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Prenatal exposure to particulate matter impairs offspring behavior via hippocampal NMDA receptor reduction: an *in vivo* and *ex vivo* study

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ABSTRACT

Accumulating epidemiological and experimental evidence implicates environmental particulate matter (PM) exposure as a risk factor for neurodevelopmental and neurodegenerative disorders. Because the developing brain is highly vulnerable to environmental toxicants, PM exposure during early pregnancy can exert disproportionate toxicity in offspring. However, systemic physiological effects of PM exposure can complicate the interpretation of neuron-specific susceptibility, and the susceptible brain cell types and molecular mechanisms underlying PM-induced neurotoxicity remain unclear. Here, we addressed this gap by investigating the neurotoxicity of PM exposure with a focus on specific brain cell populations. Prenatal intranasal administration of urban PM (200 and 400 µg/kg) induced abnormal offspring behaviors, including cognitive deficits, hyperactivity, and anxiety. Notably, exposure to 200 µg/kg PM elicited neurobehavioral abnormalities without affecting development or lung function. Transcriptomic analyses showed that PM altered hippocampal gene expression related to neuronal development and synaptic organization, potentially contributing to these behavioral deficits. Furthermore, PM reduced hippocampal expression of N-methyl-D-aspartate (NMDA) receptors, key glutamatergic receptors essential for neuronal development and function. *Ex vivo* hippocampal neuron cultures demonstrated that prenatal PM exposure (200 µg/kg) reduced NMDA receptor expression and disrupted spontaneous neuronal activity and synaptic networks, as revealed by imaging of pHluorin-tagged NMDA receptor subunits and electrophysiological recordings. By contrast, glial inflammatory responses remained unchanged. These adverse effects were recapitulated by prenatal exposure to benzo[a]pyrene (BaP), one of the most toxic polycyclic aromatic hydrocarbons in PM. Importantly, application of NMDA restored aberrant neuronal activity in PM- and BaP-exposed neurons. Our findings demonstrate the heightened vulnerability of the developing brain to PM exposure and underscore the need for effective strategies to mitigate PM-induced neurotoxicity.

1. Introduction

Ambient fine particulate matter (PM) is a major environmental and public health concern worldwide, consistently linked to human disease (Anderson et al., 2012) and implicated in millions of deaths annually (Collaborators, 2017). The World Health Organization (WHO) reports that more than 90% of the global population lives in areas where PM

concentrations exceed the WHO air quality guideline of 5 µg/m³ (WHO, 2021; WHO, 2024).

PM is classified by aerodynamic diameter: PM₁₀ (<10 µm), PM_{2.5} (<2.5 µm), PM_{1.0} (<1 µm), and ultrafine PM (<0.1 µm). PM readily traverses biological membranes and distributes systemically, where it induces toxicity and contributes to a range of pathophysiological conditions, including respiratory, cardiovascular, and cerebrovascular

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diseases (Li et al., 2019a; Mannucci et al., 2019; Yu et al., 2022). Because smaller particles can cross the blood–brain barrier (BBB), recent studies have focused on their neurotoxic effects and the underlying mechanisms in the brain.

Epidemiological studies worldwide demonstrate strong associations between PM exposure and neurodevelopmental, neurological, and neurodegenerative disorders, including cognitive decline (Fu et al., 2019; Lee et al., 2023). The developing brain is especially susceptible to environmental pollutants, and maternal PM exposure during pregnancy or childhood has been linked to significant brain damage in offspring (Clifford et al., 2016; Sram et al., 2017). Epidemiological evidence further indicates that PM exposure is an environmental risk factor for neurodevelopmental disorders such as autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), and anxiety (Lin et al., 2022), as well as behavioral problems in children (Mortamais et al., 2019). Additionally, prenatal or childhood PM exposure impairs intelligence and cognitive performance (Chiu et al., 2016; Morgan et al., 2023; Sunyer et al., 2015) and induces neurobehavioral deficits accompanied by structural brain changes (Cserbik et al., 2020).

Distinguishing direct neurotoxic effects from secondary systemic impacts and elucidating cell type-specific toxicity is crucial for identifying underlying mechanisms. However, biological evidence remains limited by the lack of fluorescently labeled PM, comparative analyses in other organs, and studies that target specific brain cell types. Recent reports have increasingly described direct adverse effects of PM on the brain. PM can deposit in the brain via the olfactory bulb (Calderon-Garciduenas et al., 2003; Maher et al., 2016) or disrupt the BBB and neurovascular unit (You et al., 2022), leading to neuroinflammation (Block and Calderon-Garciduenas, 2009; Calderon-Garciduenas et al., 2008a). PM exposure also induces mitochondrial dysfunction (Wang et al., 2019), causes ultrastructural myelin changes (Zhang et al., 2018b), and alters the expression of transcription factors required for synaptic development (Hou et al., 2023).

In mice, the BBB matures by gestational day (GD) 15.5 (Haddad-Tovoli et al., 2017), suggesting that PM exposure during early pregnancy (before GD 15) may allow coarse PM to penetrate the BBB and exert neurotoxic effects. Recent studies indicate that even oral ingestion of PM can induce neurotoxic effects in offspring (Ruiz-Sobremazas et al., 2025; Ruiz-Sobremazas et al., 2024), indicating that multiple exposure routes contribute to PM-induced neurotoxicity. Nevertheless, it remains unclear whether PM-induced brain damage results from direct neurotoxicity or indirect effects of systemic inflammation (You et al., 2022). Moreover, the molecular targets underlying PM neurotoxicity are largely undefined (Liang et al., 2023), underscoring the need to identify specific mechanisms and develop strategies to prevent PM-induced neurodevelopmental and neurodegenerative disorders (Lee et al., 2023).

NMDA receptors are essential for brain development and function, including synaptogenesis, synaptic plasticity and maturation, long-term potentiation (LTP), cognitive function, and neural network formation (Dupuis et al., 2023). Many mammalian neurons pass through a developmental stage that depends critically on NMDA receptor activation (Ikonomidou et al., 1999). In the developing brain, glutamatergic synapses predominantly express NMDA receptors and few AMPA receptors; therefore, synaptic activity development is mainly mediated by NMDA receptors (Isaac et al., 1997; Liao et al., 1999). Reductions in NMDA receptors or mutations in NMDA receptor genes are associated with abnormal brain or synaptic development and neurodevelopmental or neurological disorders, such as intellectual disability, developmental delay, epilepsy, autism spectrum disorder, and schizophrenia (Tumdam et al., 2024; Zhang et al., 2022). In this study, we investigated the toxic effects of PM on the developing brain through comparative analyses of the brain and peripheral organs. We also examined underlying mechanisms *in vivo* and *ex vivo*, focusing on hippocampal NMDA receptor expression.

2. Materials and methods

2.1. Animal handling and treatment

All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Chung-Ang University (protocol number: 2024–01030022). C57BL/6J mice (GD 8) were purchased from DBL (Chungchungbuk-do, Korea) and housed under standard laboratory conditions (temperature 22 ± 3 °C, humidity $55 \pm 5\%$) with a 12 h light/dark cycle and *ad libitum* access to food and water. Animal numbers and group sizes were determined based on ethical, welfare, and statistical considerations. Pregnant mice were randomly assigned to treatment groups and intranasally instilled with reagents ($n = 15$ for 10 μ L normal saline [vehicle, Veh], $n = 15$ for PM 200 μ g/kg in 10 μ L saline, $n = 15$ for PM 400 μ g/kg in 10 μ L saline) once at GD 9, 12, and 15, a critical window for neurodevelopment and susceptibility to PM exposure during gestation (Meredith, 2015; Perera et al., 2024).

PM doses, exposure frequency, and schedules were based on previous studies (Onoda et al., 2021; Tosevska et al., 2022). Approximately, the PM dose range of 100–200 μ g/m³ was selected based on PM concentrations reported in polluted cities (Lv et al., 2017) and indoor environments from a literature review (Morawska et al., 2017), where concentrations often exceed 300 μ g/m³ (Patel et al., 2020). Mice have a respiratory frequency of 163 (84–230) breaths/min and a tidal volume of 0.15 (0.09–0.23) mL (Guyton, 1947); accordingly, the daily ventilated PM mass is 3.5–7.0 μ g per mouse. Therefore, the estimated PM exposure in mice is 140–280 μ g/kg (assuming a 25 g body weight). In our study, the low-dose exposure (200 μ g/kg) approximates the assumed relevant human exposure range, whereas the high-dose exposure (400 μ g/kg) remains within the maximum levels reported for polluted outdoor or indoor environments.

Randomly assigned pregnant mice were orally administered benzo[a]pyrene (BaP) ($n = 10$ for 100 μ L DMSO [Veh], $n = 10$ for BaP 100 μ g/kg in 100 μ L DMSO, $n = 10$ for BaP 200 μ g/kg in 100 μ L DMSO) once at GD 9, 12, and 15. The exposure route and doses were selected based on previous studies that investigated neurotoxic effects of prenatal BaP exposure (Liu et al., 2020; McCallister et al., 2008; Yang et al., 2022).

Pregnant mice delivered 6–10 pups on GD 21, and no pup mortality was observed following PM or BaP exposure (pups born alive; Veh, $n = 297$; PM 200 μ g/kg, $n = 213$; PM 400 μ g/kg, $n = 184$; BaP 100 μ g/kg, $n = 66$; BaP 200 μ g/kg, $n = 63$). From each litter, one male and one female offspring were selected and assigned to groups for behavioral analyses, as outlined in Fig. 1A or Fig. 7A. Behavioral data were collected from 10 independent litters per group (a total of 10 males and 10 females). Each dot represents the mean value from an independent litter. After completion of behavioral testing at 20 weeks of age, mice were euthanized, and hippocampal tissues were harvested for protein expression and RNA-sequencing analyses. Animals were deeply anesthetized with isoflurane and rapidly decapitated. Hippocampi were dissected and stored at -80 °C until analysis. Each data point represents the mean of independent litters, individual animals, or cultures.

To assess BaP concentrations in the fetal brain, pregnant mice were exposed to PM (200 μ g/kg in 10 μ L saline, $n = 12$) via intranasal instillation or to BaP (200 μ g/kg in 100 μ L DMSO, $n = 12$) via oral gavage on GD 9, 12, and 15. Whole fetal brains were collected on GD 18 for BaP quantification.

2.2. Preparation and characterization of urban PM

2.2.1. Preparation of PM

Certified reference material (CRM) for urban PM was obtained from the Korea Research Institute of Standards and Science (KRISS), Daejeon, Republic of Korea. Raw material was collected from air filtration systems of large buildings and facilities in the Seoul and Gyeonggi

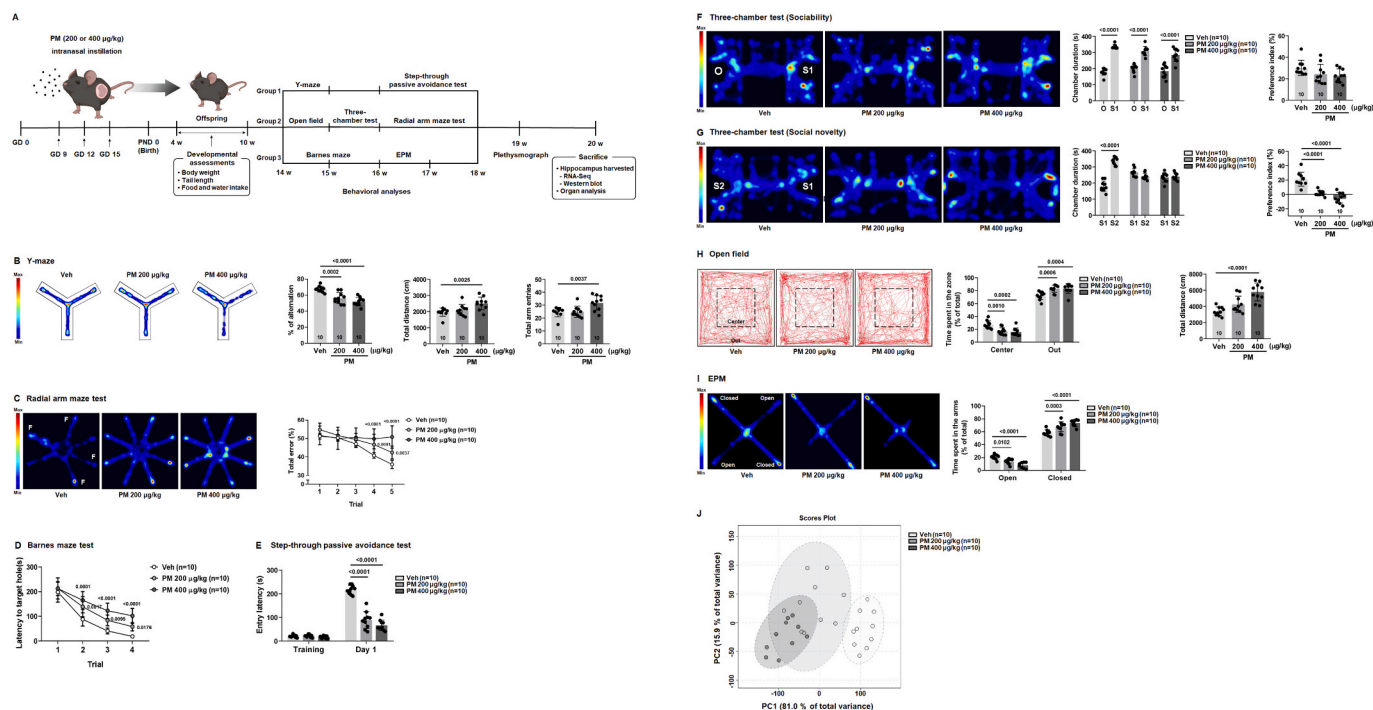


Fig. 1. Behavioral analyses in offspring exposed to PM during pregnancy. (A) Experimental schedule schematic. Pregnant mice received daily intranasal instillations of normal saline or PM (200 or 400 µg/kg) on gestational days 9, 12, and 15. Offspring underwent developmental assessments (4–10 weeks), behavioral analyses (14–18 weeks), and breathing pattern assessments (19 weeks), followed by sacrifice for RNA-seq, hippocampal protein expression, and organ analyses (20 weeks). (B) Y-maze results. Representative heat maps (left) and quantification (right) of alternation percentage, distance traveled, and total arm entries in offspring prenatally exposed to saline (Veh) or PM. Low-dose PM (200 µg/kg) reduced alternation rates without altering total distance or arm entries. High-dose PM (400 µg/kg) significantly affected alternation rate, total distance, and arm entries. One-way ANOVA with Tukey's *post hoc* test. (C) Radial arm maze test. Representative heat maps for each group (left) and quantification of total errors made while consuming food pellets (right). The Veh group showed a gradual decrease in errors across repeated trials, whereas PM exposure increased errors relative to Veh. Two-way ANOVA with Tukey's multiple comparisons test. (D) Barnes maze test. Latency to locate the escape hole was quantified. PM exposure increased latency relative to Veh. Two-way ANOVA with Tukey's multiple comparisons test. (E) Fear memory assessment by step-through passive avoidance test. PM exposure shortened the latency to enter the dark chamber compared to Veh. Two-way ANOVA with Tukey's multiple comparisons test. (F) Sociability assessment using three-chamber tests. Representative heat maps, time spent in the O and S1 chambers, and the sociability preference index are shown. Offspring spent significantly more time in S1 than in O, and all groups exhibited similar sociability preference indices. Two-way ANOVA with Tukey's multiple comparisons test for chamber duration; one-way ANOVA with Tukey's *post hoc* test for preference index. (G) Social novelty test, as in (F). Representative heat maps, time spent in the S1 and S2 chambers, and the social novelty preference index are shown. Veh offspring spent significantly more time in S2 than in S1, whereas PM exposure decreased the social novelty preference index. Two-way ANOVA with Tukey's multiple comparisons test for chamber duration; one-way ANOVA with Tukey's *post hoc* test for preference index. (H) Open field. Mouse tracking, time spent in center and outer zones, and total distance traveled were measured. Two-way ANOVA with Tukey's multiple comparisons test for time spent in zones; one-way ANOVA with Tukey's *post hoc* test for total distance. (I) Elevated arm maze tests. Representative heat maps and time spent in open and closed arms were measured. Two-way ANOVA with Tukey's multiple comparisons test. (J) PCA plot generated from various behavioral tests. Larger dots denote group means. Each dot represents the litter mean derived from one male and one female offspring. Numbers indicate independent litters used: Veh (n = 10, total 10 male and 10 female offspring), PM 200 µg/kg (n = 10, total 10 male and 10 female offspring), PM 400 µg/kg (n = 10, total 10 male and 10 female offspring).

metropolitan area between 2019 and 2020. The pooled material was homogenized, and fine dust particles (<20 µm) were obtained by multi-stage sieving to remove coarse particles. The final product contained less than 3% moisture. PM was aliquoted and stored as a powder at 4 °C in the dark, and vials were gently re-homogenized before subsampling. The CRM (KRISS CRM 109-02-004) is certified for elemental and polycyclic aromatic hydrocarbon (PAH) mass fractions in urban PM.

2.2.2. Scanning electron microscopy (SEM)

Samples were deposited on a highly doped Si wafer, air dried, and imaged without a conductive coating. Images were acquired in secondary electron mode at low accelerating voltages (3–5 kV) and low probe currents to minimize charging. When local charging was observed, a variable-pressure mode was applied to stabilize the image.

2.2.3. Laser diffraction particle-size analysis

Particle size was measured with a PSA 1090 LD particle size analyzer (Anton Paar, Switzerland) operated in liquid-suspension mode. A 0.1% (mass fraction) suspension of the particulate sample was prepared in

0.001% (v/v) Triton X-100 in water and ultrasonicated for 1 h before measurement. The instrument software calculated volume-weighted size distributions using optical parameters of refractive index 1.50 and absorption 0.10. Results are reported as volume-weighted statistics: the volume-weighted mean diameter was 12.9 µm, and D10, D50, and D90 were 2.0 µm, 10.3 µm, and 25.5 µm, respectively. D10, D50, and D90 denote the particle diameters below which 10%, 50%, and 90% of the total particle volume are present.

