Journal of Alzheimer's Disease xx (20xx) x–xx
DOI 10.3233/JAD-200377
IOS Press

Review

Air Pollution Neurotoxicity in the Adult Brain: Emerging Concepts from Experimental Findings

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Accepted 13 May 2020

Abstract. Epidemiological studies are associating elevated exposure to air pollution with increased risk of Alzheimer's disease and other neurodegenerative disorders. In effect, air pollution accelerates many aging conditions that promote cognitive declines of aging. The underlying mechanisms and scale of effects remain largely unknown due to its chemical and physical complexity. Moreover, individual responses to air pollution are shaped by an intricate interface of pollutant mixture with the biological features of the exposed individual such as age, sex, genetic background, underlying diseases, and nutrition, but also other environmental factors including exposure to cigarette smoke. Resolving this complex manifold requires more detailed environmental and lifestyle data on diverse populations, and a systematic experimental approach. Our review aims to summarize the modest existing literature on experimental studies on air pollution neurotoxicity for adult rodents and identify key gaps and emerging challenges as we go forward. It is timely for experimental biologists to critically understand prior findings and develop innovative approaches to this urgent global problem. We hope to increase recognition of the importance of air pollution on brain aging by our colleagues in the neurosciences and in biomedical gerontology, and to support the immediate translation of the findings into public health guidelines for the regulation of remedial environmental factors that accelerate aging processes.

Keywords: Air pollution, Alzheimer's disease, rodent models, O_3, particulate matter

BACKGROUND

Air pollution is considered among the leading global risk factors of mortality and morbidity throughout the human lifespan [1, 2] (Table 1). Air pollution is a markedly variable mixture combining gases (e.g., O_3) and suspended particulate matter (PM). Even at a single location, the composition must vary with diurnal cycles of temperature and ultraviolet, as well as from ingression of gases and PM from other sources. Despite this intrinsic variability in composition, air pollution has proven to be a neurotoxicant and teratogen in global populations. In many countries, air pollution is strongly associated with increased risk of several neurodevelopmental and neurodegenerative diseases including autism spectrum disorders [3, 4], accelerated cognitive aging and Alzheimer's disease (AD) [5–7] and Parkinson's disease [5, 8]. Unfortunately, our limited understanding of air pollution neurotoxicity has grossly underestimated the global burden of air pollution on neurological disorders. For example, most

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Table 1
Mortality and morbidity of airborne toxicants

	Annual excess mortality (millions)	Life expectancy loss (years)	Disability-adjusted life-years (millions)
Air pollution	6.5 [1]–9 [111]	1.8 [112]	103.1 [113]
PM2.5	3–4.2 [113, 114]		
O_3	0.5 [114]		
Household	1.6 [114]–3.8 [115]		
Cigarette smoke	8 [116]		148.6 [117]
Secondhand	0.65 [117]		
Influenza associated respiratory death	0.2–0.52 [118]		

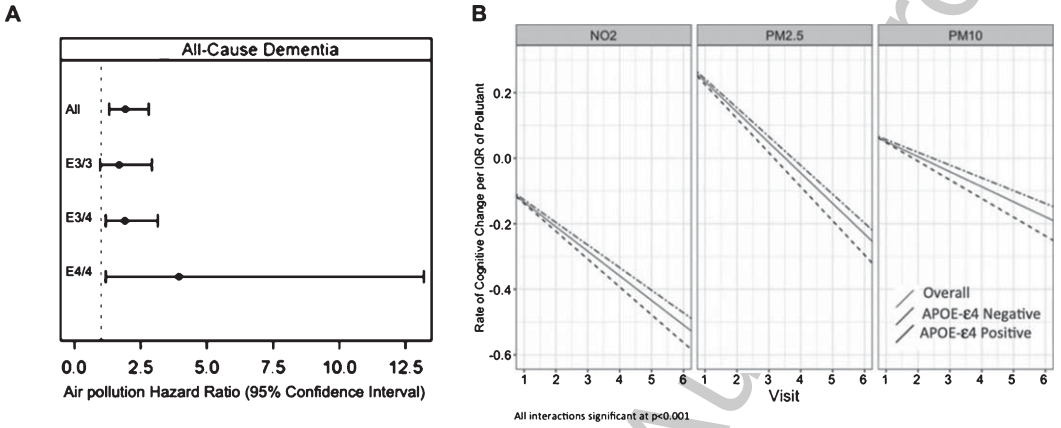

Fig. 1. Air pollution increase the risk of dementia and accelerate cognitive aging particularly in *APOE4* carriers. A) Hazard ratio of air pollution for dementia in Women Health Initiative Study [31]. B) Air pollution mediated cognitive decline in The Washington Heights Inwood Community Aging Project [6]. Figures adapted from [31] and [6].

studies rely on incomplete air pollution data with limited longitudinal observations. However, air pollution exposure starts in our grandmother's uterus, where oocytes form in our maternal ovaries before her birth [9]. Thus, the full life history of environmental neurotoxicity must consider at least two generations before birth and continue until the last day of the lifespan. This gap of understanding may be approached by a multidisciplinary approach to the life history of exposures, combining experimental biology with follow-up validation in human population data. Gradually, the findings should be translated into specific regulations to control air pollution hazards in future generations.

The first step in designing the experiments is to properly define the air pollution environment and interacting variables. Air pollution toxicity is shaped by an interface of the chemical composition of air pollution mixture with biological features of the exposed individuals. The chemical and physical characteristics of air pollution components are highly dependent on the source, location, humidity, and weather. In addition to this complex composition, air pollution effects could have individual variations dependent on sex, age, genetic structure, and even interactions with other environmental factors such as nutrition and cigarette smoke (Fig. 1). Thus, these complex relationships should be studied in a series of systemic experiments.

Current estimates of air pollution toxicity are based on individual components such as PM or O_3, with little consideration of potential interactions. Even in PM toxicity, the causative toxicants are still obscure. PM has a wide range of size distribution and carries numerous toxic chemicals including transition metals, organic compounds, polycyclic aromatic hydrocarbons, and microbial endotoxins. It is still unclear which of these components contributes to neurotoxicity and how combining these components alters the toxic effects. Air pollution and cigarette smoke interact synergistically approaching 2-fold for cognitive decline (Table 2).

The current body of literature suggests O_3 and PM, separately or together, can cause oxidative

Table 2
Air pollution and cigarette smoke interacts in their toxicity

Interaction of air pollution and cigarette smoke	Study	Synergy (fold-excess above additivity)
Neurodegeneration cognitive decline	Meta-analysis of 12 studies (109,838 mother-child pairs) [119]	1.6
	Health and Retirement Survey, 2004 : 18575,>50 y [120]	1.9
Cardiovascular mortality	ACS Prevention Study II: 429406 adults [121]	1.1
Lung cancer	ACS Prevention Study II: 1.2 million adults [92]	2.2
Body mass index	Southern California Children's Health Study: 3318 children, 10–18 y [122]	1.3

Note: Table adapted from [123].

damage, neuroinflammation, neuropathology, and affect cognition and behavior (Table 3). However, the underlying mechanisms are still obscure, which frustrates identification of specific targets for intervention. The known targets include NF-κB activation [10, 11], but we do not know the details of how they are activated in the brain. Moreover, the interaction of air pollution with other risk factors of neurodegenerative diseases such as sex, genetic variations, and age is still obscure. This review aims to summarize the current experimental findings of air pollution neurotoxicity in the adult brain and identify critical gaps of knowledge that require urgent attention in future studies.

DIVERSE EXPERIMENTAL MODELS FOR AIR POLLUTION EXPOSURE

Before summarizing the findings on air pollution neurotoxicity in adult brains, we describe the experimental limitations that could confound the conclusions. Studying air pollution neurotoxicity in adult rodents began only two decades ago, yielding about 50 publications to which we and our collaborators have contributed about one-third. In contrast, the epidemiology literature includes 140 PubMed entries. Notably, few experimentalists have focused on this aspect of environmental neurotoxicity. These relatively few studies indicate the early stage of air pollution neurotoxicity and the urgent need to expand research on this global dilemma. We need to find greater consensus in experimental exposure models and methods. The variety of air pollution exposures (ambient traffic air to diesel exhaust particles to pure ozone) frustrates comparisons of findings in the current body of literature. Tobacco toxicity research in contrast was greatly facilitated in 1969 by adoption of a standard cigarette [12]. We briefly summarize the common experimental methods and limitations that require urgent attention.

Air pollution delivery to rodent models is done by different methods such as inhalation, intranasal instillation, intra-tracheal instillation, oropharyngeal, or intraperitoneal (IP) injection. Since there are few direct comparisons of these methods, it is still unclear how air pollution inhalation toxicity differs from other delivery methods. One study compared the brain accumulation of uranium oxide particles (<2.5 μm dia., P2.5) after inhalation exposure or IP injection [13], which increased uranium oxide accumulation in the olfactory bulb, tubercles, frontal cortex, and hypothalamus but not in other brain regions. Thus, inhalation exposure may aggravate PM neurotoxicity compared to other delivery methods. However, it is problematic to deliver similar particle concentration by these different methods. Due to these challenges, most studies used inhalation delivery of air pollution to have comparability with real-life conditions.

