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IMMEDIATE COMMUNICATION

Air pollution impairs cognition, provokes depressive-like behaviors and alters hippocampal cytokine expression and morphology

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Particulate matter air pollution is a pervasive global risk factor implicated in the genesis of pulmonary and cardiovascular disease. Although the effects of prolonged exposure to air pollution are well characterized with respect to pulmonary and cardiovascular function, comparatively little is known about the impact of particulate matter on affective and cognitive processes. The central nervous system may be adversely affected by activation of reactive oxygen species and pro-inflammatory pathways that accompany particulate matter pollution. Thus, we investigated whether long-term exposure to ambient fine airborne particulate matter (< 2.5 μm ($PM_{2.5}$)) affects cognition, affective responses, hippocampal inflammatory cytokines and neuronal morphology. Male mice were exposed to either $PM_{2.5}$ or filtered air (FA) for 10 months. $PM_{2.5}$ mice displayed more depressive-like responses and impairments in spatial learning and memory as compared with mice exposed to FA. Hippocampal pro-inflammatory cytokine expression was elevated among $PM_{2.5}$ mice. Apical dendritic spine density and dendritic branching were decreased in the hippocampal CA1 and CA3 regions, respectively, of $PM_{2.5}$ mice. Taken together, these data suggest that long-term exposure to particulate air pollution levels typical of exposure in major cities around the globe can alter affective responses and impair cognition.
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Introduction

Air pollution is an environmental toxicant that comprises a complex mixture of particulate matter, gases, metals and organic compounds. Particulate matter pollution is heavily implicated in the potentiation of cardiopulmonary morbidity and mortality and remains a major modifiable global public health threat.[1,2] In many regions of the world, individuals are exposed to particulate air-pollution over their entire lives. Life-time exposure to environmental toxicants is difficult to study in humans, especially when environmental factors interact with other common and highly prevalent risk factors to potentiate disease. This can result in outcomes indistinguishable from other chronic disorders. Thus, the true health burden of particulate matter exposure may be largely underappreciated.

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Fine particles are characterized as < 2.5 μm ($PM_{2.5}$) and are emitted from combustion engine exhaust and industrial sources. The effects of $PM_{2.5}$ on peripheral systems have been extensively studied with both short- and long-term exposure implicated in major adverse cardiovascular events.[1,2] However, there are limited studies exploring the effect of long-term exposure to particulate matter in the context of the central nervous system (CNS). Activation of reactive oxygen species and pro-inflammatory pathways by $PM_{2.5}$ are thought to instigate maladaptive responses that may in turn adversely affect organ function. The CNS is likely not immune to the effects of $PM_{2.5}$,[3,4] suggesting investigation of the impact of $PM_{2.5}$ on the brain is warranted.

Peripheral immune activation[1,2] may lead to neuroinflammation in individuals exposed to $PM_{2.5}$ as multiple pathways exist through which inflammatory signals can be communicated from the periphery to the brain.[5] Inflammatory reactions in the brain are then mediated by cytokines, chemokines or oxidative stress and can result in aberrant protein aggregation, impaired neurotransmitter and neurotrophin signaling, neuronal remodeling, and neurodegeneration.[5]

The hippocampus is a brain region that is disproportionately vulnerable to injury and inflammation with abundant receptors for pro-inflammatory cytokines such as interleukin (IL)1β, IL6 and tumor necrosis factor α (TNFα), suggesting it may be a target of prolonged $PM_{2.5}$ exposure.[6] Long-term exposure to particulate matter may result in tonic upregulation of inflammatory markers exacerbating pre-existing neurodegenerative disorders such as Alzheimer disease.[7,8] Furthermore, emerging data support involvement of inflammation in the brain in the pathogenesis of affective disorders and impaired cognition.[9,10] For example, exaggerated inflammatory cytokine response in the hippocampus of aged mice after intraperitoneal injection of LPS is associated with architectural changes to dendrites of pyramidal neurons in the CA1 hippocampus.[11] Changes in CA1 morphology are associated with depression[12] and learning and memory.[13]

We hypothesized that long-term exposure to particulate matter would induce low grade, but prolonged, brain inflammation and adjustments in neuronal morphology and behavior. We employed an extended period of $PM_{2.5}$ exposure to evaluate how lifetime $PM_{2.5}$ may impact individuals as long-term exposure activates immune measures.[14] Four-week-old male C57BL/6 mice were ordered from Jackson Laboratories (Bar Harbor, ME, USA) and were exposed to either ambient concentrated $PM_{2.5}$ or filtered air (FA) for 6 h per day, 5 days per week from April 2009 to January 2010 in Columbus, Ohio, USA in a mobile trailer exposure system, 'Ohio Air Pollution Exposure System for Interrogation of Systemic Effects (OASIS) 1'.[15–17] Following 10-months of $PM_{2.5}$ or FA exposure, mice underwent a battery of behavioral tests assessing physical abilities, sensorimotor responses, learning and memory, and affective responses. One month after the conclusion of behavioral testing, brains were collected and processed for Golgi or flash frozen for PCR analyses.

Materials and methods

Animals

Four-week-old male C57BL/6 mice were ordered from Jackson Laboratories and were provided regular rodent chow and filtered water *ad libitum*. The Committee on Use and Care of Animals of the Ohio State University approved all experimental procedures.

