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Gestational B-vitamin supplementation alleviates $PM_{2.5}$-induced autism-like behavior and hippocampal neurodevelopmental impairment in mice offspring

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ABSTRACT

Gestational exposure to $PM_{2.5}$ is a worldwide environmental issue associated with long-lasting behavior abnormalities and neurodevelopmental impairments in the hippocampus of offspring. $PM_{2.5}$ may induce hippocampus injury and lead to autism-like behavior such as social communication deficits and stereotyped repetitive behavior in children through neuroinflammation and neurodegeneration. Here, we investigated the preventive effect of B-vitamin on $PM_{2.5}$-induced deleterious effects by focusing on anti-inflammation, antioxidant, synaptic remodeling and neurodevelopment. Pregnant mice were randomly divided into three groups including control group (mice subject to PBS only), model group (mice subject to both 30 μL $PM_{2.5}$ of 3.456 μg/μL and 10 mL/(kg·d) PBS), and intervention group (mice subject to both 30 μL $PM_{2.5}$ of 3.456 μg/μL and 10 mL/(kg·d) B-vitamin supplementation (folic acid, vitamin B6 and vitamin B12 with concentrations at 0.06, 1.14 and 0.02 mg/mL, respectively)). In the current study B-vitamin significantly alleviated neurobehavioral impairment reflected in reduced social communication disorders, stereotyped repetitive behavior, along with learning and spatial memory impairment in $PM_{2.5}$-stimulated mice offspring. Next, B-vitamin corrected synaptic loss and reduced mitochondrial damage in hippocampus of mice offspring, demonstrated by normalized synapse quantity, synaptic cleft, postsynaptic density (PSD) thickness and length of synaptic active area. Furthermore, significantly down-regulated expression of pro-inflammatory cytokines including NF-κB, TNF-α and IL-1β, and lipid peroxidation were found. We observed elevated levels of oxidant-related genes (SOD, GSH and GSH-Px). Moreover, decreased cleaved caspase-3 and TUNEL-positive cells suggested inhibited $PM_{2.5}$-induced apoptosis by B-vitamin. Furthermore, B-vitamin increased neurogenesis by increasing EdU-positive cells in the subgranular zone (SGZ) of offspring. Collectively, our results suggest that B-vitamin supplementation exerts preventive effect on autism-like behavior and neurodevelopmental impairment in hippocampus of mice offspring gestationally exposed to $PM_{2.5}$, to which alleviated mitochondrial damage, increased anti-inflammatory and antioxidant capacity and synaptic efficiency, reduced neuronal apoptosis and improved hippocampal neurogenesis may contribute.

1. Introduction

Air pollution has been an important health issue with increasing global concern. Some new developed technologies for CO_2 removal such as (biomass) NOB intensification process have been applied to prevent air pollution (Sepehri and Sarrafzadeh, 2019). An epidemiological study suggests pregnant women exposed to high levels of air pollution are twice as likely to have autistic babies compared to those living in low-level pollution areas (Roberts et al., 2013). Pregnancy is an extremely sensitive period, and perinatal exposure to ambient air

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Abbreviations

TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
PBS	phosphate buffered solution
RT-qPCR	real-time quantitative PCR
MDA	malondialdehyde
SOD	superoxide dismutases
GSH	glutathione
GSH-Px	glutathione peroxidase
MWM	Morris water maze
$PM_{2.5}$	particulate matter 2.5
ASD	autism spectrum disorder
EdU	thymidine analogue 5-ethynyl-2′-deoxyuridine
DDW	deuterium depleted water
SPF	specific pathogen-free
SGZ	subgranular zone
DG	dentate gyrus
ROS	reactive oxygen species
PSD	postsynaptic density

pollution significantly increases the risk of fetal neurodevelopmental disorders such as autism spectrum disorder (ASD) (Talbott et al., 2015). The particulate matters 2.5 ($PM_{2.5}$) is an important component of air pollutants. The fetal period is vital for brain development and fetus is vulnerable to exposure to intrauterine particulate matters. There is growing concern that gestational exposure to $PM_{2.5}$ may be closely related to the adverse outcomes of fetal neurodevelopmental disorders. Particularly, $PM_{2.5}$ can penetrate maternal blood-air and placental barriers after entering the lung. The influence of particulate matters during pregnancy can be transmitted to fetus via changing uterine environment and placental function (Teng et al., 2016). $PM_{2.5}$ may exert serious and long-term impact on brain structure and function, resulting in neurodevelopmental and cognitive impairment in childhood (Guxens et al., 2018). Moreover, animal studies also demonstrated the special vulnerability during pregnancy. Gestational exposure to $PM_{2.5}$ can cause genetic changes in hippocampus of fetal mice, thus affecting intellectual development as well as inhibiting learning ability and motor coordination (Chao et al., 2017).

The hippocampus is an important brain structure for substantial plasticity and newly-generated neurons in the subgranular zone (SGZ) of the dentate gyrus (DG) (Nam et al., 2017; Hall et al., 2018). The deficits in hippocampal neurogenesis are closely related to neurodevelopmental disorders such as ASD (Abookasis et al., 2018). Improving hippocampal neurogenesis triggering plasticity contributes to the restoration of normal social behavioral phenotypes (Cai et al., 2019). Possible neurobiological mechanisms of ASD may involve neuroinflammation, oxidative stress, neurogenesis or synaptic plasticity (Gilbert and Man, 2017; Kern et al., 2015). Neuroinflammation and oxidative stress are two widely recognized biological mechanisms of $PM_{2.5}$-induced brain injury in children and adults (Cory-Slechta et al., 2018; Allen et al., 2013). It has been found that exposure to $PM_{2.5}$ can induce mitochondrial dysfunction, oxidative stress and inflammation, leading to nerve damage (Piao et al., 2018; Xu et al., 2016). Exposure to $PM_{2.5}$ during pregnancy, in particular, can induce oxidative stress and inflammation in both mother and fetus, resulting in adverse pregnancy outcomes (Liu et al., 2017). Vitamin deficiency will increase the risk of ASD in their offspring in the case of mothers short of vitamins during pregnancy (Schmidt et al., 2011). The levels of folic acid and vitamin B6 in children with ASD were significantly lower than those in normal ones (Bjorklund et al., 2019). Pregnant women who took folic acid and multivitamin supplements before and during pregnancy had a lower risk of ASD in their offspring compared with offspring from mothers who did not (Levine et al., 2018).

B-vitamin (such as vitamin B6, vitamin B12 and folic acid) is essential nutrients for the human body. They participate in many physiological functions based on their anti-inflammatory and anti-oxidative properties. B-vitamin is essential for neuronal function and thus play an important role in brain development and function maintenance. B vitamin deficiency is associated with increased risk for neurodevelopmental disorders, psychosis and dementia (Wang et al., 2018b). It has been found that perinatal regulation of maternal folic acid status can reduce ASD risk caused by $PM_{2.5}$ exposure during pregnancy (Goodrich et al., 2018). Clinical trials exhibited that B-vitamin supplementation can slow down brain degeneration and reduce shrinkage of specific brain regions in patients with Alzheimer's disease (Douaud et al., 2013). Low intake of vitamin B6 is associated with increased risk of Parkinson's disease (Murakami et al., 2010). Vitamin B12 combined with omega-3 fatty acids can improve oxidative stress, lipid metabolism and intestinal microbial composition in autistic animal models (Alfawaz et al., 2018). Vitamin B12 also acts as an intestinal microbial ecological regulator of symbiosis between host and microflora, thus improving gastrointestinal function (Degnan et al., 2014). Long-term exposure to $PM_{2.5}$ can lead to imbalanced intestinal microflora (Wang et al., 2018a; Mutlu et al., 2018), while maternal intestinal microflora determines the risk of autism and other neurodevelopmental disorders in children (Lammert et al., 2018). Compared with typically developed children, the characteristics of intestinal microflora in those with ASD changed (Zhang et al., 2018a). Regulation of intestinal flora can improve neurodevelopmental disorders such as communication, stereotyping, anxiety and sensorimotor behavioral deficits (Hsiao et al., 2013). Thus, B-vitamin may alleviate the negative impact of air pollution.

To the best of our knowledge, little attention has been paid to the antagonism of B-vitamin on the neurodevelopmental toxicity of gestational exposure to $PM_{2.5}$. We speculate that B-vitamin may alleviate the adverse effects on neurodevelopment of offspring induced by $PM_{2.5}$ exposure by inhibiting inflammation and oxidative stress, thus providing a nutritional intervention against air pollution. Neurobehavioral, ultrastructural and biochemical studies were performed in a previously reported mouse model for gestational exposure to $PM_{2.5}$ in the present work.

2. Materials and methods

2.1. $PM_{2.5}$ sampling and suspension preparation

The method of $PM_{2.5}$ sampling and suspension preparation was based on a previous study (Zhang et al., 2018b). Briefly, the atmospheric particulate sampler was used to collect atmospheric $PM_{2.5}$ in a city of northern China in the winter of 2017. $PM_{2.5}$ samples collected in the same batch were used, and $PM_{2.5}$ suspensions (pH = 6.5) used in each group were uniformly prepared in this experiment. $PM_{2.5}$ of 3.456 μg/μL corresponds to the highest value of 1000 $\mu g/m^3$ in the winter of 2016 in a certain area. Our previous study suggested that $PM_{2.5}$ at this concentration caused the most serious brain damage in mice offspring.

2.2. Animal and treatment

All mice and experimental procedures were conducted using protocols approved by the Animal Experimental Ethics Committee of Weifang Medical University (approval code: 2015266; approval date: December 2015) and conducted according to the guidelines for the Care and Use of Laboratory Animals from National Institutes of Health. Specific pathogen-free (SPF) normal ICR mice (28–32 g), aged 8–9 weeks, were purchased from Jinan Animal Experimental Center (Shandong, China). The mice adaptively fed for one week were housed

in a SPF facility and kept in an air-conditioned room at 25 °C, with 12 h day/night cycles. The standard laboratory food and water were available all the time. After being adaptively fed for one week, female and male mice were crossbred in a ratio of 2:1 and the next day when the vaginal embolus appeared was noted as embryonic day 0.5 (E0.5). After vaginal plug appeared, pregnant mice were housed in conventional cages, with aspen sawdust, plastic tubing and domes enriched (mice in each litter were distributed as equally as possible among the 3 groups).