Only a few ambient components have been tested in adult rodent models (Table 3). These components included both gas phase (e.g., O_3, NO_2, SO_2, combustion smoke, and toluene) and solid phase (e.g., ambient PM, diesel exhaust particles (DEP), dust, ammonium sulfate, iron soot, MnO_2, Ni, and uranium oxide particles). For particles, the used size range varied from diameters less than 0.05, 0.1, 0.2, 1, 2.5, or 10 μm. Nano-sized PM (nPM, filtered-eluted urban PM0.2) with 13 published papers is the most studied air pollution component. The next ranks are comprised of urban PM2.5 (12 studies), O_3 (3), and DEP0.2 (4). At present, the neurotoxicity of air pollution components is understudied, and the causative effects of several components of air pollution are either unknown or only studied once in the rodent models. Thus, the interaction of these components during air pollution neurotoxicity presents

Table 3
Summary of experimental studies on air pollution toxicity in adult rodent brain

Air pollution component	Size class (diameter μm)	Classification based on collection [Refs]	N studies	Range of conc.	Exposure paradigms, hour (h), day (d), week (w), month (m)	Animal models	Age (m)	Sex Male (M), Female (F)	Studied effects
Total air pollution		[33]	1	PM2.5∼50 μg/m^3	7 m continuous	Mouse: C57BL/6J	2 m	F	Alz
Ambient particulate matter	PM0.2	nPM (filtered-collected) [10, 11, 15, 21, 22, 24–27, 31, 41, 69, 124]	13	100–350 μg/m^3	5 h/d, 3 d/w, 3–15 w	Mouse: C57BL/6J, LDLR–/–, E3FAD, E4FAD, J20	3, or 18	M, or F	NI, NV, NT, NP, M, B, Alz, Ox, NG, S, T
		sPM (aerosol to liquid) [15]	1	100–350 μg/m^3	5 h/d, 3 d/w, 3 w	C57BL/6J	3	M	NI
		concentrated aerosol [125]	1	65 μg/m^3	5 h/d, 4 d/w, 0.5, 1, 3, 10 m	Rat: Fischer	1–2	M	T
	PM0.5	filter-collected [126]	2	5, 50 mg/m^3	4 h	Rat: Fischer	unknown	M	NI, NT
	PM1	real-time [61]	1	16.3 μg/m^3	3–6 m continuous	Rat: Sprague-Dawley	6	M	NI, M, Ox, S
	PM2.5	concentrated aerosol [46, 47, 56, 58, 70, 76, 125]	7	27–500 μg/m^3	8 h–12 w continuous	Rat: Brown Norway, Fischer, JCR/LA, Sprague-Dawley; Mouse: Kkay	1–8	M	NI, NV, NT, Ox, S
							1–2	M	
		filter-collected [55, 57, 71]	4	0.193–3 mg/kg, 90–115 μg/m^3	constant: 3–12 w, intermittent: 4–24 w	Mouse: C57BL/6J	1,2,4,10	M, or F	NI, NV, B, Ox, S, BA
	PM10	filter-collected [127]	1	100 μg	once, intra-tracheal	Mouse: BALB/C	2	M	Ox, S
Diesel particulate matter	DEP0.2	[18, 36, 43, 63, 77]	4	100–950 μg/m^3	only 6 h, intermittent for 3 m (5 h/d, 5 d/w)	Mouse: C57BL/6J, NMRI, 5xFAD	1–2	M, or F	NI, NP, NT, M, B, Ox, NG, Alz
	DEP0.2	filter-collected [128]	1	4 mg/kg	Once	Mouse: ICR	1–2	M	S
	DEP2.5	[19]	1	250–300 μg/m^3	6 h	Mouse: C57BL/6J, GCLM+/–, GCLM–/–	3	M and F	NI, Ox
Dust	unknown	[60, 65]	2	150–8000 μg/m^3	30–60 min/d, 2 d, for 4 w (intermittent)	Rat: Wistar	unknown	M	NI, M, B

O_3		[29, 30, 37, 44, 49, 126, 129, 130]	7	0.12–2 ppm	4 h–90 d (4 h/d)	Rat: Wistar, Fischer; Mouse: C57BL/6, E3TR, E4TR, APP/PS1	1–2 2–3, 17	M, or F M and F	NI, NV, NT, M, B, Ox, NG, S, Alz
Combustion smoke		[66]	1	CO: 2200–2500, $O_2 > 19\%$	0.5, 3, 24, 72 h, 7 d, 14 d	Rat: Sprague-Dawley	unknown	M	NI
Ammonium sulfate	P2.5	[45]	1	500 $\mu g/m^3$	2 h/d, for 28 d	Rat: Sprague-Dawley	10	M	NG
Iron-soot	P0.1	[82]	1	40, 200 $\mu g/m^3$	6 h/d, 5 d/w, for 5 w	Mouse: C57BL/6	2	F	NI, BA
MnO2	P0.1	[59]	1	2.63, 5.26 mg/kg	5 d/w, for 3, 6, 9 w	Rat: Wistar	2	M	B, BA, S
Ni	P0.05	[38]	1	1 mg/m^3	3 h	Mouse: FVBN	2	M and F	Alz
NO2		[32]	1	2.5, 5 mg/m^3	5 h/d, for 4 w	Mouse: C57BL/6	2	unknown	NI, NP, NT, Alz, S
SO2		[131]	1	7, 14, 28 mg/m^3	6 h/d, for 7 days	Rat: Wistar	unknown	M	NI
Toluen		[14]	1	90 ppm	30 min/d, 6 d/1 m	Mouse: C3H	unknown	M	S
Uranium oxide	P1–2.5	[13, 62, 80, 81]	4	190–545 mg/m^3	30 min – 3 w (30 min/d, 4 d/w)	Rat: Sprague-Dawley	3–4	M	M, B, BA

Summary of studied targets: neuroinflammation (NI), neurovascular (NV), neurotransmitters (NT), neuropathology (NP), memory (M), behavior (B), Alzheimer's processes (Alz), oxidative stress response (Ox), neurogenesis (NG), systemic or metabolic effects (S), brain accumulation (BA), transcriptome (T).

a complex puzzle. In one study, exposure to DEP (<0.2 μm diameter, DEP0.2) with O_3 pretreatment (DEP with secondary organic aerosols, DEP-SOA) aggravated the air pollution effects and caused memory decline and hippocampal glutamatergic changes in mouse [14]. Thus, although studying the individual components provides mechanistic insight, it does not adequately model the effects of the mixtures in real-life air pollution.

Another challenge for experimental modeling of air pollution neurotoxicity is the exposure paradigms and selection of a realistic dosage. Even after two decades, there is still no standard exposure paradigm or concentration range. A major challenge for experimental studies is that the real-life air pollution concentration and composition is highly dynamic and variable, which cannot be easily modeled for replicable experiments. Our lab exposures have used a range off re-aerosolized PM 100, 200, or 300 $\mu g/m^3$ concentration for 5 h/day, 3 days/week, for 3–15 weeks depending on the research question [15]. At 15 h per week, the total PM inhaled at these levels approximates the range experienced in many cities 9–27 $\mu g/m^3$ PM. For laboratory mice, exposure for 10 weeks approximates 10% of typical lifespan of 2.5 years, which is equivalent to 10 years of the human lifespan. Other research groups used a wide range of particle concentration (e.g., 27 $\mu g/m^3$ up to the extreme dosage of 545 mg/m^3 uranium oxide PM) and widely varying exposure durations (e.g., 30 min, 5 h exposures triweekly, or constant for 12 weeks). For O_3, exposures ranged from acute 1 ppm (4 h) to chronic 0.25 ppm (4 h/day for 3 months) in rats. This give equivalent exposure to 1 ppm O_3 for 6 days, to 0.12 ppm O_3 for 8 h per day for 10 years relative to a realistic exposure in human. The current EPA standard level for O_3 is 0.07 ppm for 8 h per day (based on an average of 3 years) [16].

The calculations for PM or O_3 are based on total delivery as a constant during the whole experiment, thus, it does not reflect the intermittent delivery in the experimental model. As mentioned before, humans also experience a dynamic air pollution surges dependent on the transportation, home or work address, occupation, distance to highway, and many other factors. A realistic comparison of human and mouse particle deposition is not trivial and requires consideration of anatomical differences between species because rodents are obligate nose breathers. For example, the tracheobronchial structure of humans is dichotomous, while mice have a monopodial structure. Other factors include airway geometry, alveolar size, tidal volume, and mode of aerosol delivery [17]. Thus, a direct comparison of rodent and human studies is confounded by particle deposition differences. Moreover, it remains unresolved if the divergences between labs for rodent studies are due to the exposure schedules; modes of administration (inhalation vs oro-pharyngeal intubation); insufficient attention to real-life exposure levels; or the sources and chemical composition of the particulate and gas phase factors.

The diversity of rodent models for air pollution neurotoxicity is another general concern for comparability of findings. The most widely used mouse is the inbred C57BL/6J ('B6' mouse, 23 studies), with minor use of BALB/c, C3H, ICR, KK-Ay, and NMRI inbred strains. For rats, the most used is the outbred Sprague-Dawley (9 studies), followed by Brown Norway (inbred), Fischer (inbred), and Wistar (outbred). Transgenic mice include human familial Alzheimer's disease (FAD) genes on B6 background (J20, APP/Psen1), or together with human *APOE* alleles (E3FAD, E4FAD on B6 backgrounds). A few studies used obesity models (JCR/LA with Brown Norway rat background), atherosclerosis models (LDLR–/– mouse with C57BL/6J background), and oxidative stress models (GCLM+/– and GCLM–/– on B6 background). Most studies used males; only two studies directly compared both sexes for air pollution effects [18, 19].

The effects of aging in exposures to air pollution in rodent brains are also understudied and have not included *in vitro* studies on aging cell models. There is little consensus on choice of younger ages: some studies used rodents as young as 1–2 months, which is equivalent to childhood and adolescence in developmental stages. Others included up to 10 months, which is middle age during reproductive decline [20]. Several studies even did not report the age or used an apparently random selection over a wide age range. To address the age gap, we examined B6 mice of later middle-age male and female (18–20 months) [21, 22]. These initial studies of aging excluded later ages approaching the 28–30-month lifespan when B6 mice have increasing prevalence of tumors and blood dyscrasias [23]. Interactions of age-related pathology with air pollution should be a major priority of future epidemiological and experimental studies that links between air pollution and neurodegenerative diseases.

The next sections discuss details of neurotoxic effects of air pollution in adult brains and identify gaps that require immediate attention. We hope that

summarizing the current experimental designs can guide the field to address gaps and encourage the adoption of standardized protocols for harmonization and comparability of the findings.

EXPERIMENTAL FINDINGS OF AIR POLLUTION NEUROTOXICITY IN ADULT BRAIN

Oxidative damage

About 20 studies suggested that particular air pollution components can induce oxidative stress responses in different brain regions. These components included nPM, PM1, PM2.5, PM10, DEP0.2, DEP2.5, and O_3. Our laboratory exposure model uses a water eluted subfraction of PM0.2, designated as nPM (Table 3). Even 5 h exposure to 300 $\mu g/m^3$ nPM sufficed to increase lipid peroxidation (4HNE) in the olfactory epithelium, but not in the brain [24]. Increased lipid peroxidation (4HNE) and nitrosative stress (protein 3-nitrotyrosine) was observed in the olfactory bulb after three weeks (5 h/day, 3 day/week) exposure to similar concentration of nPM [24]. Longer exposure to nPM (>8 weeks) could induce further cerebral cortex [25] and systemic oxidative damage, with increased paraoxonase activity, LDL oxidation, and free oxidized fatty acids [26]. Subcellular changes include increased 20S proteasome and mitochondrial Lon protease activity [27]. The J20 Alzheimer mouse responded with increased 4-HNE in cerebral cortex lipid raft compartments, together with increased amyloid peptides $A\beta_{42}$ and $A\beta_{40}$, lipid raft AβPP expression, and fibrillary Aβ deposits [25].

