

Urban Air Pollution Nanoparticles from Los Angeles: Recently Decreased Neurotoxicity

Hongqiao Zhang^a, Carla D'Agostino^a, Henry Jay Forman^a, Mafalda Cacciottolo^a, Max Thorwald^a, William J. Mack^c, Qinghai Liu^c, Kristina Shkirkova^c, Krista Lamorie-Foote^c, Constantinos Sioutas^b, Milad Pirhadi^b, Wendy Jean Mack^c, Todd E. Morgan^a and Caleb E. Finch^{a,d,*}

^aLeonard Davis School of Gerontology, University of Southern California, Los Angeles, CA, USA

^bViterbi School of Engineering, University of Southern California, Los Angeles, CA, USA

^cKeck School of Medicine, University of Southern California, Los Angeles, CA, USA

^dDepartment of Neurobiology, Dornsife College, University of Southern California, Los Angeles, CA, USA

Accepted 13 April 2021

Pre-press 5 May 2021

Abstract.

Background: Air pollution is widely associated with accelerated cognitive decline at later ages and risk of Alzheimer's disease (AD). Correspondingly, rodent models demonstrate the neurotoxicity of ambient air pollution and its components. Our studies with nano-sized particulate matter (nPM) from urban Los Angeles collected since 2009 have shown pro-amyloidogenic and pro-inflammatory responses. However, recent batches of nPM have diminished induction of the glutamate receptor GluA1 subunit, Iba1, TNF α , A β ₄₂ peptide, and white matter damage. The same methods, materials, and mouse genotypes were used throughout.

Objective: Expand the nPM batch comparisons and evaluate archived brain samples to identify the earliest change in nPM potency.

Methods: Batches of nPM were analyzed by *in vitro* cell assays for NF- κ B and Nrf2 induction for comparison with *in vivo* responses of mouse brain regions from mice exposed to these batches, analyzed by PCR and western blot.

Results: Five older nPM batches (2009–2017) and four recent nPM batches (2018, 2019) for NF- κ B and Nrf2 induction showed declines in nPM potency after 2017 that paralleled declines of *in vivo* activity from independent exposures in different years.

Conclusion: Transcription-based *in vitro* assays of nPM corresponded to the loss of *in vivo* potency for inflammatory and oxidative responses. These recent decreases of nPM neurotoxicity give a rationale for evaluating possible benefits to the risk of dementia and stroke in Los Angeles populations.

Keywords: Air pollution, Alzheimer's disease, microglia, mouse brain, ultrafine particulate matter

INTRODUCTION

Exposure to particulate matter (PM) in urban air pollution is associated with accelerated cognitive decline and increased risk of Alzheimer's disease (AD) in many populations [1–4]. Urban airborne

*Correspondence to: Caleb E. Finch, Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA 90089, USA. E-mail: cefinch@usc.edu.

PM is a mixture from diverse sources that can vary widely in chemical composition and size distribution. Rodent models for the neurotoxicity of urban air document neurite atrophy, glial activation, and oxidative damage in association with increased production of amyloid peptides, inflammatory responses, and Nrf2-mediated antioxidant response [2, 5–7]. Experimental studies have used PM from diverse sources: concentrated total ambient urban PM [8, 9], ambient air from a traffic tunnel [10], size-selected PM collected on filters [11–13], and dust-storm PM [14]. Air pollution components include exposure to diesel exhaust particles [5, 15, 16], zinc nanoPM [17], iron-coated silica nanoPM [18], carbon-nanotubes [19], and ozone [7, 20].

Protocols used by our laboratory since 2010 [21] expose rodents to nPM, a subfraction of ultrafine PM_{0.2} [1, 11, 21–35]. Urban ultrafine PM (PM_{0.2}) are continuously collected on filters from the same urban freeway corridor in Los Angeles. Filters are sonicated in water to yield an eluate we designated as nPM in distinction from total ultrafine PM [21]. The nPM subfraction of PM_{0.2} has equivalent oxidative activity to total PM_{0.2} assayed *in vitro* [36], despite its lower content of transition metals and the absence of polycyclic aromatic hydrocarbons [11]. Exposure of mice to nPM for 3–12 weeks can activate microglia in multiple brain regions [25, 28, 35]; induce pro-inflammatory cytokines in brain (TNF α and IL-1 β) [21, 25, 28]; decrease neuronal glutamate receptor subunit GluA1 in hippocampus and cortex [1, 21, 28]; and cause atrophy of hippocampal CA1 neurites [1, 28]. The nPM and other air pollution components are also pro-amyloidogenic in AD-transgenic mice [1, 7, 10] and in wild-type rodents: the A β ₄₂ peptide was increased by diesel exhaust in F344 rats [37] and by nPM in C57BL/6 mice [36]. The combination of nPM with chronic cerebral hypoperfusion from bilateral carotid artery stenosis (BCAS) caused increased white matter damage and activated microglia [27].

We recently characterized seven nPM batches collected from 2016 to 2018 for inflammatory cytokine responses and lipid peroxidation in cultured monocytes [36]. Several recent batches of nPM showed lower activity for NF- κ B induction and lipid peroxidation that corresponded to decreased *in vivo* microglial activation and amyloidogenic response [36]. Here, we extend these findings to a wider time range of nPM batches, 2009–2020. The nPM batches are compared for *in vivo* inflammatory responses, glutamate signaling, amyloid production, and white matter damage in mouse models. A new *in vitro* assay

was developed for simultaneously assessing the activation of Nrf2 and NF- κ B in monocytes, which are increased in lung by chronic exposure to air pollution [38].

METHODS AND MATERIALS

Reagents

RPMI 1640 cell culture media and TRIzol reagent were from Thermal Fisher (Rockford, IL). QUANTI-Blue was from InvivoGen (San Diego, CA); reverse transcription reagent and qPCR master mixture were from BioPioneer Inc (San Diego, CA); other chemicals, were from Sigma-Aldrich (St. Louis, MO). Antibodies of Iba1, MAG, and dMBP were from Abcam (Cambridge, MA). A β ₄₂ was assayed by ELISA Meso Scale Discovery (Rockville, MD).

Collection and preparation of nPM

All nPM batches were collected from Los Angeles city within 150 m downwind of the I-110 Freeway [39]. These aerosols represent a mix of fresh ambient PM mostly from vehicular traffic. PM_{0.2} was collected on Zeflour PTFE filters (Pall Life Sciences, Ann Arbor, MI) with a High-Volume Ultrafine Particle Sampler [39] at 400 L/min flow. Filter-deposited nPM was eluted by sonication into deionized sterile water and stored in –20°C. Table 1 shows the collection dates of nPM batches.