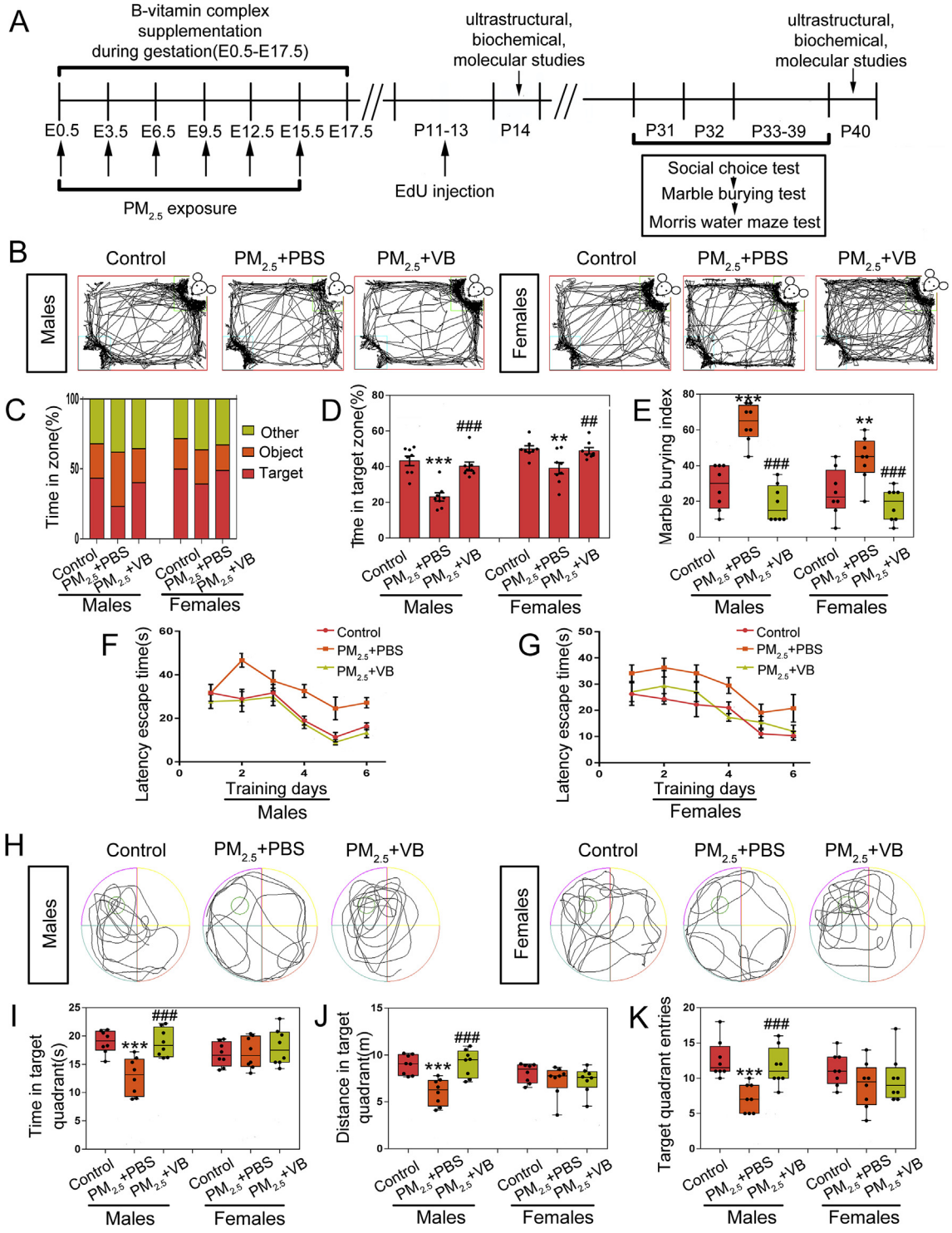

Fig. 1. B-vitamin intervention corrects autism-like behaviors in mice offspring gestationally exposed to $PM_{2.5}$. (A) Time line of experimental design. (B–D) Social choice test. (B) Representative exploration tracks. (C) Time % of mice in various zone. (D) Time % of mice in target zone. (E) Marble burying test. (J) Tail suspension experiment. (F–K) MWM test. (F–G) Changes of escape latency time in directional navigation experiment. (H) Representative swim traces in the probe test of the water maze. (I) Swimming times of mice within target quadrant in the probe test of the water maze. (J) Swimming distances of mice within target quadrant in the probe test of the water maze. (K) Times mice entered the quadrant in the probe test of the water maze. $^{**}P < 0.01$,$^{***}P < 0.001$ p.m.$_{2.5}$+PBS compared with control mice, $\#\#P < 0.01$, $\#\#\#P < 0.001$ p.m.$_{2.5}$+VB compared with $PM_{2.5}$+PBS mice, analyzed by Sidak multiple comparisons. Graphs indicate mean ± SEM (n = 8/group).

Additionally, according to completely randomized grouping method, pregnant mice were randomly divided into three groups by digital method at random (n = 12/group): control group, model group ($PM_{2.5}$ + PBS), and intervention group ($PM_{2.5}$ + VB). The overall study design was shown in Fig. 1A. The mice from model group were subject to 30 μL $PM_{2.5}$ suspension of 3.456 μg/μL and 10ml/(kg·d) PBS. The mice from intervention group were subject to 30 μL $PM_{2.5}$ suspension of 3.456 μg/μL and 10ml/(kg·d) B-vitamin mixed solution (Folic acid of 0.6 mg/(kg·d), vitamin B6 of 11.4 mg/(kg·d) and vitamin B12 of 0.2mg/(kg·d). Control group was treated using the same volume of PBS. Control group was treated using the same volume of PBS. From the first day of pregnancy to delivery, the tracheal drip was performed once every three days for a total of 6 times (i.e. performed on E0.5, E3.5, E6.5, E9.5, E12.5 and E15.5, respectively). Dams were treated with oral gavage at 6–7 p.m. every day during gestation (E0.5-E17.5). Folic acid (Sigma, USA, purity ≥ 97%), vitamin B6 (Sigma, USA, purity ≥ 98%) and vitamin B12 (Sigma, USA, purity ≥ 98%) were respectively dissolved in deionized water , with final concentrations at 0.06, 1.14 and 0.02 mg/mL, respectively, to prepare B-vitamin mixed solution. B-vitamin mixed solution concentration was selected based on previous studies (Supplemental Fig. 1). All pups stayed with their mothers until weaning on postnatal day 28 (P28). Mice offspring of 14- and 40-day-old were randomly selected for morphology and molecular biological analyses, with the latter randomly selected for the subsequent behavioral tests. Experimenters and data analysts were blind to treatment.

2.3. Behavioral assays

Blinding and both sexes were employed in all the behavioral experiments. Eight 40-day-old mice offspring from eight different pregnant mice in each group were randomly selected for the behavioral experiment. Mice were kept in the experimental room for at least 30 min for acclimatization prior to tests. The same sets of animals were used in all behavioral tests. Less stressful behavior tests were performed first, with more stressful ones done subsequently. The order of tests was as following: social choice, marble burying, and Morris water maze (MWM).

2.3.1. Social choice test

The social interaction in open-field test was performed as previously described (Chen et al., 2015). The test was performed in the open-field arena (arena size: 40 × 70 cm^2). A young stranger mouse (P40 male or female) in a wire mesh cage (arena size: 6 × 6 cm^2) was used as a social cue. A caged object and stranger mouse were placed simultaneously on the opposite side of the arena. The test mouse was then allowed to explore either the object or caged stranger mouse and its movement was videotaped. The recorded video file was analyzed by SMART video tracking system (SMART v3.0, Panlab, Spain). Time spent in the corner proximal to the stranger cage was measured.

2.3.2. Marble burying test

The percentage of marbles buried in the marble burying test is used as an index of stereotyped behavior. The marble burying test was performed as previously described (Kim et al., 2017). Mice offspring were placed in a testing arena (arena size: 40 × 20 cm^2, bedding depth: 3 cm) containing 20 glass marbles (equidistant from each other in an arrangement style of 4 × 5). At the end of the 15-min exploration period, mice were gently removed from the testing cages and the number of buried marbles was recorded. Criteria for a buried marble was > 50% marble covered by bedding material.

2.3.3. Morris water maze (MWM) test

MWM, widely used to assess spatial learning and memory, was conducted in a round black pool (diameter and height as 150 and 50 cm, respectively) (Zheng et al., 2018). The escape platform, 1 cm beneath the water surface, was placed in the center of target quadrant of the pool. The platform location remained unchanged throughout the training and was removed from the pool during the probe test.

Platform navigation test lasted 6 days. Mice offspring, having four trials to find the platform each day, were randomly placed into water facing the wall in four different quadrants. The mice offspring that reached the platform within 60 s were allowed to remain on the platform for 5 s, or else were gently guided onto it and allowed to stay for 20 s. The time taken to find the platform was considered to be the escape latency. On the 7th day of spatial probe test, the platform was removed. Mice were placed into water from the opposite quadrant where the platform used to be and allowed to swim freely for 60 s. Data were recorded and analyzed using the SMART video tracking system.

2.4. Ultrastructural characterization

Six 14- and 40-day-old mice offspring from six different pregnant mice in each group were randomly selected for the experiment. The ultrastructural characterization was performed as described previously (Zheng et al., 2018). The offspring were anesthetized with 5% chloral hydrate. The hippocampus (tissue size: 1 mm^3) was quickly collected and fixed in 2.5% glutaraldehyde, followed by being fixed in 1% osmium acid. Subsequently, the samples were rinsed using 0.1 M phosphoric acid and dehydrated step by step using ethanol before being embedded and sliced. The ultrastructural changes of neurons were observed under the transmission electron microscope (HT 7700, Hitachi, Tokyo, Japan) after the sections were counterstained by 3% uranium acetate-citric acid.

2.5. Immunocytochemistry

2.5.1. Tissue preparation

Six 14- and 40-day-old mice offspring from six different pregnant mice in each group were randomly selected for the experiment. Mice were anesthetized with 5% chloral hydrate, and the cardiacs were perfused into 50 mL 0.9% saline and 50 mL 4% paraformaldehyde (0.1M PBS as solvent, pH = 7.4) successively. After perfusion, the brains were collected and immersed in sucrose solutions of 20%, 25% and 30%, respectively and preserved at 4 °C. After the brains were embedded in Tissue Tek O.C.T. compound (Sakura Finetek, Torrance, CA), the continuous coronal slices (15 μm) were prepared for immunofluorescence.

2.5.2. Terminal deoxynucleotidyl transferase-mediated dutp nick-end labeling (TUNEL)

After antigen retrieval, the slices were blocked using 10% sheep serum. The slices were incubated with TUNEL reaction mixture (Roche, Shanghai, China). Next, sections were stained using Hoechst (1:1000, Thermo, Logan, UT, USA), followed by anti-quenching mounting. Apoptotic index (AI) was calculated as (TUNEL-positive cell number)/(total cell number) × 100%.

2.5.3. Thymidine analogue 5-ethynyl-2′-deoxyuridine (EdU) treatment and immunofluorescence analysis in the SGZ

A stock solution of 0.5 mg/mL EdU (Thermo) was prepared in 0.9% saline. Each mouse received a dosage of 5 mg/kg EdU by intraperitoneal injection twice a day for 3 consecutive days (P11-13) and sampling at P14. After antigen retrieval and blocking using 10% sheep serum, slices were incubated overnight at 4 °C with primary antibodies of murine monoclonal anti-NeuN (1:200, Abcam, Cambridge, MA, USA), anti-GFAP (1:200, CST, Danvers, MA, USA) and anti-nestin (1:100, Millipone, Billerica, MA, USA), respectively, to perform the immunofluorescence analyses. Then the slices were incubated with alexa 594 anti-mouse IgG (1:200, Invitrogen, Grand Island, NY, USA). The slices mounted on glass slides were then incubated with EdU reaction mixture (Thermo), followed by incubation with Hoechst mixture. Images were taken using laser confocal microscopy (SP8, Leica,

Mannheim, Germany) and analyzed by Image-Pro Plus software (version 6.0, Media Cybernetics Inc, Rockville, MD). Five images with high magnification were taken for quantification of each sample. Experimenters and data analysts were blinded to conditions throughout.

2.6. Real-time quantitative PCR (RT-qPCR) analysis

Six 14- and 40-day-old mice offspring from six different pregnant mice in each group were randomly selected for the experiment. Total RNA was extracted from hippocampi of mice offspring using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and cDNA was synthesized using a first-strand cDNA synthesis kit (Roche, Basel, Swiss) according to the supplier's instructions. The cDNA products were kept at −20 °C prior to use. The mRNA levels were quantified in triplicate by RT-qPCR with SYBR premix (Takara, Otsu, Japan) on a CFX Manager 3.1 system (Bio-Rad, Hercules, California, USA). Changes in mRNA levels were evaluated by the $2^{-\triangle\triangle Ct}$ method. The primers used for RT-qPCR were presented in Table 1.

2.7. Western blotting analysis

Five 14- and 40-day-old mice offspring from five different pregnant mice in each group were randomly selected for the experiment. Total proteins were isolated from the hippocampi of mice offspring using RIPA lysate supplemented with PMSF (final concentration at 3 μg/μL). Proteins were loaded and separated by 12% SDS-PAGE. Then the proteins were transferred onto PVDF membrane (Millipore, Billerica, MA, USA). The membrane was blocked using 5% skim milk dissolved in TBST (20 mM Tris-HCl of pH 7.5, 0.5 M NaCl, 0.1% Tween 20, 0.5% Triton X-100) for 2 h and incubated with rabbit anti-cleaved caspase-3 polyclonal antibody (1:500, CST) at 4 °C overnight. Then the membrane was washed three times (5 min each time) using TBST and incubated with goat-anti-rabbit IgG (1:2000, Millipore) at room temperature for 2 h, followed by analyses using a chemiluminescence detection kit (Thermo). Target band density was analyzed by Image-Pro Plus software and normalized against GAPDH (1:2000, Proteintech, Rosemont, IL, USA) which was used as an internal reference.