Nrf2 mediated oxidative stress responses were robust in cerebellum [21] as well as liver, lung, and heart of the nPM exposed animals [27]. Some of the Nrf2 associated genes that responded to nPM included GCLC, GCLM, HO1, NQO1, Bach1, and cMyc [21, 27]. Nrf2 oxidative stress response seems to partially determine the air pollution mediated damage. GCLM+/– and GCLM–/– mouse lines showed aggravated oxidative damage and neuroinflammatory responses to inhaled DEP2.5 [19]. We introduced the nematode *Caenorhabditis elegans* as a model for air pollution that allows rapid development of genetic models [28]. The C. *elegans* Nrf2 equivalent skn-1 was not essential for surviving the oxidative damage of nPM; however, this adaptive response contributed to long term developmental changes after acute nPM exposure [28].

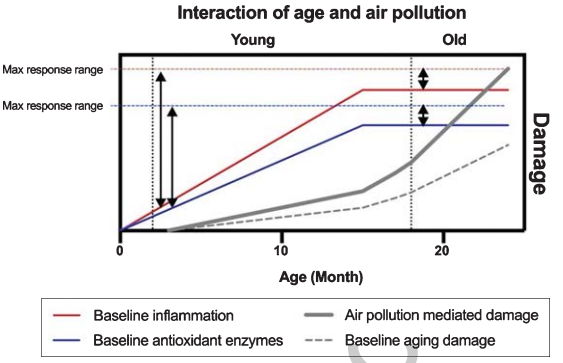

Fig. 2. Aging interacts with air pollution mediated oxidative stress, inflammation, and associated damage. Aging cause a ceiling effect on responses to air pollution, but increases the damage.

Aging may fundamentally alter the systemic and brain Nrf2 responses to air pollution (Fig. 2). Compared to 3-month-old (young adult) male and female, the 18-month-old (middle-aged) mice had a higher baseline in the expression of Nrf2 associated genes; their unexpected lack of responses to nPM in cerebellum, liver, and lung [21, 27] suggests a ceiling effect of aging responses to nPM (Fig. 3A). Another study by the laboratory of Dr. Rui-Ming Liu exposed the 3- and 17-month-old *APOE4*-targeted replacement (E4TR) male mice to ozone (O_3 and reported similar ceiling effects in thioredoxin 1 activity, glutaredoxin 1, astrogliosis, and microgliosis in the hippocampus (Fig. 4B, C) [29]. As opposed to E4TR, the 17-month-old E3TR was still responsive to nPM for the mentioned outcomes, and was the only group to show O_3 induced memory decline in the water maze test [29]. This study suggests that *APOE* genotype can alter age-dependent oxidative stress responses to air pollution components. It looks like *APOE4* genotype also cause a ceiling effect on air pollution responses (Fig. 4A). Further studies are needed to evaluate the generality of ceiling effects for other environmental stressors.

Sex also influences oxidative responses against air pollution. A direct comparison of male and female APP/PSEN1 AD models revealed a higher female baseline in glutathione, glutathione disulfide, and ascorbate, and a lack of responsiveness to O_3 than males [30]. We further discuss the relationship of sex and air pollution in a separate section.

Neuroinflammation

Neuroinflammation is another consistent response between different air pollution components using a

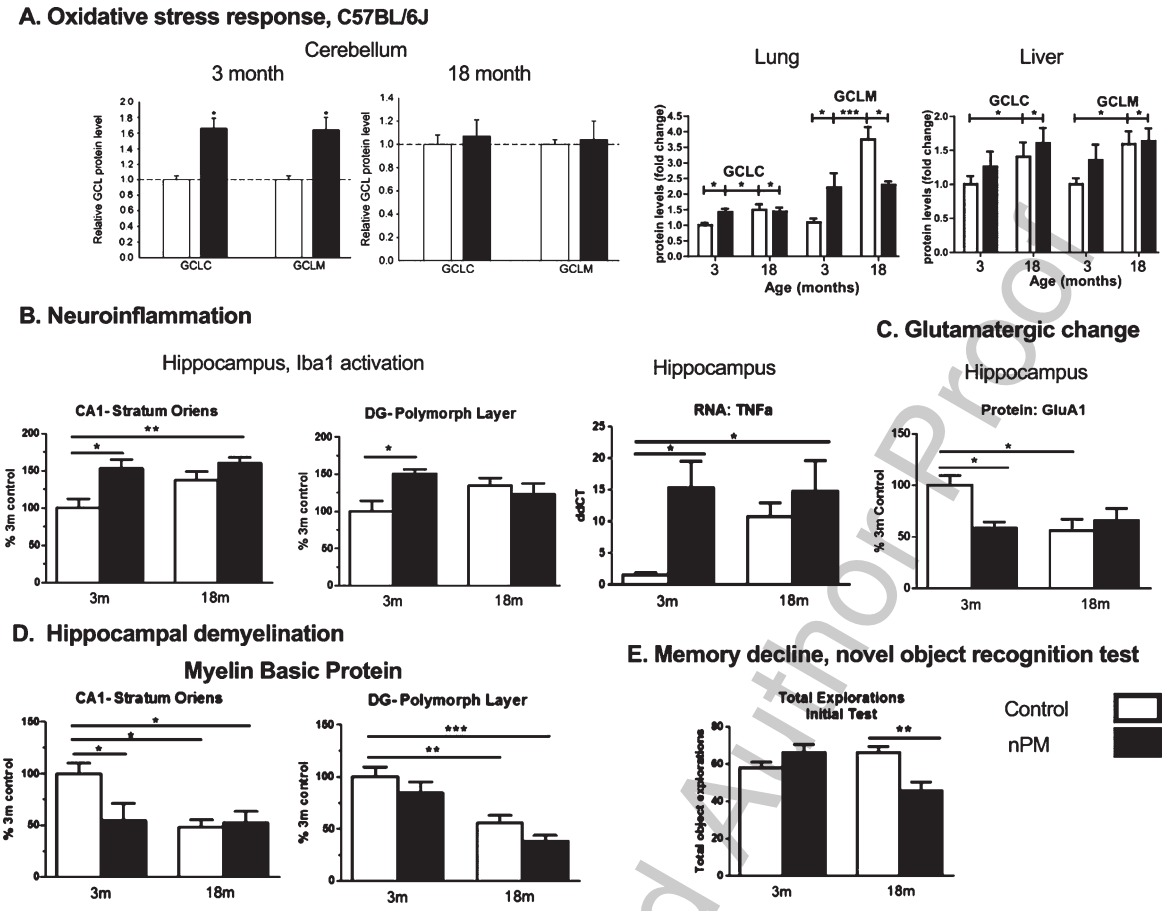

Fig. 3. nPM effects in young (3 months), and aged (18 months) B6 mice. A) Antioxidant responsive proteins (GCLC and GCLM) only responded to nPM in cerebellum, lung, and liver of the young [21, 27]. B) nPM mediated neuroinflammation: Microglial activation and increase of proinflammatory cytokines in young animals [22]. C) nPM cause decrease of hippocampal GluA1 receptor [22]. D) Decrease of myelin base protein in the hippocampus of nPM exposed young mice [22]. E) nPM mainly caused memory decline in the old mice [22]. All measured molecular and cellular responses showed an age ceiling effect on nPM responses. Figures adapted from [21, 22, 27].

diverse range of exposure paradigms. Neuroinflammation is commonly defined by microglial activation, astrogliosis, increased IL1, IL6, and TNFα (proinflammatory cytokines), and activation of NF-κB and other innate immune pathways. Around 25 independent studies reported that inhalation or intranasal instillation of air pollution particles or gases can lead to neuroinflammation in different brain regions (Table 3). A direct comparison of these findings is not possible due to diversity in the exposure paradigms and lack of proper characterization of the used air pollution components.

Using mixed-glial (astrocytes+microglia) culture, we showed that nPM induce damage associated inflammation through the TLR4 pathway and increase NF-κB mediated production of proinflammatory cytokines [11]. The knockdown of TLR4 partially attenuated the increase of inflammatory cytokines *in vitro*. However, we must consider the caveat that *in vitro* cell models use direct exposure to nPM at much higher concentrations than could reach the brain by inhalation. Thus, *in vitro* responses might be distinctly different from the brain, which receives systemic influences, 'lung to brain' (see below). Thus, we also examined these inflammatory responses *in vivo*. In mice, a short term 3-week exposure to 300 $\mu g/m^3$ nPM, but not lower concentrations, sufficed to decrease TLR4 and MyD88 mRNA, induce NF-κB localization to the nucleus, and increase proinflammatory cytokines IFNγ and IL1β in the cerebral cortex [15]. Another experiment showed that initial responses to inhalation of 300 $\mu g/m^3$ nPM begin as early as three weeks for induction of TNFα protein in the olfactory bulb, cere-

