα (Fig. 1), together with more Aβ deposits (Fig. 3) and more microbleeds [18], we hypothesize that EFAD will show female excess of BBB disruption by nPM exposure with ensuing microbleeds, and CA1 neuron and myelin damage. With the ApoE4 exacerbation of progressive BBB leakiness during normal human aging [61], E4FAD mice are predicted to accumulate more microbleeds and show more BBB leakage than E3FAD.

Hippocampal subregions may show different ApoE allele effects by sex. Hippocampal-mediated behaviors differ between control female E4- and E3FAD (Fig. 2). Based on these findings, the most vulnerable sex and ApoE allele of EFAD based on myelin and neuritic damage will be examined by Core B2 (Exp 4) for effects of chronic nPM on in vivo cerebral blood flow and postmortem for BBB leakage and tractography by ultra-highfield (DTI)-MRI.

Little is known about aging of EFAD mice beyond 8 months. Because APP (Tg2576) mice have 20% shorter lifespans) and develop hydrocephalus not found in wildtype B6 [62], we anticipate that EFAD will also die prematurely. Also, other APPtgs show sex differences in lifespan [63]. The postmortem diagnosis of hydrocephalus in 15% in Tg2576 was not described in detail (sex not stated) and could involve complications of cerebral microbleeds *which show female excess (+)*. Reproductive organs will also be characterized: females for vaginal cytology and uterine wet weight, which become atrophic at later ages [53]; males for weight of testes and seminal vesicles. Despite the increasing use of EFAD mice, little is known about interactions of reproductive aging with AD phenotypes at ages 6-10 months. Because of the strong impact of sex steroids on brain amyloid shown by the Pike Lab [36], this is a critical gap. For brains from Project 4, we anticipate that chronic cerebral hypoperfusion (CCH) will increase microbleed frequency. However, we predict female excess, because of female excess of microbleeds in EFAD mice.
Caveats: If EFAD have poor survival to 18 mo, paralleling the shorter lifespans of APP (Tg2576)[62], aging studies will be restricted to 10 mo. We also note a major divergence of mouse and human for sex differences in cerebrovascular pathology. In contrast to mice, humans have male excess of microbleeds in AD samples from ADNI and KIDS [18] and male excess of CAA (Mayo Clinic) [64]. These species differences could establish the EFAD mouse as a model for sex-ApoE interactions with cerebrovascular pathology.
Future Directions: In breeding of EFAD, the crosses yield similar numbers of non-FAD transgenic ApoE carriers, which could be valuable comparisons for ApoE allele different response to nPM on hippocampal subregions independent of human Aβ.

Aim 3 (*Major revision):* *We replaced TNFR1-ko studies in Y3-5 with new studies of the TLR4 pathway in TRAP, which were based on bioinformatics analysis of nPM RNA responses[33]. New data (Fig 6) shows that nPM exposure increased mRNA of TLR4, CD36 and MyD88, a key TLR adapter protein, as well as TNFa. The nPM induced MyD88-dependent pathway activates NF-κB, with ensuing induction of TNFa and other cytokines[24].*

We propose studies of TLR4 with a new i-mTLR4-ko model targeted for microglia made by CJ Pike, co-Investigator of this Proj 3, for adult-inducible excision of TLR4 ($mCre^{+/-}$, $fl^{+/+}$) by tamoxifen. TLR4 knockout (i-mTLR4-ko), CX_3CR1^{CreER} mice (JAX020940) were crossed with $TRL4^{loxP/loxP}$ mice (JAX024872). The CX_3CR1^{CreER} mouse provides cell-specificity as Cre expression is under control of the CX_3CR1 promoter. CX_3CR1-expressing cells in adult mice are limited to microglia in brain and macrophages in gut, kidney [65, 66]. Cre is inducible by its linkage to an estrogen receptor mutant that is activated by treatment with tamoxifen. The $TRL4^{loxP/loxP}$ mouse has TLR4 flanked with two loxP sequences (TLR4 flox). The first generation (F1) mice are 100% heterozygous ($mCre^{+/-}$, $fl^{+/-}$) and are currently breeding. F2 mice composed of 25% experimental animals ($mCre^{+/-}$, $fl^{+/+}$), 25% control group ($mCre^{-/-}$, $fl^{+/+}$) will be characterized for expression and functional efficacy of the TLR4 deletion, 3 months after tamoxifen treatment (75 mg/Kg, i.p. for 5 days or vehicle). The characterization will be completed in early 2018.

We will chose the age and sex from Aim 1 that gives maximum wildtype B6 response to nPM (Aim 1). After tamoxifen treatment both genotypes of mice will be exposed to our standard 150 hours of nPM: 2 genotypes ($mCre^{+/-}$, $fl^{+/+}$; $mCre^{-/-}$, $fl^{+/+}$) +/- tamoxifen exposed to nPM or filtered air (eight groups of N=10; 80 mice). We will prepare and process the brain as described in Aim 1. Our initial targets include myelin integrity and corpus callosum width, hippocampal CA1 and dentate gyrus neurite atrophy, myelin basic protein, and microglial Iba1, and Westerns (Core C3).

If the i-mTLR4-ko attenuates neurite atrophy and or myelin degeneration, the next experiment will examine cognitive behaviors that are sensitive to nPM (Fig. 5). Unless the control genotype ($mCre^{-/-}$, $fl^{+/+}$) show different responses to nPM than wildtype B6 from Aim 1, we will only examine the $mCre^{+/-}$, $fl^{+/+}$ mice (+/- tamoxifen). We do not have sufficient budget to examine these mice for Imaging by Core B2.

We will evaluate relationships between myelin and neurite atrophy in the interconnected hippocampal regions, e.g. subfield. If TRF4-ko attenuates neurite atrophy and or myelin degeneration, the next experiment will examine cognitive behaviors that are sensitive to nPM (Fig. 5).

Collaboration with Project 4: We will also provide i-mTLR4-ko to test the hypothesis that microglial TLR4-ko will lessen post-ischemic neurodegeneration. Our nPM exposed i-mTLR4-ko mice will serve as controls for CCH mice in Project 4.

Expected Results: We predict that microglial TLR4-ko will decrease myelin degeneration and neuroinflammation based on our data showing TLR4 involvement in the nPM response (Fig. 6 above and [33]) and because airway inflammation was attenuated by whole genomeTLR4-ko[35]. Furthermore, we anticipate that neurite atrophy is dependent on microglial TLR4 pathways. Because APP processing is largely intra-neuronal, we do not anticipate that i-mTLR4 will alter nPM effects on sAPPa:b, which we showed in wildtype B6 [1].

Caveats Because we do not know how the genetic makeup of these transgenic mice will alter brain responses to nPM exposure, therefore, we must perform careful analyses of the brains from control mice ($mCre^{-/-}$, $fl^{+/+}$) to ascertain whether they respond similar to wildtype B6. The stimulation of TLR4 inflammatory pathways in the brain by TRAP components may be mediated by systemic influences emanating from the lung [67]. The wide range of ligands that stimulate TLR4 include oxidized lipids[32] which we showed are increased in blood during nPM exposure[68]. Although there may be some direct entry from olfactory neurons, we argue that this cannot reasonably explain the brain-wide inflammatory responses to nPM [69].

Future Directions: If the microglial deletion of TLR4 results in smaller nPM-induced myelin degeneration and neuroinflammation, we will consider crossing these mice with hAPP transgenics. These experiments are complicated by the fact that lifelong, systemic TLR4-ko show increased fibrillary and soluble amyloid levels in two ADtg mice [29-31]. Because nPM induces Aβ deposition (Fig. 3 and [1]), these studies could identify unique and shared Aβ regulating pathways of TLR4 and nPM. We could also time the tamoxifen excision of microglial TLR4 before or after the nPM exposure to address possible prevention versus treatment questions. These studies would give a basis for considering the TLR4 pathway in neuroprotection for air pollution.

C3. Detailed Methods

nPM exposure (Core C2): The paradigm for rodent exposure to air pollution was developed by Prof Constantinos Sioutas, who is a leader in analysis of air pollution. Briefly, ultrafine PM are continuously collected on filters at a typical urban site in Los Angeles, 150 m east of I-110 freeway. The ultrafine PM (<0.2 μm) are transferred into aqueous suspension by vortexing and sonication. Because this re-suspension is enriched in water-soluble organic carbon, but depleted in black carbon, it is designated as nPM to distinguish it from the total ultrafine PM [22]. The nPM are re-aerosolized for rodent exposure at 300 μg/m^3 for 5 h/d, 3 d/wk to simulate intermittent exposure to high-density urban traffic. *The mouse exposure to PM0.2 at 300 ug/m^3 for 150 h (1% of the lifespan) delivers 2.25 mg/kg. For humans, exposure to PM0.2 at 30 ug/m^3 for 1% of lifespan yields 2.5 mg/kg, assuming inhalation rates: mouse, 0.025 L/min; human, 12 L/min.* Control cages receive particle-free filtered air, nPM <1 μg/m^3. For nPM exposure, rodents are transferred from home cages into whole-body exposure chambers allowing free movement. All procedures comply with animal welfare laws, guidelines, and policy, approved by USC Institutional Animal Care and Use Committee.

EFAD mice: EFAD mice were generously given by Mary Jo LaDu (Univ. Illinois at Chicago). E3FAD and E4FAD mice were generated by crossing 5XFAD to homozygous APOE3-, and APOE4-TR (non-FAD carriers) 5xFAD mice are transgenic for 5 distinct FAD mutations (APP K670N/M671L+ I716V+ V717I and PS1 M146L+L286V) controlled by the neuron-specific mouse Thy-1 promoter [70].

Ovarian activity during nPM exposure will be monitored by vaginal cytology (daily vaginal smears). The comparison of females aged 3, 10, and 18 mo for 12 weeks of nPM exposure spans major changes in ovarian steroids that could interact with brain inflammatory responses to nPM exposure. *Lab rodents are good models for corresponding STRAW Stages of human menopause*[5]. *Project Investigators (Finch, Pike, Morgan, Wendy Mack) have published on middle-aged mice as models for the human peri-menopause [71-73]. As we showed in detail for B6 mice, after 7 mo, fertility declines sharply and ovarian cycles become irregular during complex transitions to acyclicity with major decline of plasma estrogens: between 18 and 21 mo mice reach the rodent equivalent of menopause, with total follicular depletion and ovariectomy levels of plasma estradiol [5, 74].* The timing of these transitions shown in vaginal cytology varies widely between individuals [6, 75, 76]. At 10 mo, mice of both sexes are routinely discarded as retired breeders, because of reduced fecundity at ages arising far below the 28-30-mo lifespan. We will not include mice older than 22 mo, when pituitary tumors (sommatotropes, mammotropes) become prevalent [76, 77]; the individual variations in plasma GH would introduce additional heterogeneity; moreover, expanding adenomas can distort deeper structures including the hippocampus) [78]. In contrast to females, B6 males maintain gonadal function and plasma testosterone into later ages [79, 80] and pituitary tumors are absent at later ages [81].

After 10 week nPM exposure, the youngest female group, now aged 6 mo, should still be actively cycling; the 10 mo group, initially chosen for constant estrus (prolonged cycles) will be 13 mo; and the 18 mo group will be 21 mo, when >90% will be anestrus (menopause equivalent with few epithelial cells in the smear). The expected ovarian aging heterogeneity will be tracked by daily vaginal smears for one week at the onset and end of the 10 week nPM. The 10 & 18 mo groups will be selected for constant estrus smears before exposure. At necropsy, uterus wet weights will confirm the vaginal cytology for low levels of estradiol (menopausal) [74] to exclude any mice with residual ovarian steroids; necropsy will also exclude mice with early onset gross pituitary tumors.

EFAD mice will also be characterized by vaginal cytology (Aim 2). Although aging studies of EFAD are not proposed, the 6-9 mo ages studied here and by others are at the onset of ovarian senescence. Because ovarian steroids strongly alter brain amyloid, as shown by coPI CJ Pike and others, it is important to know if female EFAD differ from B6 in basic patterns of ovarian aging.

Hippocampal-mediated cognitive function (Pike Lab): *Object recognition in context (NOIC) and contextual fear conditioning (CFC) will use computer-assisted video tracking and analysis. The NOR and CFC tests were chosen because of their hippocampal dependence [82, 83]. nPM exposure impaired NOIC performance (Fig. 5).* Contextual fear conditioning (CFC) is assessed by the Contextual NIR Video Fear Conditioning System (Med Associates, St Albans, VT) with video recordings 30 frames/sec of freezing response complex, including whisker twitch, tail flick, and lack of movement except respiration. The delay fear-conditioning task measures hippocampus-dependent memory. The fear conditioning setup consists of 4 sound-proofed chambers (Ugo Basile) and a video tracking system using Noldus EthoVision software. During the training protocol on day 1, mice are exposed to 5 trials of pairing the conditioned stimulus (CS) with the unconditioned stimulus (US). Each trial is composed of 120 sec of habituation to the chamber/ inter-trial-interval, followed by 20 sec CS (tone, 75dB) and 2 sec of US (shock, 0.5mA). Freezing behavior is defined by the absence of movement other

than respiration, and is scored during a baseline period of 120 sec to account for differences in locomotor and exploratory activities between groups. The learning rate is determined by comparing freezing behavior between groups during each trial. On day 2, the mice are tested for freezing to the CS in an altered context with different chamber walls, floor, and smell. The CS is presented 3 times during day 2; freezing behavior is scored to test for classical conditioning. On day 3, the mice are tested for contextual memory by placing them into the same chamber as in day 1, but without a tone or shock, and quantifying freezing behavior for 480 sec.
Novel Object in Context Recognition (NOIC): NOIC is used to assess hippocampal-mediated object and context recognition [43]. Mice are habituated to novel object recognition chambers with no objects, in two separate locations, on days one and two, for five min./day. On day three, mice are exposed to two distinct objects (A and B) for five min. in the first location. Mice show no preference between objects on day three, and objects A and B are counterbalanced between trials. 24 hr later, mice are placed in location two, with duplicates of object A, again for five min. The following day mice are again placed in location two, this time with objects A and B, again for five min. Time spent investigating the object is recorded, with blinded experiments evaluating behavior on a live video feed. Discrimination index is calculated by exploration time of object B on the final day, divided by total time spent exploring both objects. A discrimination index of 0.5 shows no preference for novel object, with higher than 0.5 indicating novel object preference. See Fig. 5.
Cerebrovascular Aβ (Pike Lab) is assessed by two measures (I), number of Aβ-IR blood vessels quantified per area in cerebral cortex. (II) Aβ-IR sections collected at high magnification for image analysis by video capture system (Olympus DP73 camera). Brain images span the cerebral cortex, from 8 brain sections spaced equidistantly across the horizontal plane. In a regular pattern, 6 non-overlapping pictures are captured per section. Using NIH ImageJ 1.48 software, the ROI is outlined to include all cortical layers and exclude underlying white matter. The gray scale pictures are thresholded to create a binary separation between positive and negative IHC. This allows for calculation of the percentage of a section area occupied by the immunoreactive label. From the total load value for the entire cortex in each picture, a separate ROI is outlined around each Aβ-immunopositive blood vessel to calculate the Abeta associated with cerebrovasculature.
Aβ Immunohistochemistry (Finch Lab): After sagittal sectioning of EFAD brains 0.5-2 mm from midline (25 um), sections are immunostained for Aβ amyloid with 4G8 (residues 17-24 at N-terminal of APP; Covance, Princeton, NJ) [84]. Sections are immersed in 70% formic acid/5 min. Endogenous peroxidases are blocked by 3% H2O2 and 10% methanol in Tris-buffered saline (TBS). Sections are permeabilized in 0.1% Triton X-100/15 min, blocked by 30 min incubation in TBS with 2% BSA and 0.1% Triton, followed by primary and then biotinylated anti-mouse secondary antibodies (1:250), and stained by ABC peroxidase and DAB substrate (Vector, Burlingame, CA). For plaque quantification, the bright field images are thresholded to highlight plaques and to diminish background by visual inspection to confirm plaque identity. Cortex sections are evaluated for total plaque number and % area covered (Aβ load) by NIH ImageJ software.
Immunohistochemistry: Myelin degeneration is assessed with antibodies for myelin basic protein (MBP) and myelin-associated glycoprotein (MAG); glial activation by IBA-1 (microglia) and GFAP (astrocytes); blood leakage for IgG and albumin. Core B2 lists additional antigens for immunohistochemistry relevant to BBB.
Prussian Blue Staining (microbleeds): Hemosiderin deposits are assayed histochemically by Prussian Blue [7]. Sections are immersed in 5% KCN/5% HCl, rinsed, and counterstained with 2% Nuclear Fast Red.
ELISA (Aβ 38,-40,-42 peptides) Cerebral half-cortexes are homogenized in DEA buffer (0.2% diethylamine, 50 mm NaCl) with protease inhibitor cocktail (Sigma) [85]. Supernates (20,800g x 30 min) are neutralized with Tris-HCl pH 6.2. Pellets are resuspended in 1% SDS-PBS and centrifuged ('SDS extract'). Aβ38,-40,-42 peptides are assayed by Peptide Panel 1 (4G8) Kit V-PLEX™ (Meso Scale Discovery, Rockville, Md).
Western Blots (Core C3): We estimate 120 brains/ year for 3 to 5 experiments per Aim, which will yield about 360 samples (3 regions, 120 brains) for CoreC3. Tissues are homogenized in RIPA buffer by a teflon pestle on ice. See Core C3 for details of Western blot analysis of 10 µg protein with infrared detection (Licor system). We estimate a yield of 300 µg total protein for each of the 2 hippocampal regions (CA1-enriched and DG-enriched) and the entorhinal cortex CA1-DG-corpus callosum and entorhinal cortex, and 300-400 µg for the cortical shell. Primary antibodies against *TLR4, CD36, MyD88; TNFα, TNFRI; IL1b, IL6, IL10; C5, C5a, C5R*; secondary antibodies conjugated with IRDye 680 (rabbit, LI-COR Biosciences) and IRDye 800 (mouse, LI-COR).
Silver staining (Finch Lab) adapted from [86]. Sections are dried overnight at room temperature and impregnated in 1 M $AgNO_3$, washed, and incubated in ammoniacal silver, and developed. Bright field images are thresholded to highlight neurites and cell bodies and to diminish background by NIH ImageJ software.

