Ambient PM2.5 induces brain injury through PI3K/AKT/FoxO1 pathway

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Ambient $PM_{2.5}$ induces brain injury through PI3K/AKT/FoxO1 pathway

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Abstract

Background Fine particulate matter ($PM_{2.5}$)-induced neurological and mental diseases, such as cognitive impairment and stroke, tend to cause disability. In this study, we plan to find the appropriate biological indicators reflecting the early effects of $PM_{2.5}$ exposure and explore the toxicity of inhaling $PM_{2.5}$ along with its pathological mechanism on nervous system. **Methods** Male C57BL/6 mice were divided into 6 groups and exposed to concentrated $PM_{2.5}$ or filtered air for 2, 4 and 6 months, respectively. Their performances of ethology were tested by Morris water maze, elevated plus maze and buried food pellet test. Weight of whole brain and olfactory bulbs were recorded when the mice were sacrificed and the brain structure was observed by HE staining. ROS, SOD, IL-6, TNF-α, IL-1β, BDNF, β-hydroxybutyric, IgG, albumin, fibrinogen and plasminogen were tested. The mRNA expressions of the cortex were detected by RNA sequencing and real-time PCR, and the corresponding proteins were detected by Western blot. **Results** The spatial learning memory ability were impaired and the mice performed anxiously after $PM_{2.5}$ exposure. Relative brain weight decreased along with age and $PM_{2.5}$ inhaled exposure exceeded the process. But neuronal morphology, inflammatory cytokines, ROS, β-hydroxybutyric, BDNF, IgG and fibrinogen did not change. Intriguingly, SOD and albumin decreased, while plasminogen increased after $PM_{2.5}$ exposure. PI3K-AKT-FoxO1 pathway was activated after 6-month $PM_{2.5}$ exposure. **Conclusion** Albumin, A/G value and plasminogen are main serum indicators of early-stage (2 month) $PM_{2.5}$ exposure, and long-term (6 month) $PM_{2.5}$ exposure induced brain injury potentially through the activation of PI3K/AKT/FoxO1 pathway.

Keywords

$PM_{2.5}$, brain injury, FoxO, oxidative stress, ethology

Introduction

The global disease burden study shows that air pollution induced 4.9 million people death in 2017, and fine particulate matter ($PM_{2.5}$) induced 2.94 million people death, an increase of 21.6% compared with 2007 [1]. Ambient particulate pollution can increase the risk of cardiovascular diseases, while it can also increase the risk of cerebrovascular diseases. The relationship between $PM_{2.5}$ and central nervous system injuries such as cognitive decline, stroke and neurodegenerative diseases has been revealed in epidemic studies [2, 3]. Both short-term [4] and long-term [2] particulate matter exposure can increase the risk of morbidity and mortality of ischemic stroke. The risk ratio of ischemic stroke is 1.21 (95% *CI* 1.04~1.41) for every 10 $\mu g/m^3$ increase of $PM_{2.5}$ [2]. Furthermore, Jordi Sunyer et al. prospectively studied 2715 children aged from 7 to 10 years in 39 schools in Barcelona, and the results showed that children attending schools with high traffic pollution had less improvement in cognition [5]. The study published in *Environmental Health Perspectives* in 2016 shows that long-term exposure to $PM_{2.5}$ can increase the risk of dementia, Alzheimer's disease and Parkinson's disease in humans [3]. In addition, exposure to $PM_{2.5}$ may also increase the risk of brain tissue atrophy and aging [6], and lead to the damage of cerebral white matter.

Neurological and mental diseases tend to cause disability and increase disability-adjusted life years (DALYs), which greatly affects quality of life and labor. In recent years, studies have shown that ultrafine particles can enter into the brain of rats directly through the olfactory bulbs [7], harming brain. Another theory claims that $PM_{2.5}$ enters into the body through the respiratory tract and causes inflammation in the nasal epithelium or lungs, triggering a systemic inflammatory response (SIR) that leads to brain impairment. To reveal the major mechanism of how $PM_{2.5}$ causes nervous system injury, a number of great progresses have been made in molecular biology researches, indicating that inflammation, oxidative stress, brain derived neurotrophic factor (BDNF) and microRNA (miRNA) work on it. However, the main pathogenesis of $PM_{2.5}$-induced brain injury is still unclear and needs further study.

Beyond that, self-recovery from $PM_{2.5}$-induced injury is also an important issue which should not be ignored since the dose-response relationship has not been established yet. Previous study has reported that mice can repair themselves after high dose of $PM_{2.5}$ exposure for 8 weeks [8]. Thus, it is necessary to evaluate $PM_{2.5}$-induced injuries along with the exposure time, which is helpful to find the early biological indicators and clarify the potential repair or exacerbation during the impairment.

In this study, we planned to find the appropriate biological indicators reflecting the early effects of $PM_{2.5}$ exposure and explore the toxicity of inhaling $PM_{2.5}$ along with its pathological mechanism on nervous system.

Results

$PM_{2.5}$ concentration

The mean concentrations of ambient $PM_{2.5}$ were 64.71 $\mu g/m^3$, 46.36 $\mu g/m^3$ and 39.76 $\mu g/m^3$ during our 2, 4 and 6 months' experiments, respectively. The mean concentrations of $PM_{2.5}$ in filtered air (FA) and PM chamber were 14.48 $\mu g/m^3$ vs 95.10 $\mu g/m^3$ (2 months), 11.32 $\mu g/m^3$ vs 75.26 $\mu g/m^3$ (4 months) and 11.83 $\mu g/m^3$ vs 63.79 $\mu g/m^3$ (6 months). The concentration of concentrated $PM_{2.5}$ chamber was approximately six-fold higher than the filtered air chamber in this study.

The spatial learning memory ability was impaired after $PM_{2.5}$ exposure

Learning dysfunction is a typical deficit after $PM_{2.5}$ exposure [9]. Morris water maze test, a classic ethological experiment, was performed to assess the spatial learning and memory of mice. During the training trials, the mice did not show a significantly longer latency to platform (Figure 1A~C), but there was the tendency of less frequencies to platform in test after $PM_{2.5}$ exposure (Figure 1F). The time in target quadrant and the speed of swimming were also detected and analyzed, but there was no significant difference among the 6 groups (Supplemental Figure 1).

$PM_{2.5}$-exposed mice were more anxious in the elevated plus maze

The elevated plus maze was used to evaluate the anxiety of mice. The frequencies of entering and the time spent in open arms and close arms were calculated. In Figure 2, the entries to open arms were comparable among the 6 groups. FA2 mice stayed longer in open arms than PM2, FA4 and FA6 mice, while there was no significant difference among other groups. Neither did the corresponding parameters of the close arms (Supplemental Figure 2). Thus, the mice performed more anxious after first 2-month $PM_{2.5}$ exposure but then they maintained the same degree of anxiety.

$PM_{2.5}$ exposure exceeded the decrease of relative brain weight

In order to explore the functional changes of olfactory bulbs which might be the entrance of metal particles into the brain [10], the buried food pellet test was performed and the durations that mice on a restricted diet used to find the buried food were not different. As important indicators of evaluating the impairment of brain, whole brain weight, olfactory bulb weight and body weight were detected. The relative brain weight (brain weight as the percentage of body weight) shown in Figure 3B deceased along with the age of mice since this parameter dropped gradually within the FA groups and PM groups, respectively. The decline of the brain weight and the increase of body weight shown in Supplemental Figure 3 might lead to the change of relative brain weight. Although $PM_{2.5}$ exposure did not affect the brain weight or the body weight individually, the relative brain weight indeed declined after 2-month and 4-month exposure of $PM_{2.5}$ and returned to the same level until 6-month exposure. It implied that $PM_{2.5}$ exposure exceeded the decrease of relative brain weight.

$PM_{2.5}$ did not influence neuronal morphology, β-hydroxybutyric and BDNF

Furthermore, in order to determine whether the structure of brain was damaged, the brain of mice was cut into slices and observed through HE staining. The structure of hippocampus and cortex were complete in Supplemental Figure 4. Thus, $PM_{2.5}$ did not influence cellular morphology in hippocampus and cortex observed by HE staining.

Consistently, the β-hydroxybutyric and BDNF, which could enter the brain from serum, remained stable in this study (Supplemental Figure 5).