Animals and nPM exposure

Animal procedures were approved by the University of Southern California (USC) Institutional Animal Care and Use Committee (IACUC). Young adult C57BL/6J male and female mice were purchased from Jackson Laboratories. Mice were exposed to nPM on the same protocol used since 2010 [11]. For each exposure, mice were transferred from home cages into sealed exposure chambers with ample ventilation. The nPM group was exposed to re-aerosolized nPM at 300 μ g/m³, while controls received filtered air in parallel chambers. Exposure schedules were 5 h/d, 3 d/wk; the duration varied by experiment, 3 to 15 wk (Table 1). Mass concentration of the re-aerosolized nPM exposure stream was gravimetrically assessed on filters in parallel to the exposure stream before and after exposures. The nebulizer maintained a similar size distribution of re-aerosolized PM_{0.2}. In one study, mice were

Table 1
nPM Batch, Exposure paradigm, and Reference to publication or current data

Batch	Collection date	Genotype and Sex (C57BL/6 and transgenics)	Exposure duration	Reference
2009	Nov-Jan, 2008-2009	M	10 wk	[21], Fig. 2B
2010a	Mar, 2010	M	10 wk	[22, 40]
2010b	Mar-Apr, 2010	F	Prenatal 10 wk	[23, 24, 28, 29, 31]
2011	Mar-Jun, 2011	M (LDL ^{-/-})	10 wk	[41]
2012	Aug-Sep, 2012	M, M (ApoE-TR), F (EFAD)	1, 4, 9 d, 15 wk, 15 wk	[1, 25, 33]
2014	Oct-Feb, 2014-2015	M	20 wk, prenatal to adult 3 mo	[32]
2015a	Oct-Dec, 2015	M	3 wk	[27, 35]
2015b	Nov-Feb, 2015-2016	M (rat)	28 wk, prenatal to adult 5 mo	[30]
2016a	Feb-Apr, 2016	M	10	Table 2, Fig. 2B
2016b	Oct-Dec, 2016	M (J20 hAPPSwe)	10	[42]
1	Apr-June, 2016		<i>In vitro</i> only	[36], Fig. 1A, B
2	June-Aug, 2016	M	prenatal, or 8 wk	[34–36, 43], Fig. 2A, C, G, H
3	May-Sep, 2016		<i>In vitro</i> only	Fig. 1A, B
4	Nov-Jan, 2016-2017		<i>Caenorhabditis elegans</i>	[44]
5	May-Jun, 2017	M	3 wk	[11, 33]
6	Jan-Mar, 2018	M, F	8 wk	[33, 36]
7	May-Jul, 2018	F	8 wk	Fig. 2G
8	Feb-Mar, 2019	F	10 wk	Fig. 2, D, E, F
9	Sep-Nov, 2019	M, F	3 wk	Table 2, Fig. 2G, 2H
		F	10 wk	
10	Jan-Mar, 2020		<i>In vitro</i> only	Fig. 1A, B

C57BL/6 mice and designated transgenic strains, and Sprague-Dawley rats were exposed to nPM at 300 $\mu\text{g}/\text{m}^3$, 5 h/d, 3 d/wk for the stated number of weeks.

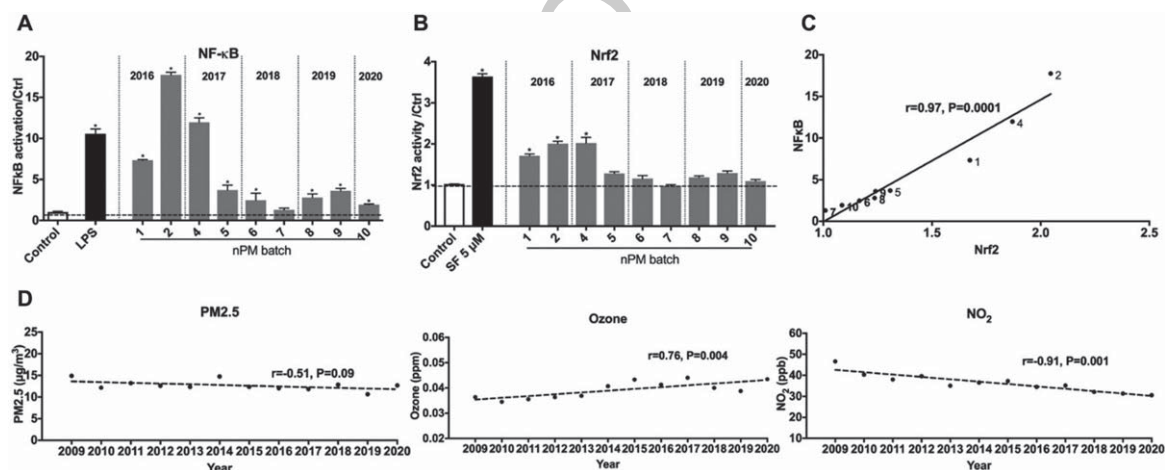


Fig. 1. *In vitro* biological activities of nPM batches. A) NF- κ B induction; LPS, lipopolysaccharide (5 ng/ml). B) Nrf2 induction; SF, sulforaphane (5 μM). * $p < 0.05$ versus control, four wells per assay plate. C) Co-plot of NF κ B and Nrf2; $R^2 = 0.94$, $p = 0.0001$; bolded numbers are batch ID since 2018 (Batch 6–10). The correlation of NF- κ B and Nrf2 was analyzed by two-tailed Pearson correlation analysis. D) Historical change of air pollutants. Levels of PM_{2.5}, ozone and NO₂ in central LA (5 miles from nPM collection site) from 2009 to 2020 showed linear trends: PM_{2.5}: $R^2 = 0.26$, $p = 0.09$; O₃: $R^2 = 0.58$, $p = 0.004$; NO₂: $R^2 = 0.83$, $p = 0.001$.

given bilateral carotid artery stenosis (BCAS) at 4 weeks before the final nPM exposure [45]. After euthanization by cardiac perfusion under anesthesia, brains were dissected, frozen on dry ice, and stored at -80°C .

mRNA

Cerebral cortex (alternate hemispheres) was extracted for RNA with TRIzol reagent, followed by qPCR with specific primers [11].