Imaging methods (see Core B2 for further details)
DCE-MRI. The DCE-MRI imaging protocol includes measurement of pre-contrast T1-values followed by a dynamic series of T1-weighted images with identical geometry and a temporal resolution of <5 seconds. A bolus dose of Gd-DTPA (Gadolinium-diethylenetriamine pentaacetic acid, Magnevist®) is injected via the tail vein and DCE images are collected. Blood-to-brain transfer K_{trans} constant maps will be constructed.
Laser-doppler flowmetry (LDF). Cerebral blood flow (CBF) responses to hind limb stimulation is measured in anesthetized mice using laser-Doppler flowmetry measured through a cranial window. CBF will be measured and averaged from 3 trials per mouse.
BBB permeability. Cortical cerebrovascular permeability is determined with rapid *in vivo* multiphoton microscopy imaging using a custom Zeiss 5MP multiphoton microscope. *In vivo* time-lapse images will be acquired every 2 min for a total of 30 min after i.v. injection of TMR-conjugated medium-sized dextran.
BBB angiography. Fluorescein-conjugated mega-dextran is injected via the left femoral vein or retroorbitally and multiphoton Z stack images are taken through the cranial window starting at 50 mm below the cortical brain surface and continuing to 500 mm deep through cortical layers II and III, at 1 μm intervals between each image. Z stacks are reconstructed with ZEN software. Capillary length is analyzed by NIH ImageJ software.
DTI-MRI. Diffusion-weighted images (DWI) are obtained from fixed brains using a conventional pulsed-gradient spin echo (PGSE) sequence. An optimized six point icosahedral encoding scheme is used for diffusion weighted acquisitions with a single un-weighted reference image for a total imaging time of ~24 hours. Imaging is performed on the entire brain, with emphasis on white matter changes in corpus callosum.
Tissue immunofluorescent and fluorescent lectin staining. Fixed brain sections are processed by standard immunofluorescence or lectin staining protocols. Images are taken on a confocal microscope (LSM 510) coupled to a Mai Tai DeepSee Ti:Sapphire, Argon 488, HeNe 543, and HeNe 633 lasers for up to four-color imaging. Regional quantification of stained cells in hippocampus and cortex uses NIH ImageJ software.
Statistical analysis (in consultation with Prof. Wendy Mack): *Sample size: Our preliminary data (Figs 3-6) show large size effects of nPM exposure: mean difference in nPM vs. control, divided by pooled SD): 1.66 (Aβ in females), 0.50 (Aβ in combined males and females), 2.50 (Aβ in ApoE4 females), 1.73 (sAPP), 1.19 (neurite density in SO), 1.19 (myelin base protein in SO), 1.25 (microglia in SO), 1.51 (NOIC cognitive task),1.71 (TLR4), 1.38 (myd88), and 2.16 (TNFα). With samples of 10 mice per group, we can detect nPM effect sizes of 1.32 and larger (80% power, 2-sided alpha=0.05), which are well within the range of effect sizes in preliminary data. Note that many of our analyses will involve nPM main effects with even larger sample sizes, e.g., combined sex, combined age groups. For such nPM main effects (assessed over the levels of another experimental factor), we will have the following minimum detectable effect sizes: 0.91 and higher (n=20 per nPM group, e.g., combined sex); 0.74 and higher (n=30 per nPM group, e.g., combined age groups); 0.63 and higher (n=40 per nPM group, e.g., combined sex/genotype groups). Analysis: Because the majority of our dependent outcome measures are evaluated at one time point per animal, the primary modeling approaches will use general linear models (or rank-based non-parametric analogues) for independent observations. Normalizing transformations will be done as needed. In all experiments, the primary independent variable is nPM vs. control. Other model factors, depending on experiment, include sex, age group (3 levels), genotype, and i-m TRL4-kockout. Interaction terms will test for differences in nPM effects (by sex, genotype, etc.). For outcomes that are evaluated multiple times within animal (e.g., Aim 1,Exp1,2,3; Aim 2, Exp1,2,3; Aim 3, Exp1; KB, IHC, Western), mixed effects linear models will be used. Besides the main effects specified above (nPM, sex, etc.), we will include additional indicator variables for measurement time; random intercept terms will be specified at the mouse level to model individual deviations from the overall population average. Depending on the distribution of each outcome, results will be presented as mean (SD, SEM) or median (IQR). To consider the multiple hypothesis testing (from multiple types of outcome variables) within experiment, multiple comparisons will be controlled at a 5% experiment-wise false discovery rate (FDR). Dr. Wendy Mack (Division of Biostatistics, Dept of Preventive Medicine) will provide statistical oversight and analytic support; she will recruit a graduate student with appropriate expertise for part-time statistical support.*
Shared data management and analysis: Projects 3 and 4. Shared data analysis will be done in Experimental Projects 3 and 4, which employ the same mouse models, nPM exposure paradigms, and common analysis of brain imaging by Core B2 and of Western blot proteins by Core C3. As part of study planning, a common REDCap database will be developed in Y1 for data entry and management. REDCap (Research Electronic Data Capture) is a web-based data application specifically developed for the data entry and management of research data; institutional support for REDCap is provided through the USC Clinical and Translational Science Institute (CTSI). REDCap data are seamlessly exported for use in all major statistical

software applications. Specific to this P01, the REDCap database will use common variable naming and coding for common outcomes across the two projects. Data for each project can be entered in the same database, using the same data structures, by adding a “study arm” (one arm for each Project’s data), that allows Project data to be maintained as separate entities (by arm), but that still can be exportable as a single database. Databases for statistical analyses of data common to the two projects can be efficiently developed.

Table 1. Timeline with Animal numbers and endpoints

															Core B2	Core C3
Year		Genotype	Detail	Groups	Geno	Sex	Age(mo)	Air	TAM	N/group	Total	Behav	KB* (prj4)	IHC*	Imaging/IHC	Western*
Y1	A1, Exp1	B6	M+F	4	1	2	2	2	n/a	10	40	0	40	40	0	40
Y1	A1, Exp2	B6	M+F	4	1	2	10	2	n/a	10	40	0	40	40	0	40
Y1	A1, Exp3	B6	M+F	4	1	2	18	2	n/a	10	40	0	40	40	0	40
Y2	A1, Exp4	B6	Imaging	4	1	2	TBD	2	n/a	10	40	0	0	0	40	0
Y2-3	A1, Exp5	B6	Age	6	1	1	2,10,18	2	n/a	10	60	60	0	0	0	0
Y2	A2, Exp1	EFAD	M+F	8	2	2	2	2	n/a	10	80	0	80	80	0	80
Y3	A2, Exp2	EFAD	M+F	8	2	2	10	2	n/a	10	80	0	80	80	0	80
Y3	A2, Exp3	EFAD	M+F	8	2	2	18	2	n/a	10	80	0	80	80	0	80
Y3-4	A2, Exp4	EFAD	Imaging	2	1	1	TBD	2	n/a	10	20	0	0	0	20	0
Y5	A2, Exp5	EFAD	Age	6	1	1	2,10,18	2	n/a	10	60	60	0	0	0	0
Y4-5	A3, Exp1	i-mTLR4-K		6	2	1	TBD	2	2	10	60	0	60	60	0	60
Y5	A3, Exp2	i-mTLR4-K		4	1	1	TBD	2	2	10	40	40	0	0	0	0

TBD, to be determined

*split tissue from same brain (see Experimental Details)

VERTEBRATE ANIMALS

1. Provide a detailed description of the proposed use of the animals in the work outlined in the Research Strategy section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.

C57BL/6 (B6)mice (male and female), EFAD transgenic (tg) mice (male and female) and TLR4 knockout (i-mTLR4-ko) mice/ littermate controls (sex to be determined from Aim 1 results) will be used in this proposal.

EFAD (E3FAD and E4FAD) mice were generated by crossing 5XFAD to homozygous APOE3-, and APOE4-TR. 5xFAD mice are transgenic for 5 distinct FAD mutations (APP K670N/M671L+ I716V+ V717I and PS1 M146L+L286V). They are provided by University of Illinois at Chicago (Mary Jo LaDu, PhD).

i-mTLR4 knockout (TLR4-ko), adult-inducible excision of TLR4 ($mCre^{+/-}$, $fl^{+/+}$) by tamoxifen, are CX_3CR1^{CreER} mice (JAX020940) crossed with $TRL4^{loxP/loxP}$ mice (JAX024872). The CX_3CR1^{CreER} mouse provides cell-specificity as Cre expression is under control of the CX_3CR1 promoter. CX_3CR1-expressing cells in adult mice are limited to microglia in brain and macrophages in gut, kidney (Jung et al 2000, PMID10805752; Yona et al 2013, PMID23273845). Cre is inducible by its linkage to an estrogen receptor mutant that is activated by treatment with tamoxifen. The $TRL4^{loxP/loxP}$ mouse has TLR4 flanked with two loxP sequences (TLR4 flox). The first generation (F1) mice are 100% heterozygous ($mCre^{+/-}$, $Fl^{+/-}$) and are currently breeding. F2 mice composed of 25% experimental animals ($mCre^{+/-}$, $fl^{+/+}$), 25% control group ($mCre^{-/-}$, $fl^{+/+}$) will be characterized for expression and functional efficacy of the TLR4 deletion, 3 months after tamoxifen treatment (75 mg/Kg, i.p. for 5 days or vehicle).

The strains, ages, sex and numbers used in each aim are listed below:

														Core B2	Core C3
	Genotype	Detail	Groups	Geno	Sex	Age(mo)	Air	TAM	N/group	Total	Behav	KB* (prj4)	IHC*	Imaging/IHC	Western*
A1, Exp1	B6	M+F	4	1	2	2	2	n/a	10	40	0	40	40	0	40
A1, Exp2	B6	M+F	4	1	2	10	2	n/a	10	40	0	40	40	0	40
A1, Exp3	B6	M+F	4	1	2	18	2	n/a	10	40	0	40	40	0	40
A1, Exp4	B6	Imaging	4	1	2	TBD	2	n/a	10	40	0	0	0	40	0
A1, Exp5	B6	Age	6	1	1	2,10,18	2	n/a	10	60	60	0	0	0	0
A2, Exp1	EFAD	M+F	8	2	2	2	2	n/a	10	80	0	80	80	0	80
A2, Exp2	EFAD	M+F	8	2	2	10	2	n/a	10	80	0	80	80	0	80
A2, Exp3	EFAD	M+F	8	2	2	18	2	n/a	10	80	0	80	80	0	80
A2, Exp4	EFAD	Imaging	2	1	1	TBD	2	n/a	10	20	0	0	0	20	0
A2, Exp5	EFAD	Age	6	1	1	2,10,18	2	n/a	10	60	60	0	0	0	0
A3, Exp1	i-mTLR4-K	)	6	2	1	TBD	2	2	10	60	0	60	60	0	60
A3, Exp2	i-mTLR4-K	)	4	1	1	TBD	2	2	10	40	40	0	0	0	0

TBD, to be determined

*split tissue from same brain (see Experimental Details)

Animals will be kept on a 12-hour light/ dark cycle with water available at all times and food ad libitum (except during deprivation for behavioral group). All animals will be euthanized at end of experiment.

Genotyping: Tissue for genetic analysis of EFAD and i-mTLR4-KO mice will be obtained by tail biopsy (tail snip). Tail biopsies are obtained using clean and sterile procedures, by cutting the tip of the tail perpendicular to the long axis with very sharp scissors about 5mm in length. Alternatively, using a scalpel or razor blade. In mice <3 weeks,
hemostasis is easily achieved by light or direct digital pressure around the tip of the tail. When necessary, hemorrhage can be controlled with the use of silver nitrate sticks, applied pressure, or Kwik Stop®.
Vaginal Cytology: Adult female mice are characterized by daily vaginal cytology obtained from lavage with a drip of saline from a firepolished glass pipette at 11am. Mice are manually restrained by gently holding at the scruff of the neck during procedure. Examination of vaginal lavage under microscope reveals the cellular composition present at each day which indicates the cycle stage: estrus, metestrus, diestrus, proestrus.
Air Pollution (nPM) Exposure: In each experimental cohort, mice are randomized into either of 2 exposure groups: 1) filtered air or 2) nanoscale particulate matter (nPM). Exposures are performed 3 days per week, 5 hours per day, for 10 weeks (150 cumulative hours). Animals are placed into exposure cages in an exhaust hood that is vented to the outside. Temperature and airflow are controlled to ensure adequate ventilation,

minimize buildup of contaminants, and to avoid thermal stresses. During each exposure, nPM suspension is aerosolized and delivered to whole-body animal exposure chambers. Filtered air animals are treated identically, but exposed to filtered air without nPM added. Between exposures, animals are returned to standard cages.

Novel Object in Context Recognition (NOIC): NOIC is used to assess hippocampal-mediated object and context recognition (Balderas 2008). Mice are habituated to novel object recognition chambers with no objects, in two separate locations, on days one and two, for five min./day. On day three, mice are exposed to two distinct objects (A and B) for five min. in the first location. Mice show no preference between objects on day three, and objects A and B are counterbalanced between trials. 24 hr later, mice are placed in location two, with duplicates of object A, again for five min. The following day mice are again placed in location two, this time with objects A and B, again for five min. Time spent investigating the object is recorded, with blinded experiments evaluating behavior on a live video feed. Discrimination index is calculated by exploration time of object B on the final day, divided by total time spent exploring both objects. A discrimination index of 0.5 shows no preference for novel object, with higher than 0.5 indicating novel object preference.

Contextual fear conditioning (CFC) is assessed by the Contextual NIR Video Fear Conditioning System (Med Associates Inc, St Albans, VT) with video recordings 30 frames/sec of freezing response complex, including whisker twitch, tail flick, and lack of movement except respiration. The delay fear-conditioning task measures hippocampus-dependent memory. The fear conditioning setup consists of 4 sound-proofed chambers (Ugo Basile) and a video tracking system using Noldus EthoVision software. During the training protocol on day 1, mice are exposed to 5 trials of pairing the conditioned stimulus (CS) with the unconditioned stimulus (US). Each trial is composed of 120s of habituation to the chamber/ inter-trial-interval, followed by 20s CS (tone, 75dB) and 2s of US (shock, 0.5mA). Freezing behavior is defined by the absence of movement other than respiration, and is scored during a baseline period of 120 s to account for differences in locomotor and exploratory activities between groups. The learning rate is determined by comparing freezing behavior between groups during each trial. On day 2, the mice are tested for freezing to the CS in an altered context in which the walls, floor, and smell of the chamber have been changed. The CS is presented 3 times during day 2 and freezing behavior is scored to test for classical conditioning. On day 3, the mice are tested for contextual memory by placing them into the same chamber as in day 1, but without a tone or shock, and quantifying freezing behavior for 480s. Novel object recognition is a measure of cognition in learning and memory that involves parahippocampal regions. Mice are habituated to the apparatus without objects for 5 minutes. Sixty minutes later, 2 identical objects are placed into the box and allowed to explore for 5 minutes (training). Either 1 hour (short-term memory) or 24 hr later (long-term memory), mice are placed again in the chamber with one familiar object and one novel object for 5 min. The time spent exploring the objects is recorded.

Imaging (Core B2): *DCE-MRI***.** The DCE-MRI imaging protocol includes measurement of pre-contrast T1-values followed by a dynamic series of T1-weighted images with identical geometry and a temporal resolution of <5 seconds. A bolus dose of Gd-DTPA (Gadolinium-diethylenetriamine pentaacetic acid, Magnevist®) is injected via the tail vein and DCE images are collected. Blood-to-brain transfer K_{trans} constant maps will be constructed. *Laser-doppler flowmetry (LDF).* Cerebral blood flow (CBF) responses to hind limb stimulation is measured in anesthetized mice using laser-Doppler flowmetry measured through a cranial window. CBF will be measured and averaged from 3 trials per mouse. *BBB permeability.* Cortical cerebrovascular permeability is determined with rapid *in vivo* multiphoton microscopy imaging using a custom Zeiss 5MP multiphoton microscope. *In vivo* time-lapse images will be acquired every 2 min for a total of 30 min after i.v. injection of TMR-conjugated medium-sized dextran. *BBB angiography.* Fluorescein-conjugated mega-dextran is injected via the left femoral vein or retroorbitally and multiphoton Z stack images are taken through the cranial window starting at 50 mm below the cortical brain surface and continuing to 500 mm deep through cortical layers II and III, at 1 µm intervals between each image. Z stacks are reconstructed with ZEN software. Capillary length is analyzed by NIH ImageJ software. *DTI-MRI.* Diffusion-weighted images (DWI) are obtained from fixed brains using a conventional pulsed-gradient spin echo (PGSE) sequence. An optimized six point icosahedral encoding scheme is used for diffusion weighted acquisitions with a single un-weighted reference image for a total imaging time of ~24 hours. Imaging is performed on the entire brain, with emphasis on white matter changes in corpus callosum.

Tissue Collection: At the end of the experiment, animals will be anesthetized by the inhalant isoflurane and blood collected through cardiac puncture using heparinized tubes. Animals will then be perfused with phosphate buffer, pH 7.4. The brain will be removed. Depending on the requirements for each particular experiment, the brain will be dissected (hippocampus, cortex) and immediately frozen or the whole brains will be immersion-fixed.

2. Justifications: Provide justification that the species are appropriate for the proposed research. Explain why the research goals cannot be accomplished using an alternative model (e.g. computational, human, invertebrate, in vitro).
In vitro studies or computer/ mathematical models cannot satisfactorily reproduce the white matter/ neuronal injury and functional deficits resulting from nPM/ CCH exposures. Due to cost, ethical concerns, and reproducibility, mice are most appropriate for these studies. We choose mice because of our experience in the models being tested, availability of applicable antibodies/ assays, and potential for future investigations. C57BL/6 mice are the standard murine strain used by most aging researchers and are available through the NIA Aging Mouse Colony. Further, all previous nPM data has been collected using C57BL/6J mice. Altering background strains for the study would likely introduce strain specific differences in exposure responses and outcomes. This variation would increase sample sizes. Male and female mice are selected to study sex influences and comply with recent NIH initiatives to balance sex in animal studies. EFAD-tg and TNFR1-KO mice are chosen (in conjunction with project 4) to study specific mechanisms (TNFα- mediated inflammation/ APOE susceptibility) relevant to nPM and AD. Power analyses are designed to limit sample size.