2.2.4. Dynamic light scattering (DLS)

Dispersions were measured on a Zetasizer Nano ZSP (Malvern Panalytical) using backscatter detection in disposable sizing cuvettes with 60 s acquisition times. The software reported the intensity-weighted size distribution, Z-average (cumulants), and PDI. Data interpretation followed best-practice guidance for polydisperse, aggregate-prone suspensions.

2.2.5. Zeta potential

Zeta potential was measured on the same Zetasizer Nano ZSP by

electrophoretic light scattering (phase analysis). Measurements were performed in clear disposable zeta cells, and electrophoretic mobility was converted to ζ using the Smoluchowski approximation. The dispersant conductivity during measurement was 0.445 mS cm^{-1} , and values are reported as mean $\pm$ standard deviation (SD) over 12 runs.

2.2.6. Constituent analysis

Elemental constituents were certified using isotope-dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) for Sb, Ca, Cu, Pb, Mg, and Sn and single-comparator instrumental neutron activation analysis (SC-INAA) for Cr and Co. Zinc was certified by isotope-dilution ID-ICP-MS and cross-checked by single-comparator SC-INAA. PAHs were quantified by isotope-dilution gas chromatography–mass spectrometry (GC–MS) following dichloromethane-based accelerated solvent extraction (ASE). All certified values are reported on a dry-mass basis. The material was stored at 4°C in the dark, rehomogenized before subsampling, and handled using recommended minimum sample masses of 10–50 mg for elemental analysis and 150 mg or greater for PAH analysis. Moisture content was corrected by drying parallel subsamples with phosphorus pentoxide (P_2O_5) for seven days to obtain a correction factor. Certified values are traceable to the SI through primary elemental and PAH standards. Expanded uncertainties are stated at the 95% confidence level and include a small between-bottle heterogeneity component constrained to a low relative standard uncertainty.

2.3. Behavioral analyses

During treatment and behavioral testing, mice were maintained under standard housing conditions with *ad libitum* access to food and water, except during the radial arm maze test. All behavioral tests were conducted under controlled lighting and minimal background noise. Behavioral assessments were performed in offspring of C57BL/6J mice (14–18 weeks old, 23–27 g), consistent with previous reports (Paing et al., 2024). Spatial memory was evaluated using Y-maze, radial arm maze, and Barnes maze tests. Fear memory was assessed with the step-through passive avoidance test. Sociability and social novelty were measured using the three-chamber test. Locomotor activity and anxiety-like behaviors were evaluated with the open field and elevated plus-maze (EPM) tests, respectively. Arenas were constructed from white acrylic. Animal movements were automatically recorded by overhead video cameras and analyzed using EthoVision software (EthoVision XT, Noldus, Wageningen, the Netherlands). Arenas were cleaned with 70% alcohol after each trial to eliminate olfactory cues.

2.3.1. Y-maze test

Spatial working memory was assessed from the percentage of spontaneous alternations in the Y-maze, following established protocols (Katz and Schmaltz, 1980). The apparatus consisted of three identical white acrylic arms ($40 \times 14 \times 4 \text{ cm}$) arranged 120° apart. During the training phase, one arm was blocked, and mice were placed in another arm to explore the maze for 15 min. After a 1 h interval, all three arms were opened for a 10 min test phase. Movements were automatically recorded by an overhead camera controlled by EthoVision software (Noldus, Wageningen, the Netherlands). The percentage of spontaneous alternations was calculated as the number of sequential entries into all three arms divided by the total possible alternations (total arm entries minus 2) multiplied by 100.

2.3.2. Radial arm maze test

The radial arm maze comprised an octagonal central platform (21 cm diameter) with eight arms (25 cm long, 7 cm wide) extending outward, each enclosed by 7-cm-high walls and elevated 80 cm above the floor. Mice were housed individually and maintained at 80–90% of their initial body weight by food restriction. Training consisted of exploring the maze for 15 min with food pellets placed at the end of each arm; the session ended when all pellets were consumed. In the test trial, pellets

were placed in four randomly selected arms, and mice retrieved pellets for 10 min. Trials ended when all pellets were consumed or the time limit elapsed. Entries into non-baited arms were recorded as errors.

2.3.3. Barnes maze test

The Barnes maze consisted of an open circular platform (70 cm diameter) with 15 evenly spaced holes (5 cm diameter). A dark escape box ($9 \times 9 \times 20 \text{ cm}$) was located beneath one hole, and distinct visual cues were placed on the surrounding walls. Mice were trained twice daily for three days, with at least 30 min between sessions, followed by a probe trial on day 4 with the escape box removed. Each trial ended when the mouse located the escape box or after 5 min. Escape latency to the target hole was measured during each trial.

2.3.4. Step-through passive avoidance test

The apparatus consisted of an acrylic chamber ($41 \times 42 \times 30 \text{ cm}$) divided into two equal compartments connected by a guillotine door. One compartment, constructed of white acrylic, was illuminated by a 60 W bulb; the other, made of black acrylic, served as the dark compartment. During training, mice were placed in the illuminated compartment and allowed to explore until they entered the dark compartment, which triggered closure of the door and delivery of a 0.3 mA shock for 3 s. The test phase was conducted 24 h later, and the latency to enter the dark compartment was measured without shock, up to a maximum of 300 s.

2.3.5. Three-chamber test

The three-chamber test was used to assess sociability and social novelty. The apparatus ($63 \times 23 \times 42 \text{ cm}$) was divided into three equal rectangular chambers, with round wire cups placed in the left and right chambers. Mice were acclimated by exploring the apparatus for 10 min. For the sociability phase, a stranger mouse (stranger 1 [S1]) was placed in one cup, whereas the other cup remained empty (O). The time the test mouse spent in each chamber was recorded for 10 min. For the social novelty phase, a novel mouse (S2) was introduced into the previously empty cup, and the time spent in the S1 or S2 compartments was recorded for another 10 min. Preference indices were calculated as follows: sociability = $(\text{Time}_{\text{S1}} - \text{Time}_{\text{O}}) \times 100 / (\text{Time}_{\text{S1}} + \text{Time}_{\text{O}})$; social novelty = $(\text{Time}_{\text{S2}} - \text{Time}_{\text{S1}}) \times 100 / (\text{Time}_{\text{S2}} + \text{Time}_{\text{S1}})$.

2.3.6. Open field test

Locomotor activity and anxiety-like behavior were assessed using the open field test. Center and outer zones were defined using EthoVision XT software, and the arena was illuminated at 100 lx. Mice were placed in the center of a square open field arena ($42 \times 42 \times 40 \text{ cm}$) with acrylic walls, and their movements were recorded for 5 min. Total distance traveled and time spent in each zone were measured.

2.3.7. EPM

Anxiety-like behavior was assessed using the EPM according to established methods (Umemura et al., 2017). The apparatus comprised a square central platform with two opposing open arms and two opposing closed arms (each $5 \times 35 \text{ cm}$); closed arms were surrounded by 30-cm-high white walls. The arms were illuminated at 100 lx and elevated 30 cm above the floor. Each mouse was placed in the center, facing a closed arm, and allowed to explore freely for 10 min. The ratio of time spent in open versus closed arms was analyzed.

2.4. Principal component analysis (PCA) of mouse behaviors

Behavioral data were collected from seven tests: Y-maze, radial arm maze, Barnes maze, step-through passive avoidance, three-chamber test, open field test, and EPM. All behavioral variables were standardized using z-score normalization before PCA, such that each variable had a mean of 0 and a standard deviation of 1. Principal components 1 (PC1, 81.0%) and 2 (PC2, 15.9%) captured most of the variance, with loadings

≤ 0.50 . PCA was performed using the scikit-learn package (v1.2.2) in Python.

2.5. Pup and dams testing

The body weight of the offspring and dams, as well as tail length, food and water intake, and organ weight in the offspring, were measured at 4 weeks and then monitored weekly until 10 weeks.

2.6. Plethysmography

Respiratory function in C57BL/6J mouse offspring (14–18 weeks old) was measured for 3 min using a Buxco non-invasive double-chamber plethysmograph (DSI NAM 2-site Station, Buxco, NC, USA) controlled by the NAM system. Lung function indicators were automatically calculated with FinePointe software (Data Sciences International, St. Paul, MN, USA).

2.7. RNA sequencing (RNA-seq) analysis

Total RNA was extracted from frozen mouse brain tissue using TRIzol (Invitrogen, CA, USA) and the easy-spin Total RNA Extraction Kit (iNtRON Biotechnology, Kyunggi-do, Korea) according to the manufacturers' instructions. RNA concentration and quality were assessed with RNA ScreenTape on the 4200 TapeStation system (Agilent Technologies, CA, USA). Ribosomal RNA was removed from 1 μ g of total RNA with RIN (RNA integrity number) values > 8.0 , and cDNA (complementary DNA) was synthesized using GoScript Reverse Transcriptase (Promega, WI, USA). Sequencing libraries were prepared with the NEBNext Ultra II RNA Library Prep Kit (New England Biolabs, MA, USA), and 100 bp paired-end sequencing was performed on a NovaSeq 6000 (Illumina, CA, USA). Three RNA samples per group were analyzed. Quality of polyadenylated (PolyA) mRNA sequencing reads was controlled using Trimmomatic (v0.36) (Bolger et al., 2014), and reads were aligned in paired-end mode to the mm10 genome using STAR (v2.7.11b) (Dobin et al., 2013). Raw counts were obtained with HTSeq (v0.9.1) (Anders et al., 2015), and further analysis was performed using R (v4.2.3; <https://www.R-project.org/>), Bioconductor (Huber et al., 2015), and DESeq2 (Love et al., 2014). The RUVSeq package (Risso et al., 2014) was used to remove confounding factors. Data were pre-filtered to retain only genes with at least ten total reads. Genes with $\log_2$ fold change > 1 or < -1 and adjusted p-value (pAdj) < 0.05 (Benjamini-Hochberg correction) were considered significantly differentially expressed. Gene enrichment analysis was performed using GSEA (<https://www.gsea-msigdb.org/gsea/index.jsp>) and Metascape (<https://metascape.org/gp/index.html#/main/step1>). Data visualization was conducted using dplyr (<https://CRAN.R-project.org/package=dplyr>) and ggplot2 (Wickham, 2009).

2.8. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the hippocampus using TRIzol and the easy-spin Total RNA Extraction Kit (iNtRON Biotechnology). A total of 0.1–1 μ g of RNA was reverse transcribed using Maxim RT PreMix (iNtRON Biotechnology). qPCR was performed using SYBR Green qPCR Master Mix (GlpBio, CA, USA) and gene-specific primers (Supplementary Table 2). DNA amplification was monitored using the QuantStudio 1 Real-Time PCR System (Thermo Fisher Scientific). Relative mRNA levels were calculated from the geometric mean of cycle threshold (Ct) values, which were normalized to the internal control GAPDH. Relative quantification of mRNA expression was calculated using the equation $RQ = 2^{-\Delta\Delta Ct}$.

2.9. Western blot

Hippocampi were lysed in RIPA (radioimmunoprecipitation assay) buffer (Biosesang, Kyunggi-do, Korea) supplemented with a protease inhibitor cocktail (Roche, CA, USA). Protein concentration was measured using the bicinchoninic acid assay. For each sample, 30 μ g of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to analyze hippocampal protein expression. Proteins were transferred to nitrocellulose membranes (Cellnest, Gyeonggi-do, Korea) and blocked with 5% non-fat milk in Tris-buffered saline containing 0.2% Tween-20 for 1 h at 22 ± 3 °C. Membranes were incubated overnight at 4 °C with the following primary antibodies: NR1 (1:2000, Thermo Fisher Scientific, MA, USA), NR2A (1:2000, Novus Biologicals, CO, USA), NR2B (1:2000, Thermo Fisher Scientific), GluR1 (1:2000, Thermo Fisher Scientific), GluR2 (1:2000, Merck, NJ, USA), PSD-95 (1:2000, Santa Cruz, CA, USA), synaptophysin (1:4000, Cell Signaling Technology, MA, USA), synaptotagmin-1 (1:4000, Thermo Fisher Scientific), GAD-65/67 (1:4000, Santa Cruz), and β -actin (1:10,000, Santa Cruz). Membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at 25 °C. Blots were developed using a chemiluminescence solution (iNtRON Biotechnology) and visualized with a chemiluminescence imaging system (Vilber, Marne-la-Vallée, France). Band intensities were quantified using ImageJ software (National Institutes of Health, USA) after subtraction of background signals surrounding the regions of interest.

2.10. Ex vivo and in vitro cultures, transfection, and treatment

Primary cultures of neurons, astrocytes, and microglia were established as previously described (Paing et al., 2024). Ex vivo brain cell cultures were prepared from postnatal day 1 mouse pups born to pregnant dams exposed to reagents as described above ($n = 20$ for normal saline or DMSO [Veh], $n = 5$ for PM 200 μ g/kg, $n = 5$ for PM 400 μ g/kg, $n = 10$ for BaP 200 μ g/kg). Pup sex was determined by genital morphology. For each culture, one to two pups of each sex were used, and data were collected from at least three independent litters. Each data point represented the mean value from an independent litter. Pups were decapitated immediately after induction of isoflurane anesthesia.

Hippocampal tissue was dissected from pup brains under an optical microscope (C-PSN, Nikon, Japan) and dissociated into single cells using trypsin. Neurons were seeded onto poly-D-lysine (PDL, Merck)-coated plates or coverslips. Typically, 100,000–150,000 cells per well were plated for multi-electrode array (MEA) analysis and 50,000–80,000 cells per well for live imaging and immunocytochemistry. Neurons were cultured in Neurobasal medium supplemented with 2% B27 (Thermo Fisher Scientific), 1% Glutamax (Thermo Fisher Scientific), and 1% penicillin/streptomycin and maintained in a humidified incubator at 37 °C with 5% CO₂.

Hippocampal neurons at day in vitro (DIV) 4 or 5 were transfected with plasmids using Lipofectamine LTX (Thermo Fisher Scientific) according to the manufacturer's protocol. DsRed2 (Addgene, #54493) was a gift from Michael Davidson. Super-ecliptic pHluorin (SEP)-NR1 (Addgene, #23999), SEP-NR2A (Addgene, #23997), and SEP-NR2B (Addgene, #23998) were gifts from Robert Malinow. Enhanced green fluorescent protein (eGFP) (Addgene, #13031) was a gift from Doug Golenbock. Fresh medium was added 4 h after transfection to minimize cellular toxicity. Typically, 4–10 neurons per coverslip (approximately 0.01–0.04% of total neurons) were transfected, consistent with a previous study (Villarreal et al., 2017). NMDA (Merck) was dissolved in DPBS and applied to developing hippocampal neurons at a final concentration of 100 pM on DIV 3. Neurons were incubated with NMDA from DIV 3 to DIV 5, after which the culture medium was replaced with fresh medium.

To culture astrocytes and microglia, cortices were dissected from pup brains and mechanically dissociated by pipetting. Cultures were maintained in Dulbecco's Modified Eagle's Medium (DMEM)/Nutrient

Mixture F12 (DMEM/F12, Thermo Fisher Scientific) with 10% fetal bovine serum (FBS, Youngin, Gyeonggi-do, Korea) in a humidified chamber for seven days. Microglia were isolated by shaking confluent cultures on an orbital shaker at 250 rpm for 1 h. The culture medium was then centrifuged for 2 min at 1200 rpm. After supernatant removal, microglia were resuspended in DMEM/F12 containing 10% FBS and 1% penicillin–streptomycin and seeded onto PDL-coated coverslips. Astrocytes were detached from confluent co-cultures with 0.25% trypsin and ethylenediaminetetraacetic acid, then suspended in DMEM/F12 with 10% FBS. Collected astrocytes were seeded onto PDL-coated coverslips and cultured for an additional 5 days.

In vitro hippocampal neurons were prepared from 1-day-old C57BL/6J mouse pups (total $n = 50$) obtained from Nara Biotech (Seoul, Republic of Korea) using the same protocol as for *ex vivo* neuronal cultures. Primary hippocampal neurons were incubated with PM from DIV 1 to DIV 5, after which the culture medium was replaced with fresh medium. Neurons expressing eGFP were treated with Dulbecco's phosphate-buffered saline (DPBS) or PM at DIV 6 to assess NMDA receptor subunit expression.

2.11. Live cell imaging

Live imaging of SEP-NMDA receptor subunits was performed on neurons at the indicated DIV14. Neurons expressing SEP-NMDA receptor subunits and DsRed2 were transferred to a Chamlide magnetic chamber (CM-B25-1, Massy, Gataca Systems, France) containing a bath solution composed of 119 mM NaCl, 2.5 mM KCl, 2 mM CaCl_2 , 2 mM MgCl_2 , 30 mM glucose, and 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4). MES (2-(N-morpholino)ethanesulfonic acid) buffer (25 mM, pH 5.5) was applied to quench SEP signals (Wu et al., 2016), and pHluorin signals were subsequently allowed to recover in fresh bath solution. Images were acquired using a confocal microscope (LSM 800, Zeiss, Oberkochen, Germany) in a light-protected room at 15–25 °C. SEP intensity was quantified in individual SEP-expressing neurons using Zen software (Zeiss).

2.12. Sholl analysis

Hippocampal neurons expressing DsRed2 were fixed at DIV 5 with 4% paraformaldehyde for 15 min at 15–25 °C. Images were acquired with a confocal microscope (LSM 800, Zeiss, Oberkochen, Germany). Neuronal complexity was quantified by Sholl analysis as previously described (Bartelt-Kirbach et al., 2016). Concentric circles at 10 μm intervals were drawn around the soma, up to a radius of 200 μm . The number of neuronal processes intersecting each circle was manually counted.

2.13. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Neuronal viability was assessed using the MTT assay. Neurons were incubated with 100 μM MTT for 1 h at 37 °C, after which formazan crystals were dissolved in DMSO (dimethyl sulfoxide). Absorbance at 540 nm was measured using a microplate reader (BioTek Instruments, VT, USA). Absorbance values were normalized to the mean of the Veh group.