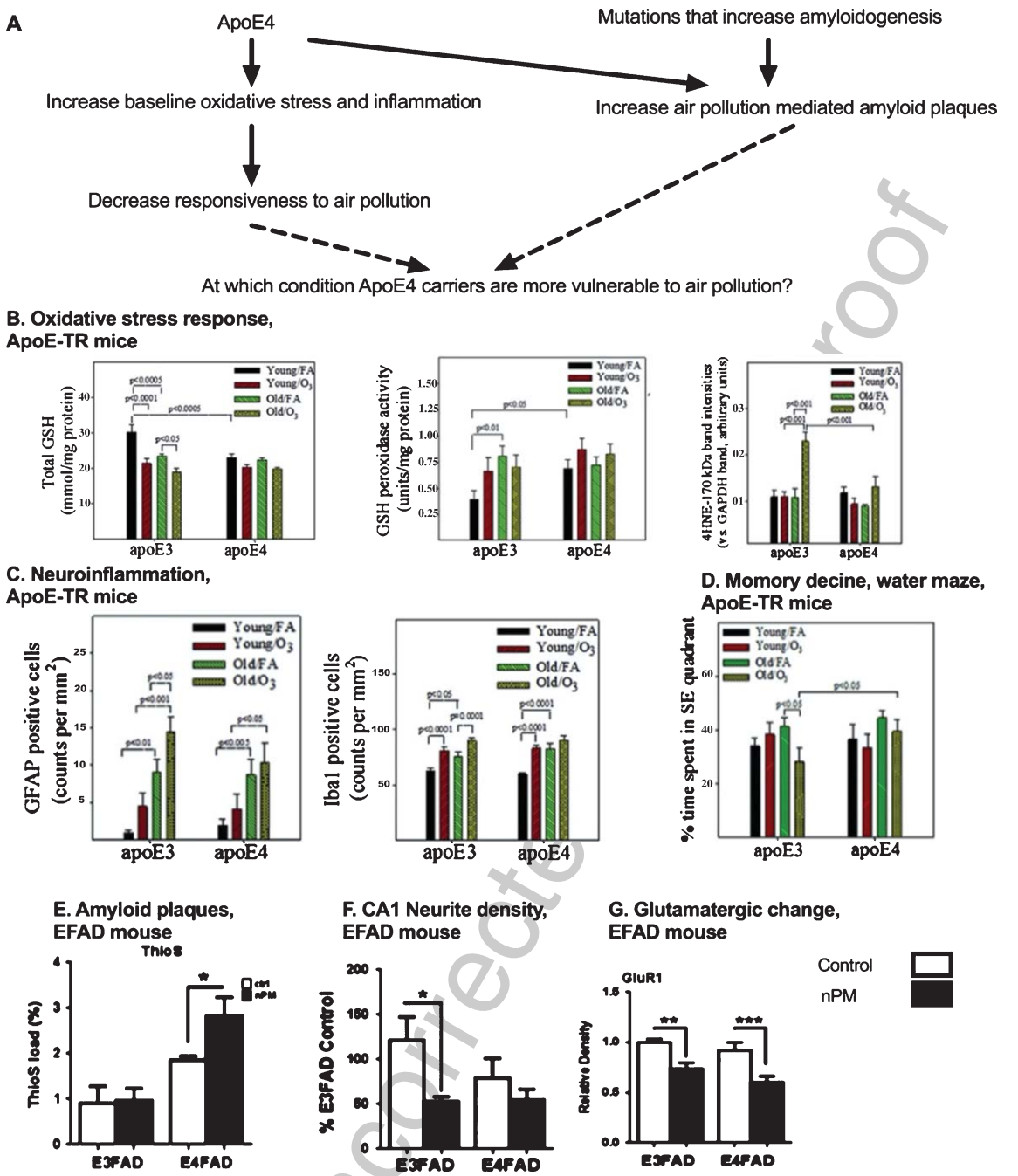

Fig. 4. *APOE* allele interacts with O_3 and nPM mediated brain responses. A) Schematic summary of findings and gaps from the two mouse studies on *APOE*-air pollution interactions [29, 31]. *APOE4* mice show an apparent ceiling effect on responses to air pollution. B) Effects of O_3 exposure on total GSH, GSH peroxidase activity, and 4-HNE protein adducts in the hippocampus of young and old *APOE3* or 4-TR mice [29]. C) O_3 mediated astrogliosis and microgliosis of the hippocampus of young and old *APOE3* or E4-TR mice [29]. D) O_3 exposed caused memory decline mainly in E3 old mice [29]. E) Inhalation exposure to nPM mainly increased amyloid plaques in E4FAD, but not E3FAD mice [31]. F) Ceiling effect of *APOE4* allele on nPM mediated decline in CA1 neurite density [31]. G) nPM mediated decrease of hippocampal GluA1 in both E3- and E4FAD mice [31]. Figures were adapted from [29, 31].

bral cortex, and cerebellum of young male mice [24]. The earliest cell responses in this experiment was an increase in microglial number in the nasal epithelium turbinate after 5 h exposure to nPM [24]. A longer-term exposure in 3-month-old female mice (10 weeks exposure to nPM plus 4 weeks recovery) caused hippocampal responses of increased mRNA for TLR4 and MyD88, but decreased NF-κB1 and TRAF6 [11]. Notably, hippocampal microgliosis was limited to CA1 stratum oriens and dentate gyrus (DG) polymorphic layer (Fig. 3B) [22]. These results indicate that innate immune responses to air pollution components are a dynamic process that depends on the exposure dosage and brain region. The vulnerability of hippocampal CA1 and DG to nPM might be a link between air pollution and risk of AD. CA1 region even more vulnerable than DG for nPM-mediated demyelination (Fig. 3D) and a decline in neurite density [22].

Air pollution and Alzheimer's disease associated genes

Only 9 studies have examined changes in known AD processes in FAD or wildtype mouse models for air pollution responses. In young adult J20 male mice, long-term exposure to 300 $\mu g/m^3$ nPM for 10 weeks caused increased levels of $A\beta_{42}$ peptides and Aβ plaques in cerebral cortex [25]. Baseline AβPP expression was also increased in lipid raft compartments, in parallel with increased lipid peroxidation (Fig. 5A, B). Another study exposed young female E3FAD and E4FAD mice to 15 weeks of 300 $\mu g/m^3$ nPM [31]. These mice carry five known familial AD mutations plus human *APOE* alleles with targeted replacement of the mouse *APOE*. Exposure to nPM caused a 50% increase in amyloid plaques only in the E4FAD mice but not E3FAD (Fig. 4E, 5C). As noted above, long-term O_3 exposure mainly caused memory decline in old E3-TR animals but not E4-TR (Fig. 4D) [29]. Another study of O_3 showed memory decline in males but not females in the APP/PSEN1 AD mouse model [30]. In both studies, O_3 did not change $A\beta_{42}$ levels of the cerebral cortex or hippocampus. However, a higher baseline of hippocampal $A\beta_{42}$ peptide and Aβ load in females than male animals was noticeable. In another study of B6 wildtype mice, at the high dose of NO_2 (5 mg/m^3, 5 h/day for 4 weeks) p-tau increased in the cerebral cortex and hippocampus [32]. Exposure of female B6 mice to total ambient air pollution (Santiago, Chile;>50 $\mu g/m^3$ PM2.5, 7 months) caused 2-fold increases of p-tau (AT8) and γ-H2A.X (DNA damage marker) in cerebral cortex [33]. Epidemiological studies suggest a complex relationship between air pollution, lifestyle, age, sex, *APOE* genotype, tauopathy, amyloidogenesis, and the degree of damage that can accelerate cognitive decline across pediatric and adult urbanites [34, 35]. Resolving the mechanism of these interactions requires a systematic experimental approach in rodent models.

A key factor in the interpretation of air pollution effects on amyloidogenesis is the duration of exposure. For example, a lack of change in Aβ plaques after O_3 exposure in APP/PSEN1 mice can be due to a ceiling effect of amyloid accumulation in the brain [30]. A study of 5xFAD females compared the effects of 3- and 13-week exposures to DEP0.2 [36]. Only the 3-week exposure to DEP mediated an increase in cerebral cortex Aβ plaque load and total brain $A\beta_{42}$. The 13-week DEP exposure increased baseline Aβ load and weakened grip strength but did not affect the memory. There was also indication of a ceiling effect.

While these studies modeled longer-term exposure, brain Aβ peptides are responsive within 24 h to brief surges of air pollution. Amyloid homolog genes are among the initial responses to nPM in our *C. elegans* nematode air pollution model (Fig. 5E) [28]. In old male B6 mice, short-term exposure to 1 ppm O_3 for only 4 h increased $A\beta_{42}$ in the cerebral cortex, with blood-brain barrier disruption [37]. In a model for industrial air pollution from nickel (Ni) refining [38], FVBM mice were exposed for 3 h to Ni nanoparticles (<0.05 μm dia., 1 mg/m^3; within EPA standards!), which increased $A\beta_{40}$ and $A\beta_{42}$ by 24 h (Fig. 5D). While high levels of Ni levels are atypical of most locations, a surge of downwind nickel from a Canadian refinery was associated with increased mortality in New York City [39]. These findings illustrate the potential hazards of short-term fluctuations of trace air pollution components. Surges of PM2.5 are associated with increased stroke admissions to Emergency Departments [40]. The pace of amyloidogenesis response to other air pollution components and lower concentrations remained for future studies. Tauopathic transgenic AD mice (e.g., PS19) also merit study for air pollution factors.

Glutamatergic and other neuronal effects of air pollution

Neurochemical specificity is indicated for glutamatergic neurons, a major class of excitatory