3. Minimization of Pain and Distress. Describe the interventions including analgesia, anesthesia, sedation, palliative care and humane endpoints to minimize discomfort, distress, pain and injury.
Discomfort, distress, pain, and injury will be minimized in all phases of the experiment. No pain or distress is expected based on our past nPM exposure studies using the same dose of nPM. We have detected subtle learning and memory deficits, mild systemic and neuroinflammation which do not appear to affect the well being of the mouse. We will monitor behavioral changes, e.g., loss of appetite, grooming and activity. Moribund criteria include one or a combination of the following: >20% weight loss, sustained hunched posture, respiratory difficulty, hypo/hyperthermia, inability of access food/water, inability to ambulate or make normal postural adjustments. Mice will be monitored for any signs of stress or pain as well as general criteria outlined by IACUC (Body Condition Score 2, BC2) and will be euthanized.

4. Euthanasia. State whether the method of euthanasia is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.
After completion of experimental procedures, all rodents will be euthanized by methods approved by the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

1. Identify the select agent(s) to be used in the proposed research.

None

2. Provide the registration status of all entities* where select agent(s) will be used.

If the performance site(s) is a foreign institution, provide the name(s) of the country or countries where select agent research will be performed.

*An “entity” is defined in 42 CFR 73.1 as “any government agency (Federal, State, or local), academic institution, corporation, company, partnership, society, association, firm, sole proprietorship, or other legal entity.”

Not applicable

3. Provide a description of all facilities where the select agent(s) will be used.

Describe the procedures that will be used to monitor possession, use and transfer of select agent(s).

Describe plans for appropriate biosafety, biocontainment, and security of the select agent(s).

Describe the biocontainment resources available at all performance sites.

Not applicable

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USC
UNIVERSITY
OF SOUTHERN
CALIFORNIA

Keck School of Medicine

Department of Preventive Medicine

Division of Biostatistics

May 20, 2017

Dear Committee, Caleb Finch and William Mack,

I am pleased to confirm my commitment to serve as a collaborator on the experimental research proposed in Projects 3 & 4 for the P01 entitled "Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms". I have reviewed and collaborated with your teams on the study design and statistical approaches, and will be actively involved in data analysis and interpretation. I have a long-standing collaboration with Professor Finch in P01 AG026572-011 Brinton (PI) 09/21/16-09/20/20, Progesterone in Brain Aging and Alzheimer's Disease and we have co-authored several articles, e.g., Yin et al 2015, PMID25921624. I have also worked with Dr. Bill Mack extensively as a co-investigator on your ongoing study (Mack; R01ES024936 Neurotoxicity of Airborne Particles: Role of Chronic Cerebral Hypoperfusion) that utilizes similar analysis methods.

In addition, I will advise P01 Projects 3 and 4 in year 1 on the development of a common REDCap (Research Electronic Data Capture) database for data entry and management. I will provide statistical consultation in project years 2-5 for analysis and reporting of the common dataset.

As you know, I am the director of the Biostatistics Resources core of the Southern California CTSI (of which Dr. Bill Mack is a KL2 scholar) as well as the Biostatistics and Data Management Core of the USC Alzheimer's Disease Research Center (of which Professor Finch is a Co-Director and Dr. Bill Mack is a pilot grant awardee). Your work is highly relevant to both of these institutes and biostatistics and data resources from these institutes will be available.

Your pilot data demonstrate a female and apoE4 bias in amyloid-beta load in response to air pollution exposure (project 3) and pilot data from Project 4 suggests a role for air pollution in white matter injury and the progression of acute ischemic stroke. I have worked with you in developing the analysis sections of the current grant proposal; the proposed research is very exciting. It incorporates a well-designed study plan to assess the influence of age and sex on the joint influence of particulate matter and underlying cerebrovascular disease. I look forward to working with you to address this topic, and heartily express my support for and commitment to collaborate with you on this research.

Sincerely,

Keck School of Medicine of the University of Southern California
2001 N. Soto St., SSB202Y
Los Angeles, California 90033
Tel: 323 442 1810
Fax: 323 442 2993

Wendy Mack, PhD
Professor
Department of Preventive Medicine
Keck School of Medicine
University of Southern California

Resource Sharing Plan

Data Sharing Plan: We will share data in accordance with NIH policy. Processed data will be made public along with publications, and will be distributed through supplementary materials or our own websites.

Sharing Model Organisms: not applicable

Genome Wide Association Studies: not applicable

All key resources for this proposal will be authenticated to enhance the reproducibility of our results, as appropriate and according to NIH policy. Key Biological Resources that will be utilized and validated in this proposal include:

Cell lines: None

Transgenic mouse strains: EFAD and TLR4 knockout (TLR4-ko) mice.

EFAD (E3FAD and E4FAD) mice were generated by crossing 5XFAD to homozygous APOE3-, and APOE4-TR. 5xFAD mice are transgenic for 5 distinct FAD mutations (APP K670N/M671L+ I716V+ V717I and PS1 M146L+L286V). They are provided by University of Illinois at Chicago (Mary Jo LaDu, PhD).

TLR4 knockout (TLR4-ko), adult-inducible excision of TLR4 ($mCre^{+/-}$, $fl^{+/+}$) by tamoxifen, are CX_3CR1^{CreER} mice (JAX020940) crossed with $TRL4^{loxP/loxP}$ mice (JAX024872). The CX_3CR1^{CreER} mouse provides cell-specificity as Cre expression is under control of the CX_3CR1 promoter. CX_3CR1-expressing cells in adult mice are limited to microglia in brain and macrophages in gut, kidney (Jung et al 2000, PMID10805752; Yona et al 2013, PMID23273845). Cre is inducible by its linkage to an estrogen receptor mutant that is activated by treatment with tamoxifen. The $TRL4^{loxP/loxP}$ mouse has TLR4 flanked with two loxP sequences (TLR4 flox). The first generation (F1) mice are 100% heterozygous ($mCre^{+/-}$, $Fl^{+/-}$) and are currently breeding. F2 mice composed of 25% experimental animals ($mCre^{+/-}$, $fl^{+/+}$), 25% control group ($mCre^{-/-}$, $fl^{+/+}$) will be characterized for expression and functional efficacy of the TLR4 deletion, 3 months after tamoxifen treatment (75 mg/Kg, i.p. for 5 days or vehicle).

Transgenic mice will be validated by genotyping.

Antibodies: Only commercially available antibodies will be used.

Air Pollution particles: Collected, characterized and validated by Core C2 (Prof C. Sioutas, Director). These nPM characteristics are reported in recent publications by members of this P01 (Liu et al 2016; Morgan et al 2011) and are used in other studies supported by our NIA grants to CE Finch (AG040753; AG040683; AG051521).

Other resources used in this proposal will be standard laboratory reagents. Should we need to generate or obtain additional unique resources in the course of this proposal, they will be authenticated using methods similar to those described above, as appropriate.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

5. APPLICANT INFORMATION **Organizational DUNS*:** 0729333930000

Legal Name*: University of Southern California
Department: Contracts and Grants
Division: 95-1642394
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701

Person to be contacted on matters involving this application

Prefix:	First Name*:	Middle Name:	Last Name*:	Suffix:
Mr.	Steven		Misuraca	

Position/Title: Contracts and Grants Officer
Street1*: 3720 South Flower Street
Street2:
City*: Los Angeles
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 90089-0701
Phone Number*: 2137408207 Fax Number: 213-740-6070 Email: misuraca@research.usc.edu

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Urban air pollution and cerebral hypoperfusion: aging and sex influences

12. PROPOSED PROJECT

Start Date*	Ending Date*
04/01/2018	03/31/2023

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Southern California
Duns Number:	0729333930000
Street1*:	3720 South Flower Street
Street2:	
City*:	Los Angeles
County:	Los Angeles
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	90089-0701
Project/Performance Site Congressional District*:	CA-037

Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: 1 2 3 4 5 6 If

NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Mack._P01_abstract.pdf
8. Project Narrative*	Mack.P01.Narrative_copy.pdf
9. Bibliography & References Cited	Mack.P01.References.pdf
10.Facilities & Other Resources	mack._Facilities_and_resources._P01.pdf
11.Equipment	mack._Equipment._P01.pdf

Joint Effects of Air Pollution and Cerebral Hypoperfusion: Age and Sex Influence

Emerging evidence suggests a strong association between traffic-related air pollution (TRAP) and cognitive aging. Clinical and experimental studies demonstrate white matter toxicity and hippocampal neuronal atrophy in the setting of particulate matter (PM) exposure. Little is known, however, about the underlying pathophysiology and selective vulnerabilities. Evidence supports a critical role for cerebral vascular dysfunction in the onset and progression of Alzheimer's disease (AD), with cortical hypoperfusion implicated in the pathogenesis of neuronal dysfunction and cognitive deficits. Studies have demonstrated increased memory impairment, hippocampal neuronal loss, and altered Aβ metabolism in APPSwInd and APP overexpressing mice exposed to chronic cerebral hypoperfusion (CCH). Individuals with AD or cognitive impairment may demonstrate increased susceptibility to deleterious effects of TRAP exposure through vascular mechanisms. This program leverages experimental models focused exclusively on the cerebrovascular contributions to AD/ cognitive decline. We hypothesize that nanoparticulate matter (nPM) exposure and CCH exhibit synergistic effects on neurodegenerative pathways from the entorhinal cortex and hippocampus including the perforant pathway and diffuse white matter tracts. Age and sex variances are evident in AD prevalence, with older women most affected. These factors also impact cerebrovascular reserve, ischemic injury response, and BBB permeability. The proposed project seeks to determine age and sex influences on nPM and CCH exposure alone, and in combination, through the following specific aims: 1) Examine age dependence for the individual/ joint effects of nPM exposure and CCH on white matter toxicity, hippocampal / entorhinal cortex neuronal injury, and neurocognition, 2) Examine sex differences in the independent/ joint effects of nPM and CCH on the above outcomes and, 3) Examine mechanistic pathways by which nPM promotes neurodegenerative processes in the setting of CCH. The nPM exposure model has been used in our group's prior studies. Near roadside urban nPM is collected by means of innovative particle samplers developed by the USC Aerosol group (Sioutas). Whole body exposures are administered. The CCH model has been refined and leveraged to examine inflammatory mediators and BBB. A factorial design will assess independent and combined effects of nPM and CCH on white matter toxicity, hippocampal/ entorhinal cortex injury, and neurocognition. When administered together, we expect these exposures to exhibit synergy. Consistent with AD pathologies, we expect older female mice to demonstrate greatest vulnerability. We hypothesize that effects are associated with inflammatory upregulation and BBB permeability. Baseline interactions established in wild type mice and data from Project 3 will be leveraged to study mechanism in EFAD-Transgenic and inducible macrophage/ microglial specific TLR4 knockout mice. Expected knowledge will advance our understanding of age and sex impact on neurotoxicity secondary to air pollution and vascular mechanisms of cognitive decline evident in AD.

PROJECT NARRATIVE

This proposal studies age dependence and sex differences in the risk of early cognitive decline and Alzheimer's disease pathology secondary to air pollution exposure. Specifically, the project investigates the interaction between air pollution and underlying cerebrovascular mechanisms of cognitive aging. By examining an isolated vascular component, findings will provide initial directions towards establishing differential risk assessments and potential targets for intervention in vulnerable populations.

FACILITIES AND RESOURCES

The **University of Southern California** provides a rich scientific environment that offers both an intellectual community and infrastructure that will contribute to the success of the proposed research project. The work will be completed at the Zilkha Neurogenetic Institute and in the Davis School of Gerontology at the University of Southern California.

The applicant is a member of institutional groups that provides robust resource support to the project described in this grant proposal:

The AirPollBrain Group at USC is an organized research group of scholars who do research on ways to promote optimal neurodevelopment in humans and healthy brain aging by better understanding environmental effects of urban air pollution and gene-environment interactions. The APB group developed from a core of USC's leadership in urban environmental health research in the Los Angeles Basin, with collaborating institutions. Taking a multi-disciplinary research, APB addresses effects of environmental pollution on the brain across the lifespan. Bimonthly meetings convene faculty and trainees on specific topics with a focus on developing new research projects and consortia.

The Southern California Environmental Health Sciences Center: SCEHSC was established in 1996 to promote environmental health research in Southern California. The Center aims to more fully characterize environmental health hazards, understand the basis for personal vulnerability, and translate research into preventive action to reduce the burden of environmentally-related diseases. This Center has played an important role in the development of the air pollution and cognition initiative, which is a focus of a significant program of research at USC.

USC Animal Facility: These facilities are maintained by the Department of Animal Resources under the supervision of Don Casebolt, DVM. The Department employs 2 additional veterinarians, a manager of animal husbandry, 2 facility supervisors and a staff of 12 trained animal care workers (certified by the American Association of Laboratory Animal Science). USC has been accredited under the American Association for Accreditation for Laboratory Animal Care, since 1966. An Institutional Animal Care and Use Committee (IACUC) reviews all applications to ensure ethical and humane treatment of animals. The Ray R Irani Hall Animal Facility was completed in 2007. The facility has a total capacity of 10,000 rodent cages within the 14,770 net square feet consisting of 20 animal holding rooms, 10 procedure rooms, 2 quarantine rooms, additional rooms for cage processing, bulk autoclave & storage, and office, locker & break rooms. The facility features a "suite" design concept of grouping 4 animal holding rooms with 2 procedure rooms into an individual security-controlled suite. Our group has exclusive use of 1 of these suites. The individually-ventilated rodent cages ensures consistent air exchange rates of 50-80 air changes per hour within each cage to reduce ammonia and carbon dioxide levels. Incoming air is high-efficiency particulate arresting (HEPA) filtered for control of airborne particles. All components of the cages and racks are autoclaved and changed weekly. Wall-mounted supply air blowers and central exhaust plenums reduce noise and vibration. Automatic watering system is supplied to each cage. The exposures for the studies will occur in these facilities.

EQUIPMENT

Laboratory: Dr. Mack's 500 square foot laboratory in the Zilkha Neurogenetic Institute (Room 221) is equipped to perform cellular physiology and molecular biology experiments, and immunohistochemistry. The laboratory also has a cell culture room. Dr. Mack's laboratory has the following equipment relevant to this project proposal:

Murine surgeries: Surgical microscope (Nikon SMZ 645) and Illuminator (Nikon NI 150), Physitemp temperature and physiology monitor (TCAT), Kent Scientific Physiosuite for operative monitoring, Periflux 5000 Laser Doppler Flowmetry system and probes, Image analysis system: digital cameras, flat bed scanner,

Tissue processing and storage: Thermo Scientific Cryotome, Zeiss Scope Stemi 2000 AM Scope, Zeiss Axio Scope, VWR Vortex Mixer, VWR mini-incubator, VWR mini- hot plate, Scientific Industries orbital genie, Eppendorf Centrifuge 5430, Thermo-Scientific 280 Series Waterbath, VWR Digital Vortex Mixer, VWR

Hybridization oven, BioRad Transblot turbo transfer system, Biorad T100 Thermal Cycler, Eppendorf Centrifuge 5810 R, New Brunswick Ultralow Temperature freezer, Thermo Scientific Refrigerator,
Behavior testing: Dr. Mack's laboratory has an 8-arm radial maze, Y-maze, and Novel Object recognition testing arena (Coulbourne Instruments). Data is collected, analyzed and archived using the Panlab tracking system with computer directed video camera and image analysis software.
Computers: Macintosh and PC (3) computers are available for data collection and analysis, word processing, and printing. In addition, a flatbed scanner and digital camera are available for scanning cerebral sections or planimetric determination of cerebral injury.
Office: In addition to a small room located within the laboratory for use by laboratory personnel, Dr. Mack maintains independent office space in close proximity to the laboratory.
Shared resources: Dr. Mack has freely available to his research program all ZNI core equipment, including high resolution confocal and light microscopy, vibratome, microtome, advanced molecular biology and biochemistry cores and a *first rate vivarium* with around the clock veterinary support and behavioral testing suite. The ZNI office provides administrative support, including pre- and post-award grant management and research account reconciliation.

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OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: William Middle Name J Last Name*: Mack Suffix: M.D.