2.14. Immunocytochemistry

Cultured neurons (DIV 14–21) expressing eGFP were immunostained for NMDA receptor subunits. Coverslips were washed with DPBS and fixed with 4% paraformaldehyde (BioPrince, Gangwon-do, Korea) for 15 min at 15–25 °C, then washed again in DPBS. Cells were permeabilized with 0.1% Triton X-100 for 10 min and incubated with primary antibodies in blocking solution (DPBS containing 1% BSA (bovine serum albumin) and 3% donkey or goat serum [Merck]) overnight at

4 °C. Primary antibodies were NR1 (1:200, Invitrogen, CA, USA), NR2A (1:200, Novus Biologicals, CO, USA), NR2B (1:200, Invitrogen, CA, USA), p65 (1:400, BD Biosciences, NJ, USA), CD86 (1:200, Abcam, MA, USA), and GFAP (1:400, Abcam). After three washes with DPBS, Alexa488- or Alexa594-conjugated secondary antibodies (1:200, Abcam) were applied for 1 h at 15–25 °C. Nuclei were counterstained with DAPI (4',6-diamidino-2-phenylindole, 0.5 $\mu\text{g}/\text{mL}$). Coverslips were mounted with gel mount solution (Biomed, CA, USA). Images were acquired with a confocal microscope (LSM 800, Zeiss), and signal intensity was quantified using Zen software (Zeiss).

2.15. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of BaP

BaP concentrations in fetal mouse brain were quantified by LC-MS/MS on an Agilent 6490 triple-quadrupole mass spectrometer (Agilent) coupled to an Agilent 1290 HPLC system. Chrysene-d12 was used as an internal standard (IS). BaP was extracted with acetonitrile and separated on an AccucoreTM C30, 2.6 μm , 2.1 $\times$ 250 mm column (Thermo Fisher Scientific) using gradient elution with 0.3% formic acid in water and acetonitrile at a column temperature of 40 °C. The mass spectrometer was operated in positive atmospheric pressure chemical ionization (APCI) mode with multiple reaction monitoring (MRM) transitions at m/z 253.1 $\rightarrow$ 252.2 for BaP and m/z 240.2 $\rightarrow$ 236.1 for the IS. Calibration standards ranged from 0.5 to 100 ng/mL. Data were acquired using MassHunter Quantitative Analysis software (Agilent). Concentrations were expressed as ng/g tissue.

2.16. MEA

Spontaneous neuronal activity and synaptic network function were recorded with an MEA system (M384-tMEA-24 W, Maestro Systems, Axion Biosystems, GA, USA). Each MEA well contained 16 electrodes arranged in a 4 $\times$ 4 grid. *Ex vivo* hippocampal neurons were seeded onto MEA plates (1 $\times$ 10⁵ cells/well). After a 15-min stabilization period, recordings were acquired at the indicated DIV at 37 °C in 5% CO₂, using a 12.5 kHz sampling rate and a 200–3000 Hz band-pass filter. Burst firing was defined as more than five spikes with an inter-spike interval of less than 100 ms; network bursts were defined as more than 25 spikes with an inter-spike interval of less than 50 ms. Data were analyzed using AxIS software (ver. 3.6.2, Axion Biosystems).

2.17. Statistical analysis

Data are presented as mean $\pm$ SD. Each dot represents the mean value from an independent litter or culture. Samples were anonymized prior to analysis. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, Inc., Boston, MA, USA). Comparisons between two groups with non-normally distributed data were performed using the Mann-Whitney *U* test. One-way ANOVA (analysis of variance) with post hoc Tukey's test or two-way ANOVA with post hoc Bonferroni's multiple comparisons test was used to assess group interactions. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Characterization of urban PM

SEM images acquired at increasing magnifications revealed a heterogeneous mixture of irregular fragments and particle aggregates, with layered, plate-like microtextures evident at the highest magnification (Supplementary Fig. 1A). This morphology is consistent with urban particulate mixtures and therefore supports subsequent analyses of size distribution and composition. Laser diffraction of the liquid dispersion yielded a volume-weighted micrometer-scale size distribution with a fine-particle tail; the volume-weighted mean was 12.9 μm , and the D10/

D50/D90 values were 2.0/10.3/25.5 μm (Supplementary Fig. 1B). Because laser diffraction and DLS quantify different size metrics—volume-weighted equivalent diameters for the laser-diffraction dispersion versus intensity-weighted hydrodynamic diameters in aqueous DLS—direct numerical agreement is not expected. Instead, the two measurements are complementary and together delineate a broad, aggregate-prone size spectrum. In water, DLS resolved an intensity-weighted mode at ~ 260 nm, whereas the Z-average was 1242 nm with a PDI of 1.00, reflecting the disproportionate influence of larger agglomerates in polydisperse suspensions (Supplementary Fig. 1C). Electrophoretic light scattering measured a zeta potential of -12.4 ± 4.0 mV (12 runs), indicating weak electrostatic stabilization relative to the ± 30 mV benchmark and consistent with the aggregate morphology observed by SEM.

3.2. Neurotoxic effects of prenatal exposure to PM in offspring

To examine the neurotoxic effects of PM, pregnant mice received daily intranasal instillations of 200 or 400 $\mu\text{g/kg}$ PM, and their offspring subsequently underwent behavioral testing (Fig. 1A). In the Y-maze, offspring prenatally exposed to PM exhibited a lower alternation rate but increased total traveled distance and arm entries (Fig. 1B). In the radial arm maze, Veh mice showed a gradual reduction in total errors across trials, whereas offspring prenatally exposed to PM did not exhibit a significant decrease (Fig. 1C). In the Barnes maze, both Veh and PM-exposed mice progressively decreased latency to locate the target hole, but latencies remained longer in PM-exposed mice than in Veh controls (Fig. 1D). Additionally, in the light–dark transition test, PM-exposed mice had a reduced latency compared to Veh controls (Fig. 1E). Together, these results indicate that prenatal PM exposure impairs both short- and long-term learning and memory in the offspring.

We further assessed the effects of prenatal PM exposure on social behavior, hyperactivity, and anxiety-like behavior in the offspring. In the three-chamber sociability test, both Veh and PM-exposed mice preferred the subject (S1) over the object (O), indicating intact sociability (Fig. 1F). In the subsequent social novelty test, PM-exposed mice spent similar time with the familiar (S1) and stranger (S2) mice, indicating impaired social novelty following PM exposure (Fig. 1G). In the open field test, PM exposure decreased the proportion of time spent in the center zone and increased time spent in the outer zone (Fig. 1H). Notably, a significant increase in total traveled distance was observed only at the higher PM dose (400 $\mu\text{g/kg}$), not at 200 $\mu\text{g/kg}$. To further evaluate anxiety-like behavior, we conducted the EPM, in which PM-exposed offspring spent less time in the open arm and more time in the closed arm (Fig. 1I), indicating increased hyperactivity and anxiety-like behavior.

PCA of the behavioral data revealed that the 400 $\mu\text{g/kg}$ PM group was distinctly separated from Veh, whereas separation for the 200 $\mu\text{g/kg}$ group was less pronounced. PC1 and PC2 accounted for 81.0% and 15.9% of the total variance, respectively. Step-through passive avoidance and Barnes maze tests contributed most to group separation (Fig. 1J). No differences in behavioral, cognitive, or social measures were detected between male and female offspring (Supplementary Fig. 2A–C). Collectively, these results demonstrate dose-dependent adverse effects of prenatal PM exposure: 200 $\mu\text{g/kg}$ induced cognitive deficits and anxiety-like behavior, whereas 400 $\mu\text{g/kg}$ resulted in more widespread behavioral impairments, including hyperactivity.

3.3. Dose-dependent adverse impact of prenatal exposure to PM in offspring

Determining whether prenatal PM exposure affects brain function directly or indirectly via systemic physiological changes is pivotal for elucidating the underlying toxicity pathways. To address this, we investigated physiological developmental parameters in offspring as described in Fig. 1A. Because maternal PM exposure is associated with

low birth weight (Kim et al., 2016), we assessed offspring development by measuring body weight, tail length, food and water intake, and organ weights. Body weight was unchanged in the 200 $\mu\text{g/kg}$ group, whereas 400 $\mu\text{g/kg}$ PM exposure reduced body weight in both male and female offspring (Fig. 2A). Similarly, tail length was reduced only in offspring exposed to 400 $\mu\text{g/kg}$ PM, but not in the 200 $\mu\text{g/kg}$ group (Fig. 2B). Food and water intake did not differ by PM exposure (Fig. 2C and D). Organ weights of the brain, kidney, liver, lung, and spleen were significantly reduced in offspring exposed to 400 $\mu\text{g/kg}$ PM (Fig. 2E). Collectively, these findings suggest that prenatal exposure to 400 $\mu\text{g/kg}$ PM adversely affects offspring development, whereas exposure to 200 $\mu\text{g/kg}$ PM does not.

We also examined respiratory function in the offspring as part of the general physiological assessment. Given that maternal PM exposure impairs lung development and function in offspring in both humans and mice (Jedrychowski et al., 2010; Zhang et al., 2024), we assessed respiratory function in the offspring using plethysmography. This allowed us to determine whether prenatal PM exposure induced non-neural physiological alterations that could confound interpretation of the observed neurobehavioral outcomes. Notably, indicators of the measured respiratory function—tidal volume, breathing frequency, minute ventilation, specific airway resistance (sRAW), and peak inspiratory and expiratory flow—remained normal in the low-dose PM group (200 $\mu\text{g/kg}$), whereas significant changes were observed in the high-dose group (400 $\mu\text{g/kg}$) (Fig. 2F). Maternal weight and the measured respiratory function remained normal following PM exposure (Supplementary Fig. 3A and B), indicating that PM exposure at these doses does not induce maternal toxicity.

Collectively, these findings indicate a dose-dependent differential influence of PM on offspring. Low-dose PM exposure (200 $\mu\text{g/kg}$) primarily induces brain dysfunction, particularly cognitive impairment and anxiety, without affecting somatic development and without detectable alterations in general physiological measures, highlighting the vulnerability of the developing brain to prenatal PM exposure. In contrast, high-dose PM exposure (400 $\mu\text{g/kg}$) impairs both brain and lung function as well as overall development, likely reflecting broader physiological disturbances.

3.4. RNA-seq analysis in hippocampus prenatally exposed to PM

To investigate the potential mechanisms underlying the susceptibility of the developing brain to PM exposure and to identify affected brain cell types, RNA-seq analysis was performed on the hippocampus—a region essential for learning, memory, and anxiety behavior (Bannerman et al., 2014; Bird and Burgess, 2008)—from offspring prenatally exposed to low-dose PM (200 $\mu\text{g/kg}$), which exhibited behavioral impairments without changes in development or lung function. PCA revealed clear separation between RNA-seq samples from the Veh and PM-exposed groups along PC1 and PC2 (Fig. 3A). A total of 4,311 differentially expressed genes (DEGs) were identified in the PM-exposed group compared to Veh (Fig. 3B; Supplementary Table 1). Of these, 1,872 genes were upregulated by more than two-fold, whereas 2,439 genes were downregulated by at least two-fold. Downregulated genes were predominantly associated with brain development, synapse organization, neuronal morphogenesis, neuronal activity, and ion channels, suggesting potential impacts on cognitive function (Fig. 3C and D). Expression of key genes involved in brain and synaptic development—such as *ErbB4*, *Usp9x*, *Robo1*, *Gabrg3*, *Cntn5*, *Slit2*, *Nyyap2*, *Nexn*, *Tet1*, *Grpr*, *Aft2*, and *Adnp*—was significantly reduced following PM exposure (Fig. 3E). Notably, genes encoding NMDA receptors (*GRIN1*, *GRIN2A/B/C*, and *GRIN3A/B*), which are linked to various neurodevelopmental disorders including epilepsy, developmental delays, movement disorders, and autism spectrum disorder (Burnashev and Szepietowski, 2015; Soto et al., 2014), were also significantly downregulated by PM (Fig. 3F). qRT-PCR confirmed the suppressed expression of NMDA receptor subunits following PM exposure (Fig. 3G).

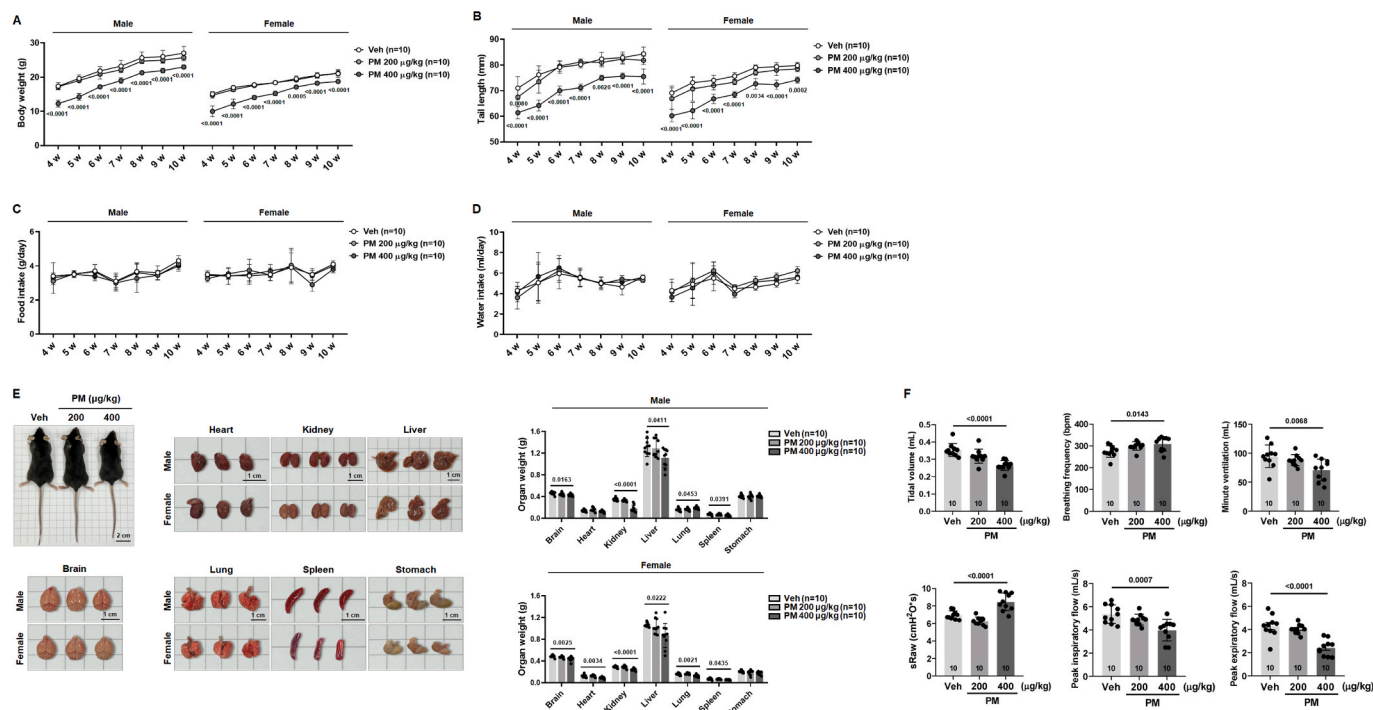


Fig. 2. Dose-dependent influence of prenatal exposure to PM on offspring. (A–D) Body weight (A), tail length (B), food intake (C), and water consumption (D) of male and female offspring. (E) Representative images of 20-week-old female offspring and organs from male and female offspring. Prenatal exposure to 400 µg/kg PM reduced body weight, tail length, and organ weights (brain, kidney, liver, lung, and spleen) in both sexes compared with the Veh group. Food and water intake remained normal with 400 µg/kg PM exposure. All developmental assessments were normal with 200 µg/kg PM exposure. (F) Assessment of breathing patterns in offspring using plethysmography. Changes in tidal volume, breathing frequency, minute ventilation, sRAW, and peak inspiratory and expiratory flow were observed at the 400 µg/kg PM dose. No significant changes were noted at 200 µg/kg. For panel F, one male and one female offspring were analyzed per litter: Veh (n = 10, total 10 male and 10 female offspring), PM 200 µg/kg (n = 10, total 10 male and 10 female offspring), PM 400 µg/kg (n = 10, total 10 male and 10 female offspring). Numbers indicate independent litters used. One-way ANOVA with Tukey's *post hoc* test (E and F) and two-way ANOVA with Tukey's multiple comparisons test (A to D).

These findings demonstrate that PM exposure impairs the transcriptional activity of genes critical for brain development and function.

3.5. Prenatal exposure to PM reduces NMDA receptor expression in the hippocampus of offspring

To further investigate the adverse effects of PM on brain function, we examined the expression of glutamatergic receptors and pre- and postsynaptic proteins, which are essential for neuronal development and activity, in the hippocampus of offspring. Prenatal PM exposure decreased the expression of NMDA receptor subunits in the hippocampus (Fig. 4). In contrast, levels of AMPA receptor subunits (GluR1 and GluR2), PSD-95, synaptophysin, synaptotagmin-1, and GAD-65/67 were unchanged compared to Veh. Quantification of western blot band intensities showed that PM exposure reduced NMDA receptor protein levels in the hippocampus without altering AMPA receptor subunits or pre- and postsynaptic markers.

Investigating the cell-specific effects of PM is essential for understanding the underlying mechanisms. Thus, *ex vivo* cultures of neural cells—neurons, microglia, and astrocytes—were prepared from brains of pups exposed to PM (200 µg/kg) during pregnancy. We first assessed the impact of PM on NMDA receptors in hippocampal neurons using NMDA receptor subunits tagged with SEP, a pH-sensitive green fluorescent protein variant widely used to study glutamate receptor dynamics and surface expression at synapses (Dupuis et al., 2014; Taylor et al., 2023). *Ex vivo* hippocampal neurons from Veh or PM-exposed offspring were transfected with SEP-NMDA receptor subunits NR1, NR2A, or NR2B, the most abundant NMDA receptor subunits in the hippocampus (Cull-Candy and Leszkiewicz, 2004). To validate SEP-tagged receptor functionality, SEP-NR1 and DsRed2-expressing neurons (DIV 14–21) were

perfused with acidic MES buffer (pH 5.5) and subsequently with bath solution, as described previously (Ashby et al., 2004). SEP signals, which colocalized with DsRed2-labeled dendritic morphology, were quenched by acidic buffer and reversibly recovered with bath solution (pH 7.4), whereas DsRed2 intensity remained stable (Fig. 5A), indicating that SEP reports functional NMDA receptors. Measurement of SEP-NR1, NR2A, and NR2B puncta demonstrated that prenatal PM exposure reduced postsynaptic NMDA receptor levels in mature hippocampal neurons (Fig. 5B). Total postsynaptic density was unchanged, whereas dendritic spine maturation was decreased by PM exposure (Supplementary Fig. 4A).