The ability of anti-oxidation was impaired after 6-month $PM_{2.5}$ exposure

Oxidative stress is an essential pathway of $PM_{2.5}$-induced impairment. Reactive oxygen species (ROS) and superoxide dismutase (SOD) are the major indicators of oxidative stress. There was no difference of ROS in either hippocampus or cortex (Supplemental Figure 6). SOD is usually treated as the item to evaluate oxidation resistance inside the body. In FA groups, the SOD activity did not change in the first 4-month exposure, while it rose sharply in 6-month exposure (Figure 3C). However, there was a bilateral change of SOD among PM groups—the SOD activity was firstly up-regulated and then down-regulated along with the time of $PM_{2.5}$ exposure. At the same time, we should not ignore the rising tendency in PM2 group when compared to FA2 group, although the post hoc test was not statistically different ($P = 0.080$). Significantly, SOD activity decreased in PM6 group compared with FA6 group.

Age reduced systematic inflammation and $PM_{2.5}$ exposure might influence the interleukin-1β (IL-1β) after 6-month exposure

IL-6 and tumor necrosis factor α (TNF-α) showed a downward trend along with the feeding time, while PM4 group exhibited lower TNF-α compared with FA4 group (Figure 3D~E). Another proinflammatory cytokine IL-1β went up firstly in both FA and PM groups and then went down only in FA groups (Figure 3F). Besides, it should not be ignored that IL-1β had a non-significant rise between FA6 and PM6 group ($P = 0.064$).

$PM_{2.5}$ reduced A/G value by decreasing albumin without increasing IgG

In Figure 4A, IgG increased rapidly with age, characterized by the difference among FA2, FA4 and FA6 as well as PM2, PM4 and PM6. However, $PM_{2.5}$ exposure did not make a difference. But when it came to albumin (Figure 4B), it was clear that this indicator went up firstly and then went down, and PM groups downregulated it from 2-

month exposure to 6-month exposure. The value of A/G is the percentage of albumin over IgG. A/G value went down along with feeding time in FA groups, while this trend disappeared among the PM groups (Figure 4C). Besides, both PM2 and PM4 group showed lower A/G when compared with corresponding FA2 and FA4 group, respectively.

$PM_{2.5}$ influenced fibrinolysis through increasing plasminogen but not fibrinogen

Fibrinogen is an important substance for coagulation synthesized by liver and it is the most common clotting factor in plasma. Fibrinogen went up along with the feeding time while the increase was disrupted by $PM_{2.5}$ in 6-month exposure group (Figure 4D). Plasminogen is an item reflecting the fibrinolysis inside the body. It rose the same way like fibrinogen in FA groups, but it only increased in 6-month PM group when compared with 4-month PM group (Figure 4E). In addition, plasminogen was obviously higher in PM2 and PM6 group when compared with the corresponding FA groups. This difference did not exist between the FA4 and PM4 group.

$PM_{2.5}$ influence mRNA expression in the cortex of mice detecting by RNA sequencing

$PM_{2.5}$ did harm to the body after 6-month exposure according to the above results including impairing the spatial learning memory ability, causing anxiety, decreasing the relative brain weight and so on. To explore how $PM_{2.5}$ worked on brain, we detected the whole-genome expression through RNA sequencing, and counts of 55281 genes were filtered. When compared with FA6 group, a total of 2719 genes (1188 upregulated and 1531 downregulated) in PM6 group were identified and visualized by volcano plot (Figure 5A). This result showed that 6-month $PM_{2.5}$ exposure influenced a number of gene expressions in the cortex of brain which could further affect the functional performances such as learning or memory, cognition and signal release (Figure 5B).

The Forkhead box O (FoxO) family is an important transcription factor including FoxO1, FoxO3a, FoxO4 and FoxO6 molecules in mammals. The phosphatidylinositol 3-

kinase/protein kinase B (PI3K/Akt) signaling pathways can phosphorylate FoxO, transport it from the nucleus to the cytoplasm, lower its transcriptional activity and inhibit the expressions of downstream genes. Combing with the results of KEGG enrichment analysis, among the genes we found that the expressions of *FoxO1* (P_{adjust}=8.70×10^{-7}) and *FoxO6* (P_{adjust}=0.000406) decreased in PM6 group, while no significant changes of *PI3K*, *AKT, FoxO3a or FoxO4* were found between FA6 and PM6 group. Since PI3K/AKT signaling pathway is closely related to cellular physiological processes, such as metabolism, aging, cancer [11] and $PM_{2.5}$-induced damages [12], PI3K/AKT/FoxO1 pathway might explain how $PM_{2.5}$ worked on nervous system.

PI3K-AKT-FoxO1 pathway was activated after 6-month $PM_{2.5}$ exposure

Since we found that PI3K/AKT/FoxO1 pathway might be an important intracellular signaling pathway in $PM_{2.5}$-induced damages from the above RNA sequencing test, the mRNA expressions in the cortex of mice were detected by RT-PCR. As shown in Figure 5 and Supplemental Figure 7, 6-month feeding sharply increased the mRNA expression of *PI3K*, *Akt* and *FoxO* family members except for *FoxO3a* when compared with the corresponding 2- or 4-month groups. Interestingly, $PM_{2.5}$ exposure increased mRNA expression of *PI3K* and *AKT*, and decreased mRNA expression of *FoxO1* only in 6-month group. Detection of other 3 molecules in FoxO family did not display any difference between the corresponding FA and PM groups.

The same trend of PI3K, p-AKT and FoxO1 were found between FA6 and PM6 group by Western Blot (Figure 6). As the major downstream target of AKT, p-FoxO1 had a higher level in PM6 group although its total FoxO1 decreased. Interestingly, *PI3K* and *AKT* showed an increase of mRNA rather than protein along with feeding time. Specifically, the increased trend of *PI3K* and *AKT* at mRNA among FA2, FA4 and FA6 groups disappeared entirely at protein, while the difference between PM2 and PM6 group still existed. Total FoxO1, the corresponding protein of *FoxO1*, performed the same as it did at mRNA among FA groups, but did not display an increase among the PM groups. In

addition, the elevated PI3K and p-AKT actually increased the p-FoxO1 in PM6 group compared with FA6 group.

Discussion

In 2019, the average annual concentration of $PM_{2.5}$ in China is 36 $\mu g/m^3$, lower than that in 2017 (43$\mu g/m^3$), but still higher than both Chinese standard (35$\mu g/m^3$) and U.S EPA standard (12 $\mu g/m^3$). As consequence, it is necessary to focus on the adverse health effects caused by chronic low-concentration exposure of $PM_{2.5}$.

$PM_{2.5}$ is prone to cause metabolic damages, such as atherosclerosis, lipids and glucose metabolism disorder, and cardiovascular diseases, the pathogenesis of which we have studied previously [13, 14]. Moreover, brain injury is also an important impairment caused by $PM_{2.5}$, including cerebrovascular injury and neurological damage, which can further lead to stroke, cognitive decline and mental diseases.

Albumin, A/G value and plasminogen are sensitive and accessible indicators reflecting effects of early-stage $PM_{2.5}$ exposure

The brain weight is partly related to the body weight because it increases with the age during puberty. Then, the brain weight decreased during the span of adult human life [15]. The loss of brain weight over the corresponding physiological value in adults is associated with brain atrophy and cognitive impairment. Loss of relative brain weight was clear in this experiment, and this might be explained by the change of both brain weight and body weight. There was no significant difference of whole brain weight or body weight between FA and PM groups. The whole brain weight of PM groups showed a trend of decline over time, and body weight of PM mice did not show the same increase over time as FA groups did, which eventually led to the different relative brain weight between FA and PM groups at 2- and 4-month. Interestingly, there was no difference between FA6 group and PM6 group, suggesting that age had a greater effect on the loss of relative brain weight in adult mice than $PM_{2.5}$. Thus, relative brain weight is an

important indicator of brain damage caused by $PM_{2.5}$, especially in mice younger than 24 weeks.

β-hydroxybutyric is a kind of ketone which can be directly used as the energy donor in the brain besides glucose, and its increase in serum can be caused by diabetic ketoacidosis, chronic hunger, malnutrition and strenuous exercise. Although acute high-concentration exposure of $PM_{2.5}$ can make an impact on the systemic energy metabolism as we studied before [16], β-hydroxybutyric did not change in this study. The reason might be that the mice did not suffer fat metabolism disorder developed by severe glucose metabolism disorder after chronic low-concentration exposure of $PM_{2.5}$.

BDNF has exactly the same amino acid sequences in humans, mice and pigs, and its structure is similar to that of nerve growth factor (NGF), which belongs to the nerve growth factor family and affects the development, differentiation and synaptic plasticity of neurons. Both epidemiological studies and animal experiments have found that $PM_{2.5}$ reduces the content of BDNF [17] [18-20]. Interestingly, the change of BDNF was not observed in this study. This may indicate that BDNF changes more after acute exposure to $PM_{2.5}$ and less for chronic exposure.