2.8. Enzyme-linked immunosorbent assay (ELISA) analysis

Six 14- and 40-day-old mice offspring from six different pregnant mice in each group were randomly selected for the experiment. Levels of NF-κB, TNF-α and IL-1β in hippocampi of mice offspring were quantified with corresponding ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the producer's instructions.

2.9. Oxidative stress analysis

Six 14- and 40-day-old mice offspring from six different pregnant mice in each group were randomly selected for the experiment. Levels of lipid peroxidation product malondialdehyde (MDA), superoxide dismutases (SOD), glutathione (GSH) and glutathione peroxidase (GSH-Px) were measured using relevant commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the producer's instructions.

2.10. Statistical analysis

Statistical analyses were performed using the SPSS software (version 22.0, SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7.0 (Cabit Information Technology Co., Ltd., Shanghai, China). Experimental data were presented as mean ± SEM. The repeated measures two-way ANOVA combined were used for the repeated measures of 6 days' average escape latency analyses in the MWM. All the other data were analyzed by two-way ANOVA. Two-way ANOVA results were reported as the F-statistics and P values and a Sidak post-hoc test was used for within group comparisons. Boxes represent interquartile ranges, with middle lines representing the medians; whiskers (error bars) above and below the box indicate the 90th and 10th percentiles, respectively; P values less than 0.05 were considered statistically significant.

3. Results

3.1. B-vitamin intervention corrects autism-like behaviors in mice offspring gestationally exposed to $PM_{2.5}$

We conducted a series of behavioral tests in 40-day-old mice offspring. We found no significant difference between male and female in behavioral experiments, with just a slight difference in spatial learning and memory processes. Firstly, we tracked and recorded the spontaneous locomotion paths of offspring (Fig. 1B). We found significant difference in social communication ability among groups (Males: Interaction $F(1,28) = 11.268$, $P = 0.002$, $PM_{2.5}$ $F(1,28) = 21.092$, $P < 0.001$; Females: Interaction $F(1,28) = 4.970$, $P = 0.034$, $PM_{2.5}$ $F(1,28) = 7.281$, $P = 0.012$; Fig. 1D). Post-hoc analysis showed that offspring from model group had significantly lower levels of social communication ability than those from control group ($P < 0.001$, see males of Fig. 1D; $P = 0.002$, see females of Fig. 1D). Furthermore, offspring from intervention group exhibited greater interaction with the target areas than those from model group in the social choice test ($P < 0.001$, see males of Fig. 1D; $P = 0.004$, see females of Fig. 1D). In order to study the stereotyped repetitive behavior of mice offspring, we analyzed their performance in marble burial test (Males: Interaction $F(1,28) = 25.095$, $P < 0.001$, $PM_{2.5}$ $F(1,28) = 10.243$, $P = 0.003$; Females: Interaction $F(1,28) = 11.548$, $P = 0.002$, $PM_{2.5}$ $F(1,28) = 5.008$, $P = 0.033$; Fig. 1E). Post-hoc analysis of B-vitamin effect confirmed that the number of buried marbles reduced and the stereotypical repetitive behavior was improved significantly in B-vitamin-treated mice offspring ($P < 0.001$, see males of Fig. 1E; $P < 0.001$, see females of Fig. 1E). Finally, to further investigate the effects of B-vitamin intervention on cognitive dysfunction of mice offspring, we performed the MWM test. Offspring from all groups after being trained for six days exhibited a decrease in escape latency (Fig. 1F–G). Regarding the probe trial, representative swim traces in each group were shown (Fig. 1H). We found that significant difference in the time needed within the target quadrant between male adult mice offspring, with no significant difference among female ones (Males: Interaction $F(1,28) = 11.201$, $P = 0.002$, $PM_{2.5}$ $F(1,28) = 12.527$, $P = 0.001$; Females: Interaction $F(1,28) = 0.291$, $P = 0.594$, $PM_{2.5}$ $F(1,28) = 0.645$, $P = 0.429$), however. Post-hoc analysis showed that after B-vitamin intervention, male adult mice offspring spent significantly more time within the target quadrant ($P < 0.001$, see males of Fig. 1I). Similar trend was also observed as to the number of target quadrant crossing and movement distance within the target quadrant (The number of target quadrant crossing: Males: Interaction $F(1,28) = 6.283$, $P = 0.018$, $PM_{2.5}$ $F(1,28) = 13.108$, $P = 0.001$; Females: Interaction $F(1,28) = 0.188$, $P = 0.668$, $PM_{2.5}$ $F(1,28) = 3.229$, $P = 0.083$. Movement distance within the target quadrant: Males: Interaction $F(1,28) = 12.111$, $P = 0.002$, $PM_{2.5}$ $F(1,28) = 9.684$, $P = 0.004$; Females: Interaction $F(1,28) = 0.007$, $P = 0.935$, $PM_{2.5}$ $F(1,28) = 3.014$, $P = 0.094$; Fig. 1J–K). Post-hoc analysis showed that

Table 1
Sequences of primers used for RT-qPCR.

Target	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
Caspase-3	CTGGACTGCGGTATTGAGAC	CCGGGTGCGGTAGAGTAAGC
NF-κB	AGGCTTCTGGGCCTTATGTG	TGCTTCTCTCGCCAGGAATAC
TNF-α	ATTCTCTACCCAGCCCCCACTCT	TCCAGGTCACTGTCCCAGCATC
IL-1β	ACCTCACAAGCAGAGCACAAGCC	AAGTCCCTTTCGCAGAAACAG
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

after B-vitamin intervention, the number of target quadrant crossing and movement distance within the target quadrant significantly elevated in male adult mice offspring ($P = 0.001$, see males of Fig. 1J; $P < 0.001$, see males of Fig. 1K). Taken together, gestational exposure to $PM_{2.5}$ affected neurobehavioral functions and B-vitamin intervention was sufficient to restore social communication ability, inhibit the occurrence of stereotyped repetitive behavior, and improve learning and spatial memory impairment.

3.2. B-vitamin intervention alleviates hippocampal oxidative stress and mitochondrial damage induced by $PM_{2.5}$

In the hippocampi of 14- and 40-day-old mice offspring (Fig. 1A-D), we found significant difference in oxidant-related genes (SOD, MDA, GSH and GSH-Px) protein levels among groups (SOD: P14: Interaction $F(1,20) = 5.466$, $P = 0.030$, $PM_{2.5}$ $F(1,20) = 10.356$, $P = 0.004$; P40: Interaction $F(1,20) = 4.644$, $P = 0.044$, $PM_{2.5}$ $F(1,20) = 6.776$, $P = 0.017$. MDA: P14: Interaction $F(1,20) = 40.252$, $P < 0.001$, $PM_{2.5}$ $F(1,20) = 25.569$, $P < 0.001$; P40: Interaction $F(1,20) = 29.766$, $P < 0.001$, $PM_{2.5}$ $F(1,20) = 36.034$, $P < 0.001$. GSH: P14: Interaction $F(1,20) = 9.776$, $P = 0.005$, $PM_{2.5}$ F

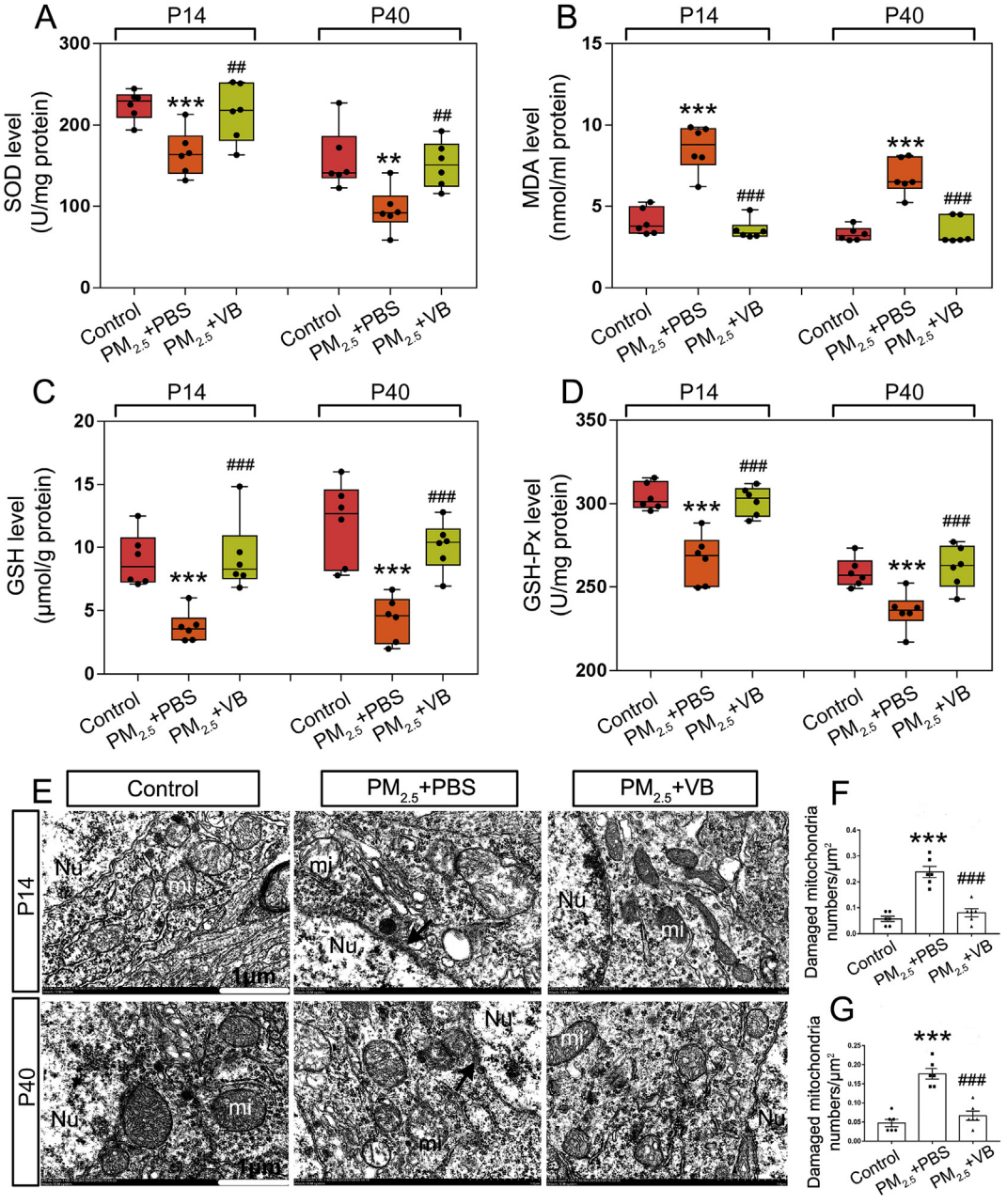

Fig. 2. Oxidative stress and mitochondrial damage induced by gestational exposure to $PM_{2.5}$ are alleviated by B-vitamin intervention in hippocampus of mice offspring. (A) Changes of SOD activity. (B) Lipid peroxidation measured by MDA concentration. (C) Changes of GSH level. (D) Changes of GSH-Px activity. (E) Effects of B-vitamin intervention on ultrastructure of neurons mitochondria in mice offspring (Magnification is 10000 × , bar = 1 μm). The arrow shows the nucleus gap widening and partial vagueness, neurons in model group exhibiting partial vagueness in mitochondrial cristae, vacuolar degeneration in mitochondrion. Nu = nucleus, mi = mitochondrion. (F–G) Numbers of damaged mitochondria per μm^2. $^{**}P < 0.01$,$^{***}P < 0.001$ p.m.$_{2.5}$+PBS compared with control mice, $\#\#P < 0.01$, $\#\#\#P < 0.001$ p.m.$_{2.5}$+VB compared with $PM_{2.5}$+PBS mice, analyzed by Sidak multiple comparisons. Graphs indicate mean ± SEM (n = 6/group).