Position/Title*: Associate Professor of Neurosurgery
Organization Name*: University of Southern California
Department: Neurosurgery
Division:
Street1*: 1937 Hospital Place
Street2:
City*: LOS ANGELES
County: Los Angeles
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 900330000

Phone Number*: 323 442 7512 Fax Number:

E-Mail*: William.Mack@med.usc.edu

Credential, e.g., agency login: WJMACK

Project Role*: Other (Specify) Other Project Role Category: Project Lead

Degree Type: MD,BA Degree Year: 2001

Attach Biographical Sketch*: File Name: Mack.Biosketch.P01.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: WENDY Middle Name JEAN Last Name*: MACK Suffix: Ph.D

Position/Title*:	Professor
Organization Name*:	University of Southern California
Department:	Preventive Medicine
Division:	Keck School of Medicine
Street1*:	2001 N Soto Street
Street2:	
City*:	LOS ANGELES
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	900330000

Phone Number*: (213) 342-1820 Fax Number: (213) 342-2993

E-Mail*: WMACK@USC.EDU

Credential, e.g., agency login: WJMACKPI

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 1989

Attach Biographical Sketch*: File Name: Wendy_Mack_Biosketch_May2017.pdf

Attach Current & Pending Support: File Name:

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	Mack.introduction._resubmission.Project_4_final.pdf
Research Plan Section	
2. Specific Aims	Mack.P01.specaims.pdf
3. Research Strategy*	finalsubmit.Mack.P01.Research_Strategy.final..final.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	Vertebrate_Animals-P01.final.pdf
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	
13. Letters of Support	Wendy_mack._letter_FINAL.pdf
14. Resource Sharing Plan(s)	Resource_Sharing_Plan.project_4.pdf
15. Authentication of Key Biological and/or Chemical Resources	Proj4.authentication_of_key_resource_plan.pdf
Appendix	
16. Appendix	

New publications related to this P01 since the prior submission: (1) Liu Q et al. Stroke Damage is exacerbated by Nano-Size Particulate Matter in a Mouse Model. PloS One 2016. (2) Babadjouni R, et al. Clinical effects of air pollution on the central nervous system: review; J Clin Neurosci 2017. (3) Liu Q et al. Experimental Chronic Cerebral Hypoperfusion Results in Decreased Pericyte Coverage and Increased Blood Brain Barrier Permeability in the Corpus Callosum. *Accepted with revisions* J Cereb Blood Flow Metab

Following the reviewers suggestions, *Project 4 R1 presents new pilot data showing synergy between nPM/ CCH exposures; develops a strengthened mechanistic approach, highlights similarities/ differences with Project 3 and concurrent work (R01ES024936); and attends to methodological suggestions. Major text changes are italicized in the proposal.*

Reviewers suggested that preliminary data made leaps regarding potential interactions between nPM and CCH. *Since the prior submission, our lab generated strong preliminary data that establishes synergistic effects of nPM and CCH on white matter degeneration with impact on working memory (C1, Fig. 4). These findings demonstrate a novel synergy, in which the combined influences of the two exposures exceed those expected by an additive model. Our new RNA seq data suggests that CCH may amplify inflammatory responses secondary to nPM toxicity (C1, Fig. 5).*

R**eviewers felt the study would benefit from further mechanistic development.** *Vascular outcome assessments (capillary density, pericyte coverage, inflammatory markers relevant to complement) are incorporated into Aims 1 and 2 to advance the hypothesis that the age "ceiling effect" (older mice do not demonstrate nPM effects beyond their younger counterparts) may not translate to nPM/CCH exposed mice as a result of diminished vascular reserve and microvascular failure secondary to CCH (A3, C3K, C4D). Studies are now designed to examine the TLR4 pathway and interplay with complement C5, a positive feedback loop that has been shown to influence other models of ischemia reperfusion injury. Our gene set enrichment analysis (corpus callosum RNA Seq) data suggests a role for TLR4 pathways/ inflammation following nPM/ CCH exposures (C1, Figure 5). Our pilot studies suggest each exposure (nPM, CCH) incites a microglial-mediated inflammatory response and complement C5 upregulation (C1). Aim 3 is redesigned. TNFR-1-ko experiments (for which the reviewers lacked enthusiasm) were eliminated and replaced by studies of an inducible macrophage/ microglial TLR4-ko mouse (i-mTLR4-ko).*

Reviewers raised questions about the similarities/ differences between projects 4 and 3. *We have clarified project differences and collaborations throughout the proposal; see sections C3, C4E, and C5E. To maximize connections between the projects and increase the overall interaction of the program grant, project 4 employs similar exposure paradigms (nPM, age, mouse strain) and outcome assessments as project 3. Project 4 is designed to assess the effects of nPM in an experimental paradigm that isolates an exclusively vascular influence/ risk factor for AD and cognitive decline (CCH). Although many of the outcomes and experimental parameters are shared with project 3, the scientific questions are independent and different. The study examines synergistic relationships between exposures in a susceptible population with unique pathophysiology (cerebral hypoperfusion; C1). As stated, critical vascular endpoints have been incorporated into project 4 to examine effects of age in nPM/ CCH exposed mice. We hypothesize that findings may be contrary to the "ceiling effect" seen in mice exposed to only nPM and described in project 3. Additionally, Project 4 Aim 3 now examines TLR4 and C5 interplay as a central mechanism of injury in nPM/CCH mice.*

Reviewers questioned similarities with the PI's funded work (R01ES024936). *The current study builds on the framework from R01ES024936, but examines mice of different age, sex, and strain to address distinct scientific questions. R01ES024936 establishes a time course for nPM associated white matter toxicity (no CCH) and studies joint effect of nPM/ CCH in young male mice. The current P01 proposal investigates age dependence (old males) and sex differences (young/old females) for individual and joint effects of NPM/ CCH and explores mechanisms of synergism through genetically modified mice (EFAD-Tg and i-mTLR4-ko). To limit total number of mice/ avoid data duplication, the current project leverages a small "control/comparison group" of young male mice from R01ES024936. Differences are clearly outlined in section C1 and described throughout the proposal*

Concerns regarding methodological details were advanced. *In the original stenosis model publication (Shibata, 2004), the 0.18 microcoil was associated with 20% mortality. We have performed 195 refined BCAS procedures with aggregate mortality of about 10%. We have accounted for this in sample size calculations. To address multiple statistical testing/ interactions, we have specified white matter toxicity as our primary outcome, and will test hypotheses related to this outcome at an experiment-wise alpha of 0.05.The remaining outcome measures will adjust for experiment-wise false discovery rate (FDR).* A graduate assistant has been budgeted to assist Professor Wendy Mack with statistical analyses.

SPECIFIC AIMS

Exposure to traffic-related air pollution (TRAP) may differentially affect susceptible individuals with pre-existing or concomitant neurological disease. Persons with AD or vascular dementia could be exquisitely sensitive to TRAP neurotoxicity secondary to combined effects and shared molecular pathways with cerebral hypoperfusion. This proposal examines individual and joint effects of: 1) *nanoparticulate matter (*nPM, PM< 0.2 μm, nanosize subfraction of TRAP PM2.5, Core C2*)* and 2) *chronic cerebral hypoperfusion* (CCH) on endpoints germane to AD pathology in a murine model. Experiments assess effects of nPM in a system isolating an exclusively vascular influence/ risk factor for AD and cognitive decline (CCH). Studies examine age and sex impact and explore putative mechanisms related to BBB permeability and inflammation involving TLR4 and complement C5 pathways. Our group has demonstrated inflammatory changes (reactive microglia, C5 deposition) in the corpus callosum following 10-week nPM exposures in young, male C57BL/6 mice. Our murine CCH model produces consistent white matter degeneration, neurocognitive decline, BBB permeability, and neuroinflammation. Older ages and longer exposure durations result in hippocampal CA1 pathology. We have previously demonstrated C5 upregulation and ischemic protection of C5 deficiency in the setting of CCH. Increased BBB permeability and decreased pericyte coverage are evident. [*Recent data suggests synergistic effects of nPM and CCH on white matter injury/ neurocognition in young male mice (Mack; R01ES024936). Gene set enrichment analysis of corpus callosum by RNA Seq supports a role for TLR4 pathways in neuro-inflammation following nPM/ CCH. We hypothesize an injury mechanism leveraging interplay between TLR4 and C5 pathways. In experimental ischemia/ reperfusion models, this positive feedback loop causes tissue damage through microvascular failure. Loss of BBB/ pericyte integrity may contribute to injury severity.]*

Age and sex are known to impact AD. Advanced age also influences hemodynamic compromise and collateral outgrowth in the setting of cerebral hypoperfusion. Our pilot data suggests that estradiol mitigates deleterious effects of CCH. *Vascular outcome assessments (capillary density, pericyte coverage, inflammatory markers) are utilized to evaluate interactions of nPM age "ceiling effects" (older mice do not demonstrate greater nPM effects, project 3) with CCH, as a result of diminished vascular reserve and microvascular failure secondary to CCH.* This project leverages data from male C57BL/6 mice exposed to 10 weeks of nPM/ CCH in our ongoing study. Ages of mice (2 and 18 month) are identical to Project 3 for comparison of controls (Project 3) with CCH/ nPM exposed mice across ages and sex. Studies assess joint effects of nPM and genetic alterations relevant to AD (EFAD; new *inducible macrophage-microglia TLR4-ko*) in the setting of CCH to further dissect neuroinflammatory and BBB-related mechanisms. Biomarkers relevant to AD pathology are examined. The following *Aims* and hypotheses are studied:

***Specific Aim 1*: Examine age dependence for independent and joint effects of nPM and CCH on white matter toxicity, hippocampal CA1/ entorhinal cortex neuronal injury, and neurocognition.** This aim will establish main effects and joint influence of nPM and CCH on white matter neurotoxicity and hippocampal/ entorhinal neuronal injury in late middle age (18-month) male C57BL/6 mice. Findings will be compared to young (2-month) male mice from our ongoing study and control/ nPM exposed mice from Project 3. We hypothesize that nPM exposure and CCH will each result in white matter damage, hippocampal CA1/ entorhinal cortex injury, *decreased capillary density*, and neurocognitive deficits. When co-administered, we expect nPM and CCH to exhibit synergy. We hypothesize that older mice will show greater vulnerability.

***Specific Aim 2*: Examine sex differences for independent and joint effects of nPM and CCH on white matter toxicity, hippocampal CA1/ entorhinal cortex neuronal injury, and neurocognition.** This aim will establish main effects and joint influence of nPM and CCH on white matter neurotoxicity and hippocampal/entorhinal neuronal injury in young and late middle age female C57BL/6 mice. Findings will be compared to Project 3 control mice. We hypothesize that nPM and CCH will each result in white matter damage, hippocampal CA1/ entorhinal cortex injury and neurocognitive deficits. When co-administered we expect the exposures to exhibit synergy. We hypothesize that older females will show greatest vulnerability.

Specific Aim 3: Examine mechanistic pathways by which nPM promotes neurodegenerative processes in the setting of CCH (major revision). This aim will study mechanisms contributing to the independent/ joint neurotoxic effects of nPM and CCH in the most vulnerable age/ sex mice (white matter injury) in *Aims 1 and 2*, and early aims of Project 3. *We hypothesize joint effects of nPM exposure and CCH are associated with increases in TLR4/ complement C5 associated inflammatory markers* and BBB permeability. BBB integrity will be assessed through multiphoton microscopy, tissue immunofluorescent/ lectin staining (Core B2). Ultra-high field MRI will examine tractography, connectivity, and BBB permeability. The interactions between nPM and APOE alleles/ *inducible genetic deletion of macrophage- microglia specific TLR4 will be tested in the setting of CCH during the later years of the program.*

RESEARCH STRATEGY

A. Significance

A1. Traffic Related Air Pollution (TRAP) exposure effects on cognition, white matter and hippocampal pathology: Epidemiologic studies have established strong links between TRAP exposure, cognitive deficits, and accelerated brain aging[1-7]. These data are reviewed in projects 1 and 2. A recent population-based cohort study revealed that living in close proximity to heavy traffic is associated with higher dementia incidence[8]. Moreover, J.C. Chen et al. showed associations between greater $PM_{2.5}$ exposures and decreased regional white matter volumes in the corpus callosum and frontal/ temporal lobes of community dwelling older women[9]. Relationships between particulate matter at fine-sized or smaller scales, cognitive decline, and white matter injury have also been recognized in experimental models. Fonken et al. found that long-term exposure to concentrated fine particles impairs spatial memory, upregulates hippocampal inflammatory cytokines, and decreases dendritic spine density in the CA1 hippocampal subregion in mice [10]. Allen et al. recently demonstrated that concentrated ambient ultrafine particle exposure in the early postnatal period is associated with microglial activation, white matter hypomyelination, and corpus callosum size reduction[11].

A2. Effects of cerebral hypoperfusion: Alzheimer's disease, cognitive impairment, and white matter injury: Data supports a critical role for cerebral vascular dysfunction in the onset and progression of Alzheimer's disease (AD). Similarly, cortical hypoperfusion is implicated in the pathogenesis of neuronal dysfunction and cognitive impairment. Radiographic studies have revealed diminished resting cerebral blood flow in Alzheimer's patients[12] and chronic cerebral hypoperfusion (CCH) has been linked with cognitive impairment and white matter hyperintensities on MR imaging[13-17]. Investigations have demonstrated that severe atherosclerotic disease is associated with a three-fold increase in AD/ dementia[18]. Murine models of CCH secondary to bilateral carotid artery stenosis (BCAS) consistently yield selective white matter injury and neurocognitive deficits[19-24]. Older ages and longer exposure durations result in hippocampal CA1 pathology[25, 26]. CCH accelerates memory impairment and hippocampal neuronal loss in transgenic AD mouse models[27-29].

A3. Potential synergies of TRAP and CCH- inflammation and BBB permeability *(revised)*: Evidence suggests that TRAP and CCH both modulate inflammatory processes and BBB permeability. ***Neuroinflammation:*** Inhalation of ambient air pollution[30-33] and diesel exhaust have been shown to elicit inflammatory changes within the brain[34, 35]. The Finch laboratory **(**Co-PI) has previously demonstrated a decrease in hippocampal neuronal glutamate receptor subunits and an induction of cortical inflammatory cytokines/ chemokines following nanoparticulate matter (nPM) exposure[36]. They established a critical role for microglial activation and TNFα-induction in nPM-mediated neuroinflammation[37]. Our group's pilot data reveals that young male mice exposed to 150 cumulative hours of nPM exhibit nearly 50 and 100 percent increases in complement C5, (C5 and C5a) deposition and reactive microglia (IBA-1), respectively, in the corpus callosum (Section C1, Fig. 1). *In vivo nPM exposure of mice induced TLR4 and downstream genes in the hippocampus (Project 3, Fig 6). Further, TLR4 knockdown in mixed glia attenuated the neuroinflammatory response to nPM[38]. These findings suggest a* ***microglia-mediated inflammatory response involving the TLR4 and complement pathways (C5 component).*** *Candidate gene association studies have suggested that cytokines and inflammation may play a critical role in the progression of cognitive decline in the elderly [39]. Tahara et al. demonstrated that an AD mouse model homozygous for a TLR4 loss-of-function mutation demonstrated increased diffuse and fibrillar Aβ deposits and buffer-soluble/ insoluble Aβ in the brain when compared with a TLR4 wild type AD mouse model[40]. Studies have demonstrated that TLR4 is induced after cerebral ischemia and that genetic TLR4 deficiency is protective in the setting of murine stroke[41, 42]. In an APP mouse model, complement C5 inhibition results in decreased Aβ plaque load and improved neurological function[43, 44]. Our findings indicate C5 upregulation after stroke and ischemic protection of C5 deficiency in the setting of CCH (section C1). As two components of the innate immune response, functional interplay between TLRs and C5 activation has been described. [45-47]. In the absence of decay accelerating factor, C5a receptor activation up-regulates TLR4 mediated IL-1, IL-6, and TNFα production[48]. Mesenteric ischemia/ reperfusion studies demonstrate significant complement activation, which is attenuated in the absence of TLR4[49]. As the TLR4 pathways and complement C5 are both induced by nPM and are implicated in cerebral ischemia/ AD, they represent a logical target for neuroprotection in the setting of joint nPM/ CCH exposures.]* ***BBB Permeability:*** In vitro studies reveal increased permeability of cultured brain endothelial cells and reduced tight junction proteins following diesel exhaust (DE) particulate exposure[50]. A recent investigation showed that mice exposed to DE had elevated extravascular IgG in the brain parenchyma, indicating BBB compromise [51]. Similarly, CCH has been shown to disrupt BBB integrity. In a study of cognitively normal elderly individuals, the presence of incidental cortical microbleeds was associated with significant and pervasive reductions in resting-state cerebral blood flow[52]. In a murine CCH model, Nakaji et al. demonstrated that matrix metalloproteinases

(MMPs) play a critical role in BBB breakdown, glial cell activation, and white matter injury in the corpus callosum. Genetic knockout and selective inhibition of MMP-2 diminished these responses[23]. Our group has demonstrated an 18-fold increase in BBB permeability (Evans Blue integrate density) with a concordant endothelial pericyte coverage decrease (40%) in the corpus callosum following experimental CCH in mice (section C1). Studies have demonstrated a critical role for cyclophilin-A (CypA)/ MMP-9 mediated BBB permeability in the setting of AD and mild cognitive impairment. Carriers of the APOE e4 allele with AD and mild cognitive deficits exhibit accumulation of cytokine CypA and MMP-9 in both pericytes and endothelial cells[53]. Using APOE transgenic mice, the Zlokovic laboratory (Core B) has shown that expression of APOE e4 is associated with pericyte-specific CypA/ MMP-9 pathway activation and resultant BBB permeability[54].
Potential synergies: nPM exposure and CCH are independently associated with neurocognitive decline. *When co-administered, the two factors may function synergistically through complementary mechanisms and shared pathways involving neuroinflammation (TLR4/C5) and BBB permeability*. Models exist for this type of interaction in the setting of AD. Using microdialysis, Takeda et al. demonstrated that peripheral administration of lipopolysaccharide (LPS) resulted in significantly elevated brain IL-6 levels and decreased locomotor activity/ impaired social behavior in an ADtg (APP) mouse vs wild type controls[55]. Studies have demonstrated greater reference memory impairment, hippocampal neuronal loss, and altered Aβ metabolism in APPSwInd and APP overexpressing mice exposed to experimental CCH[27, 28]. These findings suggest that AD-related and/or vascular pathways impacting inflammation and BBB permeability could potentiate the neurotoxicity of inhaled nPM. *Similarly, our preliminary data suggest synergistic effects of nPM and CCH on white matter injury and working memory in a murine model (Section C1).*

A4. Neuroinflammation and BBB permeability: Putative mechanisms for age/ sex related cognitive changes (revised): *[In elderly humans, Wellenius et al. [56] demonstrated associations between $PM_{2.5}$ exposure and higher resting cerebrovascular resistance and lower cerebral blood flow velocities. Older stroke patients have increased mortality and more-severe disability compared to their younger counterparts for similar spectrums of stroke severity [57, 58]. The lower restorative brain capacity in older patients implies a decreased ischemia threshold from which clinical recovery is possible. Advanced age influences hemodynamic compromise and collateral outgrowth in the setting of cerebral hypoperfusion[59]. These processes may limit vascular reserve, regional perfusion, and salvage of "at risk" tissue following joint nPM/CCH exposures. Vascular outcomes of CCH will examine interactions with an apparent ceiling effect during aging, in which neuroinflammatory responses of middle-aged mice to nPM did not increase above baseline aging elevations[60, 61]. If aging mice also have the age-decreased vascular reserve shown in elderly humans, the combination of nPM and CCH should have intensified glial hyperactivity and myelin degeneration in aging mice, this would suggest a new neurodegenerative synergy in the aging brain.*