Oxidative stress and inflammatory response are important mechanisms of diseases caused by $PM_{2.5}$, and they can jointly promote the pathological processes. In this study, neither inflammatory factors nor ROS, an indicator of oxidative stress, changed due to $PM_{2.5}$. However, SOD, an antioxidant in vivo, significantly decreased after 6-month $PM_{2.5}$ exposure. The results suggested that long-term exposure to moderate concentrations of $PM_{2.5}$ can indeed affect balance of oxidation and antioxidation, which is consistent with previous study [21].

Albumin is an important protein synthesized by the liver, which is responsible for maintaining a stable plasma colloid osmotic pressure. Furthermore, human serum albumin (HSA) effectively protects neurons after cerebral ischemia or related injuries [22], and regulates metabolism of nervous system during development [23]. Albumin is significantly reduced after exposure to $PM_{2.5}$, and the reasons might be associated with

the decreased albumin synthesis caused by $PM_{2.5}$-induced liver damage or the increased albumin leakage caused by $PM_{2.5}$-induced vascular diseases. Additionally, IgG, an important immunoglobulin, showed an increasing trend in both FA and PM mice since 4-month feeding, indicating that the immune response was indeed enhanced in the mice after 4-month feeding. However, $PM_{2.5}$ had little effect on IgG. At the same time, due to the high IgG in older mice, the absolute value of albumin better reflects the impact of $PM_{2.5}$ exposure than the A/G value.

Another two serum indicators, collagen and plasminogen, can reflect the health of blood system and were affected by age in this experiment. But only plasminogen was able to reflect the impact of 2-month and 6-month $PM_{2.5}$ exposure.

Combining with above sections, $PM_{2.5}$ can delay or accelerate the effects of age on some indicators. Although the relative brain weight is sensitive to $PM_{2.5}$, it is hard to elevate it without sacrificing the mice. The best serum indicators observed in the early stage (2 month) are albumin, A/G value and plasminogen, which are more sensitive to $PM_{2.5}$ and highly accessible, especially for humans.

Self-recovery might happen after 4-month $PM_{2.5}$ exposure

Limited studies focus on the following recovery after $PM_{2.5}$ exposure. Thus, we detected a number of biological indicators at 3 different time to figure out whether self-recovery exists during $PM_{2.5}$ exposure. SOD, TNF-α and plasminogen, which represents the ability of antioxidation, inflammation and fibrinolysis, respectively. All these 3 dimensions are the major pathological mechanisms of $PM_{2.5}$-induced injuries. In this study, the activity of SOD in serum was downregulated in PM4 group, which might be due to its consumption for resisting oxidative stress. TNF-α decreased in PM4 group and was lower than that of FA4 group, but other inflammatory cytokines, IL-6 and IL-1β, did not show this pattern. This indicates a partial downregulation of inflammation after 4-month $PM_{2.5}$ exposure. Plasminogen in PM4 group did not exhibit a significant increase when compared with FA4 or PM2 group. It might come to the conclusion that fibrinolysis was

rapidly impaired by 2-month $PM_{2.5}$ exposure, recovered from 4 month and kept going worse after 6-month $PM_{2.5}$ exposure.

Previous study has shown that after 4, 8, and 12 weeks of consecutive exposure to high levels of $PM_{2.5}$ in male C57BL/6 mice, the mice exhibit a damage-repair-imbalance response, which ultimately led to depressive behavior and upregulation of pro-inflammatory cytokines [8]. Consistently, pro-inflammatory cytokines and chemokines elevated after 4-week $PM_{2.5}$ exposure, and reversed to normal levels in the following 2 week [24]. Combining with our research, self-recovery from $PM_{2.5}$ exposure might exist in partial physiological processes, such as antioxidation, inflammation and fibrinolysis, but it still needs further studies to figure out whether and when self-recovery happens in other $PM_{2.5}$-induced impairments.

Ambient $PM_{2.5}$ induces brain injury through PI3K/AKT/FoxO1 pathway

FoxO, which is present in most of the tissues in mammals, regulates multiple physiological and pathological processes such as oxidative stress, inflammatory response, energy metabolism and apoptosis which are also closely related to the pathogenic mechanism of $PM_{2.5}$. FoxO factors are key downstream targets of insulin, growth factor, nutrient, and oxidative stress stimuli that coordinate a wide range of cellular functions. Post-translational modification is the major regulation of FoxO. It can be phosphorylated by PI3K/AKT pathway which is active after $PM_{2.5}$ exposure [12]. FoxO1 is inactivated through phosphorylation by AKT at Thr24, Ser256 and Ser319 resulting in nuclear export and inhibition of transcription factor activity [25]. However, it can also be phosphorylated by JNK at Thr 447 and Thr 451 which active the transcription factor activity [26]. Active Foxo1 can promote SOD [27] and downregulate plasminogen activator inhibitor 1 (PAI-1), which can promote the transformation of plasminogen into plasmin working on fibrinolysis. In this study, PI3K/AKT/FoxO1 pathway in the brain tissue was activated after long-term (6-month) $PM_{2.5}$ exposure by transcription, translation and post-translation modification, resulting in the reduction of FoxO1 activity, down-regulation of SOD and albumin, and increased plasminogen. It further reduced the

ability of antioxidation, repairment and fibrinolysis in nervous system, in turn affected learning and memory, consequently increased the risk of cerebrovascular damage and stroke. This might be due to the cumulative effect of consecutive PM2.5 exposure since the pathway was not activated before 6-month exposure. Therefore, to improve the expression and function of FoxO1 may be a feasible method to prevent and treat $PM_{2.5}$-induced brain damage.

In addition, result of RNA sequencing showed differences of a large number of genes after 6-month $PM_{2.5}$ exposure. Although some differences were not detected by RT-PCR, such as *FoxO6*, a gene that regulates memory consolidation and synaptic function in mammals, it still provided reliable genetic targets for further studies of brain injuries caused by long-term $PM_{2.5}$ exposure.

Conclusions

In conclusion, albumin, A/G value and plasminogen are great serum indicators of early-stage (2 month) $PM_{2.5}$ exposure, and long-term (6 month) $PM_{2.5}$ exposure induces brain injury potentially through the activation of PI3K/AKT/FoxO1 pathway.

Methods

Animals

Male C57BL/6 mice were bought from Shanghai JSJ Laboratory Animal Company (Shanghai, China) and equilibrated for 2 weeks before experimental enrollment in the animal center in Fudan University. The mice were randomly divided into 6 groups (FA2, FA4, FA6, PM2, PM4 and PM6 group) and exposed to concentrated $PM_{2.5}$ or FA using Shanghai Meteorological and Environmental Animal Exposure System (Shanghai-METAS) [13, 14] for 2, 4 or 6 months, respectively (10 hours per day, 7 days per week with food and water freely). The study was approved by the Fudan University Animal

Care and Use Committee and all animal procedures were treated in accordance with the National Institutes of Health guide for the care and use of Laboratory animals.

$PM_{2.5}$ exposure

As our previous studies [13, 14], "real-world" exposure to ambient $PM_{2.5}$ was carried out in animals through the use of Shanghai-METAS. Two $PM_{2.5}$ monitors (pDR-1500, Thermo Scientific, Waltham, MA, USA) were connected to the air inlet of the exposure and control chambers for the use of measuring the real-time concentration of $PM_{2.5}$.

Morris water maze

The experimental equipment consists of a circular water pool (120 cm in diameter, 40 cm in height), containing water (21 ± 1 °C) to a depth of 15.5 cm. A platform (4.5 cm in diameter, 14.5 cm in height) was placed at the target quadrant and submerged 1 cm below the water surface. A video camera was set in the ceiling and connected to an analysis-management system Ethovision XT version 8.5 (Noldus, Wageningen, Netherlands). The pool was surrounded by 4 visual cues at different locations: N, E, W, S. Each mouse was trained 4 times per day for 5 consecutive days. Latency to platform was observed and calculated. On Day 6, the platform was removed and the mouse was allowed to swim freely for 60 s in the same pool. The latency and frequencies to the hidden platform and target quadrant were measured.

Elevated plus maze

The elevated plus maze apparatus consists of two open arms with no sides and two close arms with sides. A video camera was set in the ceiling and connected to the analysis-management system Ethovision XT version 8.5 (Noldus, Wageningen, Netherlands). Mice were placed on the middle of the maze and allowed for exploring the maze freely for 5 min during which entries to and time spent in the open arms and closed arms were measured.