(1,20) = 8.022, $P = 0.010$; P40: Interaction F(1,20) = 7.109, $P = 0.015$, $PM_{2.5}$ F(1,20) = 18.757, $P < 0.001$. GSH-Px: P14: Interaction F(1,20) = 17.140, $P = 0.001$, $PM_{2.5}$ F(1,20) = 22.650, $P < 0.001$; P40: Interaction F(1,20) = 9.543, $P = 0.006$, $PM_{2.5}$ F (1,20) = 5.251, $P = 0.033$). Post-hoc analysis showed that exposure to $PM_{2.5}$ during pregnancy resulted in increased MDA but decreased SOD, GSH-Px activity and GSH level in hippocampi of 14- and 40-day-old mice offspring ($P = 0.001$, see P14 of Fig. 2A; $P = 0.003$, see P40 of Fig. 2A; $P < 0.001$, see P14 of Fig. 2B; $P < 0.001$, see P40 of Fig. 2B; $P < 0.001$, see P14 of Fig. 2C; $P < 0.001$, see P40 of Fig. 2C; $P < 0.001$, see P14 of Fig. 2D; $P = 0.001$, see P40 of Fig. 2D). However, the oxidative stress induced by $PM_{2.5}$ was corrected by B-vitamin supplementation ($P = 0.004$, see P14 of Fig. 2A; $P = 0.006$, see P40 of Fig. 2A; $P < 0.001$, see P14 of Fig. 2B; $P < 0.001$, see P40 of Fig. 2B; $P < 0.001$, see P14 of Fig. 2C; $P = 0.001$, see P40 of Fig. 2C; $P < 0.001$, see P14 of Fig. 2D; $P < 0.001$, see P40 of Fig. 2D).

Regarding the vital role of mitochondrion in chain of oxidative stress, we further evaluated the ultrastructural changes of mitochondria in hippocampal neurons of mice offspring. The hippocampal neurons mainly showed matrix-type mitochondrial swelling which was characterized by blurred mitochondrial ridge, vacuolar degeneration of mitochondria, and widening of nuclear membrane gap. On the contrary, it was found that the morphology of neurons in hippocampus of mice offspring after B-vitamin intervention was normal, with abundant mitochondria and intact nuclear membrane. Moreover, there was no significant difference between the intervention and control groups (Fig. 2E). Finally, the number of mitochondria per unit area was calculated and a significant interaction effect was found by two-way ANOVA (P14: Interaction F(1,20) = 36.253, $P < 0.030$, $PM_{2.5}$ F (1,20) = 59.911, $P < 0.004$; P40: Interaction F(1,20) = 23.407, $P < 0.001$, $PM_{2.5}$ F(1,20) = 42.522, $P < 0.001$). Post-hoc analysis revealed that the number of damaged mitochondria in model group increased significantly ($P < 0.001$, see Fig. 2F; $P < 0.001$, see Fig. 2F), while it decreased significantly after B-vitamin intervention ($P < 0.001$, see Fig. 2G; $P < 0.001$, see Fig. 2G). The hippocampus of 14- and 40-day-old offspring showed the same trend (Fig. 2F–G). Therefore, B-vitamin intervention alleviated both oxidative and mitochondrial damage in hippocampus of mice offspring induced by gestational exposure to $PM_{2.5}$.

3.3. B-vitamin intervention alleviates hippocampal neuroinflammation induced by $PM_{2.5}$

Given the beneficial effects of B-vitamin intervention on $PM_{2.5}$-induced oxidative damage and mitochondrial damage, we infer that B-vitamin may alleviate the neuroinflammation induced by gestational exposure to $PM_{2.5}$ through its anti-inflammatory property. $PM_{2.5}$, however, did lead to a significant increase in the expression of inflammatory mediators (NF-κB, TNF-α and IL-1β) in protein level, with a significant interaction effect between $PM_{2.5}$ exposure and B-vitamin treatment (NF-κB: P14: Interaction F(1,20) = 9.372, $P = 0.006$, $PM_{2.5}$ F (1,20) = 32.776, $P < 0.001$; P40: Interaction F(1,20) = 4.867, $P = 0.039$, $PM_{2.5}$ F(1,20) = 22.822, $P < 0.001$. TNF-α: P14: Interaction F(1,20) = 11.525, $P = 0.003$, $PM_{2.5}$ F(1,20) = 31.004, $P < 0.001$; P40: Interaction F(1,20) = 6.542, $P = 0.019$, $PM_{2.5}$ F (1,20) = 32.520, $P < 0.001$. IL-1β: P14: Interaction F(1,20) = 17.965, $P < 0.001$, $PM_{2.5}$ F(1,20) = 41.400, $P < 0.001$; P40: Interaction F (1,20) = 7.089, $P = 0.015$, $PM_{2.5}$ F(1,20) = 24.302, $P < 0.001$). Similar results were also seen for the mRNA level of NF-κB, TNF-α and IL-1β (NF-κB: P14: Interaction F(1,20) = 4.406, $P = 0.049$, $PM_{2.5}$ F (1,20) = 6.851, $P = 0.016$; P40: Interaction F(1,20) = 6.791, $P = 0.017$, $PM_{2.5}$ F(1,20) = 5.504, $P = 0.029$. TNF-α: P14: Interaction F(1,20) = 4.737, $P = 0.042$, $PM_{2.5}$ F(1,20) = 7.092, $P = 0.015$; P40: Interaction F(1,20) = 5.073, $P = 0.036$, $PM_{2.5}$ F(1,20) = 5.278, $P = 0.033$. IL-1β: P14: Interaction F(1,20) = 10.017, $P = 0.005$, $PM_{2.5}$ F(1,20) = 8.185, $P = 0.010$; P40: Interaction F(1,20) = 20.858, $P < 0.001$, $PM_{2.5}$ F(1,20) = 31.462, $P < 0.001$). Post-hoc analysis showed that after B-vitamin intervention, the expression of these

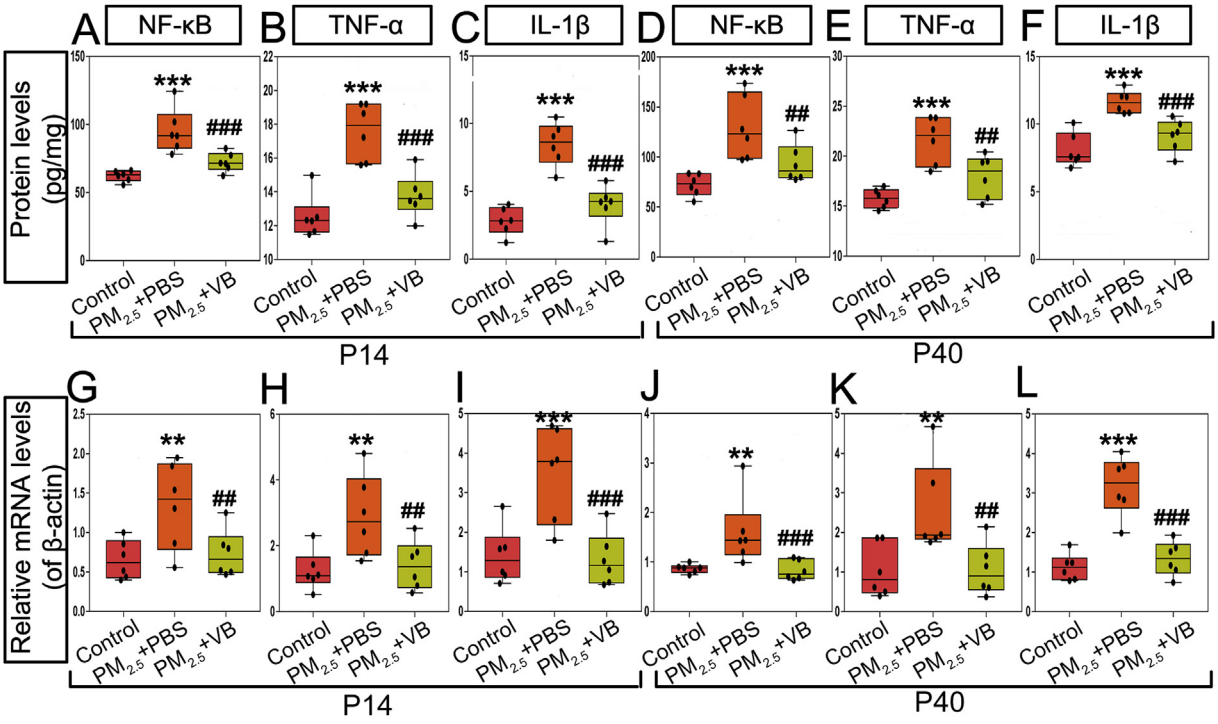

Fig. 3. Neuroinflammation induced by gestational exposure to $PM_{2.5}$ is alleviated by B-vitamin intervention in hippocampus of mice offspring. (A-L)Protein and mRNA level changes of NF-κB, TNF-α and IL-1β, respectively. All mRNA levels were normalized to β-actin, respectively. $^{**}P < 0.01$,$^{***}P < 0.001$ p.m.$_{2.5}$ + PBS compared with control mice, $\#\#P < 0.01$, $\#\#\#P < 0.001$ p.m.$_{2.5}$ + VB compared with $PM_{2.5}$ + PBS mice, analyzed by Sidak multiple comparisons. Graphs indicate mean ± SEM (n = 6/group).

inflammatory mediators such as NF-κB, TNF-α and IL-1β in hippocampi of mice offspring significantly decreased ($P < 0.001$, see Fig. 3A; $P < 0.001$, see Fig. 3B; $P < 0.001$, see Fig. 3C; $P = 0.005$, see Fig. 3D; $P = 0.002$, see Fig. 3E; $P = 0.001$, see Fig. 3F; $P = 0.008$, see Fig. 3G; $P = 0.006$, see Fig. 3H; $P < 0.001$, see Fig. 3I; $P = 0.001$, see Fig. 3J; $P = 0.005$, see Fig. 3K; $P < 0.001$, see Fig. 3L). Then, we used hematoxylin-eosin (HE) staining to observe the pathological structure of the brain in mice offspring (Supplemental Fig. 2). Whereas, after the intervention of vitamin B, the nerve cells were arranged closely and orderly, and the nucleus was clearly visible. There was no significant difference between the intervention group and control groups. The results also showed that the dosage of B-vitamin was appropriate and exerted no toxic side effects.

3.4. B-vitamin alleviates hippocampal synaptic dysfunction induced by $PM_{2.5}$

We observed the ultrastructure of synapses in hippocampus neurons of 14- and 40-day-old mice offspring by transmission electron microscopy (Fig. 4A). Analysis by two-way ANOVA found no effect of $PM_{2.5}$ exposure on the number of synapses in 14- and 40-day-old mice offspring (P14: Interaction $F(1,20) = 5.324$, $P = 0.032$, $PM_{2.5}$ $F(1,20) = 1.195$, $P = 0.287$; P40: Interaction $F(1,20) = 1.433$, $P = 0.245$, $PM_{2.5}$ $F(1,20) = 1.433$, $P = 0.245$). In the hippocampi of 14- and 40-day-old mice offspring, we found significant difference in synaptic cleft, PSD thickness and length of synaptic active area among groups (Synaptic cleft: P14: Interaction $F(1,20) = 4.552$, $P = 0.045$, $PM_{2.5}$ $F(1,20) = 4.572$, $P = 0.045$; P40: Interaction $F(1,20) = 5.093$, $P = 0.035$, $PM_{2.5}$ $F(1,20) = 10.511$, $P = 0.004$. PSD thickness: P14: Interaction $F(1,20) = 7.182$, $P = 0.014$, $PM_{2.5}$ $F(1,20) = 7.028$, $P = 0.015$; P40: Interaction $F(1,20) = 4.909$, $P = 0.038$, $PM_{2.5}$ F