Particulate matter exposure

Mice were randomly assigned a group and were exposed to either ambient concentrated $PM_{2.5}$ or FA during the light phase for 6 h per day, 5 days per week from April 2009 to January 2010 in Columbus, Ohio, USA in a mobile trailer exposure system, 'Ohio Air Pollution Exposure System for Interrogation of Systemic Effects (OASIS) 1'.[15–17] $PM_{2.5}$ and FA exposure were then discontinued for 3 weeks while mice underwent behavioral testing in order to eliminate any impact daily handling may have had on behavioral results. Following behavioral testing the mice were re-exposed to the respective air conditions for 1 month before tissue collection. The mean (s.d.) daily $PM_{2.5}$ concentration at the study site in Columbus, OH, was 12.66 (8.36) $\mu g\,m^{-3}$. The mean concentration of $PM_{2.5}$ in the exposure chamber was 94.38 $\mu g\,m^{-3}$ (~sevenfold higher concentration than ambient Columbus, Ohio levels). Because the mice were exposed for 6 h per day, 5 days per week, the equivalent $PM_{2.5}$ concentration to which the mice were exposed to in the chamber normalized over the 10-month period was 16.85 $\mu g\,m^{-3}$.

Behavioral testing

Physical measurements. Before behavioral testing mice underwent an initial assessment during which body weight, body length, vibrissae, eye appearance and muscle tone were evaluated. The two groups did not differ with respect to any of these variables and all mice appeared healthy (Supplementary Table S1). Following the initial assessment, sensory motor reflexes were tested. Visual acuity was evaluated by slowly lowering each mouse towards the edge of a table. A positive score of 1 was recorded if the animal extended its forepaws before touching the table, three trials were conducted and combined. Auditory orientation was evaluated by sounding a clicker 15 cm behind the mouse's head. A positive score was recorded if the animal turned its head towards the sound. Contact placing and orienting to touch of vibrissae was tested by lowering the mouse slowly towards the edge of a table until the vibrissae touch the edge. This test was conducted in a room illuminated by dim red light (3 lux) to prevent the use of visual cues. The reaction of the animal to a touch on the vibrissae was scored on a scale of 0 (no response) to 3 (maximal response).

Olfactory abilities were tested in a 2-day trial. On day one the mice were habituated to a novel cage with 2 cm of corn cob bedding with a piece of cookie hidden beneath. Mice were left to explore the cage for 10 min after which time the cookie was revealed if it had not been located. Mice were then food deprived for 24 h, after which time they were placed back in the chamber and tested for the latency to find the cookie (Supplementary Table S1).

Forelimb grip strength was assessed by suspending the mice by their forelimbs on a wire 60 cm above a foam pillow. The time (sec) until the mouse fell or 90 s passed was recorded (a score of zero was assigned to animals that fall immediately; a score of 90 is given to mice that do not fall). This test was repeated three times (Supplementary Table S1).

Motor skills were measured by testing the ability of each mouse to turn on an inclined screen. The mouse was placed at the center of a wire mesh screen (35 cm^2) that was tilted at a 45° angle. The mouse was placed on the screen facing downward, and the time taken by the mouse to turn and face upward was recorded within a 2 min period (Supplementary Table S1).

Mice were then trained and tested in a rotarod task. Mice were habituated to the rod while it slowly rotated (5.0 r.p.m.—rotations per min). Three 2-min habituation sessions were given, with 10 s between sessions. Mice were replaced on the rod if they fell off before 2 min passed. Testing consisted of three-trial sessions (two sessions per day); each session had a progressively increasing speed of rod rotation. On each of the three trials per session, the mouse was placed on the rod and left there until either 60 s elapsed or until the mouse fell off. There was a 10 s break between trials, and a 2 h break between sessions. Performance was measured as total time on the rod for the three-trial session at each speed (Supplementary Table S2).

Affective responses. To assess locomotor behavior and anxiety-like responses, mice were placed in a 40×40 cm clear acrylic chamber lined with clean corncob bedding, inside a ventilated cabinet (Med Associates, St Albans, VT, USA). Test chambers were rinsed with 70% ethanol between tests. A frame at the base of the chamber consisting of 32 photobeams in a 16×16 arrangement, in addition to a row of beams above, detected the location of horizontal movements and rearing, respectively (Open Field Photobeam Activity System, San Diego Instruments, San Diego, CA, USA). Total movement was tracked for 8 min and analyzed for the following: (1) the percentage of beam breaks in the center (inside 30×30 cm) of the open field, (2) number of rears and (3) total locomotor behavior.

To assess anxiety-like responses further, mice were tested in an elevated-plus maze apparatus. The maze is elevated 1 m above the floor and made of dark-tinted acrylic and consists of two open arms bisected by two arms enclosed by walls. Mice were placed in the central maze area facing a closed arm and recorded for 5 min. The maze was wiped with mild soapy water between tests. An open arm entry was scored when the mouse's entire body entered an open arm. A condition-blind observer using Observer software (Noldus, Leesburg, VA, USA) scored tapes for the following: (1) total time spent in the open arms and (2) number of open arm entries.

To assess depressive-like responses, mice were evaluated in the forced swim task. Mice were placed in room-temperature ($22 \pm 1\,^{\circ}$C) water $\sim$17 cm deep, within an opaque, cylindrical tank (diameter = 24 cm, height = 53 cm). Swimming behavior was recorded on video for 5 min and scored by a condition-blind observer with the Observer software for latency to float, total number of floating bouts and total time spent floating.

Learning and memory. Learning and memory was assessed in the Barnes maze as described previously.[18] Mice were placed in the center of a brightly lit arena and given 2 min to find an escape hole leading to a dark box. Brightly lit open spaces are generally considered aversive to mice, which motivates their escape. The maze has 18 evenly spaced holes but only one leads to the dark box. On the first day of testing, mice were acclimated to the maze; a bright light and loud fan were turned on and mice were guided from the center of the maze to the target hole. After entering the dark box the bright light and fan were turned off and the mouse was left undisturbed for 2 min. Mice received 5 days of training trials, consisting of one daily session of three trials. Each trial lasted 2 min or until the mouse found the escape hole where they were left for 1 min. After each trial the mouse was placed in its home cage for 10 min before beginning the next trial. During the training trials, latency to find the target hole and number of errors were scored. At 24 h after the last training trial, mice were given a 90 s probe trial in which the escape box was blocked off. Percent of visits to the target hole and latency to first reach the target hole were quantified.