Buried food pellet test

The mice were on a restricted diet (1g food per day per mouse were given for 3 consecutive days) 3 days before the test and they were allowed to stay in the testing cage for 10 min 24 h before the test. The test was conducted in a clean mice cage filling with usual bedding, and the food pellet (1.0 cm×1.0 cm×0.5 cm) was randomly buried 1 cm deep in the bedding. Then, the mouse was placed into the cage to explore for up to 5 min, and the latency to find the pellet was calculated.

Weight of body, brain and olfactory bulbs

The body weight of mouse was recorded every week throughout the experiment period. The weight of brain and olfactory bulbs were measured immediately after the mouse was sacrificed.

Histopathology

Brain of the mouse was perfused with 4% paraformaldehyde and embedded with paraffin. The sections were stained with hematoxylin & eosin (HE) and visualized using optical microscope.

RNA sequencing

The preparation of whole transcriptome libraries and deep sequencing were performed by Novogene (Beijing, China). 100 mg of cortical tissues were collected and used to extract RNA. Then, mRNA was enriched by Oligo(dT) beads, fragmented into short fragments , and used as templates for cDNA generation. cDNA fragments were purified, added with poly(A), ligated to Illumina sequencing adapters, and sequenced by using Illumina HiSeq™ 2500. The read counts were expressed as fragments per kilobase of exon per million mapped reads (FPKM), estimating gene expression level. Principal component analysis (PCA) was performed for sample relationship analysis, and DESeq2 was used to identify genes with $P_{value}<0.05$ and $|log2FoldChange|>0$ as significant genes. The

different mRNAs were submitted to KEGG database for pathway analysis and GO database for GO category analysis.

ELISA

Inflammatory cytokines including IL-1β, IL-6 and TNF-α, IgG, albumin, fibrinogen, plasminogen, β-hydroxybutyric and BDNF in serum were measured using ELISA kit (Biosource international, Thermo, USA) following the manufacturer's instructions. After adding the stop solution, the absorbance was measured within 15 min.

SOD activity

The serum was used to measure SOD activity by using the SOD reagent kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instruction.

Flow cytometry

The tissues of hippocampus and cortex were digested into single-cell suspension (1×10^6 cells/ml) and stained with ROS Assay Kit (Beyotime, Shanghai, China). The cells were detected by flow cytometry.

Real-time PCR

PI3K, *AKT*, *FoxO1*, *FoxO3a*, *FoxO4*, *FoxO6* and *GAPDH* mRNA expression in cortex were detected by the real-time PCR (RT-PCR) method. A total of 20 mg cortex tissues were taken to extract the total RNA by Trizol (Sigma-Aldrich, St. Louis, MO). The cDNA was synthesized using PrimeScript RT Reagent Kit (Takara, Japan) according to the manufacturer's protocol. Quantitative RT-PCR was performed using SuperReal PreMix Reagent Kit (Tiangen Biotech, China). The reaction system was 10 μl and the value of $-^{\triangle\triangle}$Ct was used for analysis. The sequences of primers were listed in the Supplemental Table 1.

Western blot

Total proteins in cortex of the mice were lysed in radio-immunoprecipitation assay lysis buffer. The proteins were separated on 8% to 10% SDS-PAGE gel and transferred to polyvinylidene difluoride membranes. The membranes were blocked for 1.5 h at room temperature with 5% bovine serum albumin (BSA), reacted with anti-PI3K (1:1000), anti-phospho-AKT (phosphorylation at Ser473) (1:1000), anti-AKT (1:1000), anti-phospho-FoxO1 (phosphorylation at Ser256) (1:1000), anti-FoxO1 (1:1000) and anti-GAPDH (1:1000) at 4°C over-night. HRP-conjugated antibody (1:5000, Cell Signaling Technology, USA) was used to incubate the membranes for 1 h at room temperature. Finally, the blots were detected by ECL (Thermo Scientific) and the band density was quantitatively analyzed through ImageJ (National Institutes of Health, USA).

Statistical analyses

The results were shown as means ±SD. Statistical analysis were performed by one-way analysis of variance (ANOVA) with LSD post hoc test by IBM SPSS statistics 22.0 software. $P<0.05$ was considered statistically significant.

List of abbreviations

$PM_{2.5}$: Fine particulate matter; DALYs: disability-adjusted life years; FA: filtered air; SIR: systemic inflammatory response; BDNF: brain derived neurotrophic factor; miRNA: microRNA; FoxO: Forkhead box O; PI3K: phosphatidylinositol 3-kinase; Akt: protein kinase B; NGF: nerve growth factor; HSA: human serum albumin; PAI-1: plasminogen activator inhibitor 1; Shanghai-METAS: Shanghai Meteorological and Environmental Animal Exposure System; HE: hematoxylin & eosin; FPKM: fragments per kilobase of exon per million mapped reads; PCA: principal component analysis; IL: interleukin; TNF-α: tumor necrosis factor α; SOD: superoxide dismutase; ROS: reactive oxygen species; RT-PCR: real-time PCR; ANOVA: one-way analysis of variance.

Declarations

Ethics approval and consent to participate

The study was approved by the Fudan University Animal Care and Use Committee and all animal procedures were treated in accordance with the National Institutes of Health guide for the care and use of Laboratory animals.

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are available but restrictions apply to the availability of these data. Data are however available from the authors upon reasonable request.

Competing interests

No potential conflicts of interest were disclosed.

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Authors' contributions

Liying Song designed and performed the experiment, and wrote the paper. Kun Pan performed the experiment and analyzed the data. Xihao Du, Shuo Jiang, Xuejiao Zeng, Jia Zhang, Lei Lei, Mengdi Zhang and Yuwen Zhang performed the experiment. Ji Zhou provide technical support of the exposure equipment. Yuquan Xie revised the paper. Jinzhuo Zhao designed the expriement and revised the paper. All authors read and approved the final manuscript.

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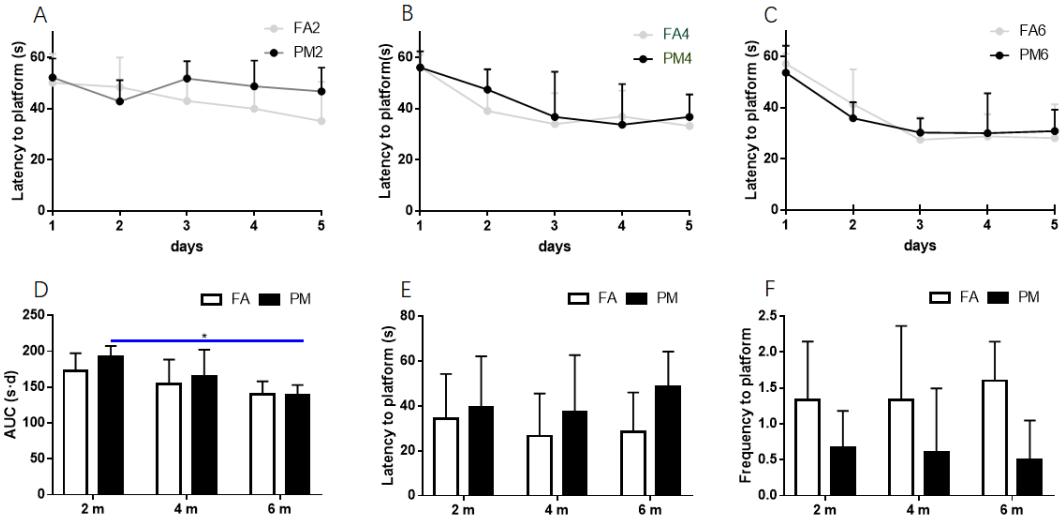
Figures and Tables



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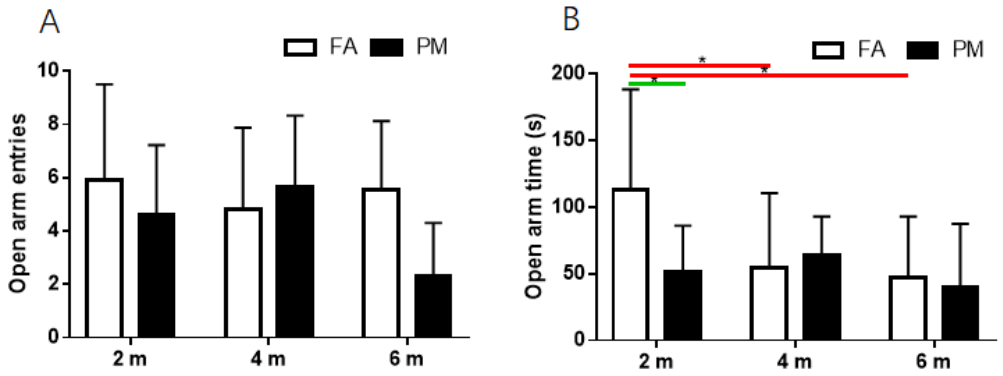