We further assessed how PM affects NMDA receptor subunits in developing neurons. SEP-NMDA subunits were strongly expressed in the somata of developing neurons (Fig. 5C). Quantification of somatic SEP signals indicated that PM exposure reduced NMDA subunit expression in developing hippocampal neurons, consistent with observations in mature neurons. PM exposure also delayed neuronal maturation (Supplementary Fig. 4B and C).

To further confirm the effect of PM on NMDA receptors, we examined *in vitro* hippocampal neuron cultures. The appropriate PM concentration was determined using an MTT assay (Supplementary Fig. 5A). Cultured developing hippocampal neurons expressing eGFP were exposed to Veh or PM and immunostained for NMDA receptor subunits. PM exposure reduced NMDA receptor expression and dendritic spine maturation in hippocampal neurons (Supplementary Fig. 5B), consistent with *ex vivo* results.

3.6. Inflammatory responses in glial cells following prenatal PM exposure

Given the link between neuroinflammation and memory deficits in

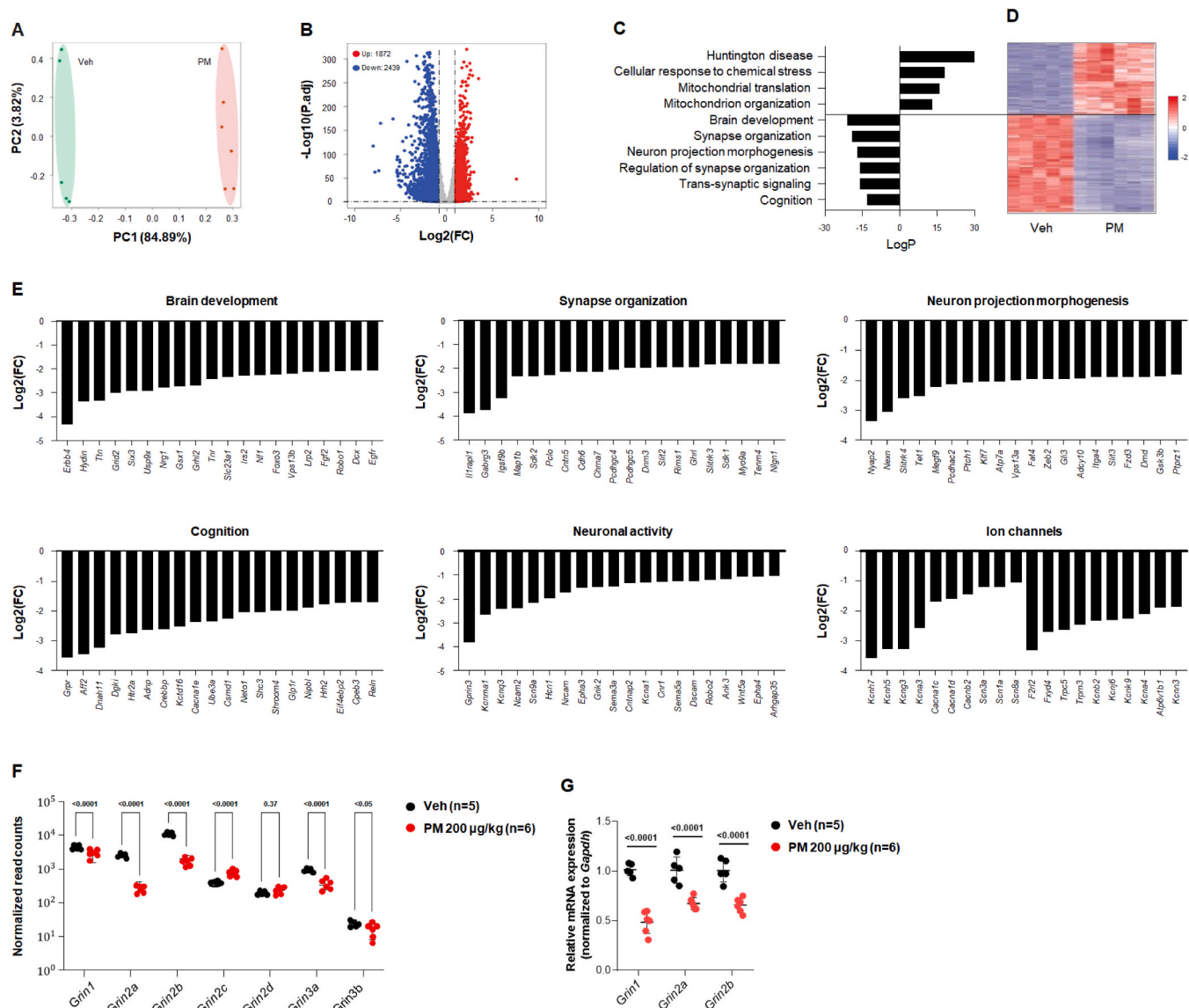


Fig. 3. Effects of PM exposure on the transcriptional regulation of brain development and function. (A) PCA of transcriptomes from Veh control (green) and PM-exposed (red) groups. The PCA depicts global gene expression variation across three experimental cohorts. Principal components 1 (PC1) and 2 (PC2), which account for the largest variance in gene expression, are shown. Data for each individual are listed in Supplementary Table 1. Each dot represents one independent offspring. Veh (n = 5, a total of 3 male and 2 female offspring), PM 200 $\mu\text{g/kg}$ (n = 6, a total of 3 male and 3 female offspring). (B) Volcano plot of differentially expressed genes (DEGs) comparing vehicle control and PM exposure. DEGs with adjusted p-value (P.adj) < 0.05 and $\log_2$ fold change (FC) > 1 or < -1 appear in red and blue, respectively. Non-significant DEGs are shown in gray. Counts of upregulated and downregulated genes are provided. (C) Gene ontology categories of significantly regulated genes in the mouse hippocampus following PM exposure compared with Veh. (D) Heat maps displaying z-scores of genes enriched in the categories shown in panel C. (E) Bar graphs illustrating relative $\log_2$ fold changes (FC) of key genes in each category. (F) Normalized read counts for *Grin* gene expression, which decreased after PM exposure. Two-way ANOVA with Tukey's multiple comparisons test. (G) Quantitative qRT-PCR analysis of NMDA receptor subunit in the hippocampus. Veh (n = 5, a total of 3 male and 2 female offspring), PM 200 $\mu\text{g/kg}$ (n = 6, a total of 3 male and 3 female offspring). Two-way ANOVA with Tukey's multiple comparisons test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PM-exposed brains (Kang et al., 2021; Song et al., 2022), we assessed immune responses in microglia and astrocytes using *ex vivo* cultures from pups prenatally exposed to PM (200 $\mu\text{g/kg}$). Nuclear translocation of p65, a marker of NF- κ B activation, is a canonical indicator of inflammatory responses in glial cells (Dresselhaus and Meffert, 2019). PM exposure did not induce nuclear p65 translocation in *ex vivo* microglia or astrocyte cultures (Fig. 6A and B). Additionally, PM exposure did not alter the expression of CD86 or GFAP, markers of activated microglia and astrocytes, respectively. In contrast, higher PM exposure (400 $\mu\text{g/kg}$) induced nuclear p65 translocation and increased CD86 expression in microglia, indicating activation (Supplementary Fig. 6A). However, PM exposure did not significantly alter p65 localization or GFAP expression

in astrocytes (Supplementary Fig. 6B). These results indicate that pre-natal PM exposure primarily causes neuronal impairment in the developing brain, whereas higher PM exposure stimulates microglial inflammatory responses that may further exacerbate cognitive impairment in offspring.

3.7. Neurotoxic effects of prenatal exposure to benzo[a]pyrene (BaP) in offspring

Epidemiological studies have indicated that the adverse environmental impacts of PM are closely associated with PAHs, as evidenced by analyses of urinary PAH metabolites in PM-exposed children (Oliveira

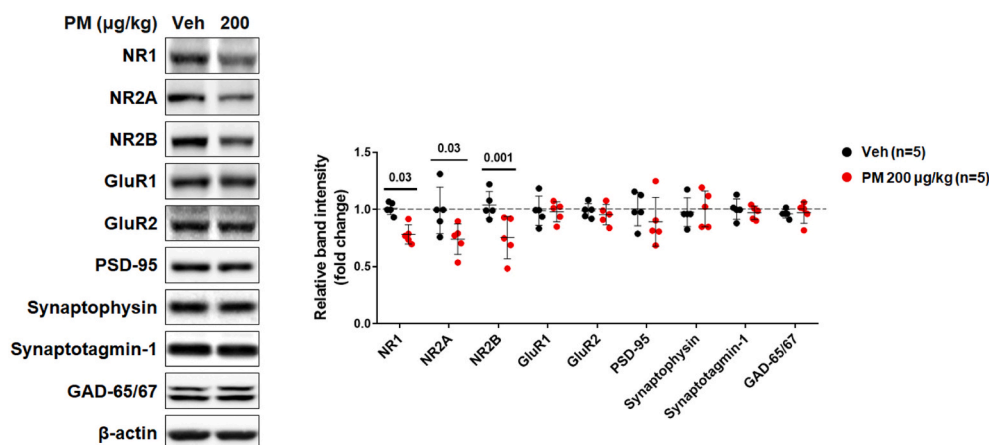


Fig. 4. Reduction of NMDA receptors in the hippocampus following prenatal PM exposure. Western blot analysis and relative band intensities of NMDA receptors, AMPA receptors, and synaptic proteins in offspring hippocampus. Each dot represents the mean value for an independent litter. Veh (n = 5, a total of 3 male and 2 female offspring), PM 200 μ g/kg (n = 5, a total of 3 male and 2 female offspring). Two-way ANOVA with Tukey's multiple comparisons test.

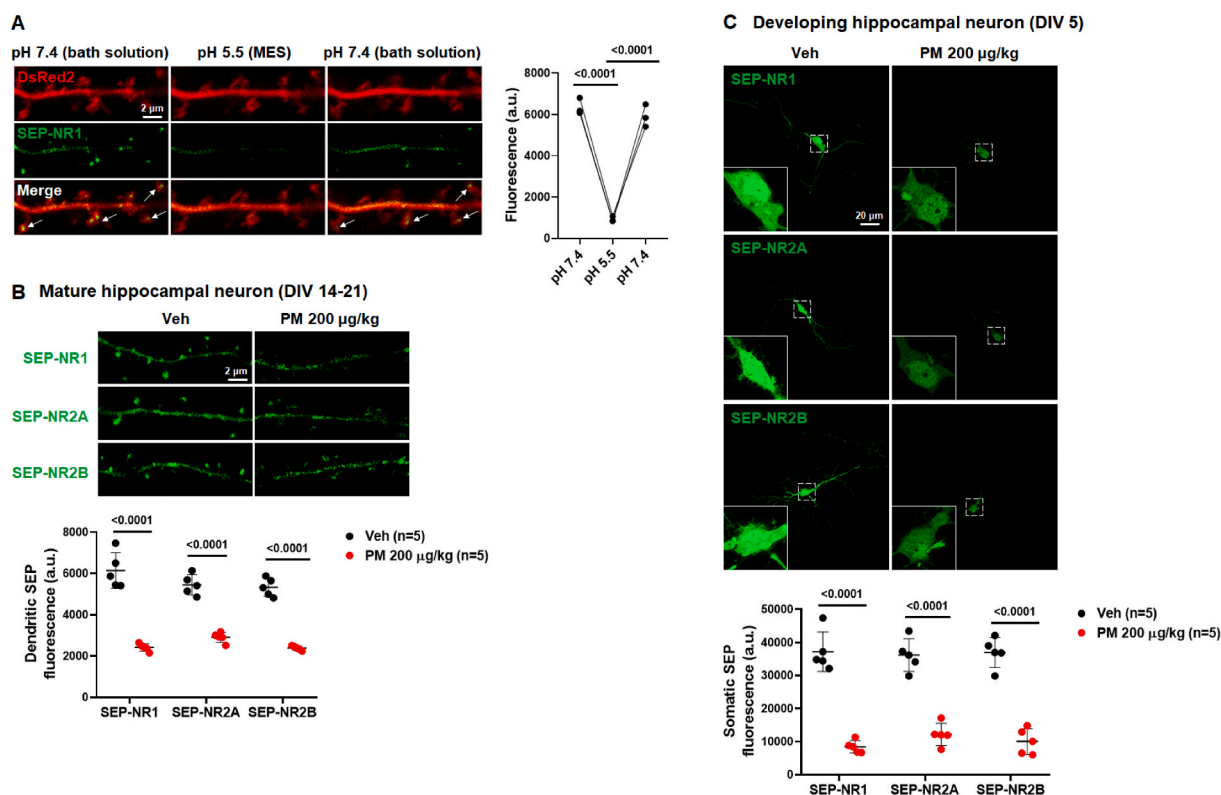


Fig. 5. Reduction of NMDA receptor subunit levels by PM exposure in *ex vivo* hippocampal neuron cultures. *Ex vivo* hippocampal neuron cultures were prepared from offspring exposed to Veh or PM (200 μ g/kg). Neurons were transfected with an SEP-NMDA receptor subunit at DIV 4 or DIV 5. SEP intensity was measured in live hippocampal neurons at the indicated DIV. Each dot represents the mean value for an independent litter. Numbers denote the number of independent cultures. (A) Validation of the SEP approach using MES buffer application. DsRed2- and SEP-NR1-expressing hippocampal neurons were incubated in bath solution (pH 7.4), perfused with MES solution (pH 5.5), and then returned to bath solution (pH 7.4). SEP-NR1 fluorescence was quenched by MES and recovered after washout, whereas DsRed2 fluorescence remained unchanged. SEP-NR1 and DsRed2 fluorescence intensities were quantified before, during, and after MES application (n = 3 independent cultures). One-way ANOVA with Tukey's *post hoc* test. (B) Representative images and quantification of SEP signals in mature hippocampal neurons demonstrate reduced NMDA receptor subunits after prenatal PM exposure. Veh (n = 5; 3 male and 2 female), PM 200 μ g/kg (n = 5; 3 male and 2 female). Two-way ANOVA with Tukey's multiple comparisons test. (C) As in (B), but in developing hippocampal neurons. Representative images and quantification of SEP signals show reduced somatic NMDA receptors in developing neurons from PM-exposed offspring. Veh (n = 5; 3 male and 2 female), PM 200 μ g/kg (n = 5; 3 male and 2 female). Two-way ANOVA with Tukey's multiple comparisons test.

et al., 2019). PAHs can cross the placenta and reach the fetal brain, and prenatal exposure to PAHs has been linked to delayed cognitive development (Perera et al., 2015; Perera et al., 2006) and cognitive deficits in children (Edwards et al., 2010; Perera et al., 2009). Therefore, PAHs are

considered potential key mediators of PM-induced neurodevelopmental disorders and neurotoxicity (Holme et al., 2024). To test whether PAHs contribute to PM-induced neurotoxicity, pregnant mice received oral doses of BaP (100 or 200 μ g/kg)—the most toxic PAH—once at GD 9, 12,

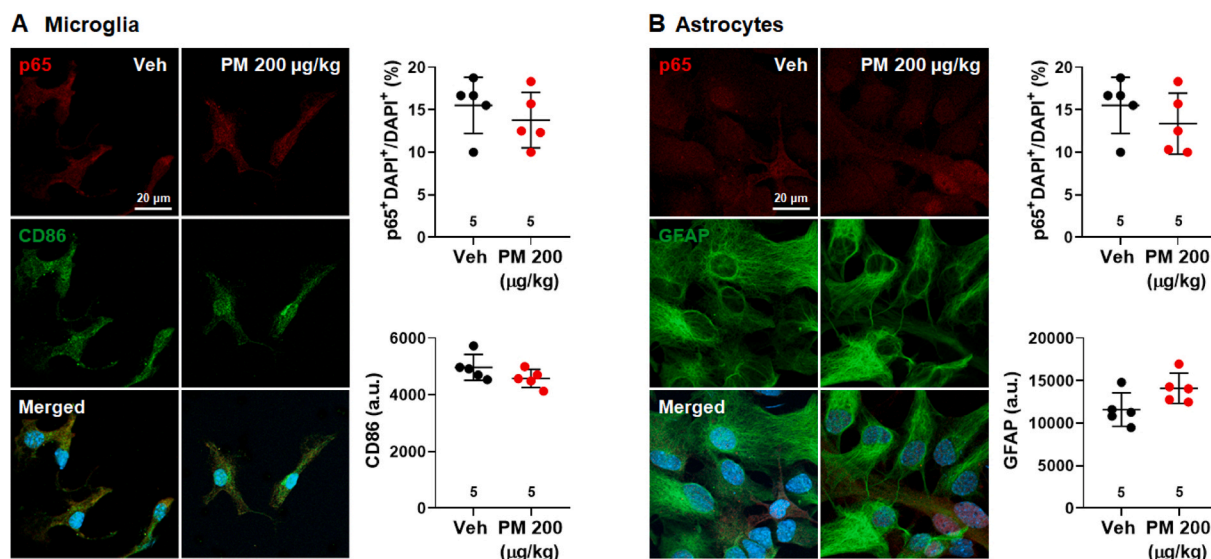


Fig. 6. Effects of prenatal PM (200 µg/kg) exposure on glial cell activation in offspring. *Ex vivo* cultures of microglia (A) and astrocytes (B) were prepared from the cortex of Veh- or PM-exposed offspring and stained for p65. CD86 and GFAP were used to assess microglia and astrocyte activation, respectively. Nuclei were stained with DAPI. Each dot represents the mean value for an independent litter. Numbers denote the number of independent cultures. (A) Representative images of microglia and quantification of nuclear p65 and CD86 intensities. (B) Representative images of astrocytes stained with GFAP and quantification of nuclear p65 in astrocytes. Veh (n = 5; 2 male and 3 female), PM 200 µg/kg (n = 5; 2 male and 3 female). Mann–Whitney *U* test.

and 15. The concentration of BaP in the brain was comparable between fetal offspring prenatally exposed to PM and those exposed to BaP (Supplementary Fig. 7). Behavioral testing in offspring and protein analyses in the hippocampus were conducted as outlined in Fig. 7A. Behavioral testing showed that prenatal exposure to BaP resulted in cognitive dysfunction and anxiety-like behavior in offspring (Fig. 7B–D) and reduced NMDA receptor expression in the hippocampus (Fig. 7E), paralleling PM exposure. Additionally, *ex vivo* hippocampal cultures showed that BaP exposure reduced SEP-NMDA subunit expression in both mature and developing neurons (Fig. 7F and G), consistent with PM results. These results suggest that PAHs may substantially contribute to the neurotoxic effects observed after prenatal PM exposure.