Hippocampal morphological analyses

Mice were all killed between 0900 and 1100 hours eastern standard time, brains were removed and divided at the hemisphere, and half of six brains per group was processed for Golgi impregnation using the FD Rapid GolgiStain Kit (FD NeuroTechnologies, Ellicott City, MD, USA) according to the manufacturer's instructions. Brains were sliced at 80 μm, thaw mounted onto gelatin-coated slides that were coded in order to remove observer bias, counterstained with cresyl violet (Sigma, St Louis, MO, USA), dehydrated and coverslipped. Brains were assessed for hippocampal cell morphology and spine density in three hippocampal subfields: dentate gyrus, CA1 and CA3. Sections were visualized using a Nikon E800 bright-field microscope (Nikon, Burlington, VT, USA) and traced using Neurolucida software (MicroBrightField, Burlington, VT, USA) at a magnification of $200\times$ for neuronal morphology and $1000\times$ for spine density. Five representative neurons were selected per area, per animal for tracing. Selected neurons were fully impregnated and lacked truncated dendrites. Whole cell traces were analyzed using NeuroExplorer software, for cell body size and perimeter and dendritic length (MicroBrightField). Sholl analyses were conducted separately for apical and basilar dendrites. From each of the five neurons selected per animal four segments >20 μm were selected in the apical and basilar areas, respectively, (except in the dentate gyrus where granule cells lack bidirectional projections). All spine segments selected were at least 50 μm distal to the cell body. Spine density (spines per 1 μm) was calculated for each trace and averaged per cell, per area and per animal.

Quantitative real time PCR

Fresh tissue was collected, flash frozen and stored at $-80\,^{\circ}$C. Brains were thawed and hippocampi dissected out in sterile saline. Total RNA was extracted using a homogenizer (Ultra-Turrax T8, IKAWorks, Wilmington, NC, USA) and an RNeasy

Mini Kit (Qiagen, Gaithersburg, MD, USA). RNA was reverse transcribed into complementary DNA with M-MLV Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Gene expression for IL1β, TNFα, IL6, HMGB1, MAC1 and heme oxygenase 1 (HO1) was determined using inventoried primer and probe assays (Applied Biosystems, Foster City, CA, USA) on an ABI 7500 Fast Real Time PCR System using Taqman Universal PCR Master Mix. The universal two-step reverse transcriptase PCR cycling conditions used were as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Relative gene expression of individual samples run in duplicate was calculated by comparison to a relative standard curve and standardized by comparison to 18S rRNA signal.

Statistical analyses

Comparisons for behavior analyses, hormone concentrations and somatic measures were conducted using a one-way analysis of variance (ANOVA). Neuronal characteristics and spine densities were averaged per animal and then analyzed using a one-way ANOVA. Sholl analyses were also averaged per animal and then analyzed using a repeated measures ANOVA with air condition as the with-in subject factor and distance from the cell body as the between subject factor. For each Barnes maze session (1–4) three trials were conducted, and variables were averaged per session for each mouse. Repeated-measures ANOVA were then conducted with air condition as the with-in subject factor and session as the between subject factor to analyze latency to find the target hole and error rate. Following a significant result on repeated measures ANOVA, single time point comparisons were made using Tukey's HSD tests. PCR results were analyzed using a one-tailed *t*-test based on *a priori* hypotheses.[19] The above statistical analyses were conducted with StatView software (v. 5.0.1, StatView Inc., Cary, NC, USA). In all cases, differences between group means were considered statistically significant if $P \leqslant 0.05$.

Results

Body mass, body length, vibrissae, eye appearance, gross olfactory abilities, muscle tone, sensorimotor responses, motor performance and serum corticosterone concentrations did not differ between $PM_{2.5}$ and FA mice ($P > 0.05$ in each comparison; Supplementary Tables S1 and 2, and Supplementary Figure S3).

Learning and memory

$PM_{2.5}$ exposure impaired spatial learning in the Barnes maze. Both FA and $PM_{2.5}$ learned the maze as indicated by a decreased latency to reach the target hole and decreased visits to incorrect holes over the course of training ($F_{3,54} = 11.730$, $F_{3,54} = 10.899$; $P < 0.05$ respectively). However, during the training trials (the learning component of the Barnes maze), $PM_{2.5}$ mice impaired performance as compared with FA mice; $PM_{2.5}$ mice increased latency to reach the target hole over the course of the training relative to FA exposed mice ($F_{1,54} = 5.091$; $P < 0.05$; Figure 1a). Furthermore, $PM_{2.5}$ mice required more visits than FA mice to incorrect holes before reaching the target hole ($F_{1,54} = 5.066$; $P < 0.05$; Figure 1b).

Spatial memory was similarly impaired by $PM_{2.5}$ exposure. Following training, mice underwent a probe trial to evaluate retention of the task (memory component), in which the target box is removed and exploratory behavior is monitored on the maze for 90 s. $PM_{2.5}$ mice increased latency to first visit where the target hole previously was, and $PM_{2.5}$ mice had a lower percentage of visits to the target hole area ($F_{1,18} = 5.361$ and $F_{1,18} = 13.478$; $P < 0.05$, Figures 1c and d) demonstrating a decrease in memory retention.

Affective behaviors

Depressive-like responses were also evaluated using a modified version of the Porsolt forced swim test,[20] revealing increased behavioral despair among $PM_{2.5}$ mice. $PM_{2.5}$ mice elevated depressive-like behaviors with elevated floating frequency and duration in the forced swim test ($F_{1,19} = 5.957$ and $F_{1,19} = 8.995$; $P < 0.05$ respectively; Figures 2a and b). Furthermore, $PM_{2.5}$ mice had a lower latency than FA mice to first float, demonstrating they more rapidly reached a state of behavioral despair ($F_{1,19} = 4.827$; $P < 0.05$; Figure 2c). Although mice did not perform differently on other measures that require high levels of cardio and lung capacity, such as total activity in the open field and performance in the rotarod testing, differences in pulmonary function previously described following $PM_{2.5}$ exposure may have affected performance in the forced swim test.