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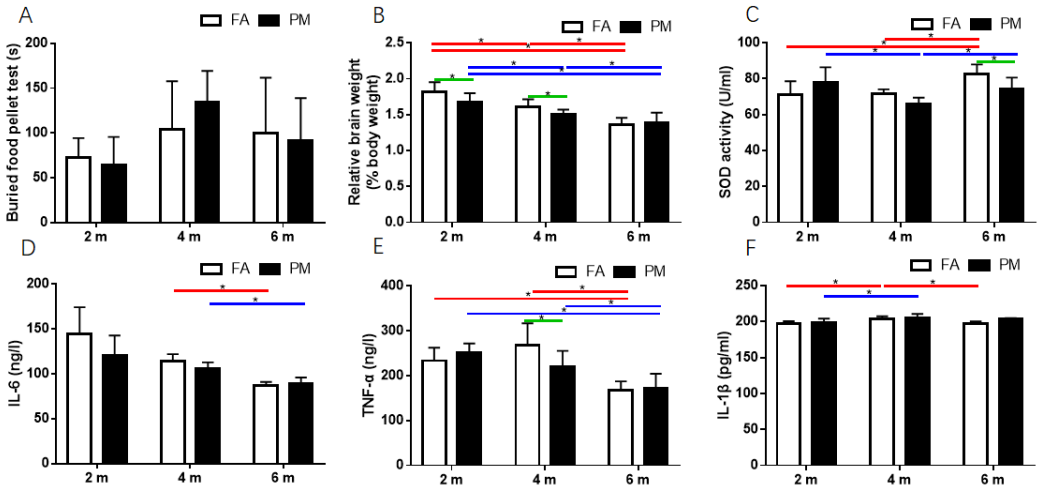

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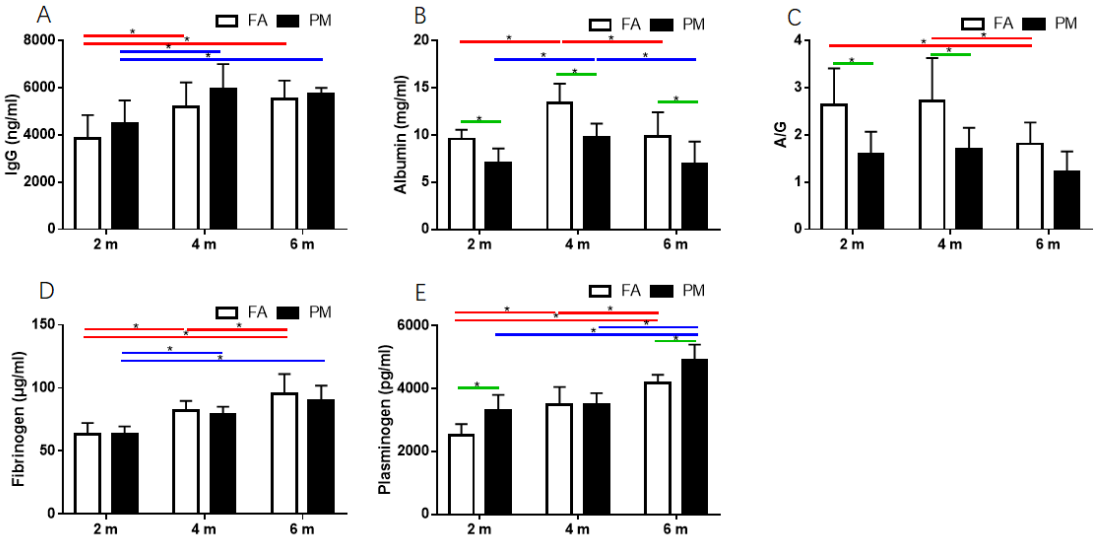

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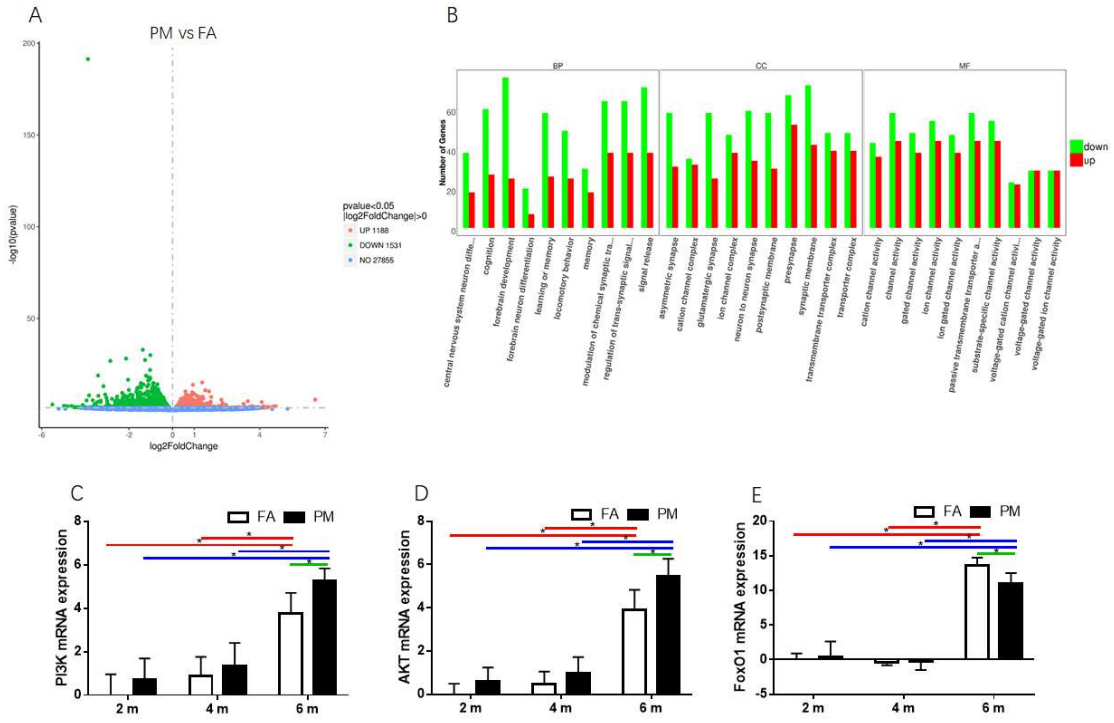

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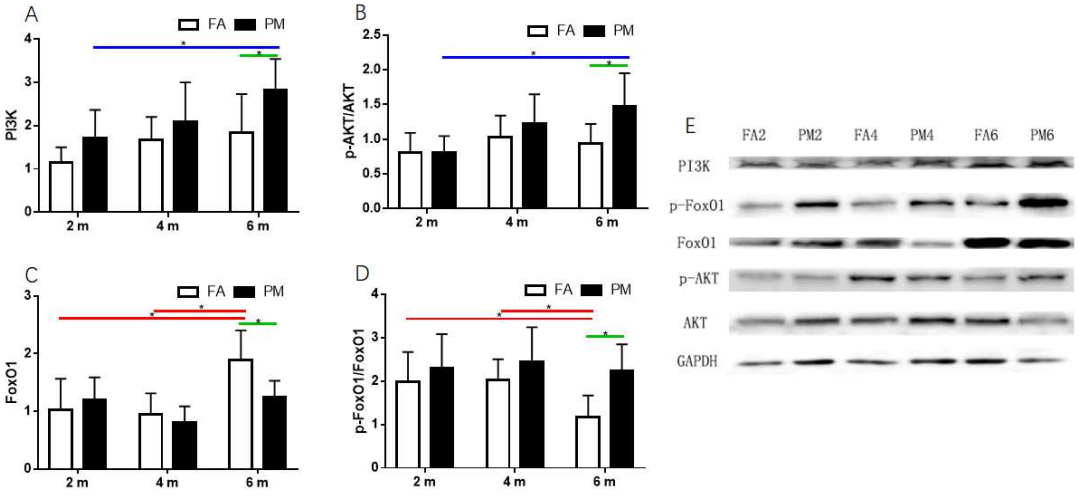