Cognitive outcomes and brain susceptibilities may demonstrate age and sex interactions in the setting of nPM and CCH. Neuroinflammation and BBB breakdown appear to contribute to sex-related differences in cognition. A distinct male prevalence for inflammatory cerebrovascular diseases exists, with relative protection in younger females[62]. While physiologic 17β-estradiol concentrations generate significant anti-inflammatory effects in the central nervous systems of young mice, a majority of studies suggest that these effects do not persist in older females [63]. Stroke models reveal that 17β-estradiol inhibits transcription of numerous pro-inflammatory cytokines including TNFα and IL-6 [64] Systemic 17β-estradiol administration prevents microglial activation and peripheral monocyte recruitment in response to intraventricular LPS injection[64]. Estradiol treatment is associated with decreased expression of MMP-9 and complement C3 receptor, and a phenotypic shift in the microglial response. Maggioli et al. examined BBB responses to peripheral LPS administration in male and female mice. Inflammation provoked acute increases in BBB permeability in males and senescent females that were not present in younger females[62]. In studies of EFAD mice, Cacciottolo et al. demonstrated a female APOE e4 excess of microbleeds, with higher CAA and Aβ levels[65]. That these strong correlations do not generalize to human AD (older females have fewer microbleeds) suggests potential APOE-sex specificity for divergent causes of cognitive decline. *The P01 PIs (Finch/ Chen) recently examined neurodegenerative effects of particulate air pollutants in older women from the Women's Health Initiative Memory Study (WHIMS) and experimental mouse models. Residing in high $PM_{2.5}$ locations was associated with increased risks of global cognitive decline and all-cause dementia. Female EFAD transgenic mice exposed to urban nPM over 15 weeks (225 hours) showed increased cerebral β-amyloid (fibrillary amyloid and Aβ deposits), both exacerbated by APOE ε4[66].*

A5. Summary: Significance of the Current Proposal: The Project 4 proposal leverages shared themes and pooled data with the other P01 projects to address several existing research gaps: 1. Isolate and characterize the vascular contributions to cognitive decline and AD in an experimental model. 2. Understand the joint effects

of nPM and CCH on well-characterized AD biomarkers and outcome measures. 3. Determine the age dependence and sex differences for the individual and joint effects of nPM and CCH. 4. Examine putative mechanisms by which air pollution may accelerate cerebrovascular damage in AD. *We hypothesize that nPM increases AD risk/ pathology by intensifying shared TLR4/ complement C5-dependent neuroinflammatory pathways with CCH*. As BBB breakdown can augment toxic effects of neuroinflammation, we also expect that joint nPM/CCH effects will be amplified in carriers of the APOE4 allele, and that effects will be most pronounced in older females. *We hypothesize that the age "ceiling effect" of nPM (older mice do not demonstrate increased nPM effects[61]; see Project 3) may not translate to nPM/CCH-exposed mice as a result of diminished vascular reserve and microvascular failure secondary to CCH.* The significance and impact of AD and cognitive impairment are profound. Findings from this study can generate a foundation for clinical and translational studies of specific targets relevant to vascular mechanisms that influence progression of AD and cognitive impairment. Dissecting the underlying age and gender interactions may provide additional insight into individual risk assessment and stratification.

B. Innovation

Within the scope of the larger urban air pollution and AD program, Project 4 isolates and studies the vascular component of white matter/ neuronal injury and cognitive decline. Few investigations focus on this critical aspect of brain aging and AD pathology. Leveraging an innovative approach, experiments employ a refined surgical model of CCH that recapitulates the pathological/ behavioral deficits seen in vascular cognitive impairment. Cerebral hypoperfusion is tested alone and in conjunction with a well-established and innovative ultrafine (nPM) collection/ exposure methodology to study outcomes and biomarkers relevant to AD. This is the first investigation to examine the joint effects/ synergy of these two exposures in at-risk populations (older females, APOE e4 carriers). *Further, the study considers putative mechanisms related to BBB permeability and inflammatory mediators involving interplay between the TLR4 and complement C5 pathways.* The comprehensive statistical analysis plan (tests for synergy, multivariable analysis) examines interactions relevant to real-world exposures in a multifactorial disease process. Advanced approaches incorporating exposures known to impact pathological processes through similar mechanisms will ultimately help to characterize individuals risk profiles for accelerated cognitive decline and AD.

C. Approach

C1. Preliminary Studies (revised): Our laboratory has devoted significant effort to studying the independent effects of both PM and CCH (BCAS model) on white matter neurotoxicity and cognitive function. The previously acquired data will serve as the foundation for the aims of the current study, which examine joint influences of these exposures. We have demonstrated inflammatory changes (activated microglia; complement C5, C5a) in the corpus callosum of young exposure (Fig 1). We have established clear interactions between nPM exposure and cerebrovascular disease. Our group utilized a mouse model to demonstrate a detrimental effect of urban airbor nPM exposure in the setting of acute ischemic stroke[67]. Following ischemia/ reperfusion, mice exposed to nPM exhibited larger infarct volumes and greater neurological deficits than those exposed to filtered air.

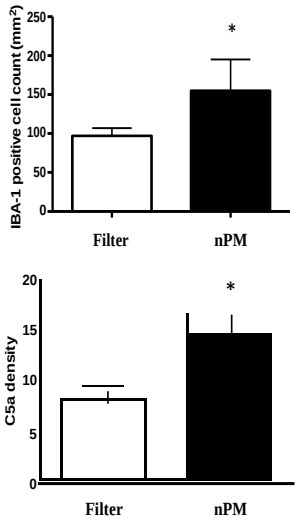


Fig 1: Top: Reactive microglia. Bottom: C5 density. *p< 0.05, (n=12/ group, mean±SEM)

Further, the mice exposed to nPM demonstrated increases in markers of inflammation (C5/C5a, C5a receptor) and reactive oxygen species (NADPH oxidase, gp91^{PHOX}) within the region of ischemia[67]. Our group and others have established that experimental murine CCH results in selective white matter injury and behavioral deficits (8 arm radial maze)[19-21]. This is accompanied by increased densities of reactive astrocytes and microglia in the region of injury (medial corpus callosum)[19]. We have also demonstrated evidence of neuronal damage in the perirhinal cortex with associated deficits in novel object recognition (NOR, Fig 2). Our data suggests that inflammation plays a prominent role in CCH-medicated white matter injury. Complement C5 protein deposition increases in the corpus callosum with a time course

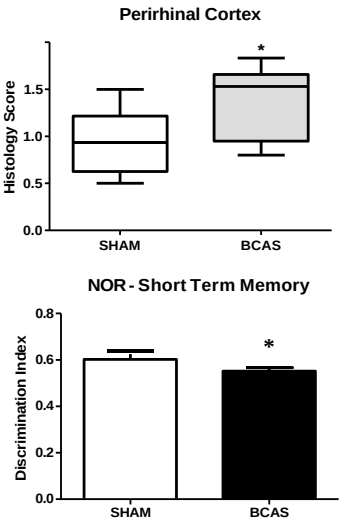


Fig 2: Top: Perirhinal neuronal injury * p<0.05 (n=6 BCAS, 7 sham, median, IQR), Bottom: NOR. P<0.05, (n=6/ group, mean±SEM) *p<0.05

temporally consistent with white matter injury. Following CCH, C5 deficiency is protective against white matter ischemia and associated with fewer reactive astrocytes/ microglia in the corpus callosum[19].

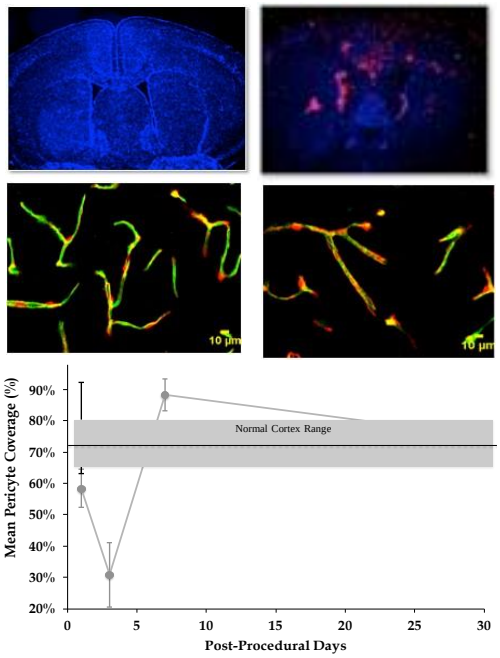
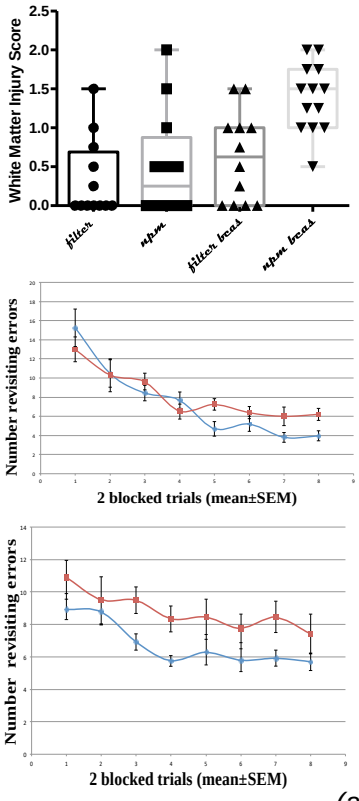


Fig 3: Evans Blue staining (above) and pericyte coverage (CD13/CD31, middle) in sham (left) and CCH (right) mice POD 3. Below: Pericyte coverage following sham (black)/CCH (grey) surgery, n=3/group mean±SEM.

Our pilot data also demonstrates decreases in BBB integrity and pericyte coverage in the white matter of CCH-exposed mice. Decreased pericyte coverage and increased BBB permeability are most pronounced on postoperative day 3 following CCH. This precedes any notable white matter injury or behavioral deficits (Fig 3).

An ongoing investigation has established the joint effects of nPM and CCH exposures in young male mice (Mack; R01ES024936). ***New data*** *indicates that, when administered together, nPM and CCH exhibit synergistic effects on white matter injury. Further, nPM/CCH exposed mice show significant working memory deficits on 8 arm radial maze testing when compared to filtered air/CCH exposed mice (Fig 4). RNA seq was performed on white matter (corpus callosum) of mice exposed to nPM and CCH. Reads were assembled and aligned to the mouse reference genome. The analysis model consisted of nPM, CCH and their interaction as independent variables. Gene set enrichment analysis of the preliminary transcriptome suggests that nPM may upregulate inflammatory pathways such as TLR4* (p<0.001). *Findings imply that CCH exposure may amplify inflammatory responses secondary to nPM toxicity. The heatmap demonstrates that the number and scope of gene expression is increased most in the setting of joint nPM/ CCH exposure (red bar, left Fig 5). The TLR4 pathway was one of the amplified upstream signals (Fig 5).* Age and sex differences are known to impact cerebrovascular reserve, BBB permeability, and response to ischemic injury. Our collaborators have demonstrated a lack of induction of phase II enzymes in older mice exposed to particulate matter[60]. Our studies suggest that sex may impact susceptibility to CCH through effects of estradiol. Pilot data demonstrates that, following experimental CCH, both male and ovariectomized female mice treated with oral 17β-estradiol (1.12 µg/day for 32 days) appear to demonstrate less white matter ischemic damage and neurocognitive deficits (NOR) than do placebo treated mice (Fig 6). *The current study builds on the framework of R01ES024936, but examines entirely distinct scientific questions. R01ES024936 establishes a time course for nPM (alone) associated white matter toxicity and studies joint effects of nPM and CCH in young male mice. This P01 investigates age dependence and sex differences for individual and joint effects of NPM and CCH and explores mechanisms of synergism.*

Fig 4: Synergistic effect of nPM / CCH on white matter injury (top, p<0.05, n=12,13/ group). No significant differences in revisiting errors between filtered air and nPM exposed mice (center). Significant differences in revisiting errors between CCH mice exposed to filtered air and nPM (bottom, p<0.05, n=12,13/ group, median, IQR)



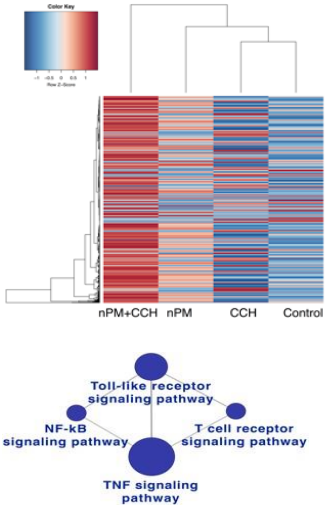


Fig 5: Transcriptome changes (corpus callosum) in mice exposed to nPM or CCH (n=7/ group). Top: Heatmap expression changes in significant genes. Cluster dendogram shows nPM causes more changes than CCH; the combination increases changes compared to control. Bottom: TLR4 and associated NF-kB/ TNF pathways are enriched from the gene set significant for nPM.

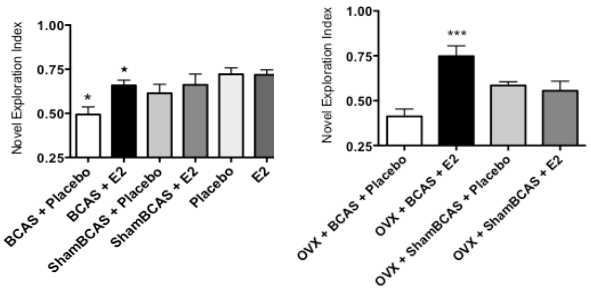


Fig 6: Effects of estradiol on male (left) and OVX female (right) mice in the setting of CCH. NOR testing: (*/***ANOVA $p < 0.01$) (n=4/group left; n=7/group right, mean±SEM)

C2. Overall Strategy: This project is designed to assess effects of nPM exposure in a system that isolates an exclusively vascular influence/ risk factor for AD and cognitive decline (CCH). We hypothesize that nPM exposure accelerates cerebrovascular damage underlying memory decline in AD and the effects are influenced by age and sex. *Aims 1* and *2* will examine independent and joint effects of nPM and CCH exposures on white matter toxicity, hippocampal/ entorhinal neuronal injury, and neurocognition. *Aim 1* will explore age dependence and *Aim 2* sex differences. A factorial design will assess independent and combined effects of nPM exposure and CCH in each aim. The primary hypothesis: synergy of the nPM and CCH will be evaluated with an interaction term. Consistency of mouse strain (C57BL/6) and exposure duration (150 cumulative hours nPM; 30 days CCH) will allow for delineation of factors affecting outcome (nPM, CCH, age, sex) in a combined, multivariable model. To limit total number of mice/ avoid duplication of available data, the current project leverages a small subset of experiments from an ongoing study that examines individual and joint effects of nPM and CCH in *young male* mice (Mack; R01ES024936). The experimental parameters of the current project are chosen to match the ongoing study so that the young male mice can be used as a reference group. *Aim 1* will examine older (18-month) male C57BL/6 mice. Findings will be compared to those of young (8-week) male mice. *Aim 2* will study young (8 week) and older (18-month) female C57BL/6 mice. Results will be compared across age and sex in the first two aims. All mice will undergo either BCAS or sham surgery. *Parallel comparison cohorts of control mice (no surgeries) will be performed for each of the aforementioned groups in Project 3 (Finch)*. Corresponding outcome measures are selected for projects 3 and 4. White matter tracts will be evaluated for myelin degeneration and hippocampal subfields/ entorhinal cortex for neurite atrophy. Neurocognition will be assessed across cohorts. One hundred ten and 220 mice will be used for *Aim 1 and 2*, respectively. *Aim 3* investigates mechanistic pathways. Neuroinflammation and BBB permeability will be assessed in the white matter tracts and hippocampus/ entorhinal cortex. Cortical cerebral microbleeds and APP processing will be examined. *Aim 3* will build upon findings generated from Project 3 and the first two aims of this project. Studies will examine independent and joint effects of nPM and CCH exposures in two groups of mice at the most vulnerable age and sex (white matter injury) from *Aims 1 and 2:* (1) EFAD-Tg, 2) inducible macrophage-microglial TLR4 knockout (i-mTLR4-ko). The above factorial design will be used; 170 mice will be used for *Aim 3*. Treatment groups are outlined according to Aim in Table 1.

TABLE 1: TREATMENT GROUPS BY SPECIFIC AIM

Strain	Sex	nPM	CCH	Assessment	Section	Exposures (number and group description)
C57BL/ 6	M	8 week	12 week	18 week	R01ES024936	4: (nPM-/CCH-, nPM+/CCH-, nPM-/ CCH+, nPM+/CCH+)
C57BL/ 6	M	18 month	18m4w	18m10w	Aim 1	4: (nPM-/CCH-, nPM+/CCH-, nPM-/ CCH+, nPM+/CCH+)
C57BL/ 6	F	8 week	12 week	18 week	Aim 2	4: (nPM-/CCH-, nPM+/CCH-, nPM-/ CCH+, nPM+/CCH+)
C57BL/ 6	F	18 month	18m4w	18m10w	Aim 2	4: (nPM-/CCH-, nPM+/CCH-, nPM-/ CCH+, nPM+/CCH+)
C57BL/ 6	F	18 month	18m4w	18m10w	Aim 3	4: (nPM-/CCH-, nPM+/CCH-, nPM-/ CCH+, nPM+/CCH+)
EFAD-Tg	F	18 month	18m4w	18m10w	Aim 3	4: (nPM-/e3, nPM-/e4, nPM+/ e3, nPM+/e4); all CCH+
i-mTLR4-ko	F	18 month	18m4w	18m10w	Aim 3	4: (nPM-/,WT, nPM-/ i-mTLR4-, nPM+/WT, nPM+/ i-mTLR4-), CCH

C3. General Methods: This section outlines the exposures, reagents, techniques, and outcomes measures to be used across aims and experiments:

C3.A. Mice: Experiments will comply with the *Guide for the Care and Use of Laboratory Animals* and stipulations of the Animal Welfare Act (7 U.S.C. S 2131 et. seq.). C57BL/6 mice will be used across aims. As C57BL/6 mice were used in the previous nPM/ CCH studies (R01ES024936), the strain was selected to maintain consistency among exposures in wild type experiments. Circle of Willis anatomy could modify the impact of CCH across mouse strains[19, 68]. Older (18 month) male and female mice will be obtained directly from NIA colonies. Female EFAD-Tg and the new i-mTLR4-ko mice will be used in *Aim 3* to study APOE alleles and TLR4 deletion. See Project 3 for breeding details of EFAD, which are provided by Dr. LaDu (University of Illinois at Chicago). For *i-mTLR4-ko mice, see Project 3 Aim 3.*

C3.B. nPM Collection: The nPM collection protocol is described in detail in Environment-Neurotoxicology Core C2 and is collected and administered according to standard methodology previously detailed[36]. Composite analysis of the particulate has been previously documented[69].