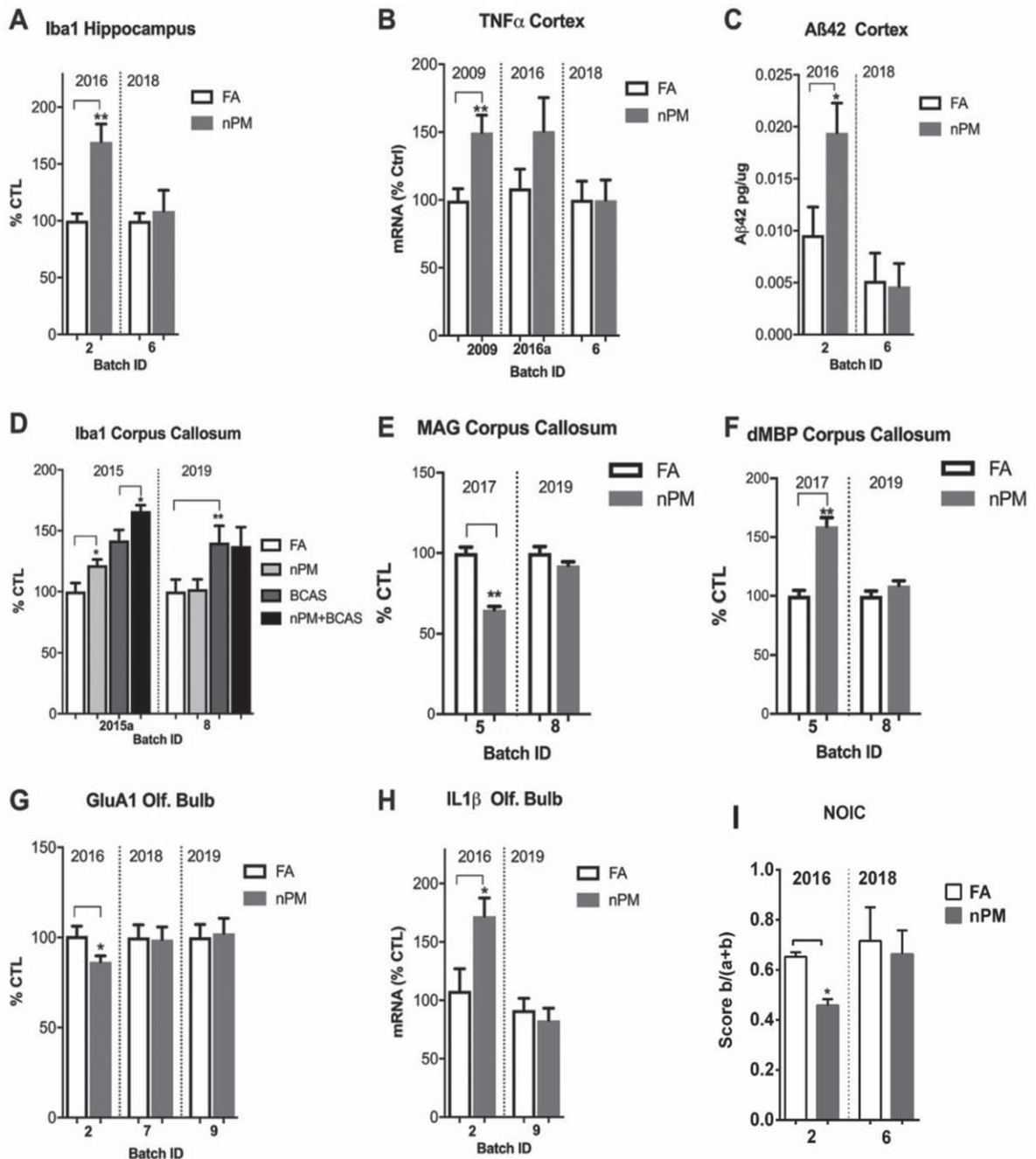


Fig. 2. *In vivo* brain responses to nPM by batch for 120 to 150 h (see Table 1 for exposure details and primary citations). A) Microglial Iba1 immunostaining in hippocampus; Batch 2 data are shown from our initial report [36]. B) TNF α mRNA in cerebral cortex; Batch 2009 [21]. C) A β ₄₂ peptide in cerebral cortex; Batch 2 data [36]. D) Microglial Iba1 immunostaining in corpus callosum; Batch 2015a [35]. E) MAG (myelin-associated glycoprotein) immunostaining in corpus callosum; Batch 5 data [Q. Liu and W.J. Mack, unpublished]. F) dMBP (debris of myelin basic protein) immunostaining in corpus callosum [Q. Liu and W.J. Mack, unpublished]. G) GluA1 in olfactory bulb; Batch 2 and Batch 9: mRNA by qPCR, Batch 7: protein by western blot. H) IL-1 β mRNA in olfactory bulb. Mean \pm SD, N = 7–10, * p < 0.05, ** p < 0.01. I) Novel Object in Context (NOIC) score for memory assessment. Batch comparisons were from exposures for 8 or 10 weeks conducted in different years. The standardized effect sizes differed across batch collection years (p = 0.001); effect sizes in 2018 and 2019 were smaller than prior years (p = 0.003).

Immunohistochemistry

Iba1 [45], myelin-associated glycoprotein (MAG), and debris of myelin basic protein (dMBP) [26] were immunostained by standard procedures in cited references.

NF- κ B and Nrf2 activity

To simultaneously assay both activities, we engineered THP-1 Blue NF- κ B reporter monocytes by transfection with a Nrf2 reporter. This THP-1 line expresses the reporter NF- κ B-SEAP (secreted embryonic alkaline phosphatase) regulated by 5-tandem I κ B cis-elements [36]. The NF- κ B reporter measures activity but cannot differentiate which NF- κ B family member causes activation. THP-1 NF- κ B/Nrf2 reporters were developed by transducing THP-1 Blue NF- κ B monocytes with ARE Luciferase Reporter Lentivirus (BPS Bioscience, San Diego, CA). The ARE Luciferase reporter is regulated by 3-tandem antioxidant-response elements. Cells were grown at density of 4×10^5 – 1×10^6 cells/ml under 5% CO₂/37°C in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and antibiotics (1% penicillin/streptomycin, 100 μ g/ml normocin, 10 μ g/ml blasticidin). Cells were treated with 5 μ g/ml nPM for 8 h; NF- κ B activity was assayed by QUANTI-Blue and Nrf2/luciferase activity by luminometry; both were calculated relative to vehicle (H₂O). This new cell line is available on request.

Behavioral testing

Novel Object in Context (NOIC) test was used to assess hippocampal dependent object and context recognition [46].