$PM_{2.5}$ mice also demonstrated increased anxiety-like behavior as compared with FA mice in one task but not another. First, to test anxiety-like responses, we assessed the amount of time spent in the center of an open field. $PM_{2.5}$ mice reduced the percentage of activity in the center of the arena compared with FA mice in the 8 min test ($F_{1,19} = 4.982$; $P < 0.05$; Supplementary Figure S1), indicating elevated anxiety-like responses.[21] Rearing behavior and total activity were comparable between groups ($P > 0.05$). There were no significant differences between groups in the elevated-plus maze ($P > 0.05$; Supplementary Figure S2). In the elevated-plus maze reduced time spent in the open arms and a decreased number of open arm entries indicate reduced anxiety-like responses.

Gene expression

$PM_{2.5}$ mice upregulated basal expression of pro-inflammatory cytokine mRNA. A subset of the brain tissue was processed for quantitative real time PCR for pro-inflammatory cytokine expression in the hippocampus or hippocampal morphology analysis using the Golgi-Cox impregnation method. The mRNA for the proinflammatory cytokine TNFα was higher in $PM_{2.5}$ mice as compared with FA mice ($t_{12} = 2.228$, $P < 0.05$, Figure 3a). IL1β gene expression

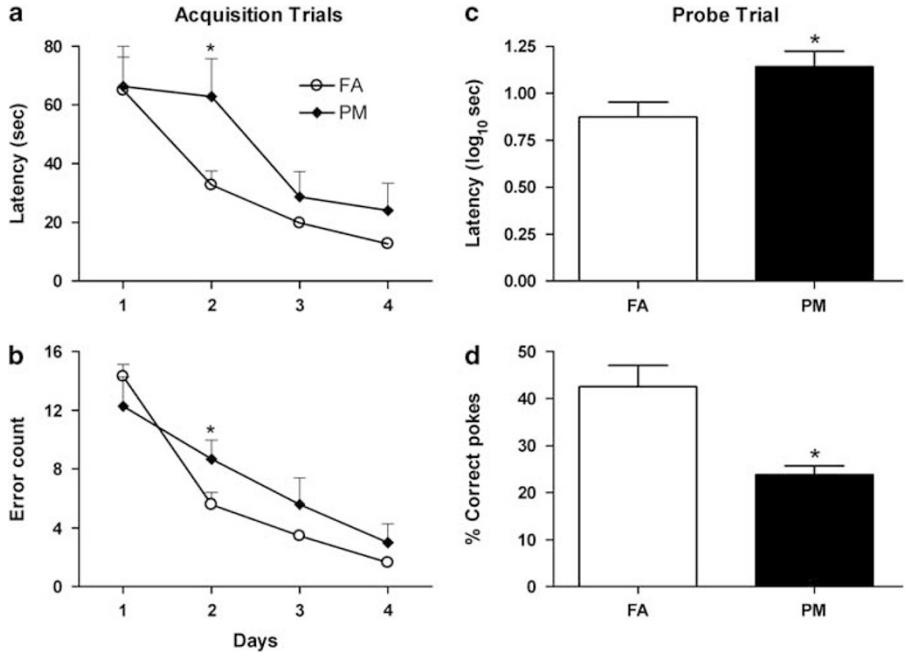

Figure 1 Effects of $PM_{2.5}$ exposure on spatial learning and memory as evaluated by the Barnes maze. (**a**) Mice exposed to $PM_{2.5}$ showed a decreased latency to reach the target hole over the course of the training trials demonstrating impaired learning ability. (**b**) During the training trials, $PM_{2.5}$ mice also had a higher number of visits than filtered air (FA) mice to incorrect holes before reaching the target hole, which is also indicative of impaired learning ability. (**c**) In the probe trial $PM_{2.5}$ mice had an increased latency to reach the target hole; a longer latency to reach the target hole in the probe trial is indicative of decreased memory retention. (**d**) Moreover, $PM_{2.5}$ mice reduced the percentage of visits to the target hole during the probe trial demonstrating a decrease in memory retention ($^*P<0.05$).

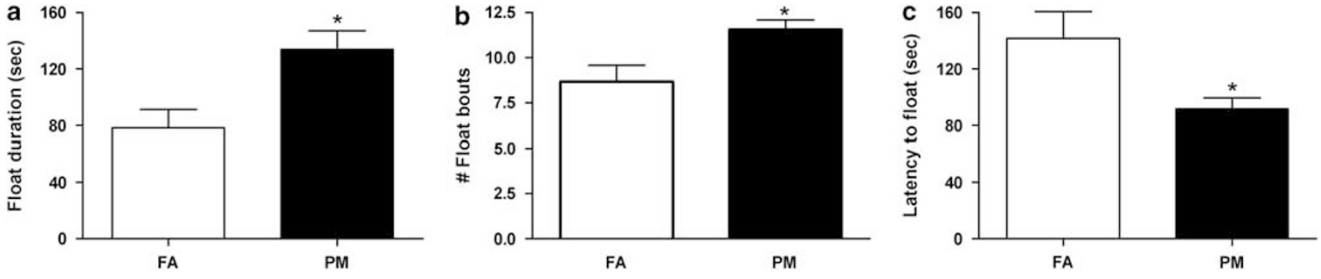

Figure 2 $PM_{2.5}$ exposure altered performance in a forced swim task. (**a**) $PM_{2.5}$ mice increased duration of time spent floating and (**b**) increased frequency of floating, which is consistent with a depressive-like state. (**c**) $PM_{2.5}$ mice also reduced the latency to first float demonstrating a rapid attainment of behavioral despair ($^*P<0.05$).

was also upregulated in $PM_{2.5}$ mice ($t_{12}=1.918$, $P<0.05$, Figure 3b). IL6 and HMGB1, other inflammatory markers, were unaffected by air quality conditions ($P>0.05$; Figures 3c and d).