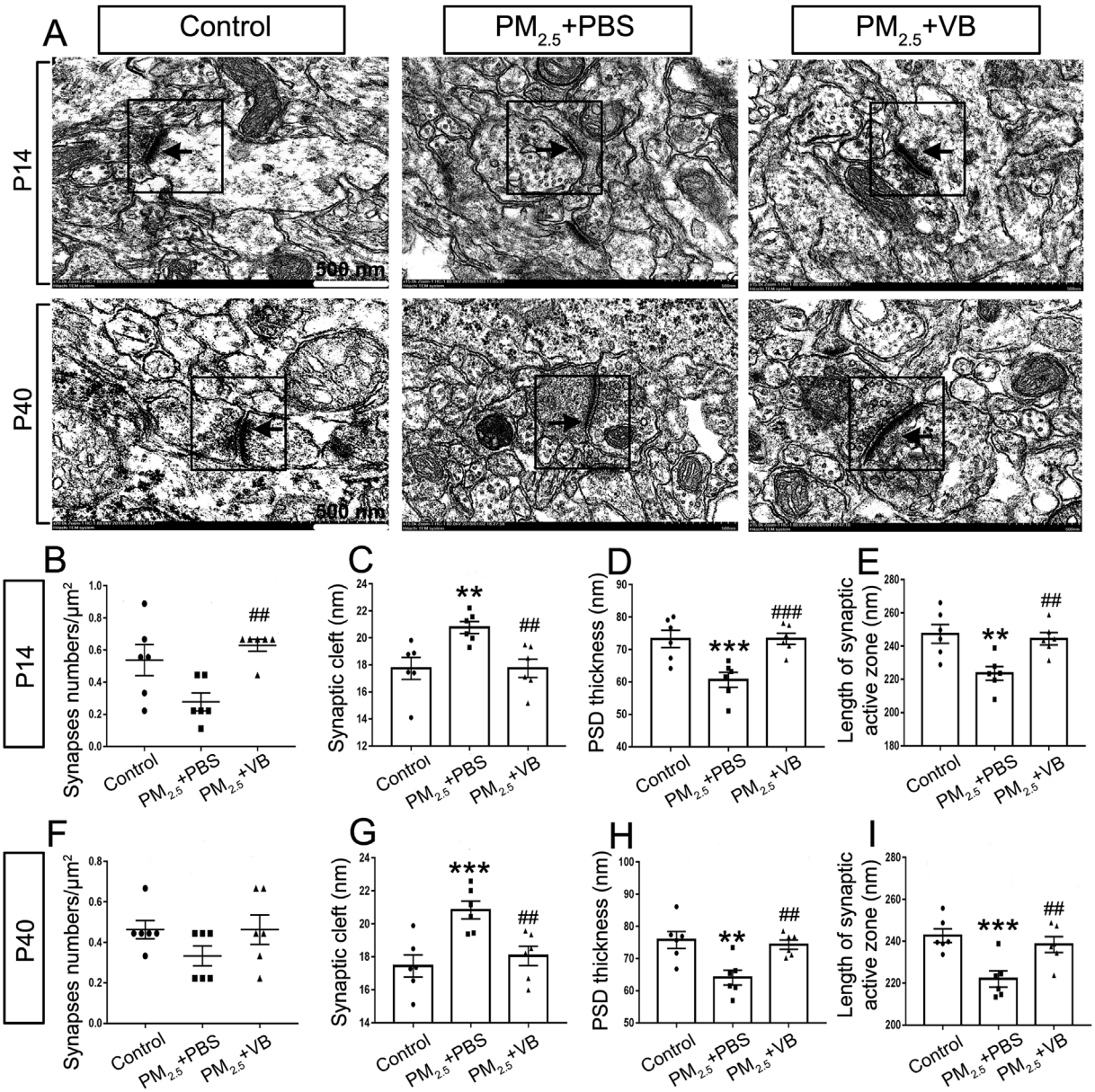

Fig. 4. Synaptic dysfunction induced by gestational exposure to $PM_{2.5}$ is alleviated by B-vitamin intervention in hippocampus of mice offspring. (A) Ultrastructure of synapses in mice offspring (Magnification = 15000 × , bar = 500 nm).(B) Numbers of synapses per μm^2. (C) Synaptic cleft. (D) PSD thickness. (E) Length of synaptic active zone. $^{**}P < 0.01$,$^{***}P < 0.001$ p.m.$_{2.5}$+PBS compared with control mice, $\#\#P < 0.01$, $\#\#\#P < 0.001$ p.m.$_{2.5}$+VB compared with $PM_{2.5}$+PBS mice, analyzed by Sidak multiple comparisons. Graphs indicate mean ± SEM (n = 6/group).

(1,20) = 8.102, $P = 0.010$. Length of synaptic active area: P14: Interaction F(1,20) = 4.579, $P = 0.045$, $PM_{2.5}$ F(1,20) = 7.595, $P = 0.012$; P40: Interaction F(1,20) = 5.356, $P = 0.031$, $PM_{2.5}$ F(1,20) = 12.459, $P = 0.002$). We found that after $PM_{2.5}$ exposure during pregnancy, synaptic cleft increased, PSD thickness thinned, and length of synaptic active area shortened in 14- and 40-day-old mice offspring ($P = 0.007$, see Fig. 4C; $P = 0.001$, see Fig. 4D; $P = 0.002$, see Fig. 4E; $P = 0.001$, see Fig. 4G; $P = 0.002$, see Fig. 4H; $P = 0.001$, see Fig. 4I). After B-vitamin intervention, synaptic efficiency increased significantly, synaptic cleft, PSD thickness and synaptic interface active area were normalized to levels ($P = 0.007$, see Fig. 4C; $P = 0.001$, see Fig. 4D; $P = 0.007$, see Fig. 4E; $P = 0.005$, see Fig. 4G; $P = 0.005$, see Fig. 4H; $P = 0.004$, see Fig. 4I). Our results suggested that B-vitamin could improve synaptic dysfunction of offspring induced by $PM_{2.5}$ exposure during pregnancy and enhance the effectiveness of neurotransmission.

3.5. B-vitamin decreases hippocampal apoptosis induced by $PM_{2.5}$

To examine the hippocampal apoptosis, we analyzed the well-known apoptotic marker of cleaved caspase-3 protein (P14: Interaction F(1,16) = 12.066, $P = 0.003$, $PM_{2.5}$ F(1,16) = 16.927, $P = 0.001$; P40: Interaction F(1,16) = 11.782, $P = 0.003$, $PM_{2.5}$ F(1,16) = 50.468, $P < 0.001$). Post-hoc analysis showed that its expression in hippocampus of 14- and 40-day-old mice offspring gestationally exposed to $PM_{2.5}$ was significantly decreased after B-vitamin intervention ($P < 0.001$, see P14 of Fig. 5B; $P < 0.001$, see P40 of Fig. 5B). The results of RT-qPCR showed that the mRNA level of caspase-3 did not change significantly among the three groups (P14: Interaction F (1,20) = 0.484, $P = 0.495$, $PM_{2.5}$ F(1,20) = 2.914, $P = 0.103$; P40: Interaction F(1,20) = 0.000, $P = 0.993$, $PM_{2.5}$ F(1,20) = 0.169, $P = 0.687$). We speculated that $PM_{2.5}$ mainly affected the caspase-3 shear activation process during the initiation of apoptosis (Fig. 5B). To further confirm that B-vitamin intervention could improve $PM_{2.5}$-induced apoptosis, TUNEL staining was performed on brain slices of 14- and 40-day-old mice offspring (Fig. 5C–D). Semi-quantitative fluorescence results showed that gestational exposure to $PM_{2.5}$ could induce significant apoptosis in hippocampus CA1, CA2, CA3 and DG cells, with a significant interaction effect between $PM_{2.5}$ exposure and B-vitamin intervention (CA1: P14: Interaction F(1,20) = 19.131, $P < 0.001$, $PM_{2.5}$ F(1,20) = 33.033, $P < 0.001$; P40: Interaction F (1,20) = 75.202, $P < 0.001$, $PM_{2.5}$ F(1,20) = 94.229, $P < 0.001$. CA2: P14: Interaction F(1,20) = 33.286, $P < 0.001$, $PM_{2.5}$ F (1,20) = 47.658, $P < 0.001$; P40: Interaction F(1,20) = 54.954, $P < 0.001$, $PM_{2.5}$ F(1,20) = 54.302, $P < 0.001$. CA3: P14: Interaction F(1,20) = 25.607, $P < 0.001$, $PM_{2.5}$ F(1,20) = 31.144, $P < 0.001$; P40: Interaction F(1,20) = 55.164, $P < 0.001$, $PM_{2.5}$ F (1,20) = 55.157, $P < 0.001$. DG: P14: Interaction F(1,20) = 16.458, $P < 0.001$, $PM_{2.5}$ F(1,20) = 18.631, $P < 0.001$; P40: Interaction F (1,20) = 57.489, $P < 0.001$, $PM_{2.5}$ F(1,20) = 56.361, $P < 0.001$). Post-hoc analysis showed that after gestational exposure to $PM_{2.5}$, mice offspring had higher number of TUNEL-positive cells in the hippocampus compared with those from control group ($P < 0.001$, see CA1 of Fig. 5E; $P < 0.001$, see CA2 of Fig. 5E; $P < 0.001$, see CA3 of Fig. 5E; $P < 0.001$, see DG of Fig. 5E; $P < 0.001$, see CA1 of Fig. 5F; $P < 0.001$, see CA2 of Fig. 5F; $P < 0.001$, see CA3 of Fig. 5F; $P < 0.001$, see DG of Fig. 5F), with an apoptotic rate of 30%–40%. In contrast, after B-vitamin intervention, $PM_{2.5}$-induced apoptosis decreased significantly ($P < 0.001$, see CA1 of Fig. 5E; $P < 0.001$, see CA2 of Fig. 5E; $P < 0.001$, see CA3 of Fig. 5E; $P < 0.001$, see DG of Fig. 5E; $P < 0.001$, see CA1 of Fig. 5F; $P < 0.001$, see CA2 of Fig. 5F; $P < 0.001$, see CA3 of Fig. 5F; $P < 0.001$, see DG of Fig. 5F). Thus, vitamins intervention decreased apoptosis induced by gestational exposure to $PM_{2.5}$ in mice offspring.

3.6. B-vitamin improves hippocampal neurogenesis induced by $PM_{2.5}$ in mice offspring

Because our TUNEL results (Fig. 5D-E) showed that gestational $PM_{2.5}$ exposure affected the niche of SGZ, which was significantly normalized by B-vitamin intervention. Therefore, we further investigated whether the neurogenesis of DG was affected. The development of new neurons in hippocampus of human brain gradually weakens with age, and stops completely in adulthood (Sorrells et al., 2018). Therefore, 14-day-old mice offspring were selected for the experiment. To quantify neurogenesis in DG, we analyzed the number of EdU-positive cells using confocal microscopy. NeuN/EdU, GFAP/EdU and Nestin/EdU double-labeling were performed to explore the effects of gestational exposure to $PM_{2.5}$ plus B-vitamin treatment on neuronal progenitors in the SGZ. We found significant difference in proliferation and differentiation of SGZ among groups (EdU-positive cells: Interaction F(1,20) = 146.757, $P < 0.001$, $PM_{2.5}$ F(1,20) = 191.034, $P < 0.001$; GFAP/EdU double-labeled cells: Interaction F (1,20) = 57.177, $P < 0.001$, $PM_{2.5}$ F(1,20) = 87.746, $P < 0.001$; NeuN/EdU double-labeled cells: Interaction F(1,20) = 59.066, $P < 0.001$, $PM_{2.5}$ F(1,20) = 64.372, $P < 0.001$; Nestin/EdU double-labeled cells: Interaction F(1,20) = 11.344, $P = 0.003$, $PM_{2.5}$ F (1,20) = 22.655, $P < 0.001$). Post-hoc analysis showed that the total number of EdU-positive cells in SGZ significantly decreased, and the proliferation of nerve cells decreased significantly due to $PM_{2.5}$ exposure ($P < 0.001$, see Fig. 6B). After B-vitamin intervention, the proliferation ability of nerve cells returned to the same level as that of the control group ($P < 0.001$, see Fig. 6B). Collectively, GFAP/EdU, NeuN/EdU and Nestin/EdU double-labeled cells in model group were significantly lower than those in control group ($P < 0.001$, see Fig. 6C; $P < 0.001$, see Fig. 6D; $P < 0.001$, see Fig. 6E), while NeuN/EdU, GFAP/EdU and Nestin/EdU double-labeled cells in B-vitamin intervention group were significantly improved to normal level ($P < 0.001$, see Fig. 6C; $P < 0.001$, see Fig. 6D; $P < 0.001$, see Fig. 6E). Therefore, B-vitamin improved the proliferation and differentiation of SGZ in mice offspring gestationally exposed to $PM_{2.5}$, subsequently affecting neurogenesis.