Statistical analysis

Data were analyzed by GraphPad Prism v.7 and Stata v.16.1 (College Station, TX). Batch group comparisons were analyzed by either Student t-test (for 2 groups) or one-way ANOVA (>2 groups). Pairwise comparisons of later batches to the first batch were corrected for multiple hypothesis testing using the False Discovery Rate of Benjamini, Krieger and Yekutieli [47]. For trend analysis, a time variable (in months since first sample batch) was calculated according to the nPM collection date, with Batch 1 represented as time 0. The date for each batch used the midpoint of the date range (Table 1); the month time

variable ranged from 0–47 months. Linear regressions evaluated NF- κ B and Nrf2 activity as dependent variables, and months as the independent variable. For effect size analysis of each data collection period in Fig. 2A-I, standardized treatment effect sizes (nPM versus control) were calculated as the mean difference between groups, divided by the pooled standard deviation. The standardized effect sizes were compared over collection year, using a mixed effects linear model specifying a fixed effect for collection year, and a random effect for study (Fig. 2A-I). Historical data 2009–2020 of PM_{2.5}, ozone and NO₂ was obtained from Chemical Speciation Network (CSN) provided by the US Environmental Protection Agency (US EPA) [48] for a site in Los Angeles city (1630 North Main St., Los Angeles, CA 90012), 5 miles from the nPM collection site (3400 South Hope St., Los Angeles, CA 90089). $p < 0.05$ was considered significant for all statistical tests.

RESULTS

In vitro activity

Inflammation and oxidative stress are two putative mechanisms of how nPM causes neurotoxicity. *In vitro* activity of nPM was assessed by its capacity to induce two key transcription factors: NF- κ B, the master transcriptional regulator of inflammatory response, and Nrf2, which controls the antioxidant response to oxidative stress [49]. The reporter genes were transfected into a monocyte cell line, as a model for respiratory tract monocyte/macrophages that directly receive inhaled PM [36]. Exposure of NF- κ B/Nrf2 reporter cells to nPM increased the activities of both NF- κ B and Nrf2, with dose-dependence over a 40-fold range (0.25–10 μ g/ml, data not shown).

The nPM batches differed in potency for inducing NF- κ B (Fig. 1A) and Nrf2 (Fig. 1B). Both transcriptional responses were strongly correlated ($r = 0.97$, Fig. 1C). The trend analysis showed a significant decrease in the induction of NF- κ B and Nrf2 by collection dates ($p < 0.01$ for both NF- κ B and Nrf2). For both NF- κ B and Nrf2, Batches 2 and 3 were significantly higher than Batch 1 ($p < 0.05$), and Batches 6–10 (2018–2019) were significantly lower than Batch 1. These data suggest that the potency of nPM to induce NF- κ B and Nrf2 varies by batch and has declined since 2017.

Trends of major air pollutants near the nPM collection site were linear during 2009–2020. PM_{2.5}

Table 2
Cerebral Cortex mRNA Response to nPM by Batch Number and Date

nPM Batch ID	Collection date	Gender	Exposure duration (wk)	GluA1	IL-1 β	NF κ B1	RelA/p65	MyD88	TLR4
2016a	Feb-Apr, 2016	M	10	0.69 \pm 0.14*	1.67 \pm 0.34*	0.71 \pm 0.05*	1.23 \pm 0.15	1.11 \pm 0.22	1.02 \pm 0.20
5	May-Jun, 2017	M	3	0.63 \pm 0.16*	0.95 \pm 0.20	0.74 \pm 0.26*	0.93 \pm 0.10	0.75 \pm 0.07*	0.80 \pm 0.12
6	Jan-Mar, 2018	M	8	0.92 \pm 0.25	1.26 \pm 0.73	1.01 \pm 0.42	1.32 \pm 0.43	0.97 \pm 0.21	1.33 \pm 0.56
6	Jan-Mar, 2018	F	10	0.81 \pm 0.40	1.15 \pm 0.72	0.80 \pm 0.26	0.97 \pm 0.18	0.90 \pm 0.16	0.95 \pm 0.20
7	Apr-Aug, 2018	F	10	1.00 \pm 0.08	1.12 \pm 0.07	0.97 \pm 0.10	0.95 \pm 0.12	1.06 \pm 0.15	0.95 \pm 0.12
8	Feb-Mar, 2019	F	10	1.21 \pm 0.29	0.96 \pm 0.37	0.81 \pm 0.09*	1.04 \pm 0.09	1.23 \pm 0.06	1.03 \pm 0.18
9	Sep-Nov, 2019	M	3	0.89 \pm 0.08	0.75 \pm 0.33	0.78 \pm 0.03*	0.91 \pm 0.09	0.82 \pm 0.13*	0.89 \pm 0.30
9	Sep-Nov, 2019	F	3	0.92 \pm 0.12	0.85 \pm 0.28	0.82 \pm 0.11*	0.94 \pm 0.14	0.95 \pm 0.10	1.13 \pm 0.23
9	Sep-Nov, 2019	F	10	0.85 \pm 0.27	0.94 \pm 0.27	0.85 \pm 0.12	0.97 \pm 0.16	0.99 \pm 0.18	1.04 \pm 0.26

Archived frozen cerebral cortex of experiments with batches from 2016–2019 was analyzed for mRNAs in the same PCR assay; numbers are fold- change (mean \pm SD) of nPM exposure versus controls (filtered air), * p < 0.05 versus control, N = 5–8.

declined weakly (p = 0.09), while the decline of NO₂ was significant (3.6%/y, p = 0.001). Opposing these declines was a significant increase of ozone (1.8%/y, p = 0.004).

In vivo responses

Five older nPM batches (2009–2017) and four recent nPM batches (2018, 2019) (Table 1) were compared from independent 8- or 10-week exposure experiments in different years (Fig. 2A-I). Each panel shows the statistically significant response of earlier nPM batches for comparison with null response to recent nPM. Microglial Iba1 (Fig. 2A) did not respond to Batch 6 (2018). TNF α mRNA (Fig. 2B) induction was robust for two nPM batches collected in 2009 and 2016 [21], contrasting with null response to Batch 6 (2018). Similarly, A β ₄₂ (Fig. 2C) responded to nPM with >70% increase for Batch 2 (2016) but did not respond to Batch 6 (2018) in an identical protocol 2 years later. In corpus callosum, white matter microglial Iba1 was increased by Batch 2015a (Fig. 2D). Partial ischemia from bilateral carotid artery stenosis (BCAS) plus nPM gave additive effects for Batch 2015a, but not for Batch 8 (2019). The increase of Iba1 from BCAS alone gives an internal control for microglial responsivity in this group of mice. Myelin damage in corpus callosum showed parallel batch differences. MAG was decreased by 35% by Batch 5 (2017) but was not changed by Batch 8 (2019); dMBP was increased by 60% by Batch 5 (2017) but did not respond to Batch 8. In olfactory bulb, the glutamate receptor subunit GluA1 was decreased and IL-1 β increased by Batch 2 (2016), but did not respond to Batch 7 (2018) and Batch 9 (2019). Moreover, Batch 2 (2016) caused memory loss assessed by Novel Object in Context

(NOIC) test, whereas Batch 6 (2018) did not impair memory. By a mixed effects model, the mean standardized effect size was smaller for nPM batches of 2018 and 2019 compared to 2009–2017 (p = 0.003). Overall, these observations indicate that recent nPM batches had less neurotoxicity.