HO1 expression was also elevated in $PM_{2.5}$ exposed mice ($t_{11}=2.340$, $P<0.05$; Figure 3e). HO1 is induced in response to environmental perturbations such as oxidative stress, hypoxia, heavy metals and cytokines. MAC1, which is a microglial marker, had comparable expression levels between FA and $PM_{2.5}$ mice ($P>0.05$; Figure 3f).

Hippocampal morphology

Prolonged exposure to $PM_{2.5}$ is associated with changes in neuronal morphology in the CA1 and CA3 regions of the hippocampus. $PM_{2.5}$ exposure altered apical spine density in the CA1 region of the hippocampus; such that, spine density was reduced among $PM_{2.5}$ mice as compared with FA mice ($F_{1,9}=12.748$; $P<0.05$; Figure 4a). Groups did not differ with respect to basilar spine density in the CA1 or spine density in the CA3 or dentate gyrus (Supplementary Table S3). Raw dendritic length, as well as dendritic complexity of pyramidal neurons in the CA3, differed between groups. $PM_{2.5}$ exposure decreased apical dendritic length ($F_{1,8}=5.945$; $P<0.05$; Figure 4c) and reduced cell complexity as determined by Sholl analysis ($F_{40,280}=2.961$; $P<0.05$; Figure 4e). Groups did not differ with respect to raw basilar dendritic length or basilar complexity.

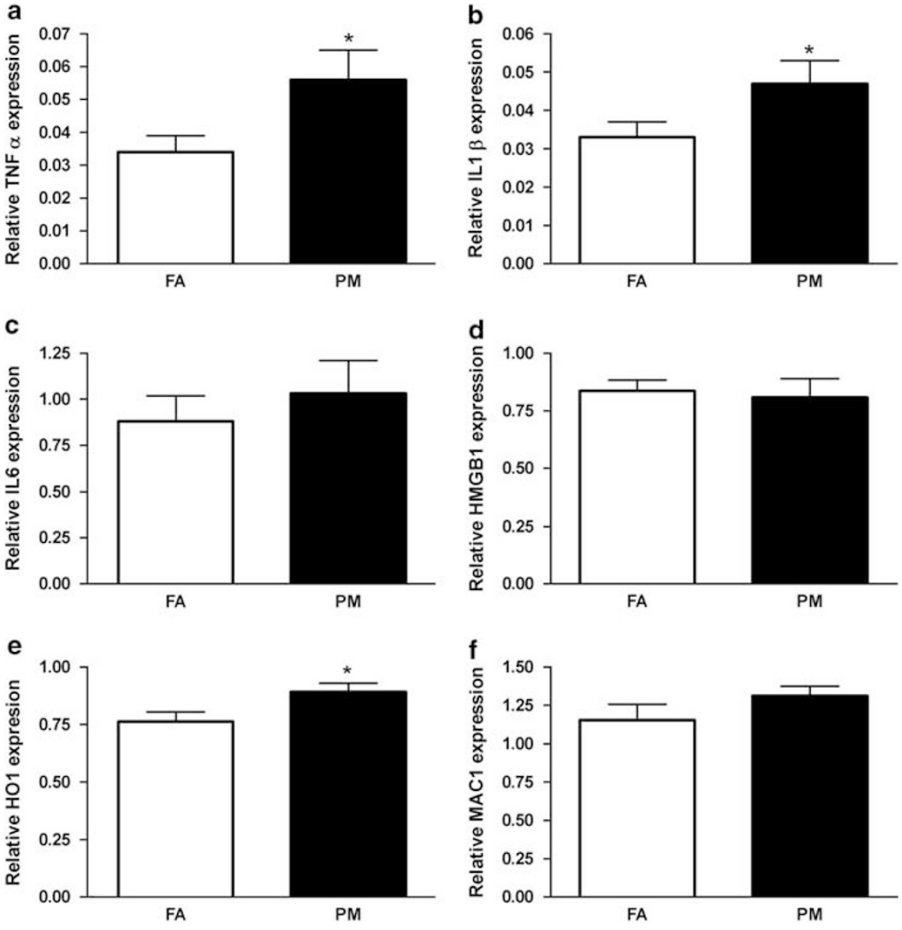

Figure 3 Effects of $PM_{2.5}$ exposure on inflammatory cytokine expression in the hippocampus. Relative gene expression of (**a**) tumor necrosis factor (TNF)-α, (**b**) interleukin (IL)1-β, (**c**) IL6, (**d**) HMGB1, (**e**) H01 and (**f**) MAC1 (*$P<0.05$).

Furthermore, there were no differences in cell body area (Supplementary Table S3).

Discussion

Here we report that 10 months of exposure to airborne fine particulate matter leads to upregulation of inflammatory markers and hippocampal structural changes resulting in depressed-like affective responses and cognitive impairment in mice. Epidemiological, as well as *in vivo* and *in vitro* exposure studies, suggest exposure to air pollutants upregulates expression of pro-inflammatory cytokines.[22–24] However, previous reports of the effects of particulate matter exposure on neuroinflammation reported differences in anterior brain[25,26] or used a double hit of pollution exposure plus an additional systemic inflammatory stimulus such as lipopolysaccharide.[4] Furthermore, more limited $PM_{2.5}$ exposure used in other studies (2–4 weeks as compared with 10 months in our study) may not have led to basal increases in inflammatory mediators.