4. Discussion

The results of the present study demonstrate that exposure to $PM_{2.5}$ during pregnancy induces persistent neurobiological effects including social communication deficits, stereotyped repetitive behavior and learning and spatial memory impairment. Furthermore, we found that $PM_{2.5}$ causes neurodevelopmental impairment in hippocampus of offspring through many ways. Hence, it is urgent to find effective interventions to alleviate the $PM_{2.5}$-induced potential harm to embryonic neurodevelopment.

A few recent studies have shown that supplementation of B-vitamin (folic acid, vitamin B6 and vitamin B12) in adults can alleviate $PM_{2.5}$-induced injury. Four weeks of administration can limit the $PM_{2.5}$ effect to 28%–76%, thus helping healthy adults resist cardiac autonomic nervous dysfunction caused by short-term $PM_{2.5}$ exposure (Zhong et al., 2017a, 2017b). Interestingly, we found that after B-vitamin intervention in pregnant mice exposed to $PM_{2.5}$, the abnormal behaviors associated with neurodevelopmental impairment in offspring were prevented. Epidemiological studies have shown that $PM_{2.5}$ exposure is closely associated with adverse neurological outcomes (Guo et al., 2018). Our results demonstrated that gestational exposure to high concentration of $PM_{2.5}$ affected the growth and development of fetal mice nervous system, manifesting as spatial learning and memory dysfunction, along with autism-like behaviors in offspring. Studies have shown that hippocampal volumes of autistic patients are significantly smaller than those of neurotypical patients (Braden et al., 2017). In the mice model of autism, long-term depression (LTD) was deregulated and synaptic plasticity was disordered (Hansel, 2019). Synapse is the key

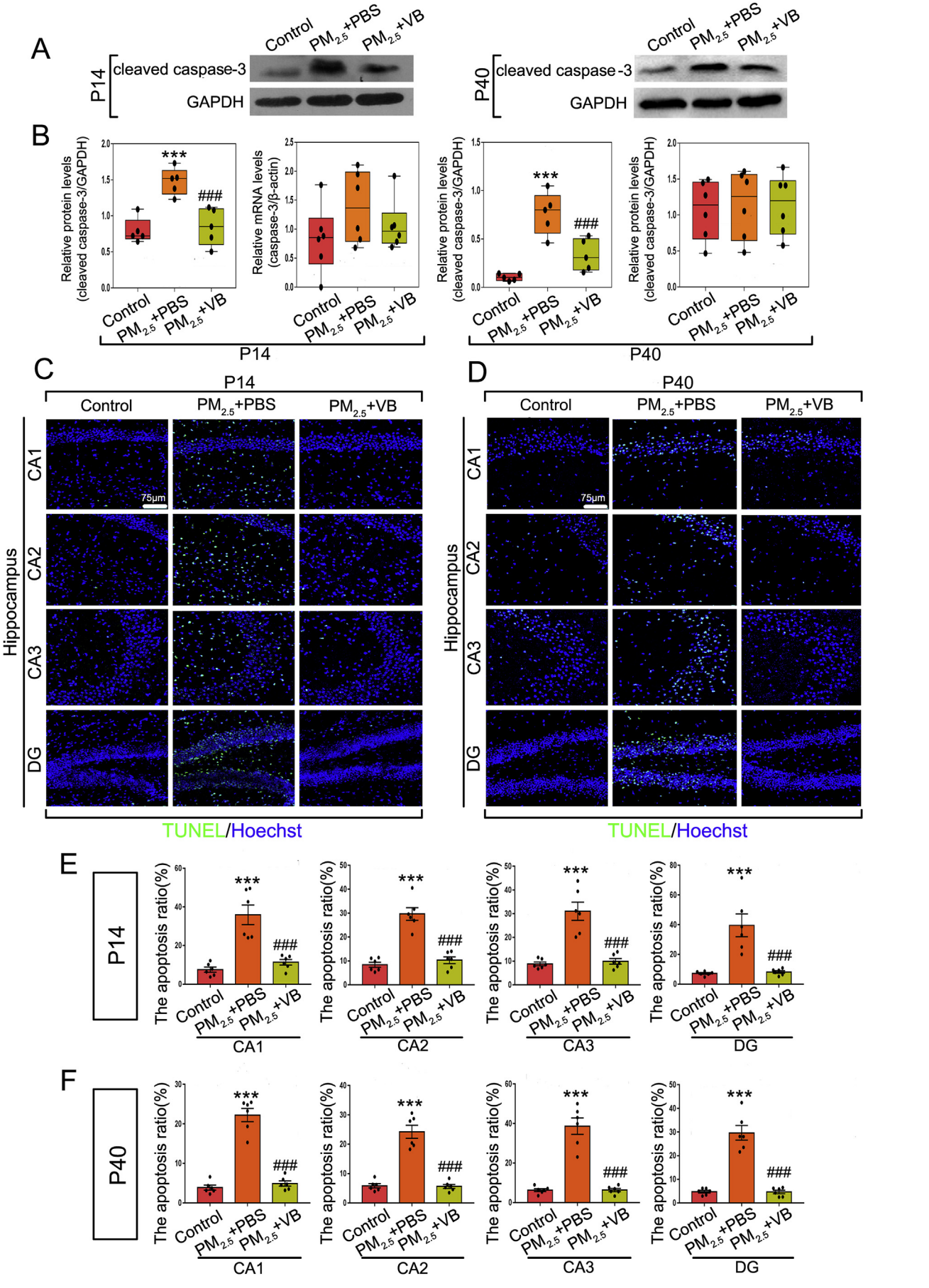

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Fig. 5. Apoptosis induced by gestational exposure to $PM_{2.5}$ in hippocampus of mice offspring is alleviated by B-vitamin intervention. (A–B) Protein level changes of cleaved caspase-3 (n = 5/group). (B) mRNA level changes of caspase-3 (n = 6/group). (C–D) Laser confocal photographs of TUNEL-positive cells in hippocampal CA1, CA2, CA3 and DG regions in 14- and 40-day-old mice offspring, respectively. TUNEL staining (green) and nuclear staining (Hoechst, blue). Magnification is 400 × , bar = 75 μm. (E) Apoptotic ratio of nerve cells in 14-day-old mice offspring (n = 6/group).(F) Apoptotic ratio of nerve cells in 40-day-old mice offspring (n = 6/group). ***P < 0.001 p.m.$_{2.5}$ + PBS compared with control mice, ###P < 0.001 p.m.$_{2.5}$ + VB compared with $PM_{2.5}$ + PBS mice, analyzed by Sidak multiple comparisons. Graphs indicate mean ± SEM.

part of nerve information transmission between neurons, and synaptic plasticity plays a key role in the development, learning and memory of the nervous system (Mansvelder et al., 2019). Synaptic plasticity (synaptic cleft, PSD thickness, and synaptic active area length) are effective indicators of synaptic connectivity and transmission efficiency. This study found that gestational exposure to $PM_{2.5}$ leads to synaptic dysfunction of hippocampus, probably related to the decrease of synaptic efficiency in hippocampal neurons induced by $PM_{2.5}$. Intervention of B-vitamin results in significantly increased synaptic efficiency, PSD thickness and synaptic active area length, decreased synaptic cleft, along with increased contact area, which was conducive to more effective binding between transmitters and receptors. Synaptic efficacy changes in hippocampal neuronal networks are the biological basis of learning and memory (https://www.ncbi.nlm.nih.gov/

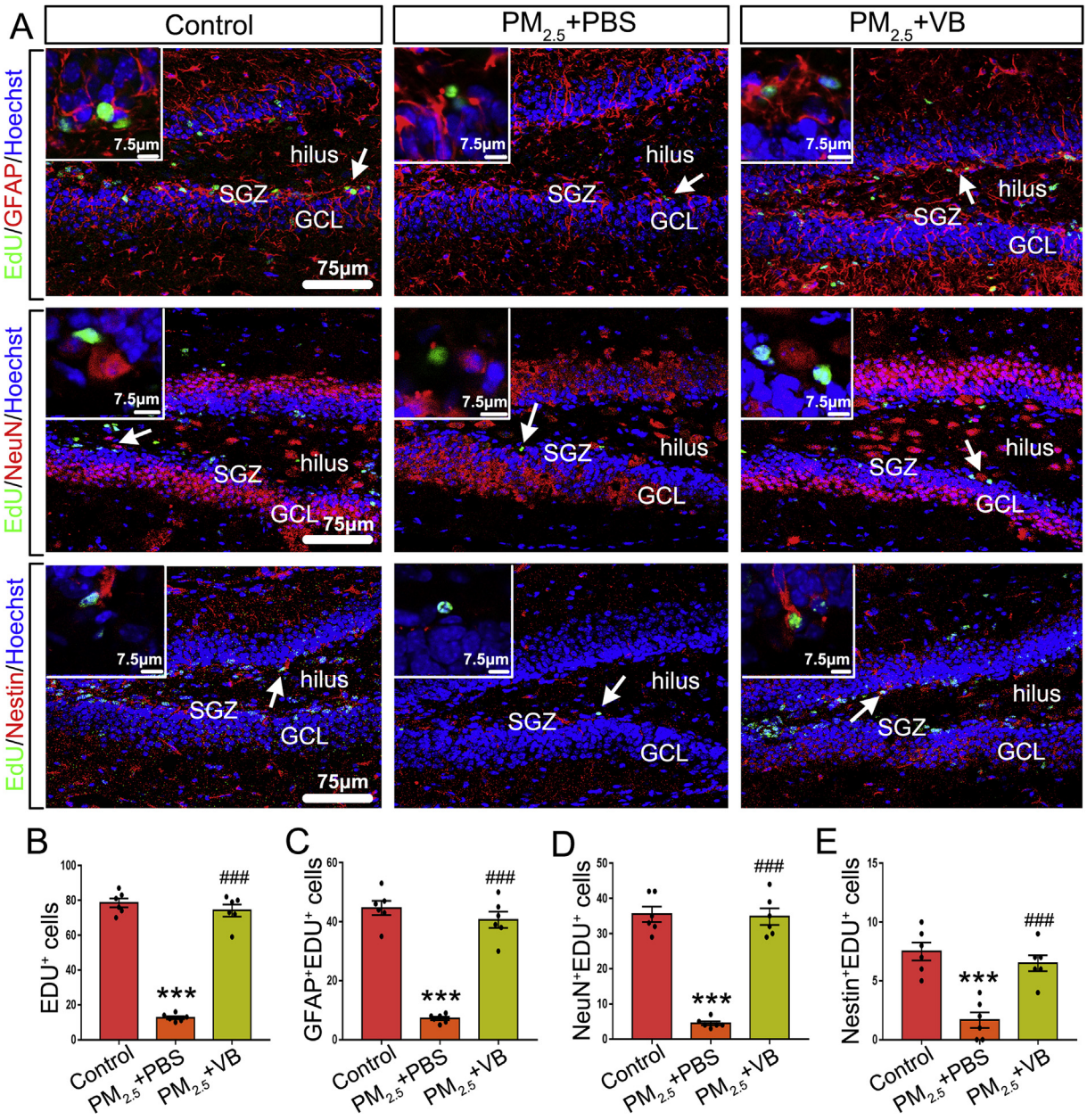

Fig. 6. B-vitamin intervention improves hippocampal neurogenesis in mice offspring after exposure to $PM_{2.5}$. (A) Laser confocal photographs of NeuN/EdU, GFAP/EdU and Nestin/EdU labeled hippocampal DG regions, respectively. NeuN staining (red), GFAP staining (red), Nestin staining (red), EdU staining (green) and nuclear staining (Hoechst, blue). Magnification is 400 × , bar = 75 μm, inset bar = 7.5 μm. (B) EdU + cells. (C) GFAP + /EdU + cells F = 91.859, P = 0.000). (D) NeuN + /EdU + cells. (E) Nestin + /EdU + cells. ***P < 0.001 p.m.$_{2.5}$ + PBS compared with control mice, ###P < 0.001 p.m.$_{2.5}$ + VB compared with $PM_{2.5}$ + PBS mice, analyzed by Sidak multiple comparisons. Graphs indicate mean ± SEM (n = 6/group).

pubmed/?term=Truchet%20B%5bAuthor%5d&cauthor=true&cauthor_uid=12440576, Truchet et al., 2002). Thus, B-vitamin supplementation enhanced learning and memory abilities of mice offspring gestationally exposed to $PM_{2.5}$, which may be closely related to the maintenance of synaptic remodeling by B-vitamin.