mRNA responses in vivo

To extend the examples of Fig. 2, we examined mRNA responses of archived cerebral cortex from mice exposed to different nPM Batches (Table 2). NF κ B1, the p105/p50 member of NF- κ B family, gave the most consistent response to nPM, and was decreased by most batches (Table 2). IL-1 β was increased by Batch 2016a but not by others. MyD88, an adaptor protein in the TLR4-NF κ B pathway, was decreased by Batches 5 and 9 in males exposed for 3 weeks, whereas MyD88 did not respond to other batches. GluA1 mRNA was decreased in cortex by Batches 2016a and 5, confirming findings in hippocampus [1, 21, 28], whereas GluA1 mRNA did not respond to recent batches. The mRNAs of RelA/p65 (NF- κ B family), and TLR4 responded minimally to all nPM batches. Taken together, Table 2 findings parallel batch responses of Fig. 1 and Fig. 2, suggesting that nPM batches collected since 2018 have decreased potency. This comparison is limited by two factors: exposures varied from 3 to 10 weeks (Table 1) and some batches were not available because of depletion in prior studies.

DISCUSSION

Air pollution PM varies widely in bioactivity/toxicity by sites of collection and season, as shown

by the DTT assay and other biochemical and *in vitro* cell models [36, 50–55]. The present data extend these findings by comparing the *in vitro* Nrf2 and NF- κ B transcriptional responses with *in vivo* neurotoxic effects caused by batches of PM_{0.2} collected over a decade at the same site in urban Los Angeles. These findings show extensive variations in the biological potency of the nPM subfraction of ambient PM_{0.2}, assessed by the same methods, materials, and mouse genotype from the same sources. The new cell-based assay for induction of Nrf2 and NF- κ B may be useful to evaluate the potency of PM collected from ambient air in animal models of air pollution.

The *in vitro* potency of nPM to induce NF- κ B and Nrf2 signaling with a monocyte reporter declined in five batches collected after 2016 (Fig. 1). NF- κ B and Nrf2 were chosen for *in vitro* assays because these transcription factors are key mediators of inflammatory, detoxification, and anti-oxidative responses. The transcriptional gene reporter for NF- κ B does not identify the relative contribution of its five subunits (RelA/p65, NF κ B1/p50, NF κ B2/p52, RelB and RelC) that act by forming hetero- or homo-dimers [54–56], which could include a decrease of the NF κ B1 mRNA as observed *in vivo*.

The *in vivo* responses of cerebral cortex were expanded to four recent batches collected after 2017 (Batches 6,7,8,9) (Fig. 2, Table 2). Batches 6–9 did not increase TNF α , microglial Iba1, A β ₄₂, and dMPB (myelin degeneration), or decrease GluA1 (glutamate receptor subunit), unlike earlier batches. The decreasing neurotoxicity of recent batches since 2016 was consistently observed in four brain regions: cerebral cortex, corpus callosum, hippocampus, and olfactory bulb. These observations further document the declining *in vitro* potency of recent batches and confirm our initial findings that nPM batches vary in the potential to cause inflammatory response and neurotoxic effects [36].

In contrast, some other cerebral cortex mRNA responses were maintained in recent batches, e.g., NF κ B1 mRNA was still decreased by Batches 8 and 9 (Table 2). Batch 5 (2017) and Batch 9 (2019) showed respectively higher and lower potency *in vitro*, nonetheless both decreased MyD88 mRNA in cortex. Moreover, a separate transcriptomic analysis of male C57BL/6 mice showed Batch 6 induced extensive responses of Nrf2 and NF- κ B mediated pathways [33]. A hierarchy of atherogenic response to air pollutants was described by Araujo and Nel [56], in which antioxidant defenses responded to lower activity of PM, followed by inflammation and

cytotoxicity at higher PM activity. The nPM batches that did not induce brain inflammatory cytokines (Fig. 2, Table 2), but still altered Nrf2 and NF- κ B [33] may be consistent with this framework. We are planning to compare Batch 6 with Batch 2 for transcriptomic responses of cerebral cortex.

Unexpectedly, the early 2012 Batch did not alter the A β ₄₂ in cerebral cortex of ApoE-TR cerebral cortex, but still decreased mRNA for amyloid production (APP, Psen1) and A β ₄₂ peptide clearance (C3, Vav3) [33]. Note that these data for transgenic mice cannot be compared with the wildtype B6 which was examined in a different study with another nPM batch (Fig. 2C). Current data do not resolve the cause of inconsistent effects of nPM on the brain and whether they are attributable to exposure duration, sex, or difference in PM_{0.2} composition. Nor do epidemiological associations of air pollution components with dementia risks [1–4] show how ubiquitous inflammatory processes may cause brain aging to go from bad to worse.

The cognitive impact of air pollution must consider multiple interacting factors and neurotoxic pathways [57] including and beyond the glial and neuronal responses being studied in this growing field. The decreased impact of nPM on glial and neuronal responses gives a rationale for examining Los Angeles elderly populations for possible benefits to cognition. The declining activity of nPM per μ g by our measures since 2016 does not parallel the modest declines of PM_{2.5} and NO₂ and is opposite to the stronger trend for increased ozone (Fig. 1D). However, the mass of the nPM fraction is less than 25% of the total PM_{2.5} at the nPM collection site [58]. We must also consider the concurrent increase of ozone 2009–2020 (Fig. 1D) [20, 59], which was specifically associated with impaired executive function of Los Angeles elderly [60].

The nPM subfraction of urban air pollution is a highly complex mixture, in which altered batch differences of trace components could alter *in vivo* potency. Batches 1–7 varied widely in composition without obvious historical trends for copper, iron, or other transition metals, or toxic organic species [36]. Hierarchical clustering by nPM batch showed weak associations of endotoxin with NF- κ B activation *in vitro* but did not identify correlations of any chemical element with *in vitro* activities [36]. A more comprehensive analysis of nPM components, including the chemical status of transition metals on particle surface, the microbial components and other factors, may explain the cause of the unequal toxicity of nPM.

We must also consider the chemical state of transition elements. For example, Los Angeles PM_{2.5} contain multiple species of iron in combination with carbonaceous material ranging from crystalline iron to several types of ferrihydrites [61]. These different iron species arise from engine combustion and from abrasion of brake linings. Additional redox active meals arise from industrial and natural sources [62, 63]. We suggest a broader discussion of urban air pollution components in experimental models that will consider particle surface chemistry as well as composition. A systematic approach could identify broadly shared anthropogenic and natural components that might serve as a standard in comparing experimental findings from the expanding research on air pollution neurotoxicity.