The mRNA for the proinflammatory cytokines TNFα and IL1β were elevated in $PM_{2.5}$ mice as compared with FA mice (Figures 3a and b). Hippocampal inflammation is associated with reduced neurogenesis and changes in neuronal morphology and may be associated with cognitive dysfunction in aging, dementia, epilepsy and other neurodegenerative disorders.[9,11,27] Furthermore, IL1 β in particular has been associated with reduced hippocampal plasticity and related changes in depressive-like behaviors.[28,29] HO1 expression was also elevated in $PM_{2.5}$-exposed mice (Figure 3e) further indicating perturbation in the immune system in the CNS of $PM_{2.5}$ mice. HO1 gene expression is very sensitive to pro-oxidant and other stressors and is responsive to cytokine induction.[30] There is also intense immunoreactivity of HO1 in neurons of the hippocampus in patients with Alzheimer disease.[31]

Peripheral immune activation in tissues such as those that comprise the pulmonary and cardiovascular systems[1,2] may lead to the neuroinflammation in mice exposed to $PM_{2.5}$. Exposure to PM results in increasing levels of circulating inflammatory mediators such as cytokines, leukocytes, C-reactive protein, fibrinogen and granulocyte macrophage colony-stimulating factor.[32,33] Recent work suggests that the pulmonary system may be responsible for potentiating these inflammatory mediators as IL6 generated in the

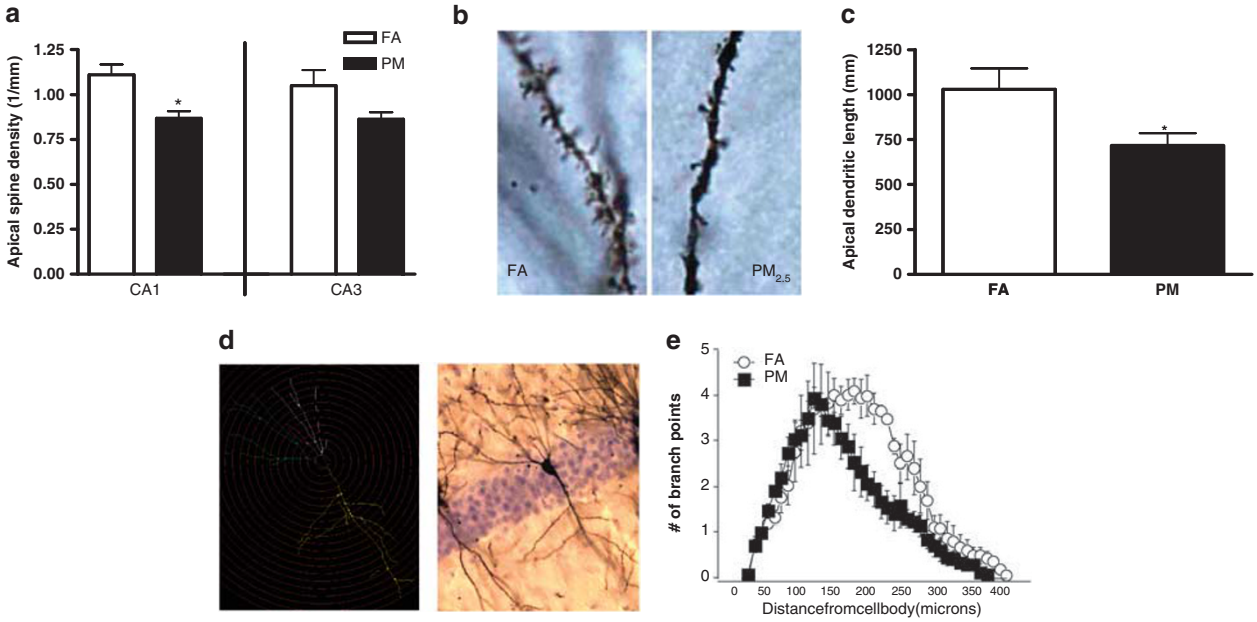

Figure 4 Effects of $PM_{2.5}$ exposure on hippocampal neuron morphology. (**a**) Spine density of apical tips of pyramidal dendrites in CA1 and CA3 regions. (**b**) Representative photomicrographs of apical spines in the CA1 of filtered air (FA) (left) and $PM_{2.5}$ (right) mice; magnification $\times 1000$. (**c**) $PM_{2.5}$ mice had reduced total apical dendritic length. (**d**) Photomicrograph of a pyramidal neuron in the CA3 and corresponding tracing with Sholl from Neuroexplorer; magnification $\times 200$. (**e**) Sholl analysis revealed that $PM_{2.5}$ mice had reduced branch points in the distal ends of apical dendrites ($^*P < 0.05$).

lungs in response to PM exposure is translocated into systemic circulation.[34] There are multiple pathways through which inflammatory signals can then be communicated from the periphery to the brain, including diffusion of cytokines across blood brain barrier (BBB)-deficient areas such as the choroid plexus, activation of BBB endothelial cells, which leads to production of soluble factors that can directly or indirectly (through microglia/astrocytes) activate neurons, or via vagal afferents.[5]

The relationship between immune activation and neural plasticity has been reported in other contexts. For instance, in aged mice and animal models of Alzheimer disease, hippocampal morphology is altered in response to peripheral immune stimulation.[11] Here, we report that prolonged exposure to $PM_{2.5}$ causes an upregulation of inflammatory gene expression that is associated with changes in neuronal morphology in the CA1 and CA3 regions of the hippocampus. Apical spine density in the CA1 region of the hippocampus was reduced among $PM_{2.5}$ mice as compared with FA mice (Figure 4a). Immune activation leads to changes in pyramidal neurons in the CA1 hippocampus and cognitive deficits,[11,35] which suggests that prolonged low-grade inflammation triggered by exposure to $PM_{2.5}$ may have contributed to the changes in the CA1. $PM_{2.5}$ exposure also decreased apical dendritic length (Figure 4c) and reduced cell complexity in the CA3 region of the hippocampus (Figure 4e). Changes in CA3 morphology have previously been reported with chronic low-grade inflammation and associated with changes in learning and memory.[36] The CA3 region of the hippocampus is particularly sensitive to glucocorticoids, with stress leading to decreased dendritic artborization, which is associated with depressive-like behaviors in animal models.[37,38]