C3.C. nPM Exposure: Mice are transferred to whole-body exposure chambers (20 mice/ chamber) consistent with our group's previous descriptions [36 70-72]. We maintain average concentration of 300 ug/m^3- roughly twice that of busy roadways [67, 73]. Temperature and airflow are controlled for adequate ventilation and to minimize

contaminants. Re-aerosolized nPM or filtered air is delivered to the sealed exposure chambers for 5 hours/day, three-days/ week[36]. Total exposure duration is 10 weeks (150 cumulative hours) for each aim. Blood pressures are recorded at 7-day intervals throughout the exposure. In prior studies, mice have not lost weight or shown signs of respiratory distress[36]. The BCAS surgery is performed 4 weeks into the exposure period for a total of 30 days (final 30 days of nPM exposure). Per protocol, neurobehavioral assessments are performed during the final 19 days of the combined exposure.

C3.D. Bilateral carotid artery stenosis (BCAS) model of CCH: Surgery is adapted/modified from a protocol described by Shibata and colleagues.[20]. Mice are deeply anesthetized with intraperitoneal Ketamine/ Xylazine and turned prone. A rectal temperature monitor is placed and temperature maintained at 36.5°C- 37.5°C throughout the procedure. A Laser Doppler probe is affixed to the bone over the left cerebral hemisphere 1mm posterior and 5mm lateral to bregma. The mouse is then laid supine and both common carotid arteries are exposed through a midline cervical incision. A microcoil (diameter 0.18 mm) is placed around each common carotid artery (Sawane, Japan) and the skin incision is closed. A PF 5010 laser Doppler Perfusion Monitoring Unit (Perimed AB, Sweden) is used to record CBF values in the supine/ prone positions prior to surgery, and following placement of each microcoil. Mice that do not demonstrate greater than 15 percent blood flow reduction are excluded from analysis. In sham-operated mice, bilateral common carotid arteries are exposed (as above), but no microcoils are applied. Blood pressure/arterial blood gasses are recorded during the procedure and blood pressure is measured at seven-day intervals and prior to euthanasia.

C3.E. Brain Harvesting: Mice are deeply anesthetized with Ketamine/Xylazine and perfused transcardially with phosphate buffered saline (PH 7.4) to clear the vasculature of blood. The brains are rapidly removed from the skull. Using an Adult Mouse Brain Slicer Matrix (Zivicinstruments), brains are divided at bregma into frontal and posterior portions. The frontal portion is post-fixed and stored for an additional 24 hours in paraformaldehyde at 4C˚, then in 70% alcohol, and prepared/ microsectioned in paraffin for KB staining. The posterior portion is hemisected. One posterior hemisphere is post-fixed first as above, then in 20% sucrose 0.1 mol/L PBS (PH 7.4). Next it is frozen, embedded with O.C.T, cryosectioned, and prepared for IHC. The other posterior hemisphere is microdissected (Finch Laboratory) into 2 hippocampal regions (CA1-enriched/ DG-enriched), entorhinal cortex, corpus callosum, and cortex. The five microdissected regions are provided fresh to Core C3 for Western protein analyses: TLR4 pathway (TLR4, CD36, MyD88), cytokines (TNFα, IL1α, IL6, IL10), TNFR1, complement factors (C5, C5a, C5R). Posterior right/ and left hemispheres are alternated.

C3.F. White Matter Ischemic Change: *Klüver-barrera (KB) staining (primary outcome):* Using the paraffin-embedded brain samples, a brain section from bregma to 3mm anterior to bregma (adjusted to mouse atlas) is sliced into 10 serial 3µm-thick coronal sections. KB staining is performed on the slice located at bregma and the slice just anterior to bregma. White matter integrity is evaluated in the medial region of the corpus callosum according to a previously described four point scale[20, 21, 74] (0: normal; 1: fiber disarrangement; 2: marked vacuoles; 3: disappearance of myelin fibers). Analysis is performed by two, blinded-to-exposure, independent observers. Each observer scores the right and left hemisphere (medial corpus callosum) separately. Scores from each observer and each side are averaged to generate a combined score for each slice. The scores from the two slices are then averaged, generating a final score. *Immunohistochemistry:* Using the frozen brain samples, a section of the brain located from bregma to 3 mm posterior to bregma (adjusted to mouse atlas) is then sliced into 10 serial 20µm-thick coronal sections. Immunohistochemistry is performed on the regions of slices corresponding to those used for KB staining (just posterior). Antigen is retrieved by microwave, dipped in 3% H2O2 for 10 min, and blocked with serum. Slides are incubated overnight with anti-myelin-associated glycoprotein (MAG,1:200, Millipore, Watford, UK), or anti-dMBP (1:1000, Millipore, Watford, UK). Subsequently, sections are treated with appropriate biotinylated secondary antibody (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) and visualized with diaminobenzidine (DAB). Photographs of immunostained slices are captured by a LAS AF microscope (Leica, Germany). Myelin integrity is assessed (anti-myelin-associated glycoprotein) by the presence of disorganized white matter fibers/ myelin debris (graded 0-3). Degraded myelin is assessed (anti-dMBP slides) by presence of irregular myelin sheaths (0-3).White matter injury is assessed in corpus callosum, external/ internal capsule, fimbria hippocampus, optic tract, and the fibers of the caudate nucleus in accordance with previous studies[75].

C3.G. Neurite Atrophy: Silver Staining of the hippocampal subfields (CA-1/ DG) and entorhinal cortex is described in detail in Project 3 methods section (Finch laboratory).

C3.H Western Blotting: See Core C3 description for full details. A total of 5 subregions (cortex, entorhinal cortex, corpus callosum, hippocampal CA1 and DG) are microdissected per section for Western blot analysis in Core C3. The following inflammatory proteins implicated in the TLR4 and/ or C5 pathway are assessed: TLR4, CD36, MyD88,TNFα, IL1α, IL6, IL10, TNFRI, C5, C5a, C5R.

C3.I. Neurobehavioral Analysis: In the BCAS model utilized for this study, 8-arm radial maze [21, 26, 75] and NOR (section C1) tests have demonstrated strong correlations with white matter ischemic change. *A battery of other neurobehavioral tests have **not** demonstrated differences between BCAS and sham mice* [21]. 8-arm radial maze and NOR testing have also been performed on mice exposed to both nPM and CCH (section C1). *8 Arm Radial Maze Test:* This test evaluates spatial working memory. The apparatus consists of an octagonal platform (20 cm diameter) with 8 radiating Plexiglas arms (47 x 7 cm). A plastic food well is located at the distal end of each arm. SMART software (Panlab, Spain) is used to control doors at the proximal end of each arm and record behavior. The maze is elevated 1 m from the floor. A camera connected to a computer is fixed on the ceiling above the central maze platform. A food deprivation protocol is used to reduce the mouse's initial body weight by 10-15% and is maintained throughout behavioral testing. The mice undergo two pre-training days to familiarize with the apparatus/ task. At the beginning of every trial (one /day) the animal is placed on the central platform with all arm doors open and allowed to make an arm choice. Once the mouse has entered one arm, the other 7 doors are closed. When the animal exits the visited arm it is confined on the central platform for 5 sec by closing the remaining door. After the delay, it is allowed to make a new choice. A trial ends when the mouse retrieves all 8 pellets or 25 minutes elapses. The testing lasts 16 days (one trial/day). For each trial, the number of correct arm entries within first 8 visits, the number of revisited arms, and time taken to complete the task are recorded. Testing is administered on 16 of the final 19 days of the nPM/ BCAS exposure (final 3 days: NOR testing). *Novel Object Recognition Test:* NOR test evaluates declarative memory. The protocol is based on 3 phases: 1) Habituation: mice are placed in an open-field black Plexiglas arena (40 x 40 x 40 cm). Each mouse is allowed to explore for 15 min. 2) Sample trial: twenty-four hours later, mice are permitted (for 15 min) to explore two identical plastic-made objects (O1 and O2). 3) Object recognition trial: mice are returned to the arena 24 h after the sample trial, and observed under equivalent conditions, in the presence of one familiar object (O3, identical to sample trial), and one novel object (N1). Location of the objects is counterbalanced across the trial. Exploration is defined as sniffing/ touching the object. Analysis of object exploration includes frequency, duration, and latency. Object recognition is calculated for each animal as novelty exploration index, with the formula: $T_{N1} / (T_{N1} + T_{O3})$, with T indicating duration of object exploration[76, 77]. The three testing phases are administered on the final three days of the exposure (nPM, CCH).

C3.J. Quantitative immunohistochemistry: neuroinflammation: Using the frozen brain samples, a section of the brain located from bregma to 3 mm posterior to bregma (adjusted according to mouse atlas) is then sliced into 10 serial 20µm-thick coronal sections. Immunohistochemistry is performed on the regions of corpus callosum slices corresponding to those used for KB staining and in the hippocampal and entorhinal cortex slices as described in Project 3. Antigen is retrieved by microwave, dipped in 3% H2O2 for 10 min, and blocked with serum. Slides are incubated overnight with rabbit anti- glial fibrillary acidic protein (GFAP) antibody (1:10,000; Dako, Denmark), rabbit anti- ionized calcium-binding adapter molecule-1 (IBA1) antibody (1:200; Wako, Japan), goat anti- Tumor Necrosis Factor-alpha (TNFα) antibody (1:50; R&D, MN), goat anti- Interleukin-6 (IL-6) antibody (1:50; R&D, MN), mouse anti- C5 antibody (1:50; Hycult, Netherlands), goat anti- C5a antibody (1:50; Bosterbio, CA), rat anti- CD88 (C5 receptor) antibody (1:200; Biolegend, CA) or goat anti- Interleukin-10 (IL-10) antibody (1:50; R&D, MN). Subsequently, sections are treated with appropriate biotinylated secondary antibody (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, California, USA) and visualized with diaminobenzidine (DAB). Photos of the immunostained slices are captured by a LAS AF microscope (Leica, Germany). Optical DAB signal density is analyzed and quantified using NIH Image J software (rsbweb.nih.gov/ij/). Images are converted to 8 bit and adjusted to threshold to count the positive cells per 0.1mm square. Protocols follow the NIH Image J user guide.

***C3.K. Capillary density**: Density of CD31-stained capillaries will be measured from multiple slices adjacent to the immunohistochemistry slices (C3.J) in the corpus callosum and hippocampal/ entorhinal cortex. Immunofluorescent CD31 (BD Pharmingen, San Jose, CA) labeling will identify regional microvessels[78, 79]. Using stereology, capillary density will be quantified as length of blood vessels less than 10 µm diameter per volume tissue. Total length of capillaries (mm) is divided by the volume of brain tissue scanned (mm^3) to obtain capillary density (length per tissue volume).*

C3.L. Blood-brain barrier permeability: In vivo cerebral blood flow, BBB permeability, and angiography measurements will be obtained by multi-photon microscopy. Confocal microscopy will be used to perform analysis of BBB integrity (pericyte coverage, IgG/ fibrin deposition, MMP-9/ Cyp-A staining) through tissue immunofluorescence and lectin staining. In later project years, ultra-highfield MRI will be leveraged to detect connectivity changes in the white matter through diffusivity and tractography maps and quantification of BBB permeability using dynamic contrast enhanced (DCE)-MRI. See Core B2 for methods description.

C3.M. Microbleeds: Hemosiderin is assayed histochemically by Prussian Blue. Brain sections are immersed in solution of 5% KCN/5% HCl, rinsed with water, and counterstained with 2% Nuclear Fast Red. This will be performed in conjunction with the Finch lab (see details Project 3)

C3.N. Cerebrovascular Aβ: In EFAD mice, cortical Aβ-IR blood vessel density will be measured by the Finch laboratory (See Project 3 methods). Total Aβ and Aβ associated with vasculature will be quantified. Cortical sections will be stained for fibrillar Aβ, total Aβ, and microglia associated with fibrillar plaques.

C3.N. Shared data management and analysis: Projects 3 and 4: We will capitalize on the harmonization of the experimental Projects 3 and 4, which share mouse strains, nPM exposures, and common analysis of proteins by Core C3. As part of study planning, a common REDCap database will be developed in Y1 for data entry and management. REDCap (Research Electronic Data Capture) is a web-based data application specifically developed for data entry/ management of research data; institutional support for REDCap is provided through the USC Clinical and Translational Science Institute (CTSI). REDCap data are seamlessly exported for use in all major statistical software applications. Specific to this P01, the REDCap database will use common variable naming and coding for common outcomes across the two projects. Data for each project can be entered in the same database, using the same data structures, by adding a "study arm" (one for each Project's data), that allows Project data to be maintained as separate entities (by arm), but that still can be exportable as a single database. Databases for statistical analyses of data common to the two projects can be efficiently developed (click of a button). Dr. Wendy Mack will advise Projects 3 and 4 in Y1 on development of this database, and will provide statistical consultation in Y2-5 for analysis and reporting of the common dataset.

C4. Specific Aims 1 and 2 (refocused outcomes): *Specific Aims 1* (age dependence) *and 2* (sex differences) each investigate individual and joint effects of nPM and CCH exposure on outcomes relevant to AD pathology. Immunohistochemical and behavioral analyses examine neurodegenerative pathways from the entorhinal cortex and hippocampus including the perforant pathway and global white matter tracts. We hypothesize that nPM exposure and experimental induction of CCH will each produce ischemic damage in white matter tracts and neuronal injury in the hippocampal CA1 subfield and entorhinal cortex. We expect that this injury will correlate with neurocognitive deficits in working memory and NOR. When administered together, we expect these exposures (nPM/CCH) to exhibit synergy. Preliminary evidence of this synergy has been demonstrated in our young, male mice (see section C1). *Specific Aims 1 and 2* share common exposure sources (nPM/CCH), experimental designs, and outcome analyses. Therefore, following independent rationale sections, shared experimental exposure and outcome analysis sections are outlined.

C4.A. Specific Aim 1: Examine age dependence for independent and joint effects of nPM and CCH on white matter toxicity, hippocampal CA1/ entorhinal cortex neuronal injury, and neurocognition Based on existing studies of inflammation and BBB permeability, we hypothesize greater effects in older male mice (lack of ceiling nPM ceiling effect, see A4, and Project 3). 18 month C57BL/6 male mice will be used for this aim. A randomized study of 4 groups will be employed to assess effects of nPM exposure and CCH. These exposures will be tested independently and together (for joint effects) in a factorial design. Outcomes of the 18-month mice (individual and combined exposures) will be compared to 8-week mice from an ongoing study with the same experimental parameters (Mack; R01ES024936).

C4.B. *Specific Aim 2: Examine sex differences independent and joint effects of nPM and CCH on white matter toxicity, hippocampal CA1/ entorhinal cortex neuronal injury, and neurocognition.* Based on literature supporting the impact of estrogen on inflammation and BBB permeability, we hypothesize a young female advantage. A) Young (8-week) and B) Older (18-month) female C57BL/6 mice will be used for this aim. For each of the two age cohorts, a randomized study of 4 groups will be employed to assess the effects of nPM exposure and CCH. These exposures will be tested independently and together (for joint effects) in a factorial design. Outcomes of the 8-week and 18-month female mice (individual and combined exposures) will be compared to one another and to the 8-week and 18-month male mice in aim 1. This will allow for testing of both age dependence and gender influence on the independent and joint effects of the exposures.

C4.C. Experimental Exposures for Specific Aims 1 & 2 (Fig 7): Each cohort of C57BL/6 mice (*Aim 1*: 18-month male; *Aim 2A*: 8-week female, *Aim 2B*: 18-month female) will be randomized to 1 of 4 study groups: 1) filtered air/ sham surgery or 2) filtered air/ CCH or 3) nPM/ sham surgery or 4) nPM/ CCH. The mice will be exposed to nPM or filtered air for 5 hours/day, 3-days/ week for 10 weeks. Thirty days from the end of the exposure, mice will undergo either CCH or sham surgery. This will allow for outcome assessments at previously validated time points (30 days CCH). Control mice that have not undergone CCH or sham surgery are prescribed an identical nPM/ filtered air exposure paradigm in Project 3.

C4.D. Outcome analysis for Specific Aims 1 and 2: Following completion of the exposures, white matter tract damage, hippocampal/ entorhinal cortical neuronal injury, capillary densities, and neurobehavioral outcome will be assessed. Two parallel, identically exposed, subgroups will be included for outcome analysis in each exposure group: 1) *Pathology subgroup*: white matter/ immunohistochemistry/ western analysis (n=16 mice); 2) *Behavioral testing subgroup*: 8-arm radial maze, NOR (n=10 mice, Fig 7). *Pathology subgroups:* 1) White matter ischemic injury will be determined by KB staining (primary outcome). MAG and dMBP staining will be performed to further characterize the white matter damage in the corpus callosum, perforant pathway, fimbria hippocampus, internal capsule, optic tract, and fiber bundles of the caudate nucleus, 2) Neurite atrophy will be quantified in the hippocampus/ entorhinal cortex by silver staining and compared between the hippocampal CA1 subregion and dentate gyrus. 3) Capillary densities will be measured in the corpus callosum and entorhinal/ hippocampal cortex. *Behavioral subgroup:* 1) Neurobehavioral outcome will be assessed by 8 arm radial maze and NOR testing.