In conclusion, we show that nPM batches from Los Angeles have recently decreased potency for neurotoxic responses relevant to neurodegeneration and AD risk in the same mouse model. The declines in ambient PM_{2.5} and NO₂ of Los Angeles air pollution 2009–2020 (Fig. 1D) in combination with decreased neurotoxicity give a rationale for examining possible parallel changes in human brain health. A decline in nPM potency could impact the hospital admission rates or discharge outcomes for patients presenting with stroke or cerebral hypoperfusion in the Los Angeles basin.

Limitations and strengths

These post hoc comparisons of nPM by batch (Table 2) were limited by availability of archived nPM and cerebral cortex tissues. The *in vivo* comparisons in Fig. 2 were from independent experiments at different times. Cognitive deficits from earlier nPM batches were not evaluated for the recent batches. Caveats recognized, the data shows progressively declining potency of nPM during the past decade of sampling in urban Los Angeles.

ACKNOWLEDGMENTS

We are grateful for support by grants from the Cure Alzheimer's Fund (CEF) and the NIH (CEF, R01-AG051521, P50-AG005142, P01-AG055367; MT, T32-AG000037, EM Crimmins; and William J. Mack, R01-ES024936), and Assistant Secretary of Defense for Health Affairs, Parkinson's Research Program Award W81XWH-17-1-0535, CEF).

Author's disclosures available online (<https://www.j-alz.com/manuscript-disclosures/20-1577r2>).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <https://dx.doi.org/10.3233/JAD-201577>.

REFERENCES

- [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 (2017) Particulate air pollutants, APOE alleles and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. *Transl Psychiatry* 7, e1022.
- [2] Calderon-Garciduenas L, Reynoso-Robles R, Gonzalez-Maciel A (2019) Combustion and friction-derived nanoparticles and industrial-sourced nanoparticles: The culprit of Alzheimer and Parkinson's diseases. *Environ Res* 176, 108574.
- [3] Kulick ER, Elkind MSV, Boehme AK, Joyce NR, Schupf N, Kaufman JD, Mayeux R, Manly JJ, Wellenius GA (2020) Long-term exposure to ambient air pollution, APOE-epsilon4 status, and cognitive decline in a cohort of older adults in northern Manhattan. *Environ Int* 136, 105440.
- [4] McGlade J, Landrigan PJ (2019) Five national academies call for global compact on air pollution and health. *Lancet* 394, 23.
- [5] Costa LG, Cole TB, Dao K, Chang YC, Coburn J, Garrick JM (2020) Effects of air pollution on the nervous system and its possible role in neurodevelopmental and neurodegenerative disorders. *Pharmacol Ther* 210, 107523.
- [6] Haghani A, Morgan TE, Forman HJ, Finch CE (2020) Air pollution neurotoxicity in the adult brain: Emerging concepts from experimental findings. *J Alzheimers Dis* 76, 773-797.
- [7] Mumaw CL, Levesque S, McGraw C, Robertson S, Lucas S, Stafflinger JE, Campen MJ, Hall P, Norenberg JP, Anderson T, Lund AK, McDonald JD, Ottens AK, Block ML (2016) Microglial priming through the lung-brain axis: The role of air pollution-induced circulating factors. *FASEB J* 30, 1880-1891.
- [8] Cory-Slechta DA, Allen JL, Conrad K, Marvin E, Sobolewski M (2018) Developmental exposure to low level ambient ultrafine particle air pollution and cognitive dysfunction. *Neurotoxicology* 69, 217-231.
- [9] Sirivelu MP, MohanKumar SM, Wagner JG, Harkema JR, MohanKumar PS (2006) Activation of the stress axis and neurochemical alterations in specific brain areas by concentrated ambient particle exposure with concomitant allergic airway disease. *Environ Health Perspect* 114, 870-874.
- [10] Patten KT, Gonzalez EA, Valenzuela A, Berg E, Wallis C, Garbow JR, Silverman JL, Bein KJ, Wexler AS, Lein PJ (2020) Effects of early life exposure to traffic-related air pollution on brain development in juvenile Sprague-Dawley rats. *Transl Psychiatry* 10, 166.
- [11] Haghani A, Johnson R, Safi N, Zhang H, Thorwald M, Mousavi A, Woodward NC, Shirmohammadi F, Coussa V, Wise JP, Jr., Forman HJ, Sioutas C, Allayee H, Morgan TE, Finch CE (2020) Toxicity of urban air pollution particulate matter in developing and adult mouse brain: Comparison of total and filter-eluted nanoparticles. *Environ Int* 136, 105510.