Our study found that $PM_{2.5}$ exposure during pregnancy was closely related to both neuroinflammation and inhibited antioxidant system in offspring. $PM_{2.5}$ reduced antioxidant enzyme activity and increased lipid peroxidation. In addition, the secretion of inflammatory mediators aforementioned increased. It has been confirmed in cell models that long-term exposure to $PM_{2.5}$ can lead to synaptic dysfunction, intracellular reactive oxygen species (ROS) production accompanied by GSH depletion and mitochondrial membrane potential loss, activation of NF-κB inflammatory pathway, stimulation of excitatory synaptic transmission, and ultimate nerve injury (Li et al., 2018). It remains unclear whether adequate B-vitamin supplementation can alleviate inflammation and oxidative damage in hippocampus of mice offspring gestationally exposed to $PM_{2.5}$. Fetus exposure to maternal inflammation increases the risk of ASD. Animal experiments exhibited how maternal inflammation during pregnancy can lead to neurodevelopmental disorders, cortical damage and behavioral abnormalities in offspring (Kim et al., 2017; Shin Yim et al., 2017). To the best of our knowledge, $PM_{2.5}$ exposure is closely related to central nervous system-related diseases including cognitive impairment, neurodegenerative diseases and some mental disorders such as schizophrenia, depression and autism (Fu et al., 2019). Mitochondrial dysfunction may also be a potential mechanism for $PM_{2.5}$-induced brain injury. Once mitochondria are destroyed, their dysfunction leads to a decrease in ATP production but an increase in ROS production. Since mitochondria play an important role in metabolism, mitochondrial dysfunction is an important cause of autism and other neurodevelopmental disorders (Hollis et al., 2017). Our study demonstrated that the mice offspring gestationally exposed to $PM_{2.5}$ exhibited oxidative damage, while the mitochondria in hippocampal neurons exhibited cristae rupture, local blurring, swelling and vacuolation. Previous study has reported that in ASD, activated microglial and astrocytes, proinflammatory cytokines, and aberrant NF-κB activity can ultimately create an environment of excessive brain inflammation, which can lead to destruction of critical brain tissue (Kern et al., 2015). Accordingly, it can be inferred that inhibition of $PM_{2.5}$-induced inflammation and oxidative stress helps to restricting $PM_{2.5}$-related brain diseases. Vitamin B6, vitamin B12 and folic acid inhibit not only the activation of many pro-inflammatory cytokines such as NF-κB, TNF-α and IL-1β but also the production of ROS in mitochondria, and reduce the inflammatory response during pregnancy (Cianciulli et al., 2016; Zhang et al., 2016; Kemse et al., 2016). Our study found that B-vitamin supplementation during pregnancy resulted in down-regulated proinflammatory mediators, significantly increased antioxidant factors, decreased lipid peroxidation, and significantly decreased number of damaged mitochondria. These results suggest that B-vitamin supplementation effectively inhibit $PM_{2.5}$-induced inflammation and oxidative damage in hippocampus of mice offspring. Inflammation and oxidative stress play an important role in the pathogenesis of schizophrenia. Patients with schizophrenia often have abnormal immune activation which leads to increased levels of pro-inflammatory cytokines and eventually functional brain damage. B-vitamin (Vitamin B6, Vitamin B12, folic acid) supplementation in different stages of schizophrenia also improves the adverse effects of $PM_{2.5}$ exposure on offspring neurodevelopment based on its anti-inflammatory and anti-oxidative activities (Mitra et al., 2017).

It was reported that $PM_{2.5}$ can induce apoptosis through inflammation, oxidative stress and mitochondrial damage (Piao et al., 2018; Bhattacharjee et al., 2016). We studied the expression of apoptosis-related genes in hippocampus of mice offspring. The results showed that $PM_{2.5}$ exposure during pregnancy induced caspase-3 activation and increased apoptosis. TUNEL staining further confirmed that the significantly increased apoptosis. Different regions of hippocampus have different division of labor. CA1 is related to various forms of memory. CA2 is crucial to social memory determining the ability of individuals to recognize other animals of the same kind. CA3 gives us the ability to recall according to sporadic clues, while the DG is responsible for distinguishing similar environments (Hsiao et al., 2013). This may further explain the impairment in learning and memory in mice offspring exposed to $PM_{2.5}$ during pregnancy. The decreased cleaved caspase-3 level and TUNEL-positive cells in the present work suggested the inhibited $PM_{2.5}$-induced apoptosis by B-vitamin. Therefore, B-vitamin supplementation may reduce neuronal injury by inhibiting abnormal neuronal apoptosis. Interestingly, more apoptotic cells were found in the SGZ of the DG of hippocampus, so it is speculated that $PM_{2.5}$ exposure during pregnancy may affect the proliferation and differentiation of the offspring in the SGZ of hippocampus. To confirm the hypothesis, we performed fluorescent staining of the SGZ in hippocampus, and found that the cell proliferation decreased and the differentiation to neurons decreased significantly. The autistic mouse model has exhibited significant abnormalities in the neurogenesis of hippocampus (Cope et al., 2016). It was found that folic acid combined with vitamin B12 could promote the growth of human neuroblastoma SH-SY5Y neuron cells and reduce the death rate of neurons, thus protecting neurons (Tripathi et al., 2016). In order to further verify the relationship between B-vitamin and the apoptosis and proliferation of neurons in offspring induced by $PM_{2.5}$ exposure during pregnancy, the mixture of vitamin B6, vitamin B12 and folic acid was employed. It was found that cleaved caspase-3 expression in hippocampus of mice offspring and apoptotic rate were down-regulated, while the number of new neurons in SGZ and the differentiation towards neurons were obviously increased, with a level comparable to the normal. These suggest that B-vitamin supplementation may alleviate the effects of $PM_{2.5}$ on the autistic-like behaviors of offspring mice by regulating apoptosis and improving neurogenesis.

B-vitamin intervention alleviates oxidative stress, neuroinflammation, mitochondrial damage, and synaptic dysfunction induced by gestational exposure to $PM_{2.5}$ in mice offspring. B-vitamin supplementation exhibits comparable preventive effects in both 14- and 40-day-old offspring, suggesting its long-term effects on improving neurodevelopmental abnormalities caused by $PM_{2.5}$ exposure during pregnancy. And it is still necessary to further elucidate the underlying mechanism of B-vitamin against the impaired neurodevelopment in offspring caused by $PM_{2.5}$ during pregnancy.

5. Conclusion

Our results suggest that B-vitamin supplementation exerts preventive effects on autism-like behavior and normalizes neurodevelopmental impairment in hippocampus of mice offspring gestationally exposed to $PM_{2.5}$, probably involved in alleviated mitochondrial damage, increased anti-inflammatory and antioxidant capacities as well as synaptic efficiency, reduced neuronal apoptosis and improved hippocampal neurogenesis. B-vitamin supplementation exhibits comparable preventive effects in both 14- and 40-day-old offspring, suggesting its long-lasting effects on improving neurodevelopmental abnormalities caused by $PM_{2.5}$ exposure during pregnancy. In conclusion, B-vitamin demonstrates promising preventive effects against the adverse outcomes induced by $PM_{2.5}$ exposure in embryonic nerve development.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

LY and YG designed the experiments, interpreted the results and

critically revised the manuscript. TW wrote the manuscript. TW and LS performed the experiments. TZ and WL analyzed the data and generated figures. CZ interpreted the results and revised the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109686.

References

Abookasis, D., Lerman, D., Roth, H., Tfilin, M., Turgeman, G., 2018. Optically derived metabolic and hemodynamic parameters predict hippocampal neurogenesis in the BTBR mouse model of autism. J. Biophot. 11, e201600322. https://doi.org/10.1002/jbio.201600322.

Alfawaz, H., Al-Onazi, M., Bukhari, S.I., Binobead, M., Othman, N., Algahtani, N., et al., 2018. The independent and combined effects of omega-3 and vitamin B12 in ameliorating propionic acid induced biochemical features in Juvenile Rats as rodent model of autism. J. Mol. Neurosci. 66, 403–413. https://doi.org/10.1007/s12031-018-1186-z.

Allen, J.L., Conrad, K., Oberdorster, G., Johnston, C.J., Sleezer, B., Cory-Slechta, D.A., 2013. Developmental exposure to concentrated ambient particles and preference for immediate reward in mice. Environ. Health Perspect. 121, 32–38. https://doi.org/10.1289/ehp.1205505.

Bjorklund, G., Waly, M.I., Al-Farsi, Y., Saad, K., Dadar, M., Rahman, M.M., et al., 2019. The role of vitamins in autism spectrum disorder: what do we know? J. Mol. Neurosci. 67, 373–387. https://doi.org/10.1007/s12031-018-1237-5.

Bhattacharjee, A., Prasad, S.K., Pal, S., Maji, B., Banerjee, A., Das, D., et al., 2016. Possible involvement of iNOS and TNF-alpha in nutritional intervention against nicotine-induced pancreatic islet cell damage. Biomed. Pharmacother. 84, 1727–1738. https://doi.org/10.1016/j.biopha.2016.10.079.

Braden, B.B., Smith, C.J., Thompson, A., Glaspy, T.K., Wood, E., Vatsa, D., et al., 2017. Executive function and functional and structural brain differences in middle-age adults with autism spectrum disorder. Autism Res. 10, 1945–1959. https://doi.org/10.1002/aur.1842.

Cai, Y., Zhong, H., Li, X., Xiao, R., Wang, L., Fan, X., 2019. The liver X receptor agonist TO901317 ameliorates behavioral deficits in two mouse models of autism. Front. Cell. Neurosci. 13, 213. https://doi.org/10.3389/fncel.2019.00213.

Chao, M.W., Yang, C.H., Lin, P.T., Yang, Y.H., Chuang, Y.C., Chung, M.C., et al., 2017. Exposure to $PM_{2.5}$ causes genetic changes in fetal rat cerebral cortex and hippocampus. Environ. Toxicol. 32, 1412–1425. https://doi.org/10.1002/tox.22335.

Chen, X., Cao, H., Saraf, A., Zweifel, L.S., Storm, D.R., 2015. Overexpression of the type 1 adenylyl cyclase in the forebrain leads to deficits of behavioral inhibition. J. Neurosci. 35, 339–351. https://doi.org/10.1523/JNEUROSCI.2478-14.2015.

Cianciulli, A., Salvatore, R., Porro, C., Trotta, T., Panaro, M.A., 2016. Folic acid is able to polarize the inflammatory response in LPS activated microglia by regulating multiple signaling pathways. Mediat. Inflamm. 2016, 5240127. https://doi.org/10.1155/2016/5240127.

Cope, E.C., Briones, B.A., Brockett, A.T., Martinez, S., Vigneron, P.A., Opendak, M., et al., 2016. Immature neurons and Radial Glia, but not astrocytes or microglia, are altered in adult Cntnap2 and Shank 3 mice, models of autism. eNeuro 3 (5). https://doi.org/10.1523/ENEURO.0196-16.2016.

Cory-Slechta, D.A., Allen, J.L., Conrad, K., Marvin, E., Sobolewski, M., 2018. Developmental exposure to low level ambient ultrafine particle air pollution and cognitive dysfunction. Neurotoxicology (Little Rock) 69, 217–231. https://doi.org/10.1016/j.neuro.2017.12.003.