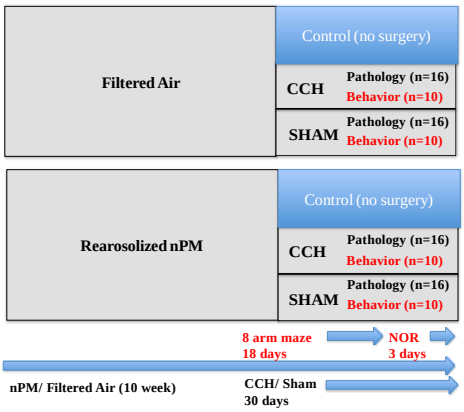


Fig 7: Exposure paradigm

C4.E. Statistical Analysis: Specific Aims 1 & 2: For each cohort of mice (older males, young females, older females) outcomes will be analyzed with a 2x2 ANOVA (nPM X CCH, Fig 8). Comparison data will be generated for young males as part of the existing (R01ES02493) study. Main effects of each exposure (nPM/CCH) will be tested. The primary hypothesis related to synergy of the nPM and CCH exposures on outcomes will be tested with the interaction term (testing hypothesis that mean outcome in the combined nPM+/CCH+ group will be greater than that predicted from summed main effects of CCH+ and nPM+). *Null hypothesis:* C1/C4= C2/C4+C3/C4. *Alternate hypothesis:* C1/C4≠ C2/C4+C3/C4. Post hoc analyses will perform pairwise group comparisons with adjustment for multiple testing. Perioperative blood pressures/ physiologic parameters will be compared between groups (mean, SD) to help differentiate vascular from direct toxic nPM effects. Next, combining all 4 groups (young males, old males, young females, old females), a general linear model will be analyzed, with white matter score (or biomarker values, see Aim3) as the dependent variable. Independent variables will include sex, age group, CCH, and nPM exposure. Association tests will include tests for main effects of each of these variables; product terms will be used to test for interactions (e.g., sex-nPM to test for sex differences in nPM effects, age-nPM to test for age differences in nPM effects).

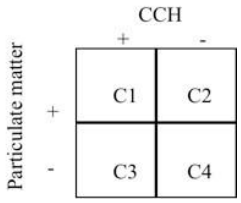


Fig 8: Analysis table for synergy between particulate matter/ CCH within each cohort

Collaboration with Project 3: Surgically intact mice of the same ages/ sex are studied in Project 3 *Aim 1*. Shared outcomes enable comparisons between these and our CCH/ sham mice. Direct collaboration will allow processing of brain sections from each project for all relevant outcomes. For reliability, each outcome will be performed in a single laboratory (Finch: CA1/ entorhinal neurons, perforant path projections; Mack: corpus callosum, white matter tracts). Specific behavioral outcome measures are unique to Project 4 (8 arm maze, NOR) as these are the only validated tests demonstrating injury in the CCH model.

C5. Specific Aim 3: To examine the mechanistic pathways by which nPM promotes neurodegenerative processes in the setting of CCH (major revision). We hypothesize that neuroinflammation and BBB permeability play critical roles in the individual and combined effects of nPM exposure and CCH. We expect that administration of nPM and CCH together substantially increase levels of biomarkers of inflammation/BBB breakdown compared to those predicted for either exposure alone. Further, we hypothesize that, in the setting of nPM exposure, the APOE4 allele (EFAD-Tg) will exacerbates CCH-AD synergies and that *an intervention attenuating the inflammatory TLR4/ TNFα pathway (i-mTLR4-ko) will decrease neurodegeneration from chronic cerebral hypoperfusion*.

C5.A. Rationale: Aim 3 concentrates on putative mechanism of injury in the most vulnerable (white matter injury) age/ sex mice from the first two aims and Project 3. We expect the largest effect size for both the independent and joint influences of nPM/ CCH in older female mice. This focus would enhance synergies with Projects 1 and 3. The brain specimens from *Specific Aims 1 or 2* (most vulnerable group) will be leveraged for the first part of this aim and each relevant biomarker (see below) assessed for independent and joint exposure effects. Planned analyses concentrate on biomarkers of inflammation and increased BBB permeability that are impacted by aging and regulated by estradiol/ estrogen receptors. Additional exposure groups will be required for BBB analysis (multiphoton microscopy), as the cranial window procedures could impact KB staining and immunohistochemical analysis. The second part of the aim explores mechanism through the use of genetically

modified mice. EFAD transgenic mice will be used to determine the susceptibility of hypoperfused mice with the APOE e4 allele to nPM exposure. *i-mTLR4-ko mice will be studied to examine/ characterize the inflammatory response to nPM in the setting of CCH.* The transgenic mouse strains are the same as those investigated for nPM effects in Project 3. Therefore, all mice will be subjected to CCH. A randomized study of 4 groups will be used to assess the effects of nPM exposure and each specific genetic alteration (TLR4 deletion or APOE allele). AD associated biomarkers will be compared in a factorial design similar to that described in *Aims 1 and 2*. Cerebral amyloid and cortical microbleeds will be quantified in EFAD mice.

C5.B. Inflammation: Immunohistochemistry will be performed on brain sections from 32 mice (n=8, each of 4 pathology subgroups) from the vulnerable group in *Aim 2.* Cells will be counted and densities quantified in the same regions of the hippocampus, entorhinal cortex, frontal cortex, corpus callosum and white matter tracts assessed for white matter/ neuronal injury in *Aim 2*. Numbers of reactive microglia (IBA-1) and astrocytes (GFAP) will be quantified as previously described [19, 20, 22, 80, 81]. *Deposition/ density of the following pro-inflammatory mediators will be determined: TNFα, IL-6, C5/C5a/C5a receptor, and IL-10. Prior studies have demonstrated that each of these TLR4 or complement pathway-related markers are influenced by PM and/or cerebral ischemia in brain tissue*[34, 35, 82-84]. Outcomes will be analyzed with a 2x2 ANOVA (nPM X CCH) for each biomarker. Main effects of each exposure (nPM, CCH) and joint influence will be tested (see C4.E)

C5.C. Microbleeds: On the 32 mice studied for inflammation, cerebral cortex will be assessed for microbleeds. Although rare/ sporadic in C57BL/6J mice, the presence of cortical microbleeds is associated with reduced cerebral blood flow in the elderly[52].

C5.D. Blood Brain Barrier Permeability (Core B2): Confocal microscopy will be utilized to analyze BBB integrity (pericyte coverage, IgG/ fibrin deposition, MMP-9/ Cyp-A staining) through tissue immunofluorescence and lectin staining in the same 32 mice studied for inflammation (C5.B). Cells will be counted and densities quantified in the regions listed above. In vivo cerebral blood flow, BBB permeability, and angiography measurements will be performed in year 3 and 4 of the project by multi-photon microscopy on an additional cohort of 32 mice [8 in each group; 1)-nPM/-CCH, 2)-nPM/+CCH, 3)+nPM/-CCH, 4) +nPM/+CCH]. These assessments will characterize alterations in cortical tissue blood flow and BBB integrity. Ultra-highfield MRI will be performed to detect connectivity changes in the white matter through diffusivity and tractography maps and quantification of BBB permeability using dynamic contrast enhanced (DCE) in 8 additional mice per group from *Specific Aim 2* (32 total mice). Regions of interest will include corpus callosum and other white matter tracts, hippocampus, and frontal cortex. New generation CCH microcoils (Sawane, Japan) are MRI compatible.

C5.E. Exploratory studies of inflammation and BBB in genetically engineered mice: Genetically engineered (EFAD-Tg or i-mTLR4-ko) mice will be exposed to nPM or filtered air for 5 hours/day, 3 days/week for 10 weeks. Thirty days from the end of the exposure *all mice will undergo CCH surgery*. They will be compared to control mice that have not undergone CCH surgery in Project 3. The experimental paradigm is similar to that described in *Aims 1 and 2* except that a single cohort (n=16/ group) will be used for pathology studies only. **EFAD-Tg studies**: Seventy, EFAD mice (most vulnerable age/ sex) exposed to CCH will be randomized to one of four study groups: 1) filtered air/ APOE e3; or 2) filtered air/ APOE e4; or 3) nPM/ APOE e3; or 4) nPM/ APOE e4. ***i-mTLR4-ko studies****: Mice will be induced for TLR4-ko prior to exposures (see details Project 3). Seventy mice (most vulnerable age/ sex) exposed to CCH will be randomized to one of four study groups: 1) filtered air/ control; or 2) filtered air/ i-mTLR4-ko; or 3) nPM/ control; or 4) nPM/ i-mTLR4-ko*.

Outcome Analysis*:* Following exposure, pathology endpoints used in *Aims 1 and 2* will be measured (white matter, hippocampal CA1/ entorhinal injury, capillary densities) as described in section C4.D. Additionally, cerebrovascular amyloid deposition and microbleeds will be quantified in the EFAD-Tg study. Studies suggest impaired vascular reactivity and resting-state cerebral blood flow is associated with cerebral amyloid angiopathy and cortical microbleeds[52, 85-88]. Inflammatory markers (C5.B) will be measured in the i-mTLR4-ko experiments. Primary outcomes will be analyzed with a 2x2 ANOVA (nPM exposure by strain type) in each section of the aim (EFAD-Tg or i-mTLR4-ko). Main effects of each exposure (nPM, strain type) and joint influence will be tested. We expect the APOE e4 allele to accelerate the deleterious effects of nPM exposure through increased BBB permeability and microhemorrhages. We anticipate these findings to be more pronounced in the CCH mice (Project 4) than controls (Project 3). We also expect that inducible TLR4 deletion will diminish the nPM-incited inflammatory response and result in injury reduction (relative neuroprotection).

Collaboration with Project 3: Transgenic experiments in the second part of this aim will be guided by the immunohistochemical studies in the first part of the aim and the results of Project 3 (EFAD-Tg; i-mTLR4-ko). Further, each group of surgically intact mice from Project 3 will serve as control comparators for the corresponding exploratory transgenic experiments described above.

C6. Limitations and alternative approaches

C6.A. nPM exposure composition: This study utilizes re-aerosolized nPM as opposed to a direct exposure. As described in our group's publications utilizing this paradigm [36, 60, 70, 72], the exposure is an aerosol with size and chemical composition characteristic of a typical urban area. The aerosol constituents are present at highly increased, yet environmentally realistic, levels. Our overall effects seen in vivo/ in vitro, at representative exposure scenarios, are well-defined. Our laboratory has demonstrated the effects of this exposure in the setting of cerebral ischemia[67]. An advantage of this approach, over particle concentrators, is maintenance of consistent aerosol size and composition across an entire exposure period. This consistency is not possible when working with real world PM, which varies diurnally, seasonally, and spatially.

C6.B. nPM mechanism of brain entry: Mechanisms and pathways by which TRAP enters the brain are not clearly defined. Potential routes include direct olfactory nerve transport, indirect influence through systemic inflammation, infiltration of peripheral monocytes, and ingestion. Our proposed studies are not constructed to directly answer these questions/ characterize portal of brain entry. Experimental design assesses end organ impact of nPM exposure (irrespective of entry route) on white matter, hippocampal/ entorhinal cortex, and neurocognition in the setting of CCH. Perioperative physiologic parameters will be compared between groups in each aim to help differentiate vascular from direct toxic PM effects. If blood pressure differences are found, carotid and peripheral arterial specimens will be examined for biomarkers of inflammation listed in *Aim 3*.

C6.C. Selection of age cohorts: The human epidemiology P01 studies examine both females (Project 1) and males (Project 2). Our older (late middle age) mice are of similar age to the individuals in the WHIMS-MRI study (Project 1) and the VETSA 1 and 2 cohorts (Project 2). Although ages of our young mice do not directly translate to the human study cohorts, they were chosen to establish baseline data for novel exposures. The mouse ages selected for investigation are identical to those in Project 3. We chose the extremes (2-3 months, 18 months) of the age range employed in project 3 (rather than 10 months) to maximize potential outcome differences among cohorts. If the 10-month mice demonstrate the greatest injury in *Aim 1* of Project 3, we will substitute this age for the older mice in our aims. Likewise, we will study the age and sex that demonstrates the most significant effects in the early aims of Project 3 and 4 for our *Aim 3* mechanistic studies.

C.6.D. Estrogen Cycling: As aging females have complex/ variable patterns of menopause, estrogen cycling will be considered in female experiments. Full rationale and protocol descriptions are reviewed in Project 3. Briefly, older (18-month) mice typically exhibit ovariectomy levels of plasma estradiol levels (menopause). Female mice will be monitored for vaginal cytology during exposures (see Project 3). At the end of the 10-week exposure, the young mice (start 8, end 18 weeks) will be actively cycling. The older group (start 18, end 21 months) will be predominantly anestrus, but younger than the typical age of pituitary tumor growth (22 months). Ovarian heterogeneity will be tracked by vaginal smear during nPM exposure. Eighteen-month mice will be selected for constant estrus smears prior to exposure. Serum will be stored for hormonal analysis.

C6.D. Modeling exposure interactions: nPM and CCH exposures are co-administered, with CCH/ sham procedures occurring 30 days prior to culmination of the nPM/ filtered air in each aim. Although the exposures do not always occur in this temporal pattern clinically, the sequence was chosen to optimize technical feasibility/ translational generalizability and represent the most common paradigm. Relevant outcome measures (white matter injury, neurocognitive testing) have been validated in the CCH model at 30 days. Generally, individuals are exposed to air pollution at younger ages and develop cerebrovascular disease later. The air pollution exposure often remains throughout the onset and progression of cerebrovascular disease. There are implicit assumptions: 1) Those with the exposure could only acquire the susceptibility factor after an extended period. From a translational standpoint, individuals could have significant white matter injury from other causes, regardless of prior air pollution exposure. 2) For the proposed biological/outcome measures, this design assumes mechanistic interactions only take place when CCH follows air pollution exposure chronologically (precluding mechanistic insights for populations who move from low to high exposures in late-life). Although assumptions limit generalizability with regards to lifetime exposure sequence, the design allows for assessment of nPM/CCH exposures in early/ later life and maximizes synergies with Project 3.

C6.E. Selection of mice for mechanistic studies (*Aim3*): To study mechanisms of a joint exposure across age ranges and sex would require a very time-intensive study with prohibitively large sample sizes. We will select the age and sex that is most vulnerable in *Aims 1 and 2* and Project 3 for use in Project 4 *Aim 3*. We expect the effect size of the joint influences (nPM/CCH) to be greatest in older females *(Aims 1 and 2*). In the EFAD and i-mTLR4-ko experiments, we expose all mice to CCH and maintain a single age and sex. This avoids incorporating up to 5 exposure variables into the study cohort (nPM, transgenic, CCH, age, sex), and rendering individual/ joint effects challenging to determine in a multivariable analysis without large sample sizes. However, we do incorporate a control cohort (no CCH) from Project 3 for comparison in each study. *Although genetic models may have less direct translational relevance than pharmacologic inhibition, the*

inducible macrophage/ microglia i-mTLR4-ko mouse enables temporally relevant induction, and restriction of genetic alteration to cell lines most relevant to nPM associated brain injury. The inducible model helps mitigate compensatory effects of longstanding TLR4 deletion.

C6.F.Behavioral testing: In *Aims 1 and 2*, parallel (separate) subgroups are used for pathology analysis and behavioral assessment to eliminate the possibility that food deprivation/ behavioral testing will impact white matter toxicity and protein expression. If older mice are not cooperative with the complex 8-arm radial maze, alternative tests used in our laboratory (nesting, burrowing, less complex y-maze testing) will be pursued.

C7. Power and Sample Size Calculations

C7.A. Specific Aim 1 and 2: We base sample size calculations on estimates of primary outcome (white matter toxicity) from our CCH and nPM studies in young males. We expect effect sizes for the older males/ females to be larger. Based on previous/ pilot studies, the white matter injury effect size for CCH is 1.5 (mean difference=0.6, SD=0.4) and for nPM exposure is 0.42 (mean difference=0.29, SD=0.695). We expect a white matter ischemia score of 1.86 in the mice exposed to both nPM/ CCH. This yields an effect size for nPM exposure in the CCH+ cohort that is 3-times larger than the nPM effect in shams (projected means: CCH-/nPM- =0.32; CCH-/nPM+ =0.64; CCH+/nPM- =0.90; CCH+/nPM+ = 1.86). To demonstrate expected mean group differences of a synergistic effect between nPM and CCH (two-sided alpha= 0.05, power=80%), roughly 16 animals will be needed per cell. A total sample size of 64 mice is therefore required for KB staining (pathology subgroup). These same mice will be used for neuronal injury quantification. Eight mice in each pathology subgroup will also undergo immunohistochemistry/ western blot protein analysis (see C7.B). An additional 10-mice/ group (behavior subgroup) will be included for neurocognitive assessment. This yields a total of 104 mice/ cohort (64 pathology, 40 behavioral subgroup). *Accounting for a roughly 10% surgical mortality (half mice undergo surgery), 110 mice are required for each individual age/ sex cohort in these aims (Table 2)*. Three hundred-thirty total mice are required for *Specific Aims 1 and 2*. **Multivariable analysis:** In the combined white matter injury analysis using 16 animals per group, each experiment (young/ old males, young/ old females) includes 64 animals, yielding 256 animals. We calculated minimum detectable correlation (min r) for a single variable in a multivariate model, testing at 2-sided alpha=0.05 and 80% power. The min r depends on amount of variability (R^2) in the outcome explained by other independent variables in the model; for 256 animals, min r ranges from 0.12-0.16 for R^2 from 15%-50%. We will be able to statistically detect correlations of this magnitude and larger.

TABLE 2: SPECIFIC AIM 1 & 2 PRIMARY OUTCOME

Cohort	n
Aim 1: White Matter injury	110
Aim 2a: White Matter Injury	110
Aim 2b: White Matter Injury	110

C7.B. Specific Aim 3: We expect to use tissue obtained from the mice in *Specific Aim 1 or 2* (most vulnerable age/ sex) for quantitative immunohistochemistry (8 mice/ pathology group). Prior CCH and nPM experiments have suggested adequate power to detect a significant difference in reactive microglia (IBA-1) between CCH and sham mice at sample sizes calculated in *Aim 2*. Specifically, the CCH effect sizes of 2.53 (mean difference=49.55, SD=19.57) in these experiments are large. nPM effect size is 2.01 (mean difference=68.99, SD=34.3). We calculated the power to detect such group differences, given 8 animals per group. Power to detect main effects of nPM and CCH will be greater than 99%. We will have sufficient power to detect synergistic relationships between CCH and nPM. We will have 86% power to detect differences in nPM effects that are 1.75 times higher in CCH+ groups vs. CCH- groups and 98% power to detect nPM effects that are 2 times greater. We believe differences and effect size will be similar for the other biomarkers. In vivo cerebral blood flow, BBB permeability, and angiography will require additional brain specimens. Based on prior experience, we believe that 8 mice per group should be sufficient to provide statistical power. *As we expect a roughly 10% surgical mortality, 34 total mice will be required for BBB studies.* **Multivariable analysis:** For combined biomarker analysis, each experiment will include 32 mice (8 per group), yielding 128 mice. Testing at 2-sided alpha=0.05; 80% power, min r will range from 0.17-0.22 for R^2 from 15%-50%. For EFAD/ i-mTLR4-ko studies, we assume sample size requirements similar to *Aims 1 and 2* (n=16 per exposure) and roughly 10% mortality. Therefore, 174 additional mice will be required for this aim (34 BBB analysis; 70 each transgenic, Table 3).