- [12] Thomson EM, Kumarathanan P, Calderon-Garciduenas L, Vincent R (2007) Air pollution alters brain and pituitary endothelin-1 and inducible nitric oxide synthase gene expression. *Environ Res* **105**, 224-233.
- [13] Farina F, Sancini G, Battaglia C, Tinaglia V, Mantecca P, Camatini M, Palestini P (2013) Milano summer particulate matter (PM10) triggers lung inflammation and extra pulmonary adverse events in mice. *PLoS One* **8**, e56636.
- [14] Hajipour S, Farbood Y, Gharib-Naseri MK, Goudarzi G, Rashno M, Maleki H, Bakhtiari N, Nesari A, Khoshnam SE, Dianat M, Sarkaki B, Sarkaki A (2020) Exposure to ambient dusty particulate matter impairs spatial memory and hippocampal LTP by increasing brain inflammation and oxidative stress in rats. *Life Sci* **242**, 117210.
- [15] Coburn JL, Cole TB, Dao KT, Costa LG (2018) Acute exposure to diesel exhaust impairs adult neurogenesis in mice: Prominence in males and protective effect of pioglitazone. *Arch Toxicol* **92**, 1815-1829.
- [16] Ehsanifar M, Tameh AA, Farzadkia M, Kalantari RR, Zavareh MS, Nikzaad H, Jafari AJ (2019) Exposure to nanoscale diesel exhaust particles: Oxidative stress, neuroinflammation, anxiety and depression on adult male mice. *Ecotoxicol Environ Saf* **168**, 338-347.
- [17] Kim SH, Knight EM, Saunders EL, Cuevas AK, Popovech M, Chen LC, Gandy S (2012) Rapid doubling of Alzheimer's amyloid-beta40 and 42 levels in brains of mice exposed to a nickel nanoparticle model of air pollution. *FI000Res* **1**, 70.
- [18] Zhang H, Zhou L, Yuen J, Birkner N, Leppert V, O'Day PA, Forman HJ (2017) Delayed Nrf2-regulated antioxidant gene induction in response to silica nanoparticles. *Free Radic Biol Med* **108**, 311-319.
- [19] Aragon MJ, Topper L, Tyler CR, Sanchez B, Zychowski K, Young T, Herbert G, Hall P, Erdely A, Eye T, Bishop L, Saunders SA, Muldoon PP, Ottens AK, Campen MJ (2017) Serum-borne bioactivity caused by pulmonary multiwalled carbon nanotubes induces neuroinflammation via blood-brain barrier impairment. *Proc Natl Acad Sci U S A* **114**, E1968-E1976.
- [20] Jiang C, Stewart LT, Kuo HC, McGilberry W, Wall SB, Liang B, van Groen T, Bailey SM, Kim YI, Tiplle TE, Jones DP, McMahon LL, Liu RM (2019) Cyclic O3 exposure synergizes with aging leading to memory impairment in male APOE epsilon3, but not APOE epsilon4, targeted replacement mice. *Neurobiol Aging* **81**, 9-21.
- [21] 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 (2011) Glutamatergic neurons in rodent models respond to nanoscale particulate urban air pollutants *in vivo* and *in vitro*. *Environ Health Perspect* **119**, 1003-1009.
- [22] Zhang H, Liu H, Davies KJ, Sioutas C, Finch CE, Morgan TE, Forman HJ (2012) 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.
- [23] Davis DA, Bortolato M, Godar SC, Sander TK, Iwata N, Pakbin P, Shih JC, Berhane K, McConnell R, Sioutas C, Finch CE, Morgan TE (2013) Prenatal exposure to urban air nanoparticles in mice causes altered neuronal differentiation and depression-like responses. *PLoS One* **8**, e64128.
- [24] Davis DA, Akopian G, Walsh JP, Sioutas C, Morgan TE, Finch CE (2013) Urban air pollutants reduce synaptic function of CA1 neurons via an NMDA/NO pathway *in vitro*. *J Neurochem* **127**, 509-519.
- [25] Cheng H, Saffari A, Sioutas C, Forman HJ, Morgan TE, Finch CE (2016) Nanoscale particulate matter from urban traffic rapidly induces oxidative stress and inflammation in olfactory epithelium with concomitant effects on brain. *Environ Health Perspect* **124**, 1537-1546.
- [26] Cheng H, Davis DA, Hasheminassab S, Sioutas C, Morgan TE, Finch CE (2016) Urban traffic-derived nanoparticulate matter reduces neurite outgrowth via TNFalpha *in vitro*. *J Neuroinflammation* **13**, 19.
- [27] 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 (2016) Stroke damage is exacerbated by nano-size particulate matter in a mouse model. *PLoS One* **11**, e0153376.
- [28] Woodward NC, Pakbin P, Saffari A, Shirmohammadi F, Haghani A, Sioutas C, Cacciottolo M, Morgan TE, Finch CE (2017) Traffic-related air pollution impact on mouse brain accelerates myelin and neuritic aging changes with specificity for CA1 neurons. *Neurobiol Aging* **53**, 48-58.
- [29] Woodward NC, Levine MC, Haghani A, Shirmohammadi F, Saffari A, Sioutas C, Morgan TE, Finch CE (2017) Toll-like receptor 4 in glial inflammatory responses to air pollution *in vitro* and *in vivo*. *J Neuroinflammation* **14**, 84.
- [30] Woodward NC, Haghani A, Johnson RG, Hsu TM, Saffari A, Sioutas C, Kanoski SE, Finch CE, Morgan TE (2018) Prenatal and early life exposure to air pollution induced hippocampal vascular leakage and impaired neurogenesis in association with behavioral deficits. *Transl Psychiatry* **8**, 261.
- [31] Pomatto LCD, Cline M, Woodward N, Pakbin P, Sioutas C, Morgan TE, Finch CE, Forman HJ, Davies KJA (2018) Aging attenuates redox adaptive homeostasis and proteostasis in female mice exposed to traffic-derived nanoparticles ('vehicular smog'). *Free Radic Biol Med* **121**, 86-97.
- [32] Woodward NC, Crow AL, Zhang Y, Epstein S, Hartiala J, Johnson R, Kocalis H, Saffari A, Sankaranarayanan I, Akbari O, Ramanathan G, Araujo JA, Finch CE, Bouret SG, Sioutas C, Morgan TE, Allayee H (2019) Exposure to nanoscale particulate matter from gestation to adulthood impairs metabolic homeostasis in mice. *Sci Rep* **9**, 1816.
- [33] Haghani A, Cacciottolo M, Doty KR, D'Agostino C, Thorwald M, Safi N, Levine ME, Sioutas C, Town TC, Forman HJ, Zhang H, Morgan TE, Finch CE (2020) Mouse brain transcriptome responses to inhaled nanoparticulate matter differed by sex and APOE in Nrf2-Nfkb interactions. *Elife* **9**, e54822.
- [34] Haghani A, Johnson RG, Woodward NC, Feinberg JI, Lewis K, Ladd-Acosta C, Safi N, Jaffe AE, Sioutas C, Allayee H, Campbell DB, Volk HE, Finch CE, Morgan TE (2020) Adult mouse hippocampal transcriptome changes associated with long-term behavioral and metabolic effects of gestational air pollution toxicity. *Transl Psychiatry* **10**, 218.
- [35] Babadjouni R, Patel A, Liu Q, Shkirkova K, Lamorie-Foote K, Connor M, Hodis DM, Cheng H, Sioutas C, Morgan TE, Finch CE, Mack WJ (2018) Nanoparticulate matter exposure results in neuroinflammatory changes in the corpus callosum. *PLoS One* **13**, e0206934.
- [36] Zhang H, Haghani A, Mousavi AH, Cacciottolo M, D'Agostino C, Safi N, Sowlat MH, Sioutas C, Morgan TE, Finch CE, Forman HJ (2019) Cell-based assays that predict *in vivo* neurotoxicity of urban ambient nano-sized particulate matter. *Free Radic Biol Med* **145**, 33-41.
- [37] Levesque S, Surace MJ, McDonald J, Block ML (2011) Air pollution & the brain: Subchronic diesel exhaust exposure causes neuroinflammation and elevates early