3.8. Reduced neuronal activity in *ex vivo* hippocampal neuron cultures from brains prenatally exposed to PM

To determine whether reduced NMDA receptor levels underlie the abnormal behavior induced by PM exposure, we measured spontaneous electrophysiological activity and network dynamics using MEA in *ex vivo* hippocampal neuron cultures from Veh- and PM (200 µg/kg)-exposed groups. PM exposure caused aberrant neuronal activity, with reduced spike and burst rates but increased burst duration and spikes per burst compared with Veh-treated neurons (Fig. 8A and B). PM exposure also disrupted network activity, decreasing network burst rate while increasing spikes per network burst. Similar impairments were observed in *ex vivo* cultures from BaP-exposed neurons (200 µg/kg). To test whether reduced NMDA receptor function mediates neuronal suppression, we challenged developing hippocampal neurons (DIV 3) prenatally exposed to PM or BaP with 100 pM NMDA. NMDA mitigated aberrant neuronal and network activity in both PM- and BaP-exposed neurons. These rescue effects were also observed in immature neurons (Supplementary Fig. 8A and B). Given that NMDA is a highly polar, ionized molecule at physiological pH and does not readily cross the plasma membrane, exogenous NMDA acts extracellularly by binding to and activating cell-surface NMDA receptors. These findings suggest that prenatal PM exposure suppresses NMDA receptor function, potentially via PAHs, thereby reducing neuronal activity and impairing network function in hippocampal neurons.

4. Discussion

Extensive animal research has demonstrated the adverse effects of PM and elucidated several neurotoxic mechanisms in the brain. Prenatal PM exposure impairs spatial learning, memory, cognitive function, and neuronal development in offspring (Hou et al., 2023; Zhang et al., 2018a; Zhang et al., 2021b). Although pathological symptoms in various organs and the complexity of PM-induced brain toxicity have been investigated, the precise molecular mechanisms mediating PM-induced neurotoxicity remain unclear. Inhalation or nasal instillation of PM induces systemic inflammation and oxidative stress in peripheral tissues, which subsequently transfer inflammatory mediators to the brain (Calderon-Garciduenas et al., 2008b) and are thought to primarily contribute to PM-induced neuropathological outcomes (Thiankhuw et al., 2022). Additionally, PM exposure increases inflammatory responses and oxidative stress in glial cells, complicating the identification of specific toxic mechanisms in the brain. Previous studies on the adverse impact of PM on the developing brain have mainly focused on oxidative stress, neuroinflammation, or glial cell activation (Umezawa et al., 2018; Woodward et al., 2018). However, neuronal mechanisms have been much less explored than glial mechanisms. To address this gap, we conducted comparative functional analyses of the brain and lung and further examined the effects of PM on individual brain cell types that regulate neural activity. In this study, we investigated the adverse effects of PM on offspring behavior and identified specific molecular targets in the brain using *in vivo* and *ex vivo* approaches. Behavioral analyses and plethysmography in the same animals revealed dose-dependent adverse effects of PM exposure on offspring, consistent with previous studies (Calderon-Garciduenas et al., 2018). Low-dose PM exposure (200 µg/kg) primarily reduced NMDA receptor expression in the hippocampus, leading to cognitive dysfunction and anxiety without detectable changes in lung function. In contrast, high-dose PM (400 µg/kg) caused detrimental effects in both the brain and lung of offspring. Furthermore, *ex vivo* cultures showed that PM exposure (200 µg/kg) reduced NMDA receptor levels in hippocampal neurons without activating inflammation in glial cells. Notably, our organ- and cell type-specific analyses revealed that PM exerts its primary toxic effects in the brain by reducing NMDA receptor expression in developing hippocampal neurons, as demonstrated by functional NMDA receptor

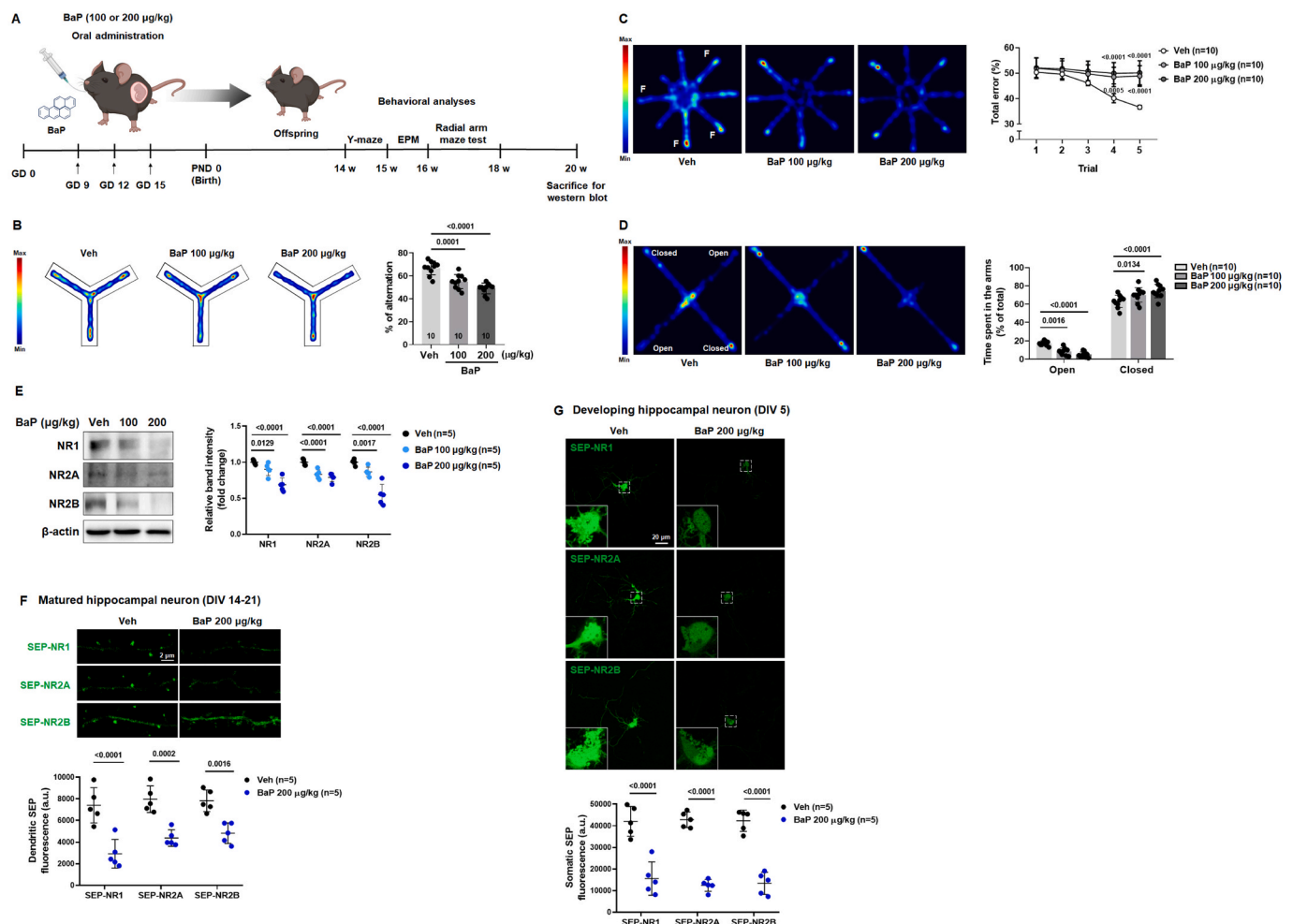


Fig. 7. Behavioral impairments and reduced NMDA receptors in offspring following prenatal BaP exposure. Panels B-D present behavioral analyses in offspring, panel E shows western blot analysis in hippocampus, and panels F and G show results from *ex vivo* hippocampal neuron cultures. Each dot represents the mean value for an independent litter or culture. (A) Experimental schedule for BaP exposure. Pregnant mice were orally treated with either Veh or BaP (100 or 200 µg/kg) once at GD 9, 12, and 15. Cognitive function and anxiety-like behavior in offspring were evaluated at 14–18 weeks of age, followed by sacrifice for hippocampal protein expression analyses. (B) Y-maze results. Representative heat maps (left) and quantification (right) of alternation percentage. Prenatally BaP-exposed mice showed reduced alternation rates compared with Veh. One-way ANOVA with Tukey's *post hoc* test. (C) Radial arm maze tests. Representative heat maps for each group (left) and quantification of total error rate to obtain food pellets (right). BaP exposure increased error rates compared with Veh. Two-way ANOVA with Tukey's multiple comparisons test. (D) EPM tests showing representative heat maps and quantification of time spent in open and closed arms. Prenatally BaP-exposed mice spent less time in open arms and more time in closed arms. Two-way ANOVA with Tukey's multiple comparisons test. Veh (n = 10; total 10 male and 10 female offspring), BaP 100 µg/kg (n = 10; total 10 male and 10 female offspring), BaP 200 µg/kg (n = 10; total 10 male and 10 female offspring). (E) Reduction of NMDA receptors in hippocampi of BaP-exposed offspring. Veh: n = 5 (3 male and 2 female offspring), BaP 100 µg/kg: n = 5 (3 male and 2 female offspring), BaP 200 µg/kg: n = 5 (3 male and 2 female offspring). Two-way ANOVA with Tukey's multiple comparisons test. (F and G) Representative images and quantification of SEP signals in mature (F) and developing (G) hippocampal neurons. NMDA receptor subunits were reduced in prenatally BaP-exposed hippocampal neurons. Each dot represents the mean value for an independent litter. Numbers denote the number of independent cultures. Veh (n = 5; 3 male and 2 female), PM 200 µg/kg (n = 5; 3 male and 2 female). Two-way ANOVA with Tukey's multiple comparisons test.

visualization using pHluorin imaging in living hippocampal neurons. Given that the developmental window spanning GD 9–15 is characterized by a substantial expansion of neuronal populations relative to glial cells in the developing brain (Vivi and Di Benedetto, 2024), the neurodevelopmental alterations observed in the present study likely reflect primarily PM-induced effects on neuronal development rather than glial responses. These results reveal key neurotoxic mechanisms of PM in neurons and highlight the susceptibility of the developing brain to environmental PM exposure.

Assessment of PM toxicity is strongly influenced by multiple experimental variables. We observed behavioral impairments in offspring at a lower PM dose (200 µg/kg) than reported in other studies (3 mg/kg) (Ku et al., 2017; Liang et al., 2023). The PM dose that produces significant toxicity varies across studies, even when the exposure route is the same (Choi et al., 2024; Hameed et al., 2020; Shou et al., 2020). The source of

PM is a key determinant of its toxic effects. PM comprises both organic and inorganic compounds, and its composition varies by season and geographic location (Perrone et al., 2018). Additionally, soluble and insoluble PM differentially affect inflammatory responses in the brain (Haghani et al., 2020). The administration route and particle size determine PM distribution in tissues and critically influence toxicity. Recent studies showed that even oral gestational exposure to PM₁₀ causes behavioral impairments in offspring (Ruiz-Sobremazas et al., 2025; Ruiz-Sobremazas et al., 2024), implying that diverse gestational exposure routes to PM can adversely affect neurodevelopment in the developing brain. The wide range of PM particle sizes also hinders quantitative analyses and reduces the reproducibility of experimental results. Therefore, providing detailed experimental information—including quantitative PM composition, particle diameter, and exposure conditions—is essential for identifying causative factors in PM

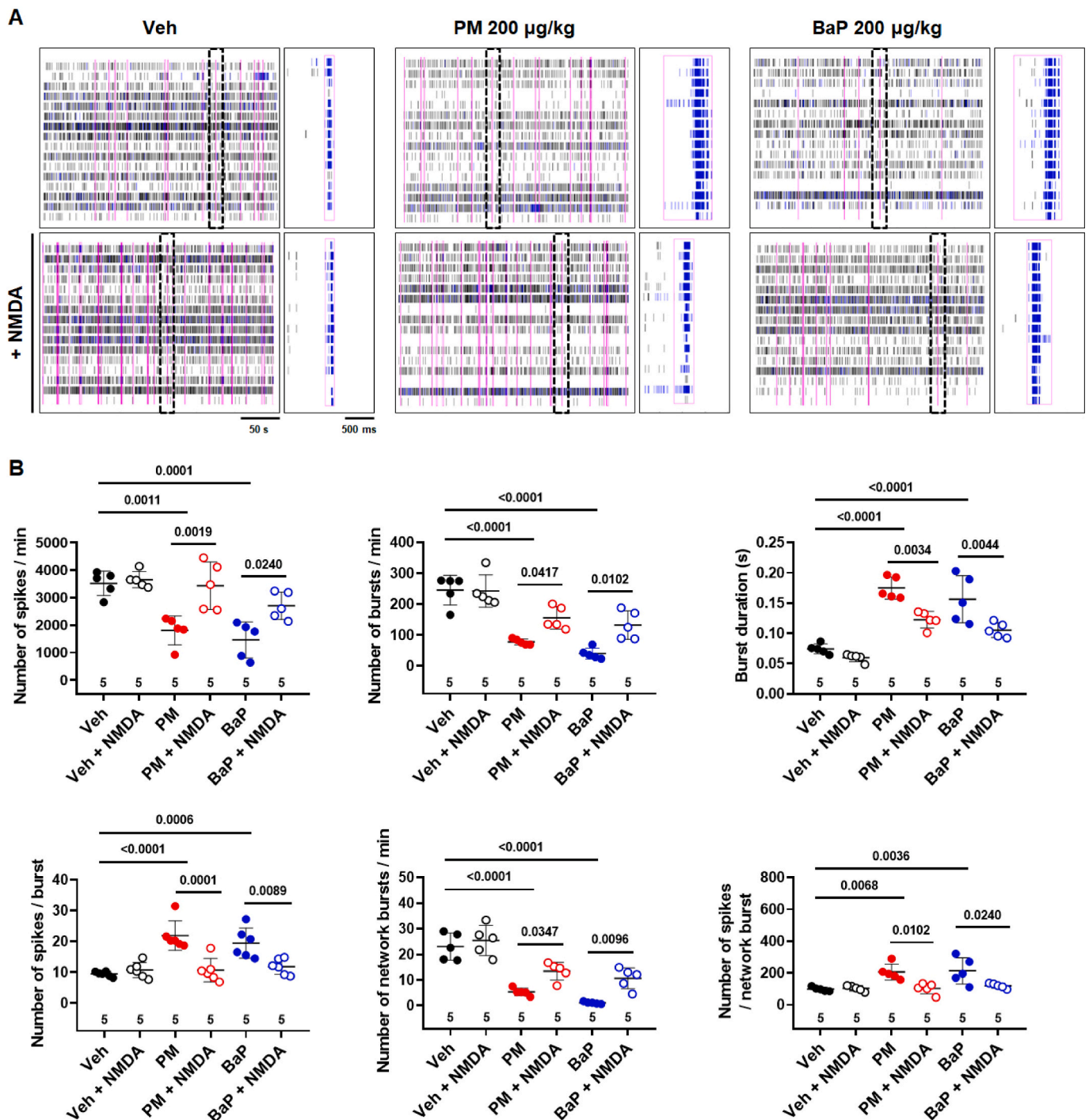


Fig. 8. Activation of NMDA receptors alleviates aberrant neuronal activity induced by prenatal exposure to PM or BaP. *Ex vivo* hippocampal neuron cultures were prepared from offspring prenatally exposed to Veh, PM (200 µg/kg), or BaP (200 µg/kg), with or without NMDA. NMDA was applied to hippocampal neurons at DIV 3. Veh refers to the application of DPBS at a volume equivalent to NMDA. Spontaneous neuronal activity and network function were measured by MEA at DIV 17. Each dot represents the mean value for an independent litter. Numbers denote the number of independent cultures. (A) Spike trace recordings from 16 MEA electrodes. Individual spikes (black), burst firing (blue), and network bursts (pink) are indicated. A magnified view of network burst firing corresponding to the dashed black box is shown. (B) Quantification of spike and burst counts, burst duration, network burst number, and spikes per burst or network burst. Veh (n = 5; 2 male and 3 female), PM 200 µg/kg (n = 5; 2 male and 3 female), BaP 200 µg/kg (n = 5; 2 male and 3 female), Veh + NMDA (n = 5; 2 male and 3 female), PM 200 µg/kg + NMDA (n = 5; 2 male and 3 female), BaP 200 µg/kg + NMDA (n = 5; 2 male and 3 female). Two-way ANOVA with Tukey's multiple comparisons test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

toxicity and for accurate risk assessment.