Degnan, P.H., Taga, M.E., Goodman, A.L., 2014. Vitamin B12 as a modulator of gut microbial ecology. Cell Metabol. 20, 769–778. https://doi.org/10.1016/j.cmet.2014.10.002.

Douaud, G., Refsum, H., de Jager, C.A., Jacoby, R., Nichols, T.E., Smith, S.M., Smith, A.D., 2013. Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment. Proc. Natl. Acad. Sci. U. S. A. 110, 9523–9528. https://doi.org/10.1073/pnas.1301816110.

Fu, P., Guo, X., Cheung, F., Yung, K., 2019. The association between $PM_{2.5}$ 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.

Gilbert, J., Man, H.Y., 2017. Fundamental elements in autism. From neurogenesis and neurite growth to synaptic plasticity. Front. Cell. Neurosci. 11, 359. https://doi.org/10.3389/fncel.2017.00359.

Goodrich, A.J., Volk, H.E., Tancredi, D.J., McConnell, R., Lurmann, F.W., Hansen, R.L., et al., 2018. Joint effects of prenatal air pollutant exposure and maternal folic acid supplementation on risk of autism spectrum disorder. Autism Res. 11, 69–80. https://doi.org/10.1002/aur.1885.

Guo, Y., Lin, H., Shi, Y., Zheng, Y., Li, X., Xiao, J., et al., 2018. Long-term exposure to ambient $PM_{2.5}$ associated with fall-related injury in six low- and middle-income countries. Environ. Pollut. 237, 961–967. https://doi.org/10.1016/j.envpol.2017.10.134.

Guxens, M., Lubczynska, M.J., Muetzel, R.L., Dalmau-Bueno, A., Jaddoe, V., Hoek, G., et al., 2018. Air pollution exposure during fetal life, brain morphology, and cognitive function in school-age children. Biol. Psychiatry 84, 295–303. https://doi.org/10.1016/j.biopsych.2018.01.016.

Hall, J.M., Gomez-Pinilla, F., Savage, L.M., 2018. Nerve growth factor is responsible for exercise-induced recovery of septohippocampal cholinergic structure and function. Front. Neurosci. 12, 773. https://doi.org/10.3389/fnins.2018.00773.

Hansel, C., 2019. Deregulation of synaptic plasticity in autism. Neurosci. Lett. 688, 58–61. https://doi.org/10.1016/j.neulet.2018.02.003.

Hollis, F., Kanellopoulos, A.K., Bagni, C., 2017. Mitochondrial dysfunction in Autism Spectrum Disorder: clinical features and perspectives. Curr. Opin. Neurobiol. 45, 178–187. https://doi.org/10.1016/j.conb.2017.05.018.

Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., et al., 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155, 1451–1463. https://doi.org/10.1016/j.cell.2013.11.024.

Kemse, N.G., Kale, A.A., Joshi, S.R., 2016. Supplementation of maternal omega-3 fatty acids to pregnancy induced hypertension Wistar rats improves IL10 and VEGF levels. Prostaglandins Leukot. Essent. Fatty Acids 104, 25–32. https://doi.org/10.1016/j.plefa.2015.11.003.

Kern, J.K., Geier, D.A., Sykes, L.K., Geier, M.R., 2015. Relevance of neuroinflammation and encephalitis in autism. Front. Cell. Neurosci. 9, 519. https://doi.org/10.3389/fncel.2015.00519.

Kim, S., Kim, H., Yim, Y.S., Ha, S., Atarashi, K., Tan, T.G., et al., 2017. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. Nature 549, 528–532. https://doi.org/10.1038/nature23910.

Lammert, C.R., Frost, E.L., Bolte, A.C., Paysour, M.J., Shaw, M.E., Bellinger, C.E., et al., 2018. Cutting edge: critical roles for microbiota-mediated regulation of the immune system in a prenatal immune activation model of autism. J. Immunol. 201, 845–850. https://doi.org/10.4049/jimmunol.1701755.

Levine, S.Z., Kodesh, A., Viktorin, A., Smith, L., Uher, R., Reichenberg, A., et al., 2018. Association of maternal use of folic acid and multivitamin supplements in the periods before and during pregnancy with the risk of autism spectrum disorder in offspring. JAMA Psychiatr. 75, 176–184. https://doi.org/10.1001/jamapsychiatry.2017.4050.

Li, B., Guo, L., Ku, T., Chen, M., Li, G., Sang, N., 2018. $PM_{2.5}$ 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.

Liu, X., Zhang, Y., Luo, C., Kang, J., Li, J., Wang, K., et al., 2017. At seeming safe concentrations, synergistic effects of $PM_{2.5}$ and formaldehyde co-exposure induces Alzheimer-like changes in mouse brain. Onco Target 8, 98567–98579. https://doi.org/10.18632/oncotarget.21637.

Mansvelder, H.D., Verhoog, M.B., Goriounova, N.A., 2019. Synaptic plasticity in human cortical circuits: cellular mechanisms of learning and memory in the human brain? Curr. Opin. Neurobiol. 54, 186–193. https://doi.org/10.1016/j.conb.2018.06.013.

Mitra, S., Natarajan, R., Ziedonis, D., Fan, X., 2017. Antioxidant and anti-inflammatory nutrient status, supplementation, and mechanisms in patients with schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatr. 78, 1–11. https://doi.org/10.1016/j.pnpbp.2017.05.005.

Murakami, K., Miyake, Y., Sasaki, S., Tanaka, K., Fukushima, W., Kiyohara, C., et al., 2010. Dietary intake of folate, vitamin B6, vitamin B12 and riboflavin and risk of Parkinson's disease: a case-control study in Japan. Br. J. Nutr. 104, 757–764. https://doi.org/10.1017/S0007114510001005.

Mutlu, E.A., Comba, I.Y., Cho, T., Engen, P.A., Yazici, C., Soberanes, S., et al., 2018. Inhalational exposure to particulate matter air pollution alters the composition of the gut microbiome. Environ. Pollut. 240, 817–830. https://doi.org/10.1016/j.envpol.2018.04.130.

Nam, S.M., Kim, J.W., Kwon, H.J., Yoo, D.Y., Jung, H.Y., Kim, D.W., et al., 2017. Differential effects of low- and high-dose zinc supplementation on synaptic plasticity and neurogenesis in the hippocampus of control and high-fat diet-fed mice. Neurochem. Res. 42, 3149–3159. https://doi.org/10.1007/s11064-017-2353-2352.

Piao, M.J., Ahn, M.J., Kang, K.A., Ryu, Y.S., Hyun, Y.J., Shilnikova, K., et al., 2018. Particulate matter 2.5 damages skin cells by inducing oxidative stress, subcellular organelle dysfunction, and apoptosis. Arch. Toxicol. 92, 2077–2091. https://doi.org/10.1007/s00204-018-2197-9.

Roberts, A.L., Lyall, K., Hart, J.E., Laden, F., Just, A.C., Bobb, J.F., et al., 2013. Perinatal air pollutant exposures and autism spectrum disorder in the children of Nurses' Health Study II participants. Environ. Health Perspect. 121 (8), 978–984. https://doi.org/10.1289/ehp.1206187.

Schmidt, R.J., Hansen, R.L., Hartiala, J., Allayee, H., Schmidt, L.C., Tancredi, D.J., et al., 2011. Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology 22, 476–485. https://doi.org/10.1097/EDE.0b013e31821d0e30.

Sepehri, A., Sarrafzadeh, M., 2019. Activity enhancement of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in activated sludge process: metabolite reduction and CO2 mitigation intensification process. Appl. Water Sci. 9, 131.

Shin Yim, Y., Park, A., Berrios, J., Lafourcade, M., Pascual, L.M., Soares, N., et al., 2017. Reversing behavioural abnormalities in mice exposed to maternal inflammation. Nature 549, 482–487. https://doi.org/10.1038/nature23909.

Sorrells, S.F., Paredes, M.F., Cebrian-Silla, A., Sandoval, K., Qi, D., Kelley, K.W., et al., 2018. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 555, 377–381. https://doi.org/10.1038/nature25975.

Talbott, E.O., Arena, V.C., Rager, J.R., Clougherty, J.E., Michanowicz, D.R., Sharma, R.K., et al., 2015. Fine particulate matter and the risk of autism spectrum disorder.

Environ. Res. 140, 414–420. https://doi.org/10.1016/j.envres.2015.04.021.
Teng, C., Wang, Z., Yan, B., 2016. Fine particle-induced birth defects: impacts of size, payload, and beyond. Birth Defects Res. C Embryo Today 108, 196–206. https://doi.org/10.1002/bdrc.21136.
Tripathi, M., Zhang, C.W., Singh, B.K., Sinha, R.A., Moe, K.T., DeSilva, D.A., et al., 2016. Hyperhomocysteinemia causes ER stress and impaired autophagy that is reversed by vitamin B supplementation. Cell Death Dis. 7, e2513. https://doi.org/10.1038/cddis.2016.374.
Truchet, B., Chaillan, F.A., Soumireu-Mourat, B., Roman, F.S., 2002. Learning and memory of cue-reward association meaning by modifications of synaptic efficacy in dentate gyrus and piriform cortex. Hippocampus 12, 600–608. https://doi.org/10.1002/hipo.10097.
Wang, W., Zhou, J., Chen, M., Huang, X., Xie, X., Li, W., et al., 2018a. Exposure to concentrated ambient $PM_{2.5}$ alters the composition of gut microbiota in a murine model. Part. Fibre Toxicol. 15, 17. https://doi.org/10.1186/s12989-018-0252-6.
Wang, X., Li, W., Li, S., Yan, J., Wilson, J.X., Huang, G., 2018b. Maternal folic acid supplementation during pregnancy improves neurobehavioral development in Rat offspring. Mol. Neurobiol. 55, 2676–2684. https://doi.org/10.1007/s12035-017-0534-2.
Xu, M.X., Zhu, Y.F., Chang, H.F., Liang, Y., 2016. Nanoceria restrains $PM_{2.5}$-induced metabolic disorder and hypothalamus inflammation by inhibition of astrocytes activation related NF-kappaB pathway in Nrf 2 deficient mice. Free Radic. Biol. Med. 99, 259–272. https://doi.org/10.1016/j.freeradbiomed.2016.08.021.
Zhang, M., Ma, W., Zhang, J., He, Y., Wang, J., 2018a. Analysis of gut microbiota profiles and microbe-disease associations in children with autism spectrum disorders in China. Sci. Rep. 8, 13981. https://doi.org/10.1038/s41598-018-32219-2.
Zhang, P., Tsuchiya, K., Kinoshita, T., Kushiyama, H., Suidasari, S., Hatakeyama, M., et al., 2016. Vitamin B6 prevents IL-1 beta protein production by inhibiting NLRP3 inflammasome activation. J. Biol. Chem. 291, 24517–24527. https://doi.org/10.1074/jbc.M116.743815.
Zhang, T., Zheng, X., Wang, X., Zhao, H., Wang, T., Zhang, H., et al., 2018b. Maternal exposure to $PM_{2.5}$ during pregnancy induces impaired development of cerebral cortex in mice offspring. Int. J. Mol. Sci. 19. https://doi.org/10.3390/ijms19010257.
Zheng, X., Wang, X., Wang, T., Zhang, H., Wu, H., Zhang, C., et al., 2018. Gestational exposure to particulate matter 2.5 ($PM_{2.5}$) leads to spatial memory dysfunction and neurodevelopmental impairment in Hippocampus of mice offspring. Front. Neurosci. 12, 1000. https://doi.org/10.3389/fnins.2018.01000.
Zhong, J., Karlsson, O., Wang, G., Li, J., Guo, Y., Lin, X., et al., 2017a. Vitamin B attenuate the epigenetic effects of ambient fine particles in a pilot human intervention trial. Proc. Natl. Acad. Sci. U. S. A. 114, 3503–3508. https://doi.org/10.1073/pnas.1618545114.
Zhong, J., Trevisi, L., Urch, B., Lin, X., Speck, M., Coull, B.A., et al., 2017b. B-vitamin supplementation mitigates effects of fine particles on cardiac autonomic dysfunction and inflammation: a pilot human intervention trial. Sci. Rep. 7, 45322. https://doi.org/10.1038/srep45322.