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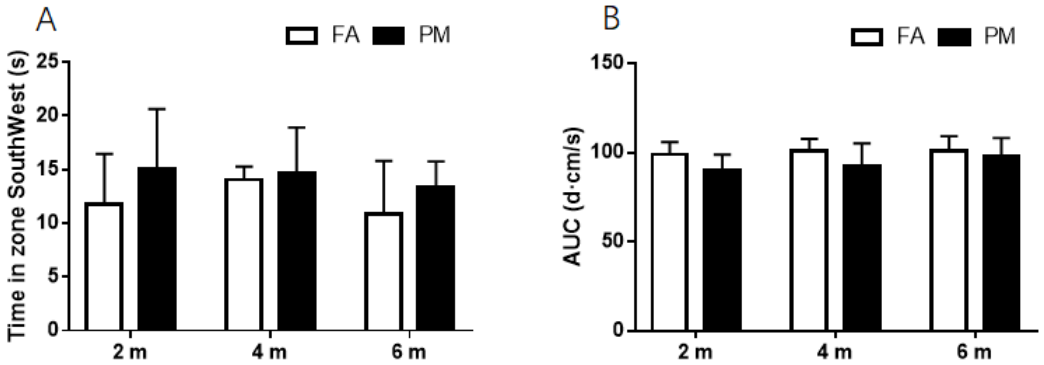
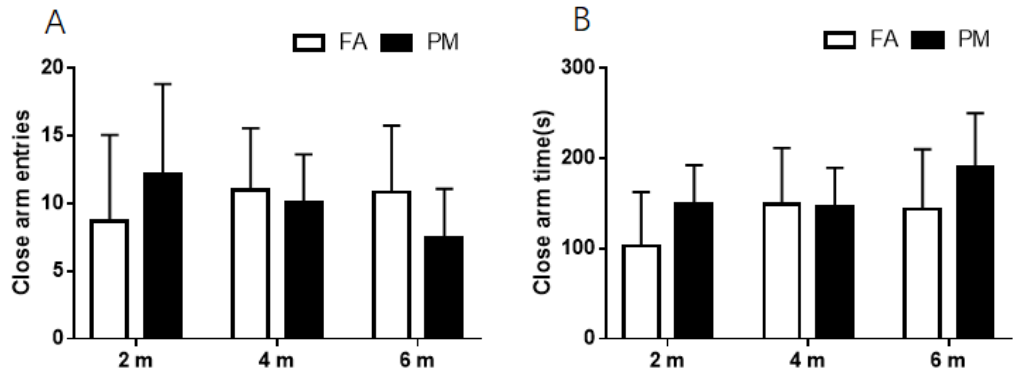
Supplemental Table 1. Sequence of qPCR primers

Gene	Sequence
PI3K	Upper: 5'- ACACCACGGTTTGGACTATGG -3'
	Lower: 5'- GGCTACAGTAGTGGGCTTGG -3'
AKT	Upper: 5'- ATGAACGACGTAGCCATTGTG -3'
	Lower: 5'- TTGTAGCCAATAAAGGTGCCAT -3'
FoxO1	Upper: 5'- GGACAGCCGCGCAAGACCAG -3'
	Lower: 5'-TTGAATTCTTCCAGCCCGCCGA -3'
Foxo3a	Upper: 5'- TGAATGTGGGGAACTTCACTG -3'
	Lower: 5'- TTGGCAAAGGGTTTTCTCTGT -3'
FoxO4	Upper: 5'- ACTTTGAGCCAGATCCCTGAGTCAC -3'
	Lower: 5'- TAAGGACAGGCCTGGCTCCACC -3'
FoxO6	Upper: 5'- AAGAGCTCCCGACGGAAC -3'
	Lower: 5'- GGGGTCTTGCCTGTCTTTC -3'
GAPDH	Upper: 5'-CAATGTGTCCGTCGTGGATCT -3'

Lower: 5’-GTCCTCAGTGTAGCCCAAGATG -3’



Supplemental Figure 1. The time mice stayed in the target quadrant and the speed of swimming in Morris water maze. **A**, The time mice stayed in the target quadrant was measured. **B**, AUC of the speed of swimming was calculated.



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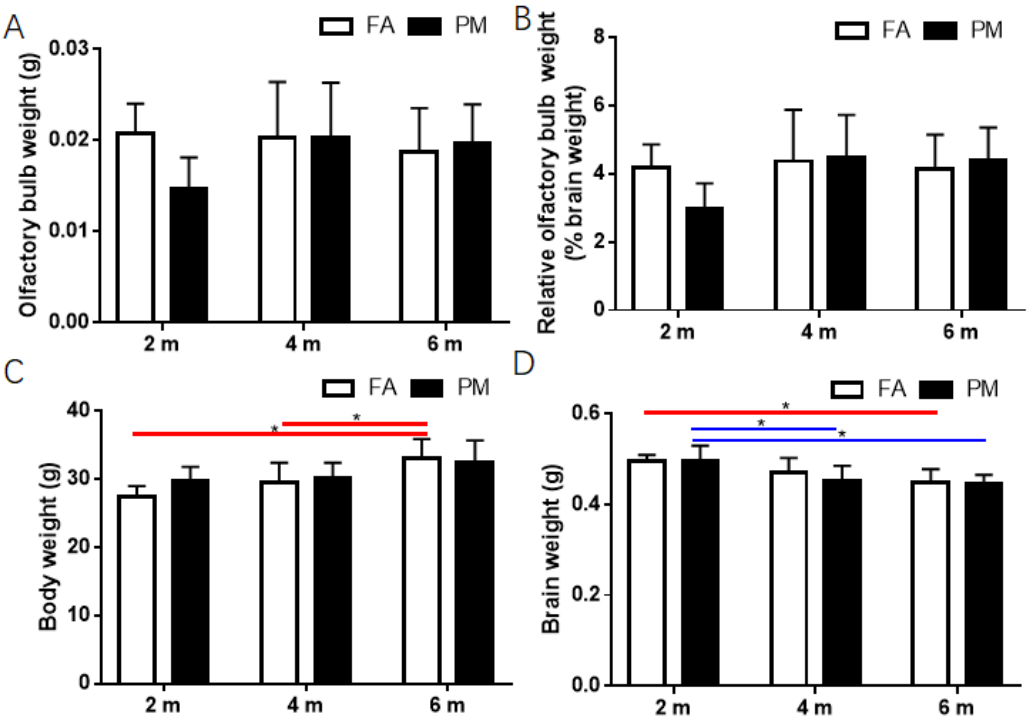

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598 **Supplemental Figure 3.** The weight of different brain structures. **A**, The weight of
599 olfactory bulbs was measured. **B**, The percentage of olfactory bulbs weight over brain
600 weight was calculated. **C~D**, The weights of body and brain were measured. **P*<0.05.

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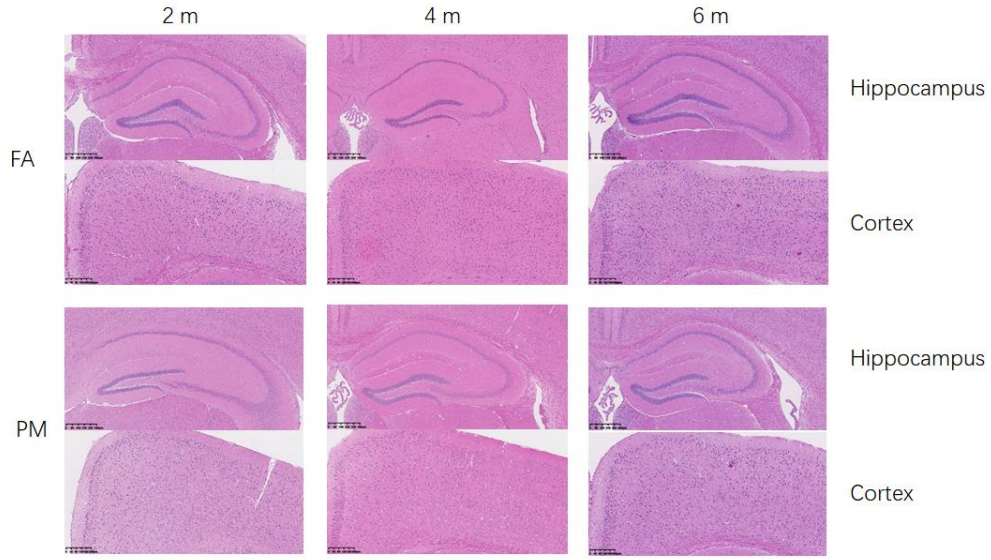

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603 **Supplemental Figure 4.** Representative images of HE staining of hippocampus and
604 cortex.