The environmental relevance of animal experiments remains debated. While humans are chronically exposed to diverse PM sizes through multiple routes, experimental animals typically receive a single PM size at high doses via one route. Additional limitations include

uncertainty about PM deposition and how much PM reaches brain tissue. Efforts to detect PM in the brain are constrained by technical and methodological barriers to reliable detection, including the need to attach dyes or fluorescent compounds to PM surfaces. Consequently, the underlying neurotoxic mechanisms of PM₁₀ in the developing brain

require further investigation to determine whether the observed adverse effects arise from direct deposition of PM₁₀ or smaller fractions such as PM_{2.5}, or from indirect effects, including the release of toxic organic and inorganic constituents, changes in microbiota (Kish et al., 2013), or metabolic dysfunction (Miranda et al., 2018). Nevertheless, because we collected PM₁₀ from the Seoul and Gyeonggi metropolitan area, which is the largest urban area in South Korea, incorporated heterogeneous PM sizes relevant to real-world exposure (Supplementary Fig. 1), and considered the high prevalence of PM₁₀ both indoors and outdoors (Bo et al., 2017; Nadali et al., 2020), our findings underscore the environmental significance of this study. Additionally, the dose range used in the present study is relevant to human exposure levels and falls within the upper range of PM concentrations reported in highly polluted urban (Garg and Gupta, 2020; Rahim et al., 2023) or indoor environments (Morawska et al., 2017; Patel et al., 2020). Furthermore, because pregnant individuals tend to spend more time at home, and 41% of home environments have PM₁₀ levels exceeding the WHO limit (Madureira et al., 2020), pregnancy may be associated with higher PM₁₀ exposure, potentially perturbing neurodevelopment in the offspring. Because a major contribution to indoor PM is infiltration of outdoor emissions (Zhang et al., 2021a), our findings underscore the importance of developing effective strategies to reduce ambient PM concentrations. We also confirmed that the PM contained both organic and inorganic compounds (KRISS CRM 109-02-004). Overall, our results align more closely with hazard characterization than with risk assessment. Future studies employing smaller PM sizes, environmentally relevant exposure levels, multiple doses and routes, and a broader dose range—including doses without significant toxicity—are needed for accurate risk assessment in humans.

Organic PM components exert more detrimental effects on the developing brain than inorganic components (Hou et al., 2023). PM contains PAHs, well-known organic toxicants that impair neurodevelopment. PAH concentrations are associated with children's brain structure and function (Peterson et al., 2015). PM-bound PAHs are ubiquitous globally, occur both outdoors and indoors, and can be transported over long distances (Jariyasopit et al., 2014). High concentrations of PM-bound PAHs have been observed in infants' rooms (Li et al., 2019b). Notably, PM-bound PAHs often include high-molecular-weight species (Yang et al., 2021), which have longer half-lives than low-molecular-weight PAHs (Ray et al., 2019), raising concerns about persistent adverse impacts on human health.

Our study showed that low-dose PM exposure (200 µg/kg) did not induce abnormal inflammatory responses in astrocytes or microglia but reduced neuronal activity in hippocampal neurons. Thus, PM exposure primarily affects neuronal activity rather than glial cell activation. However, because oxidative stress and neuroinflammation can exacerbate PM-induced excitotoxicity (Li et al., 2018a), we cannot fully exclude contributions from glial activation to PM-induced brain dysfunction.

The effects of PM on glutamatergic receptor expression remain controversial. While PM exposure increases NMDA receptors in the adult hippocampus and enhances excitotoxicity (Davis et al., 2013; Ehsanifar et al., 2021), prenatal PM exposure decreases glutamatergic receptors and synaptic gene expression (Haghani et al., 2020), suggesting that the effects of PM differ by age at exposure. Significant reductions in NMDA receptors induced by various types of PM, including PM₁₀ (Ruiz-Sobremazas et al., 2024), PM_{2.5} (Li et al., 2018b), and diesel exhaust particles (Godoy-Lugo et al., 2025), may indicate that suppression of NMDA receptors is a contributor to neurotoxicity caused by PM exposure (Jaiswal and Singh, 2024). Consequently, PM-induced reductions in NMDA receptors can impair neuronal development.

Experimental studies indicate that PAHs contribute to PM-related effects on NMDA receptors. Exposure to an aqueous PM slurry containing PAHs reduces glutamatergic gene expression, whereas PM lacking PAHs does not (Haghani et al., 2020). Chronic BaP administration causes cognitive deficits and reduces NMDA receptor expression in

the hippocampus via transcriptional and epigenetic mechanisms (Grova et al., 2008; Grova et al., 2007; Zhang et al., 2016). Additionally, BaP binds to the aryl hydrocarbon receptor (AhR) and regulates NMDAR subunit transcription in the hippocampus (Chepelev et al., 2016). In the present study, we also found that prenatal exposure to BaP induces behavioral changes and reduces NMDA receptors, consistent with the effects of PM. Additionally, reductions in NMDA receptors occur in the offspring hippocampus following prenatal exposure to PM_{2.5} containing PAHs, but not after exposure to PM_{2.5} lacking PAHs (data not shown). Thus, it is plausible that PAHs in PM repress NMDA receptor expression in the developing brain, leading to behavioral and neurodevelopmental impairments.

One limitation of this study is that PM and BaP were administered by different routes, which may affect tissue distribution and pharmacokinetics. Nevertheless, comparable BaP concentrations detected in fetal brain tissue suggest that fetal exposure levels were similar, strengthening the validity of this comparison. The lack of investigation into the differential effects of organic and inorganic compounds on NMDA receptors is another limitation of the present study. Given the predominance of inorganic compounds in PM (Park et al., 2024) and their effects on cognitive function (Wurth et al., 2018), as well as the influence of metals on NMDA receptors (Krall et al., 2022; Neal et al., 2011), we cannot exclude the potential neurotoxicity of inorganic compounds in the developing brain.

In particular, the distribution of NMDA receptors at synapses—compartmentalized structures critical for synaptic transmission and plasticity—plays a key role (Kellermayer et al., 2018; Zeng et al., 2016). Because NMDA receptors undergo dynamic regulation through exocytosis, endocytosis, local dendritic synthesis, and lateral diffusion (Petit-Pedrol and Groc, 2021), visualization of surface NMDA receptors in living neurons provides experimental evidence for PM's toxic mechanisms.

pHluorin has been widely used to monitor surface insertion and trafficking of synaptic proteins in live neurons. It enables real-time visualization of endogenous pre- and postsynaptic protein dynamics (Kopeck et al., 2006; Pan et al., 2015), supports studies of synaptic protein expression and release (Wu et al., 2016; Lituma et al., 2021), and tracks synaptic vesicle exo- and endocytosis (Royle et al., 2008). Because pHluorin fluorescence is selectively detected in the neutral extracellular environment at the plasma membrane, it provides a sensitive readout of protein expression, surface localization, and trafficking dynamics. SEP-NMDA receptor subunits, therefore, offer a precise method to visualize functional NMDA receptors in living neurons (Elmasri et al., 2022; Kopeck et al., 2006). However, intracellular acidification during neuronal activation can alter pHluorin signals and introduce artifacts in studies of glutamatergic receptor trafficking (Rathje et al., 2013). Therefore, we measured only surface NMDA receptor expression in resting neurons and did not analyze receptor dynamics. Our *in vivo*, *ex vivo*, and *in vitro* results indicate that PM primarily reduces NMDA receptor subunits in hippocampal neurons. Because glutamatergic neurons are enriched in the hippocampus and are critical for learning and memory, cognitive function is likely to be particularly vulnerable to PM exposure via NMDA receptor reduction.

5. Conclusions

Accumulating epidemiological and experimental evidence indicates that ambient PM poses a risk for neurodevelopmental disorders. Despite increasing research into PM neurotoxicity, the adverse effects of PM on the developing brain and the underlying mechanisms remain poorly understood. This study demonstrates that maternal PM exposure during pregnancy contributes to cognitive deficits and anxiety-like behavior in offspring. Specifically, PM exposure reduces NMDA receptor expression and neuronal activity in the hippocampus, leading to behavioral impairments without significantly stimulating neuroinflammation in glial cells. The neurotoxic effects of PM may be mediated by the release of

PAHs carried by the particles. Our study addresses the toxicological implications of PM in the developing brain by identifying specific molecular targets in developing neurons and highlighting the susceptibility of the developing brain to maternal exposure to environmental toxicants. Because developmental toxicants can cause pathological consequences later in life, future longitudinal studies in aged animals will be essential to determine whether prenatal PM exposure increases susceptibility to neurodegenerative disorders. These findings provide a comprehensive toxicological perspective and underscore the need to mitigate PM exposure during pregnancy to prevent potential neurotoxicity in offspring that may contribute to neurodevelopmental disorders.

6. Environmental implications

Ambient PM represents a significant environmental risk factor for neurodevelopmental disorders and behavioral problems in children. Although the developing brain is highly susceptible to environmental toxicants, the adverse effects of PM, its cellular targets, and the precise molecular mechanisms involved remain incompletely defined. Our *in vivo* and *ex vivo* data indicate that prenatal PM exposure may contribute to behavioral deficits in offspring, primarily by reducing NMDA receptor expression in hippocampal neurons. PAHs in PM may be critical contributors to PM-induced neurotoxicity. These findings highlight the health risks associated with PM exposure in children and emphasize the urgent need for regulatory strategies to mitigate PM pollution and protect public health.

CRediT authorship contribution statement

Yunkyung Eom: Methodology, Investigation, Formal analysis. **Hye Kyung Lee:** Validation, Formal analysis. **Yeong-Kyeong Kim:** Formal analysis. **Seonwoo Yang:** Methodology, Investigation. **Yong-Hyeon Yim:** Methodology, Investigation. **Soyoung Shin:** Supervision, Formal analysis. **Jung-Woong Kim:** Supervision, Conceptualization. **Hee Min Yoo:** Supervision, Conceptualization. **Sung Hoon Lee:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2026.110270>.

Data availability

The datasets generated and/or analyzed during this study are available from the corresponding author upon reasonable request. RNA-seq data produced in this study have been deposited in the Gene Expression Omnibus (GEO) under accession GSE292770 (secure token: azivkqsufstjql).

References

- Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* 31, 166–169. <https://doi.org/10.1093/bioinformatics/btu638>.
- Anderson, J.O., Thundiyil, J.G., Stolbach, A., 2012. Clearing the air: a review of the effects of particulate matter air pollution on human health. *J. Med. Toxicol.* 8, 166–175. <https://doi.org/10.1007/s13181-011-0203-1>.
- Ashby, M.C., De La Rue, S.A., Ralph, G.S., Uney, J., Collingridge, G.L., Henley, J.M., 2004. Removal of AMPA receptors (AMPA) from synapses is preceded by transient endocytosis of extrasynaptic AMPARs. *J. Neurosci.* 24, 5172–5176. <https://doi.org/10.1523/JNEUROSCI.1042-04.2004>.
- Bannerman, D.M., Sprengel, R., Sanderson, D.J., McHugh, S.B., Rawlins, J.N., Monyer, H., Seeburg, P.H., 2014. Hippocampal synaptic plasticity, spatial memory and anxiety. *Nat. Rev. Neurosci.* 15, 181–192. <https://doi.org/10.1038/nrn3677>.
- Bartelt-Kirbach, B., Moron, M., Glomb, M., Beck, C.M., Weller, M.P., Golenhofen, N., 2016. HspB5/alphaB-crystallin increases dendritic complexity and protects the dendritic arbor during heat shock in cultured rat hippocampal neurons. *Cell. Mol. Life Sci.* 73, 3761–3775. <https://doi.org/10.1007/s00018-016-2219-9>.
- Bird, C.M., Burgess, N., 2008. The hippocampus and memory: insights from spatial processing. *Nat. Rev. Neurosci.* 9, 182–194. <https://doi.org/10.1038/nrn2335>.
- Block, M.L., Calderon-Garciduenas, L., 2009. Air pollution: mechanisms of neuroinflammation and CNS disease. *Trends Neurosci.* 32, 506–516. <https://doi.org/10.1016/j.tins.2009.05.009>.
- Bo, M., Salizzoni, P., Clerico, M., Buccolieri, R., 2017. Assessment of indoor-outdoor particulate matter air pollution: a review. *Atmosphere-Basel* 8, 136. <https://doi.org/10.3390/atmos8080136>.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Burnashev, N., Szepietowski, P., 2015. NMDA receptor subunit mutations in neurodevelopmental disorders. *Curr. Opin. Pharmacol.* 20, 73–82. <https://doi.org/10.1016/j.coph.2014.11.008>.
- Calderon-Garciduenas, L., Gonzalez-Maciell, A., Reynoso-Robles, R., Delgado-Chavez, R., Mukherjee, P.S., Kulesza, R.J., Torres-Jardon, R., Avila-Ramirez, J., Villarreal-Rios, R., 2018. Hallmarks of Alzheimer disease are evolving relentlessly in Metropolitan Mexico City infants, children and young adults. APOE4 carriers have higher suicide risk and higher odds of reaching NFT stage V at <= 40 years of age. *Environ. Res.* 164, 475–487. <https://doi.org/10.1016/j.envres.2018.03.023>.
- Calderon-Garciduenas, L., Maronpot, R.R., Torres-Jardon, R., Henriquez-Roldan, C., Schoonhoven, R., Acuna-Ayala, H., Villarreal-Calderon, A., Nakamura, J., Fernando, R., Reed, W., Azzarelli, B., Swenberg, J.A., 2003. DNA damage in nasal and brain tissues of canines exposed to air pollutants is associated with evidence of chronic brain inflammation and neurodegeneration. *Toxicol. Pathol.* 31, 524–538. <https://doi.org/10.1080/01926230390226645>.
- Calderon-Garciduenas, L., Solt, A.C., Henriquez-Roldan, C., Torres-Jardon, R., Nuse, B., Herritt, L., Villarreal-Calderon, R., Osnaya, N., Stone, I., Garcia, R., Brooks, D.M., Gonzalez-Maciell, A., Reynoso-Robles, R., Delgado-Chavez, R., Reed, W., 2008a. 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.* 36, 289–310. <https://doi.org/10.1177/0192623307313011>.
- Calderon-Garciduenas, L., Villarreal-Calderon, R., Valencia-Salazar, G., Henriquez-Roldan, C., Gutierrez-Castrellon, P., Osnaya-Brizuela, N., Romero, L., Torres-Jardon, R., Solt, A., Reed, W., 2008b. Systemic inflammation, endothelial dysfunction, and activation in clinically healthy children exposed to air pollutants. *Inhal. Toxicol.* 20, 499–506. <https://doi.org/10.1080/08958370701864797>.
- Chepelev, N.L., Long, A.S., Bowers, W.J., Gagné, R., Williams, A., Kuo, B., Phillips, D.H., Arlt, V.M., White, P.A., Yauk, C.L., 2016. Transcriptional profiling of the mouse hippocampus supports an NMDAR-mediated neurotoxic mode of action for benzo[a]pyrene. *Environ. Mol. Mutagen.* 57, 350–363. <https://doi.org/10.1002/em.22020>.