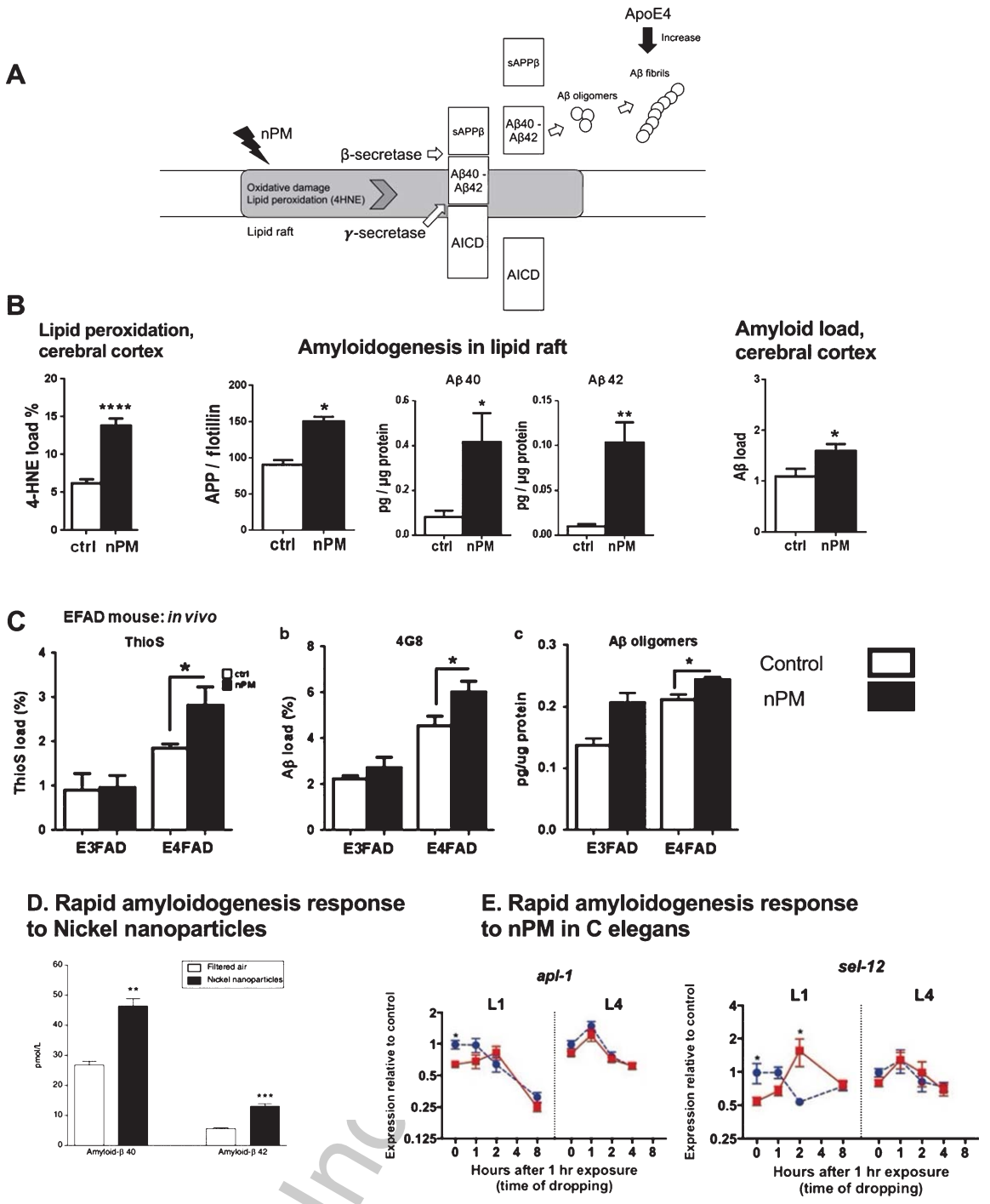

Fig. 5. Chronic and acute air pollution exposure induce amyloidogenesis responses. A) Schematic summary of the current results on nPM effects on amyloidogenesis. B) Inhalation exposure to 300 µg/m^3 nPM (3 h/d, 3 d/w, 10 weeks) in J20 mice increased lipid peroxidation, lipid raft amyloidogenesis, and amyloid plaques load in the cerebral cortex [25]. C) nPM increased cerebral cortex amyloid plaques only in E4FAD animals (300 µg/m^3, 3 h/d, 3 d/w, 15 weeks) [31]. D) A 3 h exposure to 1 mg/m^3 of nickel nanoparticles caused around a 2-fold increase of brain Aβ_{40} and Aβ_{42} peptides in FVBM mice [38]. E) AD genes were among the acute responses to nPM in C. elegans: apl-1/APP homolog, sel-12/Psen 1 homolog [28]. Figures adapted from [25, 28, 31, 38].

transmission for inhalation exposure to nPM, NO_2, and DEP0.2. Consistently, long-term nPM exposure (300 $\mu g/m^3$/10 weeks) caused a decrease of hippocampal GluA1 protein in young male and female C57BL/6J, and of young female EFAD mice for both *APOE3* and E4 alleles [22, 31, 41]. Short-term exposures to nPM (300 $\mu g/m^3$, only 3 weeks), decreased GluA1 mRNA, but not protein in the cerebral cortex [15].

Glutamatergic effects of nPM on the hippocampus were accompanied by a selective decrease of neurite density and myelin base protein in the hippocampal CA1 or DG (Fig. 3) [22, 31]. Recall from above that these hippocampal subregions were also vulnerable to nPM mediated microgliosis [22]. The shared decreases of hippocampal GluA1, neurite density, and myelin basic protein also showed age-ceiling effect in 18-month-old female mice [22]. These findings suggest that air pollution intensifies aging processes that accelerate the 'normal' baseline trajectory of cognitive aging. The individual components of baseline cognitive aging show linear trends after age 30 in humans for slower information processing and loss of synapses, increased levels of soluble and fibrillary amyloid, and increased astrocyte volume and microglial activation [42]. We do not know how air pollution components interact with each of these changes and their multilevel crosstalk.

Nitrogen dioxide (NO_2) is represented by only one study. Exposure to NO_2 at the high level of 5 mg/m^3, 5 h/day, for 4 weeks caused 25% decrease of GluA1, GluA2, GRIN2A, and GRIN2B proteins and of post-synaptic marker, PSD-95, in the cerebral cortex and hippocampus of B6 mice [32]. Exposure to 100 $\mu g/m^3 m^3$ diesel exhaust particles (DEP-SOA) for 3 months caused increase of GRIN1, and a decrease of GRIN2A mRNA in the hippocampus of male B6 mice [43].

Neurogenesis in the adult brain was also impacted in several studies. Long-term ozone exposure (4 h/day, 60 or 90 days/0.25 ppm O_3) of male adult rats caused 30–80% decrease of newly formed cells in the dentate gyrus subventricular zone (doublecortin (DCX) positive cells) [44]. Shorter exposure to O_3 (15 days) had minimal effects on neurogenesis. In another study, exposure to ammonium sulfate particles (PM2.5 μm dia.; 500 $\mu g/m^3$; 28 days, 2 h/day) caused a decrease of DCX positive cells of the hippocampus but with no change in the number of new BrdU+ cells in 10-month-old male rats [45]. While DCX labels the neuronal precursor cells, BrdU+ identifies recent DNA replication in all cell types. Air pollution effects on neurogenesis may differ by the tested component, sex and genotype, and sex. Rostral neurogenesis also merits study. A recent short-term exposure of 6 h/250 $\mu g/m^3$ DEP0.2 in 2-month-old mice showed male-specific reduction of Ki67 + cells in the hippocampal subgranular and subventricular zones [18]. The decrease of these proliferative precursor cells was accompanied by a male-specific reduction in newly developed neurons (BrdU+/NeuN+) cells (Fig. 6A). In contrast, BrdU+/NeuN+cells were depleted in the olfactory bulb of both male and female mice. This study is the first to show regional and sex difference in adult neurogenesis.

Air pollution, acute stress response, and systemic metabolic changes

Several studies showed that acute exposure to air pollution can activate both sympathetic-adrenal-medullary (SAM) and hypothalamus-pituitary-adrenal (HPA) axis stress responses. Stress signals received by the hypothalamus, which responds by secretion of corticotropin-releasing factor (CRF), that in turn releases adrenocorticotropic hormone from the pituitary gland, and then to the adrenal cortex for glucocorticoid secretion. Concurrently, the hypothalamus and other brain regions may activate both the sympathetic nervous system (through secretion of epinephrine and norepinephrine from the adrenal medulla and sympathetic nerves). Thus, the neuroendocrine stress responses are an intricate interplay of SAM (releasing of epinephrine and norepinephrine) and HPA (glucocorticoids). Air pollution exposure can induce both SAM and HPA responses. Short-term exposure to PM2.5 ($\sim$500 $\mu g/m^3$ for 8 h or 1 day) induced a 3-fold CRF increase in hypothalamus median eminence (Fig. 6C), around 2-fold increased norepinephrine and 5-hydroxyindole acetic acid in the paraventricular nucleus, and also 2-fold increase of serum corticosterone [46, 47]. Ozone exposure also induced a transient increase of serum corticosterone in rats [48, 49]. In contrast, serum epinephrine may remain high during prolonged exposure to O_3 [50].

One study examined the effects of SAM and HPA responses during acute O_3 toxicity [51]. Inhibition of SAM (by 7 days pretreatment with propranolol, a non-selective β adrenergic receptor antagonist that blocks epinephrine receptors, 10 mg/kg, i.p.) could attenuate O_3 mediated pulmonary vascular leakage and lung inflammation. Inhibition of HPA (by 7

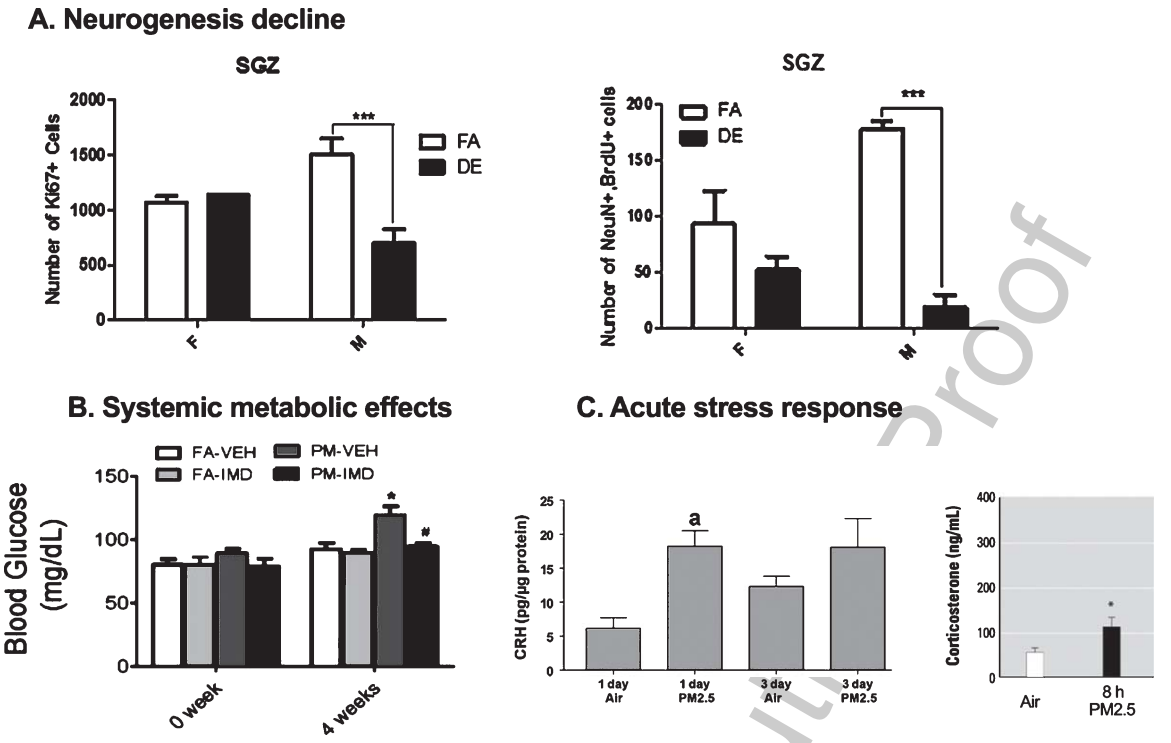

Fig. 6. Air pollution effects on neurogenesis, systemic metabolism, and acute stress responses. A) A short-term inhalation exposure (6 h) to 300 μg/m3 DEP0.2 caused a male-specific decline in the number of proliferative precursor cells (Ki67+) and newly developed neurons in the of the sub granular zone (SGZ) of the dentate gyrus [18]. B) Inhalation exposure to 100 μg/m3 PM2.5 for 4 weeks (6 h/day, 5 days/week) caused hyperglycemia and increased serum insulin (not shown) [56]. Intracerebroventricular injection of IMD-0354 (IKK2 inhibitor, an anti-inflammatory drug) attenuated the systemic metabolic effects of PM2.5 [56]. FA-VEH, exposed to filtered air, injected with the vehicle; FA-IMD, exposed to filtered air, injected with IMD-0354; PM-VEH, exposed to PM2.5, injected with the vehicle; PM-IMD, exposed to PM2.5, injected with IMD-0354. C) Acute air pollution exposure activated hypothalamus-pituitary-adrenal stress responses. Exposure to PM2.5 causes a transient increase in corticotrophin-releasing hormone (CRH) in median eminence [46] and serum corticosterone [47]. Figures adapted from [18, 46, 47, 56].