- markers of neurodegenerative disease. *J Neuroinflammation* **8**, 105.
- [38] Gangwar RS, Vinayachandran V, Rengasamy P, Chan R, Park B, Diamond-Zaluski R, Cara EA, Cha A, Das L, Asase C, Maiseyeu A, Deilulis J, Zhong J, Mitzner W, Biswal S, Rajagopalan S (2020) Differential contribution of bone marrow-derived infiltrating monocytes and resident macrophages to persistent lung inflammation in chronic air pollution exposure. *Sci Rep* **10**, 14348.
- [39] Misra C, Kim S, Shen S, Sioutas C (2002) A high flow rate, very low pressure drop impactor for inertial separation of ultrafine from accumulation mode particles. *J Aerosol Sci* **33**, 735-752.
- [40] Chepelev NL, Zhang H, Liu H, McBride S, Seal AJ, Morgan TE, Finch CE, Willmore WG, Davies KJ, Forman HJ (2013) Competition of nuclear factor-erythroid 2 factors related transcription factor isoforms, Nrf1 and Nrf2, in antioxidant enzyme induction. *Redox Biol* **1**, 183-189.
- [41] Li R, Navab M, Pakbin P, Ning Z, Navab K, Hough G, Morgan TE, Finch CE, Araujo JA, Fogelman AM, Sioutas C, Hsiai T (2013) Ambient ultrafine particles alter lipid metabolism and HDL anti-oxidant capacity in LDLR-null mice. *J Lipid Res* **54**, 1608-1615.
- [42] Cacciottolo M, Morgan TE, Saffari AA, Shirmohammadi F, Forman HJ, Sioutas C, Finch CE (2020) Traffic-related air pollutants (TRAP-PM) promote neuronal amyloidogenesis through oxidative damage to lipid rafts. *Free Radic Biol Med* **147**, 242-251.
- [43] Breton CV, Song AY, Xiao J, Kim SJ, Mehta HH, Wan J, Yen K, Sioutas C, Lurmann F, Xue S, Morgan TE, Zhang J, Cohen P (2019) Effects of air pollution on mitochondrial function, mitochondrial DNA methylation, and mitochondrial peptide expression. *Mitochondrion* **46**, 22-29.
- [44] Haghani A, Dalton HM, Safi N, Shirmohammadi F, Sioutas C, Morgan TE, Finch CE, Curran SP (2019) Air pollution alters caenorhabditis elegans development and lifespan: Responses to traffic-related nanoparticulate matter. *J Gerontol A Biol Sci Med Sci* **74**, 1189-1197.
- [45] Liu Q, He S, Groysman L, Shaked D, Russin J, Scotton TC, Cen S, Mack WJ (2013) White matter injury due to experimental chronic cerebral hypoperfusion is associated with C5 deposition. *PLoS One* **8**, e84802.
- [46] Balderas I, Rodriguez-Ortiz CJ, Salgado-Tonda P, Chavez-Hurtado J, McGaugh JL, Bermudez-Rattoni F (2008) The consolidation of object and context recognition memory involve different regions of the temporal lobe. *Learn Mem* **15**, 618-624.
- [47] Benjamini Y, Krieger AM, Yekutieli D (2006) Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* **93**, 17.
- [48] Altuwayjiri A, Pirhadi M, Taghvae S, Sioutas C (2020) Long-term trends in the contribution of PM2.5 sources to organic carbon (OC) in the Los Angeles basin and the effect of PM emission regulations. *Faraday Discuss* **226**, 74-79.
- [49] Zhang H, Davies KJA, Forman HJ (2015) Oxidative stress response and Nrf2 signaling in aging. *Free Radic Biol Med* **88**, 314-336.
- [50] Xia T, Kovochich M, Brant J, Hotze M, Sempf J, Oberley T, Sioutas C, Yeh JI, Wiesner MR, Nel AE (2006) Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Lett* **6**, 1794-1807.
- [51] Vidrio E, Phuah CH, Dillner AM, Anastasio C (2009) Generation of hydroxyl radicals from ambient fine particles in a surrogate lung fluid solution. *Environ Sci Technol* **43**, 922-927.
- [52] Saffari A, Daher N, Shafer MM, Schauer JJ, Sioutas C (2014) Global perspective on the oxidative potential of airborne particulate matter: A synthesis of research findings. *Environ Sci Technol* **48**, 7576-7583.
- [53] Guastadisegni C, Kelly FJ, Cassee FR, Gerlofs-Nijland ME, Janssen NA, Pozzi R, Brunekreef B, Sandstrom T, Mudway I (2010) Determinants of the proinflammatory action of ambient particulate matter in immortalized murine macrophages. *Environ Health Perspect* **118**, 1728-1734.
- [54] Sun X, Wei H, Young DE, Bein KJ, Smiley-Jewell SM, Zhang Q, Fulgar CCB, Castaneda AR, Pham AK, Li W, Pinkerton KE (2017) Differential pulmonary effects of wintertime California and China particulate matter in healthy young mice. *Toxicol Lett* **278**, 1-8.
- [55] Saffari A, Daher N, Shafer MM, Schauer JJ, Sioutas C (2013) Seasonal and spatial variation of trace elements and metals in quasi-ultrafine (PM0.25) particles in the Los Angeles metropolitan area and characterization of their sources. *Environ Pollut* **181**, 14-23.
- [56] Araujo JA, Nel AE (2009) Particulate matter and atherosclerosis: Role of particle size, composition and oxidative stress. *Part Fibre Toxicol* **6**, 24.
- [57] Allen JL, Klocke C, Morris-Schaffer K, Conrad K, Sobolewski M, Cory-Slechta DA (2017) Cognitive effects of air pollution exposures and potential mechanistic underpinnings. *Curr Environ Health Rep* **4**, 180-191.
- [58] Shirmohammadi F, Hasheminassab S, Saffari A, Schauer JJ, Delfino RJ, Sioutas C (2016) Fine and ultrafine particulate organic carbon in the Los Angeles basin: Trends in sources and composition. *Sci Total Environ* **541**, 1083-1096.
- [59] Rivas-Arancibia S, Guevara-Guzman R, Lopez-Vidal Y, Rodriguez-Martinez E, Zanardo-Gomes M, Angoa-Perez M, Raisman-Vozari R (2010) Oxidative stress caused by ozone exposure induces loss of brain repair in the hippocampus of adult rats. *Toxicol Sci* **113**, 187-197.
- [60] Gatto NM, Henderson VW, Hodis HN, St John JA, Lurmann F, Chen JC, Mack WJ (2014) Components of air pollution and cognitive function in middle-aged and older adults in Los Angeles. *Neurotoxicology* **40**, 1-7.
- [61] Pattammattel A, Leppert VJ, Aronstein P, Robinson M, Mousavi A, Sioutas C, Forman HJ, O'Day PA (2021) Iron speciation in particulate matter (PM2.5) from urban Los Angeles using spectro-microscopy methods. *Atmos Environ* **245**, 14.
- [62] Marris H, Deboudt K, Flament P, Grobety B, Giere R (2013) Fe and Mn oxidation states by TEM-EELS in fine-particle emissions from a Fe-Mn alloy making plant. *Environ Sci Technol* **47**, 10832-10840.
- [63] Salazar JR, Pfothhauer DJ, Leresche F, Rosario-Ortiz FL, Hannigan MP, Fakra SC, Majestic BJ (2020) Iron speciation in PM2.5 from urban, agriculture, and mixed environments. *Earth Space Sci* **7**, 10.