- Chiu, Y.H., Hsu, H.H., Coull, B.A., Bellinger, D.C., Kloog, I., Schwartz, J., Wright, R.O., Wright, R.J., 2016. Prenatal particulate air pollution and neurodevelopment in urban children: Examining sensitive windows and sex-specific associations. *Environ. Int.* 87, 56–65. <https://doi.org/10.1016/j.envint.2015.11.010>.
- Choi, S.H., Bae, H.J., Kim, S.Y., Mony, T.J., Kim, H.J., Cho, Y.E., Choi, Y.Y., An, J.Y., Cho, S.Y., Kim, D.H., Park, S.J., 2024. Particulate matter (PM10) exacerbates on MK-801-induced schizophrenia-like behaviors through the inhibition of ERK-CREB-BDNF signaling pathway. *Ecotoxicol. Environ. Saf.* 276, 116294. <https://doi.org/10.1016/j.ecoenv.2024.116294>.
- Clifford, A., Lang, L., Chen, R., Anstey, K.J., Seaton, A., 2016. Exposure to air pollution and cognitive functioning across the life course—a systematic literature review. *Environ. Res.* 147, 383–398. <https://doi.org/10.1016/j.envres.2016.01.018>.
- Collaborators, G.B.D.R.F., 2017. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 390, 1345–1422. [https://doi.org/10.1016/S0140-6736\(17\)32366-8](https://doi.org/10.1016/S0140-6736(17)32366-8).
- Cserbik, D., Chen, J.C., McConnell, R., Berhane, K., Sowell, E.R., Schwartz, J., Hackman, D.A., Kan, E., Fan, C.C., Herting, M.M., 2020. Fine particulate matter exposure during childhood relates to hemispheric-specific differences in brain structure. *Environ. Int.* 143, 105933. <https://doi.org/10.1016/j.envint.2020.105933>.
- Cull-Candy, S. G., Leszkiewicz, D. N., 2004. Role of distinct NMDA receptor subtypes at central synapses. *Sci STKE*. 2004, re16. Doi: 10.1126/stke.2552004re16.
- Davis, D.A., Akopian, G., Walsh, J.P., Sioutas, C., Morgan, T.E., Finch, C.E., 2013. Urban air pollutants reduce synaptic function of CA1 neurons via an NMDA/NO pathway in vitro. *J. Neurochem.* 127, 509–519. <https://doi.org/10.1111/jnc.12395>.
- Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21. <https://doi.org/10.1093/bioinformatics/bts635>.
- Dresselhaus, E.C., Meffert, M.K., 2019. Cellular specificity of NF-kappaB function in the nervous system. *Front. Immunol.* 10, 1043. <https://doi.org/10.3389/fimmu.2019.01043>.
- Dupuis, J.P., Ladepeche, L., Seth, H., Bard, L., Varela, J., Mikasova, L., Bouchet, D., Rogemond, V., Honnorat, J., Hanse, E., Groc, L., 2014. Surface dynamics of GluN2B-NMDA receptors controls plasticity of maturing glutamate synapses. *EMBO J.* 33, 842–861. <https://doi.org/10.1002/emboj.201386356>.
- Dupuis, J.P., Nicole, O., Groc, L., 2023. NMDA receptor functions in health and disease: old actor, new dimensions. *Neuron* 111, 2312–2328. <https://doi.org/10.1016/j.neuron.2023.05.002>.
- Edwards, S.C., Jedrychowski, W., Butscher, M., Camann, D., Kielyka, A., Mroz, E., Flak, E., Li, Z.G., Wang, S.A., Rauh, V., Perera, F., 2010. Prenatal exposure to airborne polycyclic aromatic hydrocarbons and Children's Intelligence at 5 Years of Age in a prospective Cohort Study in Poland. *Environ Health Persp.* 118, 1326–1331. <https://doi.org/10.1289/ehp.0901070>.
- Ehsanifar, M., Jafari, A.J., Montazeri, Z., Kalantari, R.R., Gholami, M., Ashtarinezhad, A., 2021. Learning and memory disorders related to hippocampal inflammation following exposure to air pollution. *J. Environ. Health Sci. Eng.* 19, 261–272. <https://doi.org/10.1007/s40201-020-00600-x>.
- Elmasri, M., Hunter, D.W., Winchester, G., Bates, E.E., Aziz, W., Van Der Does, D.M., Karachaliou, E., Sakimura, K., Penn, A.C., 2022. Common synaptic phenotypes arising from diverse mutations in the human NMDA receptor subunit GluN2A. *Commun. Biol.* 5, 174. <https://doi.org/10.1038/s42003-022-03115-3>.
- Fu, P., Guo, X., Cheung, F.M.H., Yung, K.K.L., 2019. The association between PM2.5 exposure and neurological disorders: a systematic review and meta-analysis. *Sci. Total Environ.* 655, 1240–1248. <https://doi.org/10.1016/j.scitotenv.2018.11.218>.
- Garg, A., Gupta, N.C., 2020. The great smog month and spatial and monthly variation in air quality in ambient air in Delhi, India. *J. Health and Pollution*. 10, 200910. <https://doi.org/10.5696/1556-9614-10.27.200910>.
- Godoy-Lugo, J.A., Hicks, D., Durra, S., Massey, E.R., Shkirkova, K., Sauri, A.I., Kerstiens, E., Chen, S., Zhao, L.F., Sioutas, C., Mack, W.J., Finch, C.E., Thorwald, M. A., 2025. Air pollution decreases postsynaptic PSD-95 and NMDA receptor subunits in synaptosomes from mouse cerebral cortex. *Environ. Pollut.* 383, 126845. <https://doi.org/10.1016/j.envpol.2025.126845>.
- Grova, N., Schroeder, H., Farinelle, S., Prodhomme, E., Valley, A., Muller, C.P., 2008. Sub-acute administration of benzo[a]pyrene (B[a]P) reduces anxiety-related behaviour in adult mice and modulates regional expression of N-methyl-D-aspartate (NMDA) receptors genes in relevant brain regions. *Chemosphere* 73, S295–S302. <https://doi.org/10.1016/j.chemosphere.2007.12.037>.
- Grova, N., Valley, A., Turner, J.D., Morel, A., Muller, C.P., Schroeder, H., 2007. Modulation of behavior and NMDA-R1 gene mRNA expression in adult female mice after sub-acute administration of benzo[a]pyrene. *Neurotoxicology* 28, 630–636. <https://doi.org/10.1016/j.neuro.2007.01.010>.
- Guyton, A.C., 1947. Measurement of the respiratory volumes of laboratory animals. *Am. J. Phys. Anthropol.* 150, 70–77. <https://doi.org/10.1152/ajplegacy.1947.150.1.70>.
- Haddad-Tovoli, R., Dragano, N.R.V., Ramalho, A.F.S., Velloso, L.A., 2017. Development and function of the blood-brain barrier in the context of metabolic control. *Front. Neurosci.* 11, 224. <https://doi.org/10.3389/fnins.2017.00224>.
- Haghani, A., Johnson, R., Safi, N., Zhang, H., Thorwald, M., Mousavi, A., Woodward, N. C., Shirmohammadi, F., Coussa, V., Wise Jr., J.P., Forman, H.J., Sioutas, C., Allayee, H., Morgan, T.E., Finch, C.E., 2020. Toxicity of urban air pollution particulate matter in developing and adult mouse brain: comparison of total and filter-eluted nanoparticles. *Environ. Int.* 136, 105510. <https://doi.org/10.1016/j.envint.2020.105510>.
- Hameed, S., Zhao, J.Z., Zare, R.N., 2020. Ambient PM particles reach mouse brain, generate ultrastructural hallmarks of neuroinflammation, and stimulate amyloid deposition, tangles, and plaque formation. *Talanta Open* 2, 100013. <https://doi.org/10.1016/j.talo.2020.100013>.
- Holme, J.A., Myhre, O., Ovrevik, J., 2024. Adverse neurodevelopment in children associated with prenatal exposure to fine particulate matter (PM2.5) - possible roles of polycyclic aromatic hydrocarbons (PAHs) and mechanisms involved. *Reprod. Toxicol.* 130, 108718. <https://doi.org/10.1016/j.reprotox.2024.108718>.
- Hou, Y., Yan, W., Guo, L., Li, G., Sang, N., 2023. Prenatal PM(2.5) exposure impairs spatial learning and memory in male mice offspring: from transcriptional regulation to neuronal morphogenesis. *Part. Fibre Toxicol.* 20, 13. <https://doi.org/10.1186/s12989-023-00520-2>.
- Huber, W., Carey, V.J., Gentleman, R., Anders, S., Carlson, M., Carvalho, B.S., Bravo, H. C., Davis, S., Gatto, L., Girke, T., Gottardo, R., Hahne, F., Hansen, K.D., Irizarry, R.A., Lawrence, M., Love, M.I., MacDonald, J., Obenchain, V., Oles, A.K., Pages, H., Reyes, A., Shannon, P., Smyth, G.K., Tenenbaum, D., Waldron, L., Morgan, M., 2015. Orchestrating high-throughput genomic analysis with Bioconductor. *Nat. Methods* 12, 115–121. <https://doi.org/10.1038/nmeth.3252>.
- Ikonomidou, C., Bosch, F., Miksa, M., Bittigau, P., Vöckler, J., Dikranian, K., Tenkova, T. I., Stefovskaya, V., Turski, L., Olney, J.W., 1999. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* 283, 70–74. <https://doi.org/10.1126/science.283.5398.70>.
- Isaac, J.T.R., Crair, M.C., Nicoll, R.A., Malenka, R.C., 1997. Silent synapses during development of thalamocortical inputs. *Neuron* 18, 269–280. [https://doi.org/10.1016/S0896-6273\(00\)80267-6](https://doi.org/10.1016/S0896-6273(00)80267-6).
- Jaiswal, C., Singh, A.K., 2024. Particulate matter exposure and its consequences on hippocampal neurogenesis and cognitive function in experimental models. *Environ. Pollut.* 363. <https://doi.org/10.1016/j.envpol.2024.125275>.
- Jariyapong, N., Zimmermann, K., Schrlau, J., Arey, J., Atkinson, R., Yu, T.W., Dashwood, R.H., Tao, S., Simonich, S.L., 2014. Heterogeneous reactions of particulate matter-bound PAHs and NPAHs with NO3/N2O5, OH radicals, and O3 under simulated long-range atmospheric transport conditions: reactivity and mutagenicity. *Environ. Sci. Technol.* 48, 10155–10164. <https://doi.org/10.1021/es5015407>.
- Jedrychowski, W.A., Perera, F.P., Maugeri, U., Mroz, E., Klimaszewska-Rembiesz, M., Flak, E., Edwards, S., Spengler, J.D., 2010. Effect of prenatal exposure to fine particulate matter on ventilatory lung function of preschool children of non-smoking mothers. *Paediatr. Perinat. Epidemiol.* 24, 492–501. <https://doi.org/10.1111/j.1365-3016.2010.01136.x>.
- Kang, Y.J., Tan, H.Y., Lee, C.Y., Cho, H., 2021. An air particulate pollutant induces neuroinflammation and neurodegeneration in human brain models. *Adv Sci (Weinh)*. 8, e2101251. <https://doi.org/10.1002/adv.202101251>.
- Katz, R.J., Schmalz, K., 1980. Dopaminergic involvement in attention. A novel animal model. *Prog. Neuropsychopharmacol.* 4, 585–590. [https://doi.org/10.1016/0364-7722\(81\)90099-0](https://doi.org/10.1016/0364-7722(81)90099-0).
- Kellermayer, B., Ferreira, J.S., Dupuis, J., Levett, F., Grillo-Bosch, D., Bard, L., Linares-Lopez, J., Bouchet, D., Choquet, D., Rusakov, D.A., Bon, P., Sibarita, J.B., Cognet, L., Sainlos, M., Carvalho, A.L., Groc, L., 2018. Differential nanoscale topography and functional role of GluN2-NMDA receptor subtypes at glutamatergic synapses. *Neuron* 100 (106–119), e107.
- Kim, E., Park, H., Park, E.A., Hong, Y.C., Ha, M., Kim, H.C., Ha, E.H., 2016. Particulate matter and early childhood body weight. *Environ. Int.* 94, 591–599. <https://doi.org/10.1016/j.envint.2016.06.021>.
- Kish, L., Hotte, N., Kaplan, G.G., Vincent, R., Tso, R., Gänzle, M., Rioux, K.P., Thiesen, A., Barkema, H.W., Wine, E., Madsen, K.L., 2013. Environmental particulate matter induces murine intestinal inflammatory responses and alters the gut microbiome. *PLoS One* 8. <https://doi.org/10.1371/journal.pone.0062220>.
- Kopeck, C.D., Li, B., Wei, W., Boehm, J., Malinow, R., 2006. Glutamate receptor exocytosis and spine enlargement during chemically induced long-term potentiation. *J. Neurosci.* 26, 2000–2009. <https://doi.org/10.1523/JNEUROSCI.3918-05.2006>.
- Krall, R., Gale, J.R., Ross, M.M., Tzounopoulos, T., Aizenman, E., 2022. Intracellular zinc signaling influences NMDA receptor function by enhancing the interaction of ZnT1 with GluN2A. *Neurosci. Lett.* 790. <https://doi.org/10.1016/j.neulet.2022.136896>.
- Ku, T., Zhang, Y., Ji, X., Li, G., Sang, N., 2017. PM2.5-bound metal metabolic distribution and coupled lipid abnormality at different developmental windows. *Environ. Pollut.* 228, 354–362. <https://doi.org/10.1016/j.envpol.2017.05.040>.
- Lee, J., Weerasinghe-Mudiyanselage, P.D.E., Kim, B., Kang, S., Kim, J.S., Moon, C., 2023. Particulate matter exposure and neurodegenerative diseases: a comprehensive update on toxicity and mechanisms. *Ecotoxicol. Environ. Saf.* 266, 115565. <https://doi.org/10.1016/j.ecoenv.2023.115565>.
- Li, B., Guo, L., Ku, T., Chen, M., Li, G., Sang, N., 2018a. PM2.5 exposure stimulates COX-2-mediated excitatory synaptic transmission via ROS-NF-kappaB pathway. *Chemosphere* 190, 124–134. <https://doi.org/10.1016/j.chemosphere.2017.09.098>.
- Li, D., Li, Y., Li, G., Zhang, Y., Li, J., Chen, H., 2019a. Fluorescent reconstitution on deposition of PM(2.5) in lung and extrapulmonary organs. *PNAS* 116, 2488–2493. <https://doi.org/10.1073/pnas.1818134116>.
- Li, H.L., Liu, L.Y., Zhang, Z.F., Ma, W.L., Sverko, E., Zhang, Z., Song, W.W., Sun, Y., Li, Y. F., 2019b. Semi-volatile organic compounds in infant homes: Levels, influence factors, partitioning, and implications for human exposure. *Environ. Pollut.* 251, 609–618. <https://doi.org/10.1016/j.envpol.2019.05.048>.
- Li, Q.Z., Zheng, J.L., Xu, S., Zhang, J.S., Cao, Y.H., Qin, Z.L., Liu, X.Q., Jiang, C.Y., 2018b. The neurotoxicity induced by PM2.5 might be strongly related to changes of the hippocampal tissue structure and neurotransmitter levels. *Toxicol Res-Uk* 7, 1144–1152. <https://doi.org/10.1039/c8tx00093j>.
- Liang, C., Jiang, Y., Zhang, T., Ji, Y., Zhang, Y., Sun, Y., Li, S., Qi, Y., Wang, Y., Cai, Y., Lai, T., Cui, L., 2023. Atmospheric particulate matter impairs cognition by modulating synaptic function via the nose-to-brain route. *Sci. Total Environ.* 857, 159600. <https://doi.org/10.1016/j.scitotenv.2022.159600>.