days pretreatment with mifepristone, a glucocorticoid receptor antagonist, 30 mg/kg, s.c.) could also attenuate O_3 mediated pulmonary vascular leakage, but with no effect on inflammation [51]. The increase of corticosterone may mediate some initial responses to air pollution in different organs. Corticosterone administration caused similar gene expression patterns in the lung, heart, liver, and spleen, but not kidney of rats briefly exposed to O_3 (0.8 ppm, 4 h) [48]. Inhibition of O_3 mediated corticosterone by metyrapone increased the inflammatory responses in plasma and lung. The potential effects of prolonged activation of SAM or HPA by air pollution in the risk of chronic diseases is still unresolved. Patients with type 2 diabetes, cognitive disorders, AD, and depression often have higher level of serum corticosterone levels [52, 53]. In AD, simultaneous high level of cerebrospinal fluid cortisol and $A\beta_{42}$, but not cortisol level alone, was associated with a clinical transition from a normal state to mild cognitive impairment or dementia [54]. The relationship of air pollution neurotoxicity and these systemic stress responses merit further attention.

Systemic metabolic responses to air pollution may be linked to hypothalamic inflammatory responses. The tested components include inhalation exposure to O_3 [50], NO_2 [32], nPM [22, 26], and PM2.5 [55–58], and intra-tracheal instillation of MnO_2 PM0.1 [59]. In general, exposure to these components for more than 4 weeks (except for O_3) impaired the normal weight gain of young rats, with corresponding deficits of liver weight; however, the lung weight was larger, consistent with inflammatory responses. Systemic changes included glucose intolerance with elevated serum insulin, and lower plasma HDL and elevated total cholesterol and LDL; these dyslipidemias and glucose dysregulation are risk factors for ischemic disease in humans. For nPM, the weight

loss was more prominent in 18-month-old animals [22]. For O_3, glucose tolerance was impaired by acute exposure (O_3 0.25–1 ppm, 6 h/day, for 1–2 d), but, surprisingly, was not altered by a 10-fold longer exposure (0.25–1 ppm, 6 h/day, 2 days/week, 13 weeks) [50]. The acute response also caused a transient increase of serum corticosterone [48, 49], indicating HPA activation of stress responses. One study examined if brain inflammatory responses to air pollution could contribute to systemic metabolic changes [56]. Intracerebroventricular injection of IMD-354, an anti-inflammatory drug (IKK2 inhibitor) had peripheral metabolic impact, with attenuation of the PM2.5 mediated glucose intolerance and fat gain in the exposed animals. Further studies should validate these findings and resolve the association of systemic metabolic changes, and stress responses with other effects of air pollution in the adult brain.

Air pollution effects on cognitive and behavior

Memory deficits were shown in eight independent studies of chronic inhalation exposure to different air pollution components. The components included DEP0.2-SOA [43], dust [60], nPM [22], PM1 [61], O_3 [29, 44], and uranium oxide PM2.5 [62]; memory was assessed by the novel object recognition [22, 43], Morris water maze [29, 60], passive avoidance [44], and Y-maze tests [62]. Air pollution mediated memory decline in young adults was only observed in male rat models [43, 44, 60–62]: dust (500–2000 μg/m3, 1 h/day, 4 days/week, 4 week) [60], O_3 (0.041 ppm, 4 h/day, 90 days) [44], PM1 (16.3 $\mu g/m^3$, daily for 3 months) [61], and uranium oxide P2.5 (190 mg/m^3, 30 in/day, 4 days/week for 3 weeks) [62]. In contrast, memory declined only in the older age mice in responses to nPM (Fig. 3E) [22] or O_3 (Fig. 4D) [29] by 3- and 17–18-month-old mice. O_3 also showed differential effects on memory between sexes. In APP/PSEN1 mice, only males showed O_3 mediated memory decline [30].

The human *APOE4* allele also enhances cognitive vulnerability to air pollution (Fig. 1), which we hypothesize is due to the greater induction of Aβ amyloid in *APOE4* carriers. As mentioned, nPM cause an increase in amyloid plaque formation mainly in E4FAD mice [31]. However, in APOE-TR mice without FAD transgenes, only the old male E3TR mice showed O_3 mediated cognitive decline (Fig. 4D) [29]. *APOE* interaction with sex and amyloids for air pollution vulnerability remained to be tested in future experiments.

Anxiety and depressive behaviors were increased in rodent exposure to air pollution in several studies. Chronic inhalation exposure to DEP0.2 [63], PM2.5 [64], dust [65], MnO_2 PM0.1 [59], and uranium oxide PM2.5 [62] increased immobility time and anxiety-like behaviors in the open field test. Other studies showed an increased depressive behaviors from PM2.5 [64] and DEP0.2 [63], measured by tail suspension and forced swim tests, respectively. Locomotor coordination deficits were shown by multiple-beam walking tests after inhalation exposure to combustion smoke (in male adult rats during the first 24 h of recovery) [66]. The brain circuits and mechanisms are amenable to optogenetics and other current technologies.

Air pollution interacts with cerebral ischemia, high fat diet, and other environmental factors

The cerebrovascular system is vulnerable to air pollution toxicity. In general, elevated air pollution levels is associated with increased hypertension, cardiovascular events, and cardiovascular mortality [67]. As noted above, short-term surges in different air pollution components are associated with increased hospital admission for ischemic stroke on the same day [68]. However, the mechanisms that connect air pollution and ischemic strokes are still unresolved. In a mouse model of cerebral ischemia, simultaneous cerebral artery occlusion and inhalation exposure to 300 $\mu g/m^3$ nPM for the short period of 3 weeks (5 h/day, 3 days/week) caused synergetic (2-fold increase) in cerebral infarct volume and 2-fold increase of inflammatory proteins (C5, C5a, Gp91phox) than ischemic mice exposed with filtered air [69]. High fat diets also increased sensitivity to PM2.5 induced arterial pathology, shown for aortic lesions [26], and for cerebrovascular oxidative damage and middle cerebral artery thickness [70]. In the blood-brain barrier, leakage was increased by simultaneous intranasal instillation of PM2.5 and formaldehyde for 7 days, together with memory impairments, neuroinflammation, and oxidative damage [71]. These findings extend the domain of air pollution associated carotid thickening observed in longitudinal studies of several populations. There may be cerebrovascular contributions to the cognitive declines associated with air pollution (Fig.1).

Intervention studies against air pollution toxic effects

Interventions for air pollution are indicated by early studies. Children living in the highly polluted Mexico City had high blood leptin and endothelin-1, with vitamin D deficiency [72]. It is still unclear if vitamin B supplements in these children can attenuate air pollution toxicity. In a crossover trial, vitamin B supplements attenuated PM2.5 mediated mitochondrial DNA depletion in blood and also DNA methylation changes in genes related to mitochondrial oxidative energy metabolism [73].

In experimental models, omega 3 fatty acid diet supplement (O3FA) partially attenuated PM2.5-induced middle cerebral artery thickening, systemic inflammation, and microvascular (not studied for specific regions) oxidative damage [58, 70, 74]. In another study, apolipoprotein A-I mimic peptide (D-4F) attenuated nPM mediated atherosclerosis legions and systemic oxidative damage [26]. We recently showed that a gamma-secretase modulator prevented nPM-induced microglial hyperactivity and increased $A\beta_{40}$ and $A\beta_{42}$ increase in the cerebral cortex [75].

Anti-inflammatory agents are also neuroprotective. In two studies, intracerebroventricular injection of IMD-0354 (an IKK2 inhibitor) attenuated the PM2.5 mediated systemic inflammation [76], microglial activation [76], and glucose intolerance [56, 76]. Pioglitazone (agonist of peroxisome proliferator-activated receptor-gamma (PPAR-γ) and used for treatment of type 2 diabetes, was broadly neuroprotective for DEP0.2. Four days pretreatment with pioglitazone via oral gavage (12.5 mg/kg) completely blocked DEP0.2-induced microglial activation, oxidative stress (levels of malondialdehyde as a marker of lipid peroxidation), neuroinflammation (e.g., TNFα mRNA) in cerebral cortex, and restored adult neurogenesis in the hippocampus [18]. Aminoguanidine (iNOS inhibitor) attenuated deficits in locomotor coordination (beam walk test) from inhalation of the combustion smoke [66].

The biome is a new factor in systemic understanding of air pollution. Probiotic supplements (Lactobacillus or VSL#3) were protective against colonic injury and inflammation from DEP0.2 ingestion [77]. These early findings give a rationale for examining potential benefits of probiotics for neurotoxic effects of air pollution.

Future studies should also differentiate between intervention effects on the brain and other systemic organs such as lung, liver, and heart. Such studies can inform us about the relationship of air pollution toxicity between the brain and other organs. Air pollution neurotoxicology field is at the stage that can systematize the potential intervention targets and the study designs to increase the comparability of the findings. Moreover, air pollution toxicity can interact with the effects of several commonly used drugs such as immunosuppressants, antioxidants, neurotransmitters, steroid hormones, metabolic hormones, and other medications for cholesterol, and cardiovascular diseases. Thus, several drugs such as antioxidant reagents (e.g., Nrf2 agonists) remained to be tested against or for potential interaction with air pollution toxicity.

Sex differences in air pollution effects

Despite the major sex differences in lifespan and the risk of AD in humans, few experimental studies have directly compared male and female responses to air pollution. Three studies indicate greater male vulnerability in different aspects of air pollution neurotoxicity [18, 19, 30]. In response to DEP, only males showed neurogenesis decline in the subgranular and subventricular zone, with greater inflammatory responses [18, 19]. In APP/PSEN1 mice, O_3 exposure mainly affected males for memory decline, increased lipid peroxidation in the cerebral cortex, antioxidant responses (ascorbate, GSSG), and increased apoptotic cells in the cerebral cortex [30]. The APP/PSEN1 transgenic model of amyloid overexpression showed a ceiling effect of females on O_3 antioxidant responses. The mechanisms of sex differences in air pollution response is still unclear, particularly in relation to age, and *APOE* alleles. Sex differences on air pollution responses might differ in human post-menopause, which involves a decline in sex hormones [20]. For humans, little is known of how biological sex and gender differences in lifestyle may alter exposure and responses to air pollution.