$PM_{2.5}$ mice exhibited impaired learning and memory perfomance as evaluated in the Barnes maze,[18] with increased latency to reach the target hole and increased errors over the course of training and in the probe trial (Figure 1). Depressive-like responses were also evaluated using a modified version of the Porsolt forced swim test,[20] revealing increased behavioral despair among $PM_{2.5}$ mice. Cognitive impairment and depression are often comorbid and related to changes in the hippocampus in both humans and rodent models.[39] These results begin to address recent findings in humans that demonstrate an interconnected relationship among air pollution exposure, asthma risk and changes in mood. PM exposure is implicated in potentiating asthma and other cardiopulmonary conditions, and high airway inflammation and asthma are associated with negative mood.[40,41] There is also a growing body of literature implicating second hand smoke exposure to depressive symptoms.[42,43] Furthermore, exposure to air pollution is directly associated with emergency department visits for suicide attempts.[44] One limitation of this study is that the effects of PM exposure were only evaluated in male mice. Future studies investigating the effects of pollution exposure on the female population are warranted, especially because depression disproportionately affects women.[45,46]

$PM_{2.5}$ mice increased anxiety-like responses compared with FA mice in one task, but not another. $PM_{2.5}$ mice reduced the percentage of activity in the center of an open field compared with FA mice, which is generally interpreted as (Supplementary Figure S1) elevated anxiety-like responses;[21] however, there were no significant differences between groups in the elevated-plus maze (Supplementary Figure S2). Although behavioral tests were conducted in an order we judged to be from least to most demanding to minimize potential order effects, testing order may have contributed to the equivocal results.[47]

In conclusion, exposure to particulate matter pollution levels over prolonged duration and at levels typical of cities in parts of the developing world and countries such as China and India may result in neuroinflammation, altered morphological characteristics in hippocampal neurons, changes in affective behaviors and decreased cognitive abilities in mice. Airborne particulate exposure has important implications for human health and characterization of its effects at regulated levels is critical. Our results suggest $PM_{2.5}$ exposure leads to a tonic upregulation of inflammatory markers that may have a contributory role in neurodegenerative disorders. Due to the complex nature of airborne particulate matter both direct and indirect mechanisms may act synergistically leading to CNS pathology.[1] Further characterization of the pathways involved in particulate matter pathology is needed in order to work towards preventing CNS damage provoked by polluted air.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Mills NL, Donaldson K, Hadoke PW, Boon NA, MacNee W, Cassee FR *et al.* Adverse cardiovascular effects of air pollution. *Nat Clin Pract Cardiovasc Med* 2009; **6**: 36–44.
2 Brook RD, Rajagopalan S, Pope III CA, Brook JR, Bhatnagar A, Diez-Roux AV *et al.* Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation* 2010; **121**: 2331–2378.
3 Campbell A, Oldham M, Becaria A, Bondy SC, Meacher D, Sioutas C *et al.* Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. *Neurotoxicology* 2005; **26**: 133–140.
4 Win-Shwe TT, Yamamoto S, Fujitani Y, Hirano S, Fujimaki H. Spatial learning and memory function-related gene expression in the hippocampus of mouse exposed to nanoparticle-rich diesel exhaust. *Neurotoxicology* 2008; **29**: 940–947.
5 Teeling JL, Perry VH. Systemic infection and inflammation in acute CNS injury and chronic neurodegeneration: underlying mechanisms. *Neuroscience* 2009; **158**: 1062–1073.
6 Maier SF, Watkins LR. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychol Rev* 1998; **105**: 83–107.
7 Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW *et al.* Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. *Faseb J* 2005; **19**: 1329–1331.
8 Cunningham C, Campion S, Lunnon K, Murray CL, Woods JF, Deacon RM *et al.* Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. *Biol Psychiatry* 2009; **65**: 304–312.
9 Clark IA, Alleva LM, Vissel B. The roles of TNF in brain dysfunction and disease. *Pharmacol Ther* 2010; **128**: 519–548.
10 Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 2006; **27**: 24–31.
11 Richwine AF, Parkin AO, Buchanan JB, Chen J, Markham JA, Juraska JM *et al.* Architectural changes to CA1 pyramidal neurons in adult and aged mice after peripheral immune stimulation. *Psychoneuroendocrinology* 2008; **33**: 1369–1377.
12 Bedrosian TA, Fonken LK, Walton JC, Haim A, Nelson RJ. Dim light at night provokes depression-like behaviors and reduces CA1 dendritic spine density in female hamsters. *Psychoneuroendocrinology* 2011 (in press).
13 Moser MB, Trommald M, Andersen P. An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc Natl Acad Sci USA* 1994; **91**: 12673–12675.
14 Xu X, Kherada N, Hong X, Quan C, Zheng L, Wang A *et al.* Diesel exhaust exposure induces angiogenesis. *Toxicol Lett* 2009; **191**: 57–68.
15 Xu X, Yavar Z, Verdin M, Ying Z, Mihai G, Kampfrath T *et al.* Effect of early particulate air pollution exposure on obesity in mice: role of p47phox. *Arterioscler Thromb Vasc Biol* 2010; **30**: 2518–2527.
16 Laing S, Wang G, Briazova T, Zhang C, Wang A, Zheng Z *et al.* Airborne particulate matter selectively activates endoplasmic reticulum stress response in the lung and liver tissues. *Am J Physiol Cell Physiol* 2010; **299**: C736–C749.
17 Ying Z, Yue P, Xu X, Zhong M, Sun Q, Mikolaj M *et al.* Air pollution and cardiac remodeling: a role for RhoA/Rho-kinase. *Am J Physiol Heart Circ Physiol* 2009; **296**: H1540–H1550.
18 Sunyer B, Patil S, Hoger H, Lubec G. Barnes maze, a useful task to assess spatial reference memory in the mice. *Nat Protoc* 2007.
19 Keppel G, Wickens T. *Design and Analysis: A Researcher's Handbook*, 4th edn, Prentice Hall: Upper Sadle River, NJ, 2004.
20 Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 1977; **229**: 327–336.
21 Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N *et al.* Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berl)* 1997; **132**: 107–124.
22 Sama P, Long TC, Hester S, Tajuba J, Parker J, Chen LC *et al.* The cellular and genomic response of an immortalized microglia cell line (BV2) to concentrated ambient particulate matter. *Inhal Toxicol* 2007; **19**: 1079–1087.
23 Calderon-Garciduenas L, Solt AC, Henríquez-Roldán C, Torres-Jardón R, Nuse B, Herritt L *et al.* Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. *Toxicol Pathol* 2009; **36**: 289–310.
24 Calderon-Garciduenas L, Reed W, Maronpot RR, Henríquez-Roldán C, Delgado-Chavez R, Calderón-Garcidueñas A *et al.* Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. *Toxicol Pathol* 2004; **32**: 650–658.
25 Tin-Tin-Win-Shwe, Mitsushima D, Yamamoto S, Fukushima A, Funabashi T, Kobayashi T *et al.* Changes in neurotransmitter levels and proinflammatory cytokine mRNA expressions in the mice olfactory bulb following nanoparticle exposure. *Toxicol Appl Pharmacol* 2008; **226**: 192–198.