- Liao, D.H., Zhang, X.Q., O'Brien, R., Ehlers, M.D., Hagan, R.L., 1999. Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nat. Neurosci.* 2, 37–43. <https://doi.org/10.1038/4540>.
- Lin, L.Z., Zhan, X.L., Jin, C.Y., Liang, J.H., Jing, J., Dong, G.H., 2022. The epidemiological evidence linking exposure to ambient particulate matter with neurodevelopmental disorders: a systematic review and meta-analysis. *Environ. Res.* 209, 112876. <https://doi.org/10.1016/j.envres.2022.112876>.
- Lituma, P.J., Kwon, H.B., Alviña, K., Luján, R., Castillo, P.E., 2021. Presynaptic NMDA receptors facilitate short-term plasticity and BDNF release at hippocampal mossy fiber synapses. *Elife* 10, e66612.
- Liu, M., Deng, T., He, J.L., Ding, Y.B., Liu, X.Q., Xu, H.T., Gao, R.F., Mu, X.Y., Geng, Y.Q., Liu, T.H., Wang, Y.X., Chen, X.M., 2020. Exposure to Benzo[a]pyrene impairs the corpus luteum vascular network in rats during early pregnancy. *Environ. Pollut.* 259. <https://doi.org/10.1016/j.envpol.2020.113915>.
- Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550. <https://doi.org/10.1186/s13059-014-0550-8>.
- Lv, B.L., Cai, J., Xu, B., Bai, Y.Q., 2017. Understanding the rising phase of the PM concentration evolution in large China Cities. *Sci. Rep.* 7. <https://doi.org/10.1038/srep46456>.
- Madureira, J., Slezakova, K., Costa, C., Pereira, M.C., Teixeira, J.P., 2020. Assessment of indoor air exposure among newborns and their mothers: Levels and sources of PM10, PM2.5 and ultrafine particles at 65 home environments. *Environ. Pollut.* 264, 114746. <https://doi.org/10.1016/j.envpol.2020.114746>.
- Maher, B.A., Ahmed, I.A., Karolukovski, V., MacLaren, D.A., Foulds, P.G., Allsop, D., Mann, D.M., Torres-Jardon, R., Calderon-Garciduenas, L., 2016. Magnetite pollution nanoparticles in the human brain. *PNAS* 113, 10797–10801. <https://doi.org/10.1073/pnas.1605941113>.
- Mannucci, P.M., Harari, S., Franchini, M., 2019. Novel evidence for a greater burden of ambient air pollution on cardiovascular disease. *Haematologica* 104, 2349–2357. <https://doi.org/10.3324/haematol.2019.225086>.
- McCallister, M.M., Maguire, M., Ramesh, A., Aimin, Q., Liu, S., Khoshbouei, H., Aschner, M., Ebner, F.F., Hood, D.B., 2008. Prenatal exposure to Benzo(a)pyrene impairs later-life cortical neuronal function. *Neurotoxicology* 29, 846–854. <https://doi.org/10.1016/j.neuro.2008.07.008>.
- Meredith, R.M., 2015. Sensitive and critical periods during neurotypical and aberrant neurodevelopment: a framework for neurodevelopmental disorders. *Neurosci. Biobehav. Rev.* 50, 180–188. <https://doi.org/10.1016/j.neubiorev.2014.12.001>.
- Miranda, R.A., Franco, C.C.D., Previate, C., Alves, V.S., Francisco, F.A., Moreira, V.M., de Moraes, A.M.P., Gomes, R.M., Picinato, M.C., Natali, M.R.M., Mathias, P.C.D., 2018. Particulate matter exposure during perinatal life results in impaired glucose metabolism in adult male rat offspring. *Cell. Physiol. Biochem.* 49, 395–405. <https://doi.org/10.1159/000492901>.
- Morawska, L., Ayoko, G.A., Bae, G.N., Buonanno, G., Chao, C.Y.H., Clifford, S., Fu, S.C., Hänninen, O., He, C., Isaxon, C., Mazaheri, M., Salthammer, T., Waring, M.S., Wierzbicka, A., 2017. Airborne particles in indoor environment of homes, schools, offices and aged care facilities: the main routes of exposure. *Environ. Int.* 108, 75–83. <https://doi.org/10.1016/j.envint.2017.07.025>.
- Morgan, Z.E.M., Bailey, M.J., Trifonova, D.I., Naik, N.C., Patterson, W.B., Lurmann, F.W., Chang, H.H., Peterson, B.S., Goran, M.I., Alderete, T.L., 2023. Prenatal exposure to ambient air pollution is associated with neurodevelopmental outcomes at 2 years of age. *Environ. Health* 22, 11. <https://doi.org/10.1186/s12940-022-00951-y>.
- Mortamais, M., Pujol, J., Martínez-Vilavella, G., Fenoll, R., Reynes, C., Sabatier, R., Rivas, I., Forns, J., Vilor-Tejedor, N., Alemany, S., Cirach, M., Alvarez-Pedrerol, M., Nieuwenhuijsen, M., Sunyer, J., 2019. Effects of prenatal exposure to particulate matter air pollution on corpus callosum and behavioral problems in children. *Environ. Res.* 178, 108734. <https://doi.org/10.1016/j.envres.2019.108734>.
- Nadali, A., Arfaeina, H., Asadgol, Z., Fahiminia, M., 2020. Indoor and outdoor concentration of PM10, PM2.5 and PM1 in residential building and evaluation of negative air ions (NAIs) in indoor PM removal. *Env. Pollut. Bioavail.* 32, 47–55. <https://doi.org/10.1080/26395940.2020.1728198>.
- Neal, A.P., Worley, P.F., Guilarte, T.R., 2011. Lead exposure during synaptogenesis alters NMDA receptor targeting via NMDA receptor inhibition. *Neurotoxicology* 32, 281–289. <https://doi.org/10.1016/j.neuro.2010.12.013>.
- Oliveira, M., Slezakova, K., Delerue-Matos, C., Pereira, M.C., Morais, S., 2019. Children environmental exposure to particulate matter and polycyclic aromatic hydrocarbons and biomonitoring in school environments: a review on indoor and outdoor exposure levels, major sources and health impacts. *Environ. Int.* 124, 180–204. <https://doi.org/10.1016/j.envint.2018.12.052>.
- Onoda, A., Okamoto, S., Shimizu, R., El-Sayed, Y.S., Watanabe, S., Ogawa, S., Abe, R., Kamimura, M., Soga, K., Tachibana, K., Takeda, K., Umezawa, M., 2021. Effect of carbon black nanoparticle on neonatal lymphoid tissues depending on the gestational period of exposure in mice. *Front. Toxicol.* 3, 700392. <https://doi.org/10.3389/ftox.2021.700392>.
- Paing, Y.M.M., Eom, Y., Song, G.B., Kim, B., Choi, M.G., Hong, S., Lee, S.H., 2024. Neurotoxic effects of polystyrene nanoplastics on memory and microglial activation: insights from in vivo and in vitro studies. *Sci. Total Environ.* 924, 171681. <https://doi.org/10.1016/j.scitotenv.2024.171681>.
- Pan, P.Y., Marrs, J., Ryan, T.A., 2015. Vesicular glutamate transporter 1 orchestrates recruitment of other synaptic vesicle cargo proteins during synaptic vesicle recycling. *J. Biol. Chem.* 290, 22593–22601. <https://doi.org/10.1074/jbc.M115.651711>.
- Park, J., Lee, K.H., Kim, H., Woo, J., Jeon, K., Lee, C.H., Yoo, C.G., Hopke, P.K., Koutrakis, P., Yi, S.M., 2024. Analysis of PM2.5 inorganic and organic constituents to resolve contributing sources in Seoul, South Korea and Beijing, China and their possible associations with cytokine IL-8. *Environ. Res.* 243, 117860. <https://doi.org/10.1016/j.envres.2023.117860>.
- Patel, S., Sankhyani, S., Boedicker, E.K., DeCarlo, P.F., Farmer, D.K., Goldstein, A.H., Katz, E.F., Nazaroff, W.W., Tian, Y.L., Vanhanen, J., Vance, M.E., 2020. Indoor particulate matter during HOMEChem: concentrations, size distributions, and exposures. *Environ. Sci. Technol.* 54, 7107–7116. <https://doi.org/10.1021/acs.est.0c00740>.
- Perera, F., Miao, Y.Q., Ross, Z., Rauh, V., Margolis, A., Hoepner, L., Riley, K.W., Herbstman, J., Wang, S., 2024. Prenatal exposure to air pollution during the early and middle stages of pregnancy is associated with adverse neurodevelopmental outcomes at ages 1 to 3 years. *Environ. Health-Glob* 23. <https://doi.org/10.1186/s12940-024-01132-9>.
- Perera, F., Phillips, D.H., Wang, Y., Roen, E., Herbstman, J., Rauh, V., Wang, S., Tang, D.L., 2015. Prenatal exposure to polycyclic aromatic hydrocarbons/aromatics, BDNF and child development. *Environ. Res.* 142, 602–608. <https://doi.org/10.1016/j.envres.2015.08.011>.
- Perera, F.P., Li, Z.G., Whyatt, R., Hoepner, L., Wang, S.A., Camann, D., Rauh, V., 2009. Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at Age 5 years. *Pediatrics* 124, E195–E202. <https://doi.org/10.1542/peds.2008-3506>.
- Perera, F.P., Rauh, V., Whyatt, R.M., Tsai, W.Y., Tang, D.L., Diaz, D., Hoepner, L., Barr, D., Tu, Y.H., Camann, D., Kinney, P., 2006. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. *Environ. Health Persp.* 114, 1287–1292. <https://doi.org/10.1289/ehp.9084>.
- Perrone, M.G., Vratolis, S., Georgieva, E., Torok, S., Segá, K., Veleva, B., Osan, J., Beslic, I., Kertesz, Z., Pernigotti, D., Eleftheriadis, K., Belis, C.A., 2018. Sources and geographic origin of particulate matter in urban areas of the Danube macro-region: the cases of Zagreb (Croatia), Budapest (Hungary) and Sofia (Bulgaria). *Sci. Total Environ.* 619–620, 1515–1529. <https://doi.org/10.1016/j.scitotenv.2017.11.092>.
- Peterson, B.S., Rauh, V.A., Bansal, R., Hao, X., Toth, Z., Nati, G., Walsh, K., Miller, R.L., Arias, F., Semanek, D., Perera, F., 2015. Effects of prenatal exposure to air pollutants (polycyclic aromatic hydrocarbons) on the development of brain white matter, cognition, and behavior in later childhood. *JAMA Psychiat.* 72, 531–540. <https://doi.org/10.1001/jamapsychiatry.2015.57>.
- Petit-Pedrol, M., Groc, L., 2021. Regulation of membrane NMDA receptors by dynamics and protein interactions. *J. Cell Biol.* 220. <https://doi.org/10.1083/jcb.202006101>.
- Rahim, N.A.A.A., Noor, N.M., Jafri, I.A.M., Ul-Saufie, A.Z., Ramli, N., Abu Seman, N.A., Kamarudaman, A.N., Zainol, M.R.R.M.A., Victor, S.A., Deak, G., 2023. Variability of PM10 level with gaseous pollutants and meteorological parameters during episodic haze event in Malaysia: domestic or solely transboundary factor? *Heliyon* 9. <https://doi.org/10.1016/j.heliyon.2023.e17472>.
- Rathje, M., Fang, H., Bachman, J.L., Anggono, V., Gether, U., Hagan, R.L., Madsen, K.L., 2013. AMPA receptor pHluorin-GluA2 reports NMDA receptor-induced intracellular acidification in hippocampal neurons. *PNAS* 110, 14426–14431. <https://doi.org/10.1073/pnas.1312982110>.
- Ray, D., Ghosh, S.K., Raha, S., 2019. Impacts of photochemical ageing on the half-lives and diagnostic ratio of polycyclic aromatic hydrocarbons intrinsic to PM(2.5) collected from 'real-world' like combustion events of wood and rice straw burning. *J. Hazard. Mater.* 366, 10–15. <https://doi.org/10.1016/j.jhazmat.2018.11.079>.
- Risso, D., Ngai, J., Speed, T.P., Dudoit, S., 2014. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nat. Biotechnol.* 32, 896–902. <https://doi.org/10.1038/nbt.2931>.
- Royle, S.J., Granseth, B., Odermatt, B., Deriev, A., Lagnado, L., 2008. Imaging phluorin-based probes at hippocampal synapses. *Methods Mol. Biol.* 457, 293–303. https://doi.org/10.1007/978-1-59745-261-8_22.
- Ruiz-Sobremazas, D., Coca, M., Morales-Navas, M., Rodulfo-Cardenas, R., Lopez-Granero, C., Colomina, M.T., Perez-Fernandez, C., Sanchez-Santed, F., 2025. The effects of oral gestational particulate matter 10 exposure: insights into neurodevelopmental milestones, inhibitory control, adult sociability, and object recognition. *Neurotoxicology* 108, 231–245. <https://doi.org/10.1016/j.neuro.2025.04.007>.
- Ruiz-Sobremazas, D., Coca, M.R., Morales-Navas, M., Rodulfo-Cardenas, R., López-Granero, C., Colomina, M.T., Perez-Fernandez, C., Sanchez-Santed, F., 2024. Neurodevelopmental consequences of gestational exposure to particulate matter 10: Ultrasonic vocalizations and gene expression analysis using a bayesian approach. *Environ. Res.* 240. <https://doi.org/10.1016/j.envres.2023.117487>.
- Shou, Y., Zhu, X., Zhu, D., Yin, H., Shi, Y., Chen, M., Lu, L., Qian, Q., Zhao, D., Hu, Y., Wang, H., 2020. Ambient PM2.5 chronic exposure leads to cognitive decline in mice: from pulmonary to neuronal inflammation. *Toxicol. Lett.* 331, 208–217. <https://doi.org/10.1016/j.toxlet.2020.06.014>.
- Song, J., Han, K., Wang, Y., Qu, R., Liu, Y., Wang, S., Wang, Y., An, Z., Li, J., Wu, H., Wu, W., 2022. Microglial activation and oxidative stress in PM2.5-induced neurodegenerative disorders. *Antioxidants (Basel)* 11. <https://doi.org/10.3390/antiox11081482>.
- Soto, D., Altafaj, X., Sindreu, C., Bayes, A., 2014. Glutamate receptor mutations in psychiatric and neurodevelopmental disorders. *Commun. Integr. Biol.* 7, e27887. <https://doi.org/10.4161/cib.27887>.
- Sram, R.J., Veleminsky Jr., M., Veleminsky Sr., M., Stejskalova, J., 2017. The impact of air pollution to central nervous system in children and adults. *Neuro Endocrinol. Lett.* 38, 389–396.
- Sunyer, J., Esnaola, M., Alvarez-Pedrerol, M., Forns, J., Rivas, I., Lopez-Vicente, M., Suades-Gonzalez, E., Foraster, M., Garcia-Esteban, R., Basagana, X., Viana, M., Cirach, M., Moreno, T., Alastuey, A., Sebastian-Galles, N., Nieuwenhuijsen, M., Querol, X., 2015. Association between traffic-related air pollution in schools and cognitive development in primary school children: a prospective cohort study. *PLoS Med.* 12, e1001792. <https://doi.org/10.1371/journal.pmed.1001792>.

- Taylor, K.R., Barron, T., Hui, A., Spitzer, A., Yalcin, B., Ivec, A.E., Geraghty, A.C., Hartmann, G.G., Arzt, M., Gillespie, S.M., Kim, Y.S., Maleki Jahan, S., Zhang, H., Sharmadani, K., Su, M., Ni, L., Du, P.P., Woo, P.J., Silva-Torres, A., Venkatesh, H.S., Mancusi, R., Ponnuswami, A., Mulinyawe, S., Keough, M.B., Chau, I., Aziz-Bose, R., Tirosh, I., Suva, M.L., Monje, M., 2023. Glioma synapses recruit mechanisms of adaptive plasticity. *Nature* 623, 366–374. <https://doi.org/10.1038/s41586-023-06678-1>.
- Thiankhan, K., Chattipakorn, N., Chattipakorn, S.C., 2022. PM2.5 exposure in association with AD-related neuropathology and cognitive outcomes. *Environ. Pollut.* 292, 118320. <https://doi.org/10.1016/j.envpol.2021.118320>.
- Tosevska, A., Ghosh, S., Ganguly, A., Cappelletti, M., Kallapur, S.G., Pellegrini, M., Devaskar, S.U., 2022. Integrated analysis of an in vivo model of intra-nasal exposure to instilled air pollutants reveals cell-type specific responses in the placenta. *Sci. Rep.* 12, 8438. <https://doi.org/10.1038/s41598-022-12340-z>.
- Tumdam, R., Hussein, Y., Garin-Shkolnik, T., Stern, S., 2024. NMDA receptors in neurodevelopmental disorders: pathophysiology and disease models. *Int. J. Mol. Sci.* 25.
- Umemura, M., Ogura, T., Matsuzaki, A., Nakano, H., Takao, K., Miyakawa, T., Takahashi, Y., 2017. Comprehensive behavioral analysis of activating transcription factor 5-deficient mice. *Front. Behav. Neurosci.* 11, 125. <https://doi.org/10.3389/fnbeh.2017.00125>.
- Umezawa, M., Onoda, A., Korshunova, I., Jensen, A.C.O., Koponen, I.K., Jensen, K.A., Khodosevich, K., Vogel, U., Hougaard, K.S., 2018. Maternal inhalation of carbon black nanoparticles induces neurodevelopmental changes in mouse offspring. *Part. Fibre Toxicol.* 15. <https://doi.org/10.1186/s12989-018-0272-2>.
- Villarreal, S., Lee, S.H., Wu, L.G., 2017. Measuring synaptic vesicle endocytosis in cultured hippocampal neurons. *Jove-J Vis Exp.*
- Vivi, E., Di Benedetto, B., 2024. Brain stars take the lead during critical periods of early postnatal brain development: relevance of astrocytes in health and mental disorders. *Mol. Psychiatr.* 29, 2821–2833. <https://doi.org/10.1038/s41380-024-02534-4>.
- Wang, Y., Zhang, M., Li, Z., Yue, J., Xu, M., Zhang, Y., Yung, K.K.L., Li, R., 2019. Fine particulate matter induces mitochondrial dysfunction and oxidative stress in human SH-SY5Y cells. *Chemosphere* 218, 577–588. <https://doi.org/10.1016/j.chemosphere.2018.11.149>.
- WHO, 2021. New WHO Global air quality guidelines: recommendations to protect public health - webinar session for the African region.
- WHO, 2024. Ambient (outdoor) air pollution.
- Wickham, H., 2009. *ggplot2: Elegant Graphics for Data Analysis*. Springer, New York, NY.
- Woodward, N.C., Haghani, A., Johnson, R.G., Hsu, T.M., Saffari, A., Sioutas, C., Kanoski, S.E., Finch, C.E., Morgan, T.E., 2018. Prenatal and early life exposure to air pollution induced hippocampal vascular leakage and impaired neurogenesis in association with behavioral deficits. *Transl. Psychiatr.* 8. <https://doi.org/10.1038/s41398-018-0317-1>.
- Wu, X.S., Lee, S.H., Sheng, J., Zhang, Z., Zhao, W.D., Wang, D., Jin, Y., Charnay, P., Ervasti, J.M., Wu, L.G., 2016. Actin is crucial for all kinetically distinguishable forms of endocytosis at synapses. *Neuron* 92, 1020–1035. <https://doi.org/10.1016/j.neuron.2016.10.014>.
- Wurth, R., Kioumourtoglou, M.A., Tucker, K.L., Griffith, J., Manjourides, J., Suh, H., 2018. Fine particle sources and cognitive function in an older Puerto Rican cohort in greater Boston. *Environ. Epidemiol.* 2, e022. <https://doi.org/10.1097/EE9.0000000000000022>.
- Yang, J., Xu, H.T., Gao, R.F., Liu, X.Q., He, J.L., Zhou, M.L., Ding, Y.B., Li, F.F., Geng, Y. Q., Mu, X.Y., Liu, T.H., Wang, Y.X., Chen, X.M., 2022. Exposure to Benzo(a)pyrene damages mitochondrial function via suppressing mitochondrial melatonin receptors in ovarian corpus luteum during early pregnancy. *Chem-Biol Interact.* 365. <https://doi.org/10.1016/j.cbi.2022.110085>.
- Yang, L., Zhang, H., Zhang, X., Xing, W., Wang, Y., Bai, P., Zhang, L., Hayakawa, K., Toriba, A., Tang, N., 2021. Exposure to atmospheric particulate matter-bound polycyclic aromatic hydrocarbons and their health effects: a review. *Int. J. Environ. Res. Public Health* 18. <https://doi.org/10.3390/ijerph18042177>.
- You, R., Ho, Y.S., Chang, R.C., 2022. The pathogenic effects of particulate matter on neurodegeneration: a review. *J. Biomed. Sci.* 29, 15. <https://doi.org/10.1186/s12929-022-00799-x>.
- Yu, Y., Sun, Q., Li, T., Ren, X., Lin, L., Sun, M., Duan, J., Sun, Z., 2022. Adverse outcome pathway of fine particulate matter leading to increased cardiovascular morbidity and mortality: an integrated perspective from toxicology and epidemiology. *J. Hazard. Mater.* 430, 128368. <https://doi.org/10.1016/j.jhazmat.2022.128368>.
- Zeng, M., Shang, Y., Araki, Y., Guo, T., Haganir, R.L., Zhang, M., 2016. Phase transition in postsynaptic densities underlies formation of synaptic complexes and synaptic plasticity. *Cell* 166 (1163–1175), e1112. <https://doi.org/10.1016/j.cell.2016.07.008>.
- Zhang, J., Cheng, H., Yevdokimova, K., Zhu, Y., Xie, S., Liu, R., Zhao, P., Li, G., Jiang, L., Shao, X., Zhang, Z., Chen, J., Rogers, L., Hao, K., 2024. Prenatal exposure to PM2.5 led to impaired respiratory function in adult mice. *Ecotoxicol. Environ. Saf.* 285, 117052. <https://doi.org/10.1016/j.ecoenv.2024.117052>.
- Zhang, L., Ou, C.J., Magana-Arachchi, D., Vithanage, M., Vanka, K.S., Palanisami, T., Masakorala, K., Wijesekara, H., Yan, Y.B., Bolan, N., Kirkham, M.B., 2021a. Indoor particulate matter in urban households: sources, pathways, characteristics, health effects, and exposure mitigation. *Int. J. Env. Res. Pub. He.* 18. <https://doi.org/10.3390/ijerph182111055>.
- Zhang, M., Liu, W., Zhou, Y., Li, Y., Qin, Y., Xu, Y., 2018a. Neurodevelopmental toxicity induced by maternal PM2.5 exposure and protective effects of quercetin and vitamin C. *Chemosphere* 213, 182–196. <https://doi.org/10.1016/j.chemosphere.2018.09.009>.
- Zhang, Q., Li, Q., Ma, J., Zhao, Y., 2018b. PM2.5 impairs neurobehavior by oxidative stress and myelin sheaths injury of brain in the rat. *Environ. Pollut.* 242, 994–1001. <https://doi.org/10.1016/j.envpol.2018.07.031>.
- Zhang, T., Sun, L., Wang, T., Liu, C., Zhang, H., Zhang, C., Yu, L., 2021b. Gestational exposure to PM(2.5) leads to cognitive dysfunction in mice offspring via promoting HMGB1-NLRP3 axis mediated hippocampal inflammation. *Ecotoxicol. Environ. Saf.* 223, 112617. <https://doi.org/10.1016/j.ecoenv.2021.112617>.
- Zhang, W., Tian, F., Zheng, J., Li, S., Qiang, M., 2016. Chronic administration of benzo(a) pyrene induces memory impairment and anxiety-like behavior and increases of NR2B DNA methylation. *PLoS One* 11, e0149574. <https://doi.org/10.1371/journal.pone.0149574>.
- Zhang, W. B., Ross, P. J., Ellis, J., Salter, M. W., 2022. Targeting NMDA receptors in neuropsychiatric disorders by drug screening on human neurons derived from pluripotent stem cells. *Transl. Psychiatr.* 12. <https://doi.org/ARTN.24310.1038/s41398-022-02010-z>.