EMERGING CHALLENGES IN EXPERIMENTAL MODELING OF AIR POLLUTION NEUROTOXICITY

Air pollution is a heterogenous ephemera of toxicants

Inflammatory responses to air pollution are highly dependent on the chemical and physical character-

istics of the components. The current air quality guidelines of the World Health Organization and EPA are limited to PM2.5, PM10, carbon monoxide, lead, nitrogen dioxide, O_3, and SO_2 [16]. There are major gaps: the ultrafine class (PM0.2) is not currently regulated, while PM2.5 and PM10 are only regulated by mass without regard to their source or chemical composition. The great heterogeneity of air pollution originates from different sources such as residential and commercial energy use, agriculture, power generation, industry, biomass burning, land traffic, and some natural sources. Moreover, the diurnal cycles of temperature and sunlight constantly modify its composition. Studies on atmospheric chemistry and general circulation evidenced a diverse distribution of pollutants from different sources globally. It is also shown that emissions from residential energy (e.g., heating and cooking), which are prevalent in India and China, have the largest impact on premature death globally [78]. Thus, the chemical composition of the air pollution and PM can affect the toxicity.

We recently documented that inflammatory activity of different collected samples per unit mass of PM0.2 varied widely *in vitro* and *in vivo* (Fig. 6) [10]. The diverse cytotoxic and inflammatory activity of different size fractioned particles also shown for PM collected in the vicinity of steel, copper, aluminum, and petrochemical industries [79]. It remains unresolved which chemical or physical characteristics of PM cause the heterogeneous toxicity of air pollution. This question was approached by comparing PM samples collected by two modes of collection: the common filter-collection (nPM), or a direct resuspension in water using the VACES system (slurry PM, sPM) [15]. The PM0.2 eluted by sonication of filters (nPM) were completely lacking in polycyclic aromatic hydrocarbons (PAH) and diminished several-fold in transition metals (e.g., Fe, Cu) and black carbon, relative to total PM0.2 directly collected as a slurry (sPM). The direct comparison of nPM and sPM findings challenge a common assumption in epidemiological toxic studies that PM mediated neuroinflammation is derived from PAH, alone or together with high levels of transition metals and total black carbon [15]. We proposed the importance of other characteristics such as surface chemistry, surface reactivity, particle morphology, or particle acidity in the bioactivity of air pollution particles. Future understanding of Air pollution toxicity requires further analysis of its activities after inhalation, including systemic responses and possible passage from lung to brain.

Does air pollution require direct contact with brain cells to cause toxicity?

Some particles can reach the brain, regardless of route of entry. Uranium particles [13, 62, 80, 81] and iron soot P0.1 [82] were detected in the olfactory neuroepithelium, olfactory bulb, and other brain regions after controlled inhalation [62, 81]. IP injection of uranium oxide PM2.5 also caused equal accumulation of the particles in the hippocampus, cerebellum, and cortex than inhalation exposure [13]. The penetrance of the particles in the brain could be through absorption by olfactory neuroepithelium, lung to brain axis, or both. Even after IP injection, TiO2 particles was accumulated in the brain after 14 days [83]. In another study, oropharyngeal instillation of PM2.5 (3 mg/kg, every other day, 4 weeks) led to an increase of metal accumulation (e.g., Pb, Cu) in cerebral cortex [57].

Lung to brain penetrance of the particles depends on the surface area surface reactivity and surface chemistry. The surface area increases the adsorb activity of the particles to opsonizing components of bronchoalveolar lining fluid [84]. This reaction is also dependent on surface chemistry. Carbon black particles with the oxidized surfaces have lower adsorption compared to the non-oxidized surface [84]. In another study, iron oxide nanoparticles coated with glucose or poly(ethylene glycol) caused the formation of different compositions of the protein corona, biodistribution (e.g., accumulation in liver, lung, kidney) and biodegradation from different organs [85]. The proteins in the corona around these particles were involved in acute phase response, immune response, transport, coagulation, albumin, and apolipoproteins. For roadside PM2.5, the oxygen content of the surface could determine the amount of the adsorption of opsonizing proteins such as phospholipids in bronchoalveolar lavage fluids [86]. Thus, lung to brain penetrance of the particles depends on the chemical and physical characteristics of the particles.

Another question is what part of the lung sees the greatest concentration of nPM or other air pollution components. According to the literature, of inhaled nPM, approximately 30% settles in the alveoli, approximately 30% settles in the trachea and bronchi, and the rest is either exhaled or swallowed [87]. An important consideration then is the surface area. In humans, the upper airway (trachea and bronchi) is 0.25 m^2 versus 102 m^2 for alveolar (408 times the alveolar surface area) [88]. Thus, if the same number of particles are present in both the upper air-

way and alveoli, the concentration per surface area of particles is 408 times. The ratio of epithelial cells in the alveoli is estimated as only about 18 times as many alveolar versus airway epithelium [88]. Because the alveolar surface is a monolayer while the epithelium is multilayered, the concentration per exposed cell is also much greater in the upper airway than alveoli. In view of other air pollution components, most particles >6 μm will be deposited in the upper airway while particles >0.5 μm diameter will not enter the alveolus. For gases, the penetrance is dependent on the reactivity. For example, while O_3 may reach and damage the small airways and proximal alveoli, hyperoxia damages the distal alveoli [89]. Thus, a challenge for experimental biologists is to characterize the air pollution components based on penetrance in the lungs, accumulation in the brain, systemic responses, and the degree of toxicity.

A key unknown is how a direct penetrance of the particles into the brain can alter the neurotoxicity. The current body of literature suggests that both gas phase (e.g., O_3) and solid phase (e.g., PM0.2) of the air pollution can cause neurotoxic damage. The direct comparison of these components *in vivo* can inform us about the role of particle penetrance into the brain.

Another unresolved issue is how the immune system deals with the accumulated solid particles. How fast the body can clear the particles and if the clearance of the particles can attenuate the damage. If air pollution were sharply diminished, would there be recovery from prior damage? Accumulated uranium oxide particles in the brain are cleared after 3 days [62]. However, it is unknown if the heterogeneous urban PM2.5 will have the same clearance rate. Besides, chemical and physical characteristics of the PM can potentially affect the biodistribution and biodegradation of the particles. Resolving these remained questions can alter the perspectives of air pollution neurotoxicity and give us new insights to design proper mechanistic and intervention experiments.

What is known about the toxicology of individual components?

A large body of literature has identified toxic contributions of individual air pollution components such as metals (lead, iron, manganese) and organic components (black carbon, PAHs). As we noted above, air pollution has an extreme heterogeneity and undergoes continual change from diurnal cycles of ultraviolet, humidity and temperature. This constantly changing nature of air pollution limits identification of most individual toxic components. A historically important exception is the airborne lead (Pb) from gasoline additives which increased blood Pb levels for several decades with neurotoxic and teratogenic impacts [90, 91]. A major unknown is the extent of synergies, such as shown for interactions of air pollution PM2.5 with cigarette smoke [92]. There are also many specific industrial hazards including welding, refineries, and agricultural products. While a full review of these toxic chemicals is beyond our scope, we summarize some key findings relevant to ambient air pollution neurotoxicity in the adult brain.

Studies with adult rodents include iron (e.g., iron oxide, iron sulfate, ^{59}Fe), manganese (MnO_2 [93–95], ^{54}Mn[93]), chromium ($Cr(OH)_3$ [93]), Pb (PbO, [96–98], Pb acetate [99], Pb sulfate [100]), carbon tetrachloride [101], and some PAHs (e.g., benzo(a)pyrene [102, 103] and 2-aminoanthracene [104]). These studies confirm their accumulation and neurotoxicity. A further finding is that interactions of these toxicants can alter their biodistribution in the body tissues and their toxicity. For example, intratracheal instillation of MnO_2 (2–4 mg/kg, once/day, 5 days/week, 4 weeks) can cause body-weight loss, brain Mn accumulation, and impaired synaptic potentiation in the cerebral cortex of adult male rats [94]. However, co-administration of MnO2 with Fe_3O_4 or $Cr(OH)_3$ will ameliorate the toxicity and weight loss. Similarly, pre-inhalation treatment of the male rats with iron oxide (100 mg/m^3, 4 h/day, 4 day/2 weeks before intratracheal instillation for labeled elements) altered biodistribution of instilled ^{54}Mn or ^{59}Fe; the accumulation was increased in lungs, but decreased in brain [93]. In general, Mn particles are rapidly distributed in the brain than Fe particles, particularly through the olfactory tract [105, 106]. A comparison of PM samples with different levels of Fe and Mn can further inform us about the contribution of these toxicants in air pollution neurotoxicity.