26 Calderon-Garciduenas L, Azzarelli B, Acuna H, Garcia R, Gambling TM, Osnaya N *et al.* Air pollution and brain damage. *Toxicol Pathol* 2002; **30**: 373–389.
27 Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O. Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci USA* 2003; **100**: 13632–13637.
28 Koo JW, Duman RS. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci USA* 2008; **105**: 751–756.
29 Goshen I, Kreisel T, Ben-Menachem-Zidon O, Licht T, Weidenfeld J, Ben-Hur T *et al.* Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol Psychiatry* 2008; **13**: 717–728.
30 Schipper HM, Song W, Zukor H, Hascalovici JR, Zeligman D. Heme oxygenase-1 and neurodegeneration: expanding frontiers of engagement. *J Neurochem* 2009; **110**: 469–485.
31 Schipper HM, Cisse S, Stopa EG. Expression of heme oxygenase-1 in the senescent and Alzheimer-diseased brain. *Ann Neurol* 1995; **37**: 758–768.
32 van Eeden SF, Hogg JC. Systemic inflammatory response induced by particulate matter air pollution: the importance of bone-marrow stimulation. *J Toxicol Environ Health A* 2002; **65**: 1597–1613.
33 van Eeden SF, Tan WC, Suwa T, Mukae H, Terashima T, Fujii T *et al.* Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). *Am J Respir Crit Care Med* 2001; **164**: 826–830.
34 Kido T, Tamagawa E, Bai N, Suda K, Yang HH, Li Y *et al.* Particulate matter induces translocation of IL-6 from the lung to the systemic circulation. *Am J Respir Cell Mol Biol* 2011; **44**: 197–204.
35 Richwine AF, Sparkman NL, Dilger RN, Buchanan JB, Johnson RW. Cognitive deficits in interleukin-10-deficient mice after peripheral injection of lipopolysaccharide. *Brain Behav Immun* 2009; **23**: 794–802.
36 Hauss-Wegrzyniak B, Dobrzanski P, Stoehr JD, Wenk GL. Chronic neuroinflammation in rats reproduces components of the neurobiology of Alzheimer's disease. *Brain Res* 1998; **780**: 294–303.
37 Magarinos AM, McEwen BS, Flugge G, Fuchs E. Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *J Neurosci* 1996; **16**: 3534–3540.
38 Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* 2008; **33**: 88–109.
39 Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS. Hippocampal volume reduction in major depression. *Am J Psychiatry* 2000; **157**: 115–118.
40 Kullowatz A, Rosenfield D, Dahme B, Magnussen H, Kanniess F, Ritz T. Stress effects on lung function in asthma are mediated by changes in airway inflammation. *Psychosom Med* 2008; **70**: 468–475.
41 de Miguel Diez J, Hernández Barrera V, Puente Maestu L, Carrasco Garrido P, Gómez García T, Jiménez García R. Psychiatric comorbidity in asthma patients. Associated factors. *J Asthma* 2011; **48**: 253–258.
42 Bandiera FC, Arheart KL, Caban-Martinez AJ, Fleming LE, McCollister K, Dietz NA *et al.* Secondhand smoke exposure and depressive symptoms. *Psychosom Med* 2011; **72**: 68–72.
43 Bandiera FC, Caban-Martinez AJ, Arheart KL, Davila EP, Fleming LE, Dietz NA *et al.* Secondhand smoke policy and the risk of depression. *Ann Behav Med* 2010; **39**: 198–203.
44 Szyszkowicz M, Willey JB, Grafstein E, Rowe BH, Colman I. Air pollution and emergency department visits for suicide attempts in vancouver, Canada. *Environ Health Insights* 2010; **4**: 79–86.
45 Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev* 2010; **35**: 565–572.
46 Kessler RC, McGonagle KA, Swartz M, Blazer DG, Nelson CB. Sex and depression in the National Comorbidity Survey. I: lifetime prevalence, chronicity and recurrence. *J Affect Disord* 1993; **29**: 85–96.
47 McIlwain KL, Merriweather MY, Yuva-Paylor LA, Paylor R. The use of behavioral test batteries: effects of training history. *Physiol Behav* 2001; **73**: 705–717.

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