TABLE 3: SPECIFIC AIM3: PRIMARY OUTCOME

Cohort	n
Aim 3: Immunohistochemistry	32 (from aim 2b)
Aim 3: BBB	34
Aim 3: MRI	32 (from aim 2b)
Aim 3: EFAD	70
Aim 3: i-mTLR4-ko	70

C7.C. Multiple testing: *We specify white matter toxicity as our primary outcome and will test related hypotheses at an experiment-wise alpha 0.05. The remaining outcome measures (neurite density, behavior, inflammation, etc.) will adjust for experiment-wise false discovery rate (FDR); raw and FDR-adjusted p-values will be reported.*

VERTEBRATE ANIMALS

1. Provide a detailed description of the proposed use of the animals in the work outlined in the Research Strategy section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.

C57 Black 6 mice (both male and female), EFAD transgenic (tg) mice and inducible macrophage-microglia TLR4 knockout (i-mTLR4 knockout) mice/ littermate controls will be used in this proposal. EFAD (E3FAD and E4FAD) mice were generated by crossing 5XFAD to homozygous APOE3-, and APOE4-TR. 5xFAD mice are transgenic for 5 distinct FAD mutations (APP K670N/M671L+ I716V+ V717I and PS1 M146L+L286V). They are provided by University of Illinois at Chicago. i-mTLR4-ko mice are generated by targeted deletion of the TLR4 gene from macrophages/ microglia. A transgenic mouse carrying an excision enzyme (Cre recombinase) in a specific cell-line (CX_3CR1 promoter, JAX020940) and a transgenic mouse expressing TLR4 flanked with two loxP sequences (JAX024872) are crossed. In order to achieve cell-specificity, Cre expression is controlled by the CX_3CR1 promoter and is inducible via linkage to an estrogen receptor mutant activated by tamoxifen. First generation (F1) litters are 100% heterozygous ($mCre^{+/-}$, $Fl^{+/-}$). F1 heterozygous mice are crossed with homozygous floxed mice ($Fl^{+/+}$). Second generation is composed of: 25% experimental animals ($mCre^{+/-}$, $fl^{+/+}$), 25% control ($mCre^{-/-}$, $fl^{+/+}$). Transgenic mice will be validated by genotyping.

The strains, ages, sex and numbers used in each aim are listed below:
Specific Aim 1: n=110, male, 18 month C57BL/6 mice
Specific Aim 2: n=110, female, 8 week C57BL/6 mice and 110, female18 month C57BL/6 mice
Specific Aim 3: n=34, most vulnerable age/ sex C57BL/6 mice
Specific Aim 3: n=70, most vulnerable age/ sex EFAD (35 E3FAD, 35 E4FAD) mice
Specific Aim 3: n=70, most vulnerable age/ sex i-mTLR4-ko/ WT, (35 ko, 35 WT) mice
By the above calculations, a total of roughly 500 mice will be used for this project

Animals will be kept on a 12-hour light/ dark cycle with water available at all times and food ad libitum (except during deprivation for behavioral group). All animals will be euthanized at end of experiment.

In Aims 1 and 2 of the study, main effects of each exposure (nPM, CCH) and joint effects will be tested. One hundred ten male, 18 month old, C57BL/6 mice will be used for Specific Aim 1. One hundred ten, 8 week old, and one hundred ten, 18 month old, female C57BL/6 mice will be used for Specific Aim 2. In Specific aim 3, thirty-four, C57BL/6 mice (most vulnerable age/ sex) will be used for in-vivo blood brain barrier analysis studies. The other thirty-two, C57BL/6 mice (most vulnerable age/ sex) mice used in Aim 3 will be the same mice used in Aim 1 or 2 (most vulnerable age/ sex). Also used in Aim 3 will be seventy (most vulnerable age/ sex) EFAD mice (35 E3FAD, 35 E4FAD) and seventy (most vulnerable age/ sex) i-mTLR4-ko mice (35 i-mTLR4-ko, 35 wild type). In each of these transgenic experiments, the independent and joint influences of nPM exposure and strain type will be assessed in the setting of CCH (all mice exposed to CCH). Neurocognitive assessment will be performed 30 days after the CCH/ sham procedure (in 10 mice from each group in Specific Aims 1 and 2). Animals will be euthanized on the same day for tissue analysis.

Air Pollution Exposure
In each experimental cohort, mice are randomized into either of 2 exposure groups: 1) filtered air or 2) nanoscale particulate matter (nPM). Exposures are performed 3 days per week, 5 hours per day, for 10 weeks (150 cumulative hours). Animals are placed into exposure cages in an exhaust hood that is vented to the outside. Temperature and airflow are controlled to ensure adequate ventilation, minimize buildup of contaminants, and to avoid thermal stresses. During each exposure, nPM suspension is delivered to whole-body animal exposure chambers. Filtered air animals are treated identically, but exposed to filtered air without nPM added. Between exposures, animals are returned to standard cages.

Bilateral Carotid Artery Stenosis (BCAS)
BCAS surgery is adapted/modified from a protocol described by Shibata and colleagues. Mice are deeply anesthetized with intraperitoneal 80-100 mg/kg Ketamine and 5-10 mg/kg Xylazine, using a 27.5 gauge needle. Paralube is applied to the eyes to prevent corneal abrasion. The mouse is turned prone. A rectal

temperature monitor is placed and temperature maintained at 36.5°C- 37.5°C throughout the procedure. A Laser Doppler probe is affixed to the bone over the left cerebral hemisphere 1mm posterior and 2mm lateral to bregma. The mouse is then laid supine. The surgical site is shaved, prepped with antiseptic, and draped. Both common carotid arteries are exposed through a midline cervical incision. A microcoil (diameter 0.18 mm) is placed around each common carotid artery (Sawane Spring Co, Japan) and the skin incision is closed. A PF 5010 laser Doppler Perfusion Monitoring Unit (Perimed AB, Sweden) is used to record CBF values in the supine/ prone positions prior to surgery, and following placement of each microcoil. Mice that do not demonstrate greater than 15 percent blood flow reduction are excluded from analysis. In sham-operated mice, bilateral common carotid arteries are exposed (as above), but no microcoils are applied. Blood pressure/arterial blood gasses are recorded during the procedure and blood pressure is measured at seven- day intervals and prior to euthanasia.

Neurocognitive Testing

In the BCAS model utilized for this study, 8-arm radial maze and NOR (unpublished data, see section C1 of research strategy) tests have demonstrated strong correlations with white matter ischemic change. An extensive battery of *other* neurobehavioral tests has demonstrated no differences between BCAS and sham-operated mice. The behavioral testing is performed on the final 19 days of the exposure (nPM/ CCH). 8-arm maze test is performed on 16 of the final 19 exposure days and the NOR testing is performed over the final three days.

8-Arm Radial Maze Test: 8-arm radial maze test evaluates spatial working memory. A food deprivation protocol is used to reduce the mouse's initial body weight by 10-15% and maintained throughout the behavioral testing. The mice undergo two pretraining days to familiarize with the apparatus and the task. On pretraining day one, food pellets are scattered around the maze and each animal is left to explore freely for 5 min. On pretraining day 2, a single food pellet is placed at the end of each arm. The mouse is placed in the central platform and allowed access to each arm in turn (controlled using the doors), and allowed to retrieve the food pellet. A trial ends when the mouse retrieves all 8 pellets or 25 minutes elapses. The testing lasts 16 days (one trial/day). For each trial, the number of correct arm entries within the first eight visits, the number of revisited arms (working memory errors) and the time taken to complete the task are recorded. The testing is administered on sixteen of the final nineteen days of the nPM/ BCAS exposure interval (the final 3 days are used for NOR testing).

Novel Object Recognition Test: NOR test evaluates declarative memory. The protocol is based on 3 phases: 1) Habituation: mice are placed in an open-field black Plexiglas arena (20 x 20 x 20 cm). Each mouse is allowed to explore for 15 min. 2) Sample trial: twenty-four hours later, mice are permitted (for 15 min) to explore two identical plastic-made objects (O1 and O2). 3) Object recognition trial: mice are returned to the arena 24 h after the sample trial, and observed under equivalent conditions, in the presence of one familiar object (O3, identical to those in sample trial), and one novel object (N1). Location of the objects is counterbalanced across the trial. Exploration is defined as sniffing/ touching the object. Analysis of object exploration includes frequency, duration, and latency. Object recognition is calculated for each animal as novelty exploration index, with the formulas: T_{N1} / (T_{N1} +T_{O3}), with T indicating duration of object exploration. The three testing phases are administered on the final three days of the exposure (nPM, CCH) in the behavior cohort.

Tissue Collection

At the end of the ten-week exposure (postoperative day 30 from the CCH/ sham surgery), the mice are humanely euthanized. The mice are deeply anesthetized with Ketamine 80 mg/kg and Xylazine 5 mg/kg intraperitoneally and perfused transcardially to clear the vasculature of blood. The brains are excised, and prepared/ stored for further analysis.

2. Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.

In vitro studies or computer/ mathematical models cannot satisfactorily reproduce the white matter/ neuronal injury and functional deficits resulting from nPM/ CCH exposures. Due to cost, ethical concerns, and reproducibility, mice are most appropriate for these studies. We choose mice because of our experience in the

models being tested, availability of applicable antibodies/ assays, and potential for future investigations. The bilateral carotid artery stenosis model (microcoil application) that we use has been validated only in mice.

To limit total number of mice, and avoid duplication of available data, the current project uses a subset of data from an ongoing study that examines the individual and joint effects of nPM exposure and CCH in *young male* mice (Mack; R01ES024936). The experimental parameters of the current project are chosen to match the ongoing study so that the young male mice can be used as a reference group for the older males. Further, the strains and exposure paradigms of Project 4 have been chosen to match those of Project 3 so that parallel, control groups of mice (no surgery) from Project 3 can serve as comparator cohorts for each aim of Project 4.

C57BL/ 6 mice will be used for baseline experiments in each aim. As the CCH model has been validated and used in multiple studies on C57BL/ 6 mice by the investigators, the strain was selected to maintain consistency across aims and exposures. Further, preliminary nPM data has been collected using C57BL/6 mice. Altering background strains for the study would likely introduce strain specific differences in exposure responses and outcomes. This variation would increase sample sizes. Male and female mice are selected to study sex influences and comply with recent NIH initiatives to balance sex in animal studies. Although older mice are more expensive and challenging to test/ maintain, incorporation is necessary to evaluate age dependent effects of the exposures. EFAD-tg and i-mTLR4-ko mice are chosen (in conjunction with project 3) to study specific mechanisms (TLR4 inflammation/ APOE susceptibility) relevant to nPM/CCH and AD. All transgenic mice are exposed to CCH (using control animals for Project 3 with identical nPM/ strain exposure parameters) to limit sample size of expensive, transgenic mice. Power analyses are designed to limit sample size.

3. Provide information on the veterinary care of the animals involved.

Use of laboratory animals will comply with the provisions of the Animal Welfare Act (7 U.S.C. S 2131 et. seq.) and the guidelines set forth in the Guide for the Care and Use of Laboratory Animals. The following excerpt is borrowed from the USC Department of Animal Resources:

“The University of Southern California (USC) is a leader in the ethical and humane use of animals for research and teaching. USC is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) and has an animal welfare assurance (number A3518-01) on file with the NIH Office of Laboratory Animal Welfare. The Institutional Animal Care and Use Committee review all applications to ensure ethical and humane treatment of animals. This body follows the Guide for the Care and Use of Laboratory Animals and all applicable government regulations including those of the U.S. Department of Agriculture and the State of California. A primary mission of the USC DAR is to provide for adequate veterinary care for our laboratory animals as required by regulatory and accrediting agencies including the United States Department of Agriculture (USDA), Public Health Services (PHS) Policy, and the American Association for Accreditation of Laboratory Animal Care.”

In addition to the animal care provided by the Dr. Mack’s team, the animals are assessed daily by veterinary staff. Food and water is provided. If an animal appears sick, the principal investigator is notified immediately. The staff discusses the case with the principal investigator. The veterinary staff makes the ultimate decision regarding the disposition of sick animals. The following excerpt is also borrowed from the USC Department of Animal Resources website:

“DAR (Department of Animal Resources) veterinarians have access to all animals in order to evaluate their health and well–being on a daily basis. In addition, the DAR veterinarians rely upon the research staff and laboratory animal technicians to report occurrences of injury or illness after their daily observations of animals. Should an animal be reported sick or injured by a laboratory animal technician, the responsible PI will be notified prior to initiation of treatment or diagnostic tests. If a veterinarian determines that immediate action is necessary, as in the case of a life threatening situation or where significant pain and distress for the animal exist, action will be taken and the PI will be notified as soon as possible. A veterinarian is on call 24 hours a day, 365 days a year to provide veterinary services to animals involved in research projects.”

4. Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is

unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.

Animal discomfort, distress, pain, and injury will be minimized in all phases of the experiment. Specifically, during each procedure, mice will be anesthetized with 80-100 mg/kg Ketamine intraperitoneally (IP) and 5-10 mg/kg xylazine, IP, as described above.

5. Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. If not, include a scientific justification for not following the recommendations.

Animals will be humanely euthanized by performing a cardiotomy with exasanguination and subsequent saline perfusion while under anesthesia with ketamine (80 mg/kg IP) and xylazine (5 mg/kg IP). Decapitation will then be performed. This sequence of methods, chemical method followed by physical method is consistent with the recommendations of the AVMA (American Veterinary Medical Association).

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Keck School of Medicine

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Division of Biostatistics

May 20, 2017

Dear Committee, Caleb Finch and William Mack,

I am pleased to confirm my commitment to serve as a collaborator on the experimental research proposed in Projects 3 & 4 for the P01 entitled "Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms". I have reviewed and collaborated with your teams on the study design and statistical approaches, and will be actively involved in data analysis and interpretation. I have a long-standing collaboration with Professor Finch in P01 AG026572-011 Brinton (PI) 09/21/16-09/20/20, Progesterone in Brain Aging and Alzheimer's Disease and we have co-authored several articles, e.g., Yin et al 2015, PMID25921624. I have also worked with Dr. Bill Mack extensively as a co-investigator on your ongoing study (Mack; R01ES024936 Neurotoxicity of Airborne Particles: Role of Chronic Cerebral Hypoperfusion) that utilizes similar analysis methods.

In addition, I will advise P01 Projects 3 and 4 in year 1 on the development of a common REDCap (Research Electronic Data Capture) database for data entry and management. I will provide statistical consultation in project years 2-5 for analysis and reporting of the common dataset.

As you know, I am the director of the Biostatistics Resources core of the Southern California CTSI (of which Dr. Bill Mack is a KL2 scholar) as well as the Biostatistics and Data Management Core of the USC Alzheimer's Disease Research Center (of which Professor Finch is a Co-Director and Dr. Bill Mack is a pilot grant awardee). Your work is highly relevant to both of these institutes and biostatistics and data resources from these institutes will be available.

Your pilot data demonstrate a female and apoE4 bias in amyloid-beta load in response to air pollution exposure (project 3) and pilot data from Project 4 suggests a role for air pollution in white matter injury and the progression of acute ischemic stroke. I have worked with you in developing the analysis sections of the current grant proposal; the proposed research is very exciting. It incorporates a well-designed study plan to assess the influence of age and sex on the joint influence of particulate matter and underlying cerebrovascular disease. I look forward to working with you to address this topic, and heartily express my support for and commitment to collaborate with you on this research.

Sincerely,

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Resource Sharing Plan

Data Sharing Plan: Project data will be shared in accordance with NIH policy. Along with presentations and publications, processed data will be made public and distributed through supplementary materials or on our available website.

Sharing Model Organisms: not applicable.

Genome Wide Association Studies: not applicable.

All key resources for this proposal will be authenticated to enhance the reproducibility of our results, as appropriate and according to NIH policy. Key Biological Resources that will be utilized and validated in this proposal include:

Transgenic mouse strains: EFAD and inducible macrophage-microglia TLR4 knockout (i-mTLR4-ko) mice. EFAD (E3FAD and E4FAD) mice were generated by crossing 5XFAD to homozygous APOE3-, and APOE4-TR. 5xFAD mice are transgenic for 5 distinct FAD mutations (APP K670N/M671L+ I716V+ V717I and PS1 M146L+L286V). They are provided by University of Illinois at Chicago (Mary Jo LaDu, PhD). i-mTLR4-ko mice are generated by targeted deletion of the TLR4 gene from macrophages/ microglia. A transgenic mouse carrying an excision enzyme (Cre recombinase) in a specific cell-line (CX_3CR1 promoter, JAX020940) and a transgenic mouse expressing TLR4 flanked with two loxP sequences (JAX024872) are crossed. In order to achieve cell-specificity, Cre expression is controlled by the CX_3CR1 promoter and is inducible via linkage to an estrogen receptor mutant activated by tamoxifen. First generation (F1) litters are 100% heterozygous ($mCre^{+/-}$, $Fl^{+/-}$). F1 heterozygous mice are crossed with homozygous floxed mice ($Fl^{+/+}$). Second generation is composed of: 25% experimental animals ($mCre^{+/-}$, $fl^{+/+}$), 25% control ($mCre^{-/-}$, $fl^{+/+}$). Transgenic mice will be validated by genotyping.

Antibodies: Only commercially available antibodies will be used.

Air Pollution particles: Collected, characterized and validated by Core C2 (Prof C. Sioutas, Director). These nPM characteristics are reported in recent publications by members of this P01 (Liu et al 2016; Morgan et al 2011) and are used in other studies supported by our NIA grants to CE Finch (AG040753; AG040683; AG051521) and NIEHS grant to WJ Mack (ES024936).

Other resources used in this proposal will be standard laboratory reagents. Should we need to generate or obtain additional unique resources in the course of this proposal, they will be authenticated using methods similar to those described above, as appropriate.