The hippocampus merits particular attention for associations of toxicants with accelerated brain aging and AD. For example, systemic benzo(a)pyrene (BaP) (i.p. 0.02–200 mg/kg) caused increased BaP and metabolites in brain with wide ranging effects: anxiety behaviors were decreased together with complex glutamatergic changes in the hippocampus and cerebellum (increased NR1, but decreased NR2A, and NR2B) [102]. Inhalation of lead oxide (39 μg/m^3, constant for 11 weeks) causes spongiform degeneration and neuronal vacuolization in hippocampal CA2, and increased necrotic neurons in hippocampal CA1

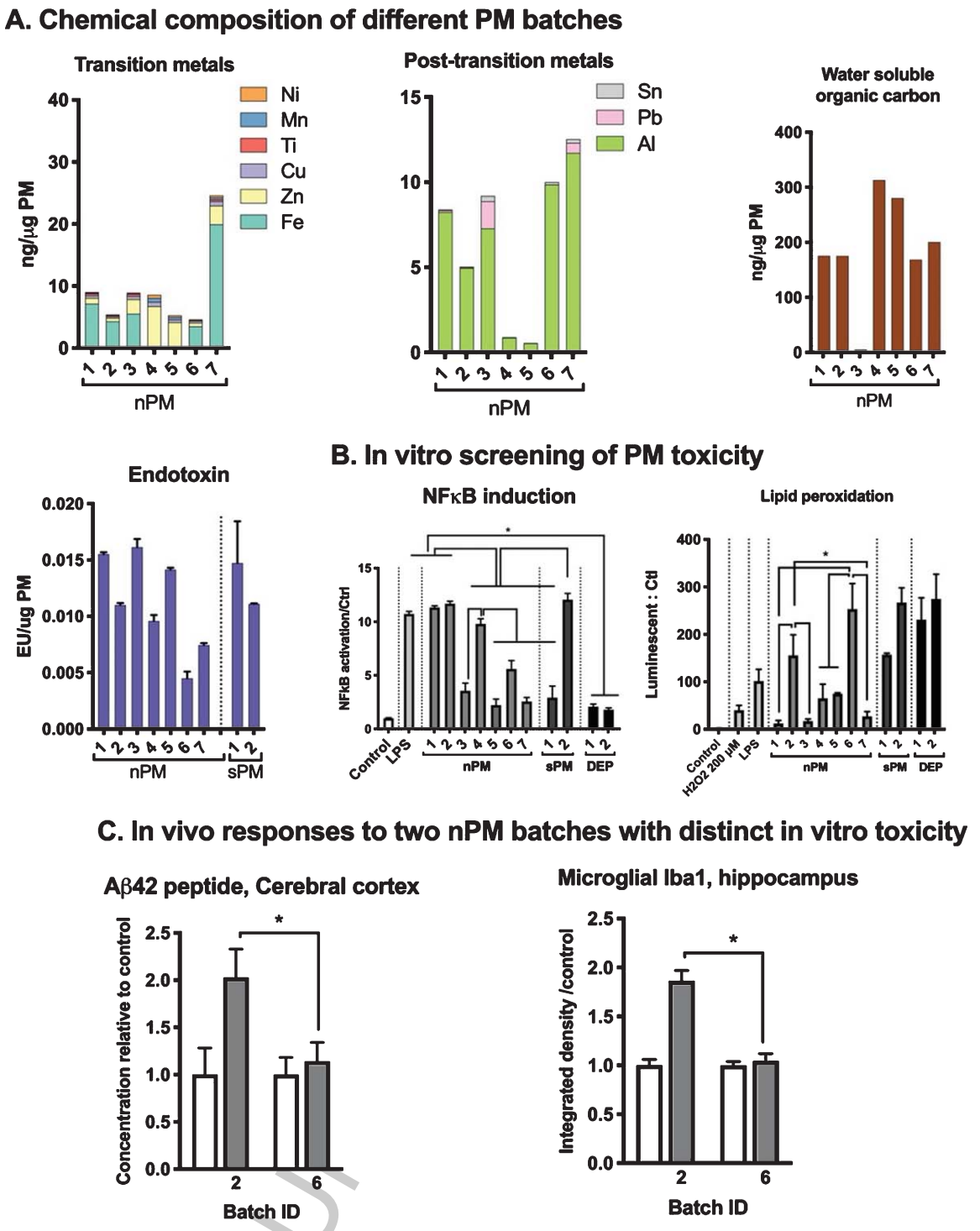

Fig. 7. PM samples are heterogenous in chemical composition, *in vitro* toxicity, and *in vivo* neurotoxic responses [10]. A) Heterogenous chemical composition of nPM batches collected from the same location at different times during 2016-2018. B) Cell-based assessment of different PM toxicity at the same mass concentration. NF-κB activity was assessed by a reporter assay in THP-1 monocyte cells. Lipid peroxidation was assessed by the DPPP assay in THP-1 monocyte. C) Responses in cerebral cortex $A\beta_{42}$ and hippocampal microgliosis after inhalation exposure to 300 μg/m3 of two different nPM batches for 8 weeks (5 h/day, 3 day/week). Figures adapted from [10].

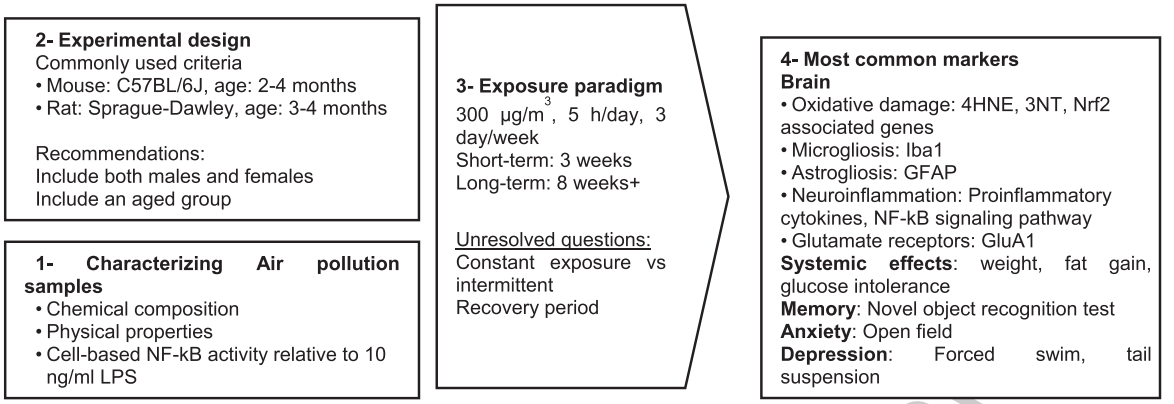

Fig. 8. Proposed standard protocol and common biomarkers to assess air pollution neurotoxicity in adult brain [10, 11, 15, 21, 22, 24–27, 31, 41, 69, 124].

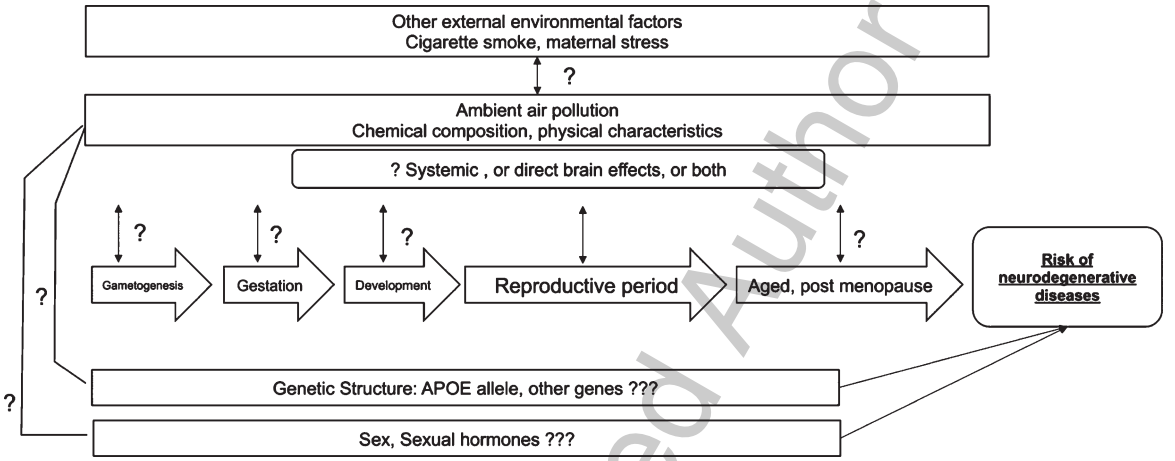

Fig. 9. A summary of complex interface of environmental pollutants, sex, genetic variants, and age in risk of neurodegenerative diseases.

of male adult mice [97]. Other effects of lead include oxidative damage, and increased lipid peroxidation in brain [96, 100], decreased vertical motility in open field test [98], and increased hippocampal L-type calcium channels [100]. Despite the exclusion of lead in automotive fuels, piston aircraft still permitted to use leaded gas in the U.S., with no safe lower level of blood lead [90]. Besides the toxicity of lead and BaP on the developing brain, we know little of their potential impact on brain aging and AD.

Air pollution neurotoxicity from the less studied sources

Much less is known about indoor air pollution, to which is attributed almost as much morbidity and mortality as outdoor air pollution and cigarettes (Table 1). The myriad indoor air pollutants include burning of charcoal wood and cow dung for cooking and heating. Biomass smokes from dung induced strong cell inflammatory responses *in vitro* [107].

Wildfire smoke is another less studied quandary with obscure neurotoxic effects. Simulation studies from the 2017 data suggested that wildfire contributed to 85% PM2.5 concentration across the Pacific Northwest during August to September [108]. The mortality estimates of the wildfire in this region indicated nearly 200 excess deaths during this period. The recently increasing frequency of wildland fires and expansion of wildland-urban interfaces also increases exposure to air pollution surges from the wildfire globally [109].

We need comprehensive comparisons by the same assays of airborne particles from coal, charcoal, cigarette, dung, and various woods. The pioneering study of Jin et al. indicated important variation

in oxidative activity between the domestic fuels and ambient air pollution in Chinese cities [110]. Thus, similar to ambient air pollution, the toxicity of biomass PM is dependent on the chemical composition of the pollutants. It is urgent that biologists design new experiments to resolve the toxic effects of these natural pollutants.

Developing a standardized exposure paradigm and experimental design

As described above, the diversity in the experimental designs confounded the direct comparability of the findings. For the first step, the field needs to have a better characterization of the air pollution samples. It is essential to develop a shared protocol to assess the bioactivity of air pollution samples prior to animal exposures. Our laboratory has added an additional screening step for NF-κB activity [10]. We compared the inflammatory activity of the collected PM samples with 10 ng/ml LPS treatment in THP-1 monocyte cells. Our result showed that the nPM batches with high *in vitro* NF-κB activity can cause greater microglial activation in the brain of the exposed animals (Fig. 7B, C). Thus, PM mass alone cannot adequately assess air pollution toxicity. Adapting this approach may increase the comparability of the findings by different research groups.

The field should also unify the experimental designs as we discussed in earlier sections. Exposure dosage, duration, delivery route, animal species, age, sex, and genotype are among the main factors that require further attention. Moreover, a selected damage marker, cognitive or behavioral tests are needed as a standard for air pollution studies. Figure 8 proposes an experimental paradigm that was piloted in our laboratory.

CONCLUSIONS

Our understanding of air pollution neurotoxicity is still immature and requires extensive research effort to resolve the complex facing questions. Air pollution neurotoxicity is shaped by a complex interface of environmental characteristics and the biological features of the affected individual (Fig. 9). We hope that summarizing the current experimental knowledge and facing gaps can help the field as a network to systematically approach the air pollution dilemma. Experimental biologists should also work closely with epidemiologists for validating the findings and accelerate the translation of our knowledge into the regulation. Moreover, understanding air pollution neurotoxicity can inform us about the underlying biological processing of aging, AD, and other neurodegenerative disorders.

DISCLOSURE STATEMENT

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/20-0377r1).

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