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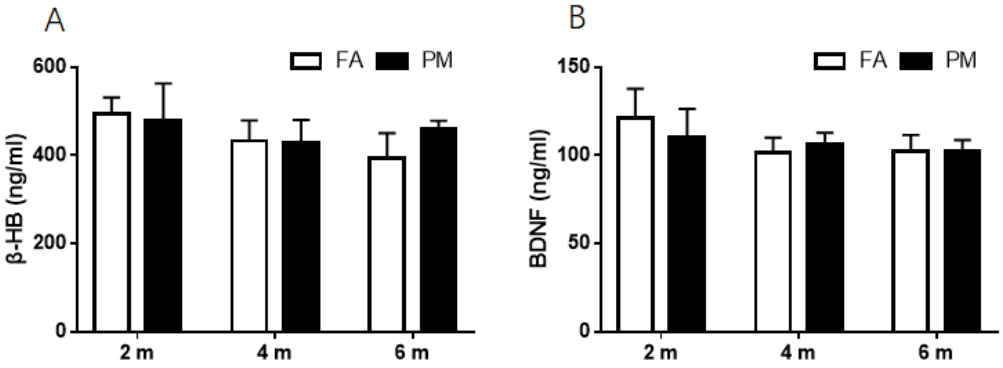

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607 **Supplemental Figure 5.** The expressions of β-hydroxybutyric (**A**) and BDNF (**B**) in
608 serum were detected by ELISA.

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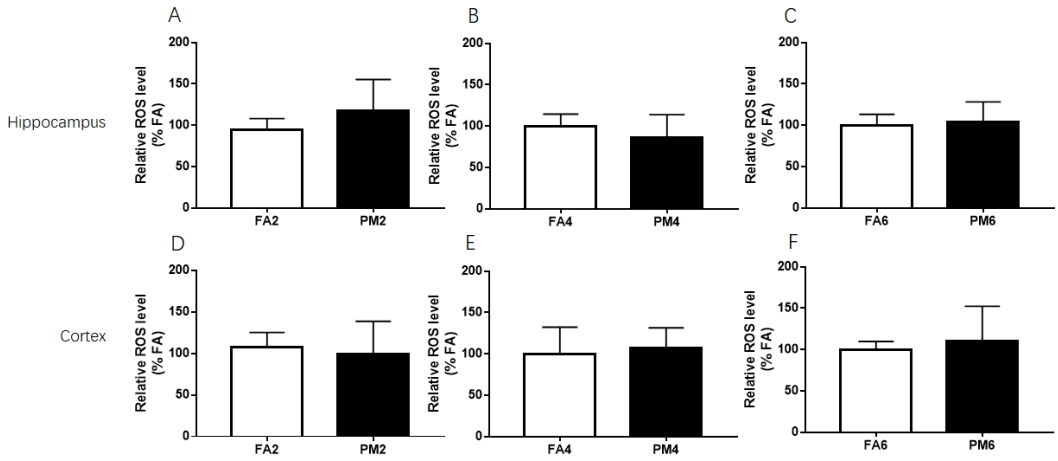

611 **Supplemental Figure 6.** ROS in hippocampus (**A~C**) and cortex (**D~F**) was detected by

612 flow cytometry.

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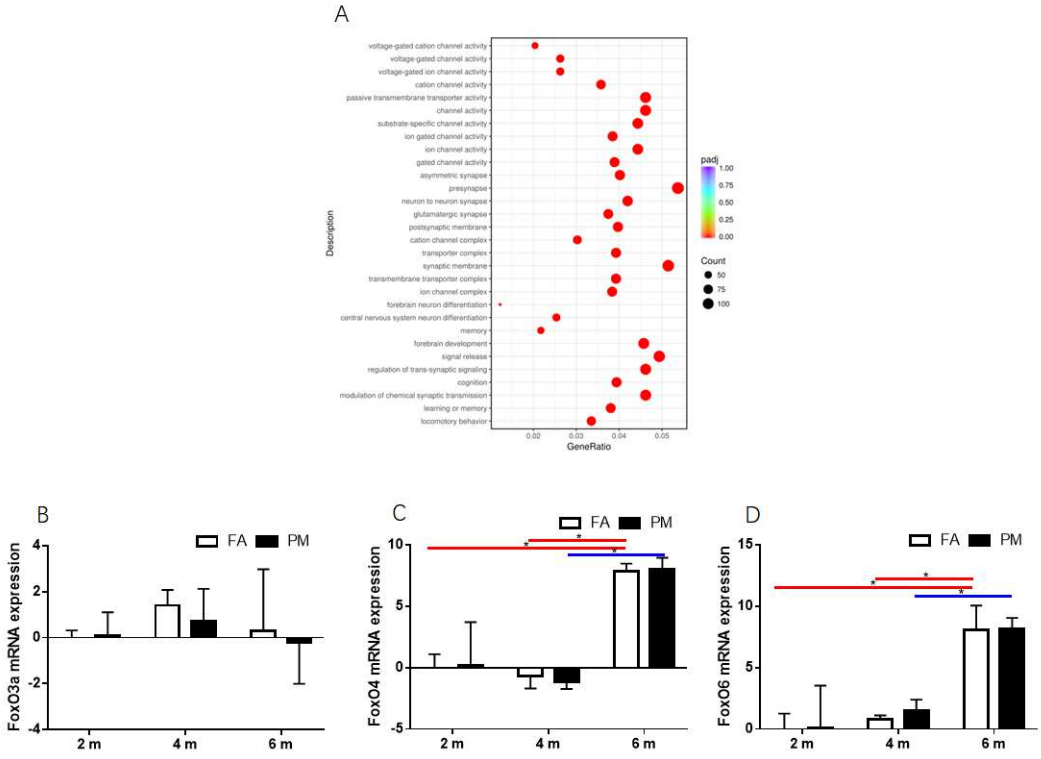

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615 **Supplemental Figure 7.** Gene expressions detected by RNA sequencing and RT-PCR.
616 **A**, Results of KEGG enrichment analysis. **B~D**, mRNA expressions of *FoxO3a*, *FoxO4*
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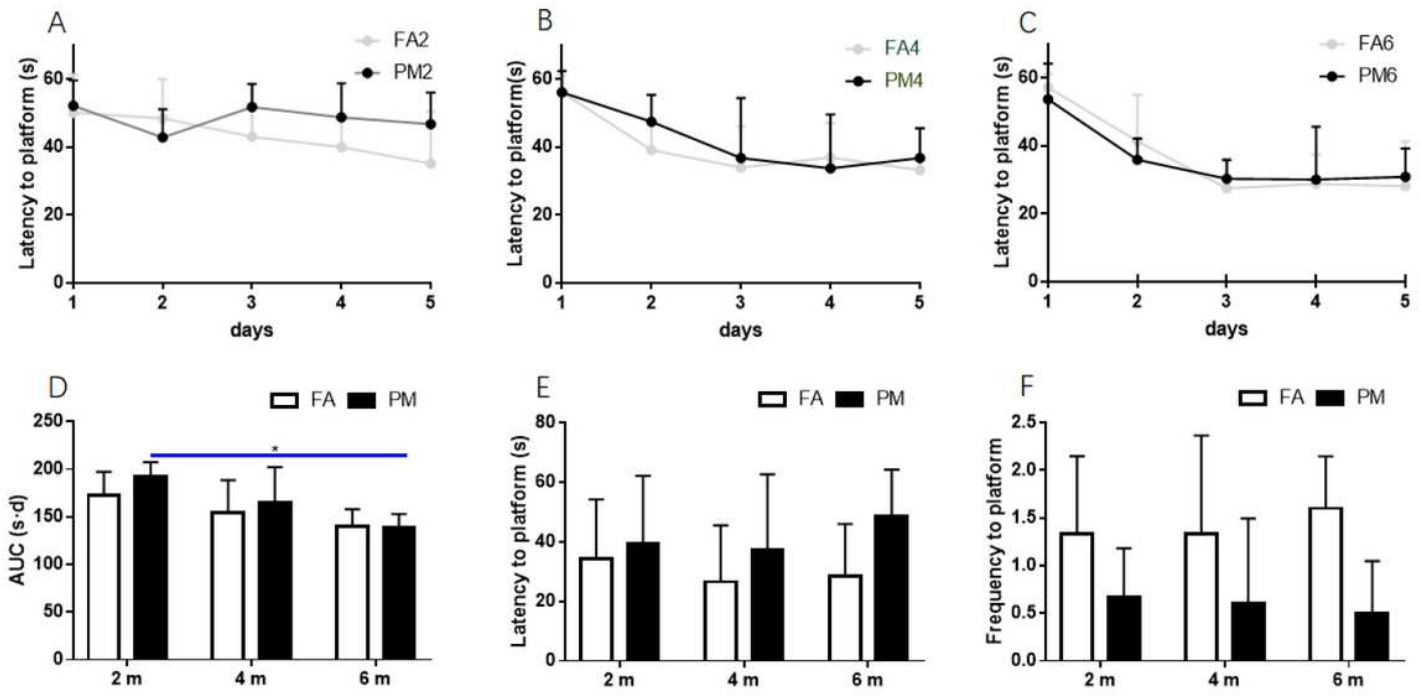
Figures



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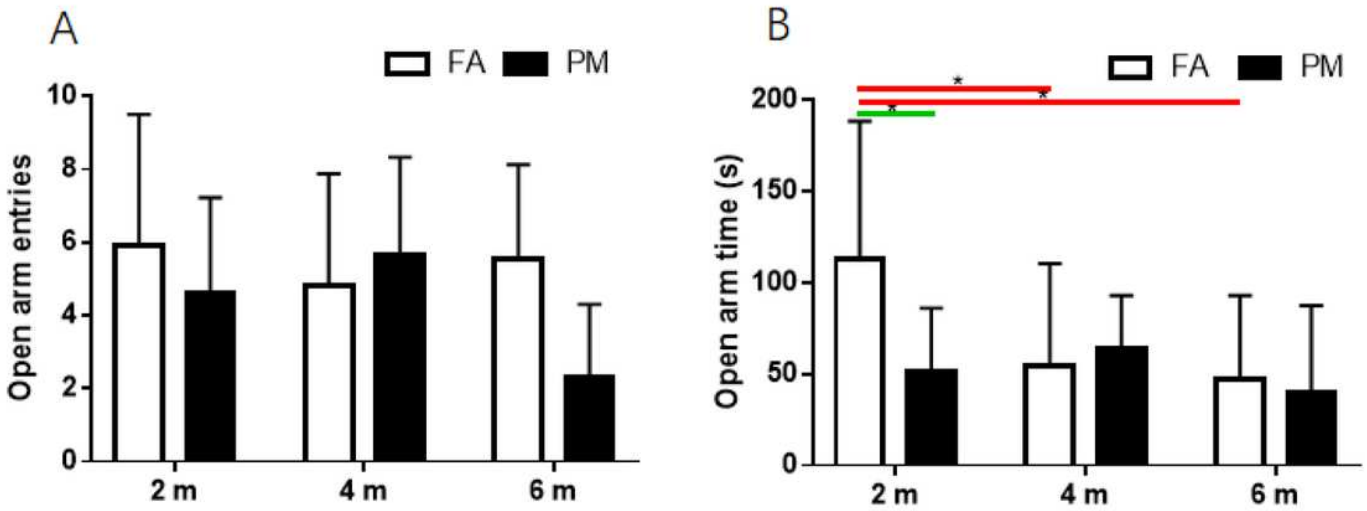

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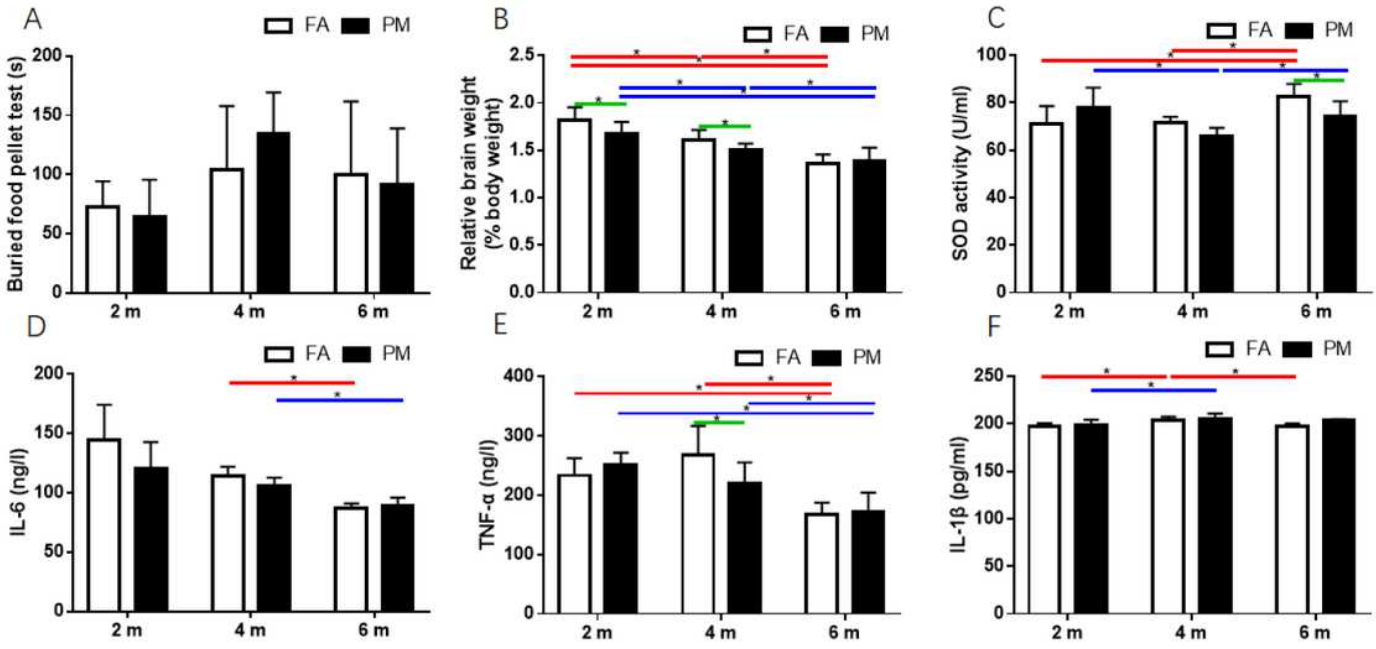

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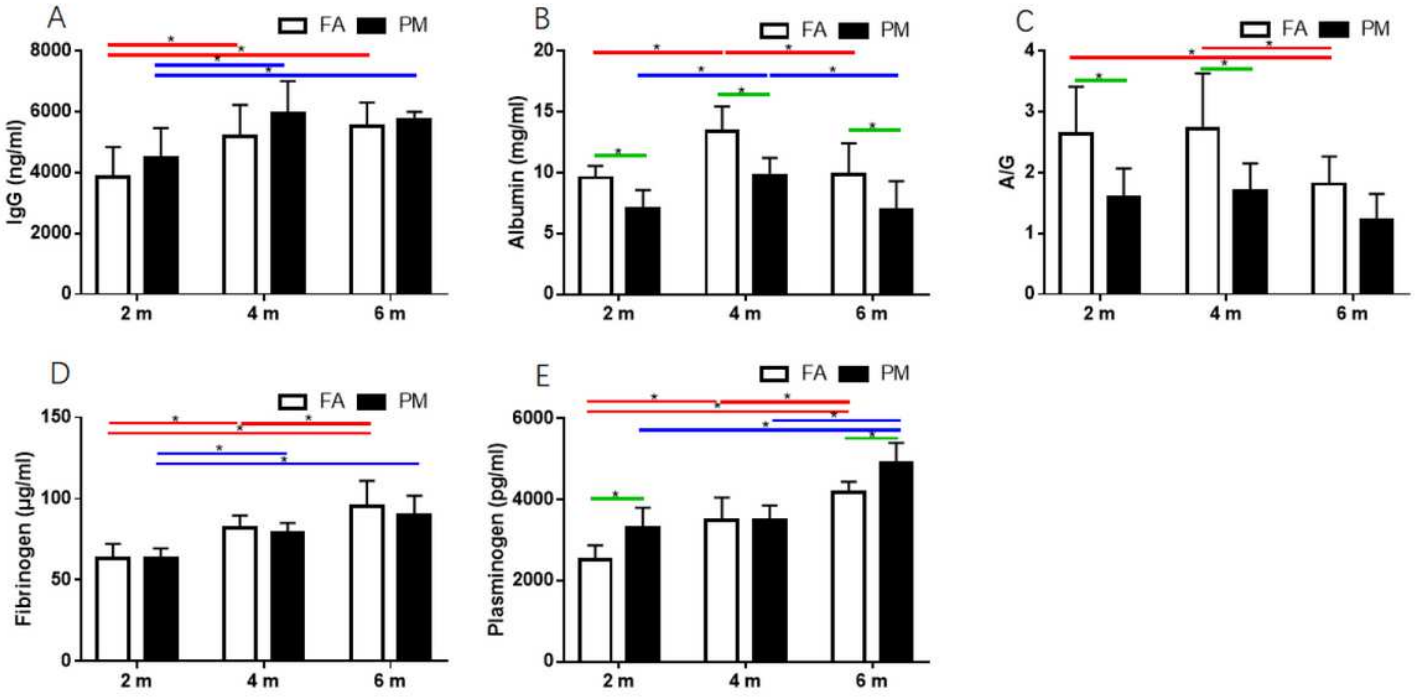

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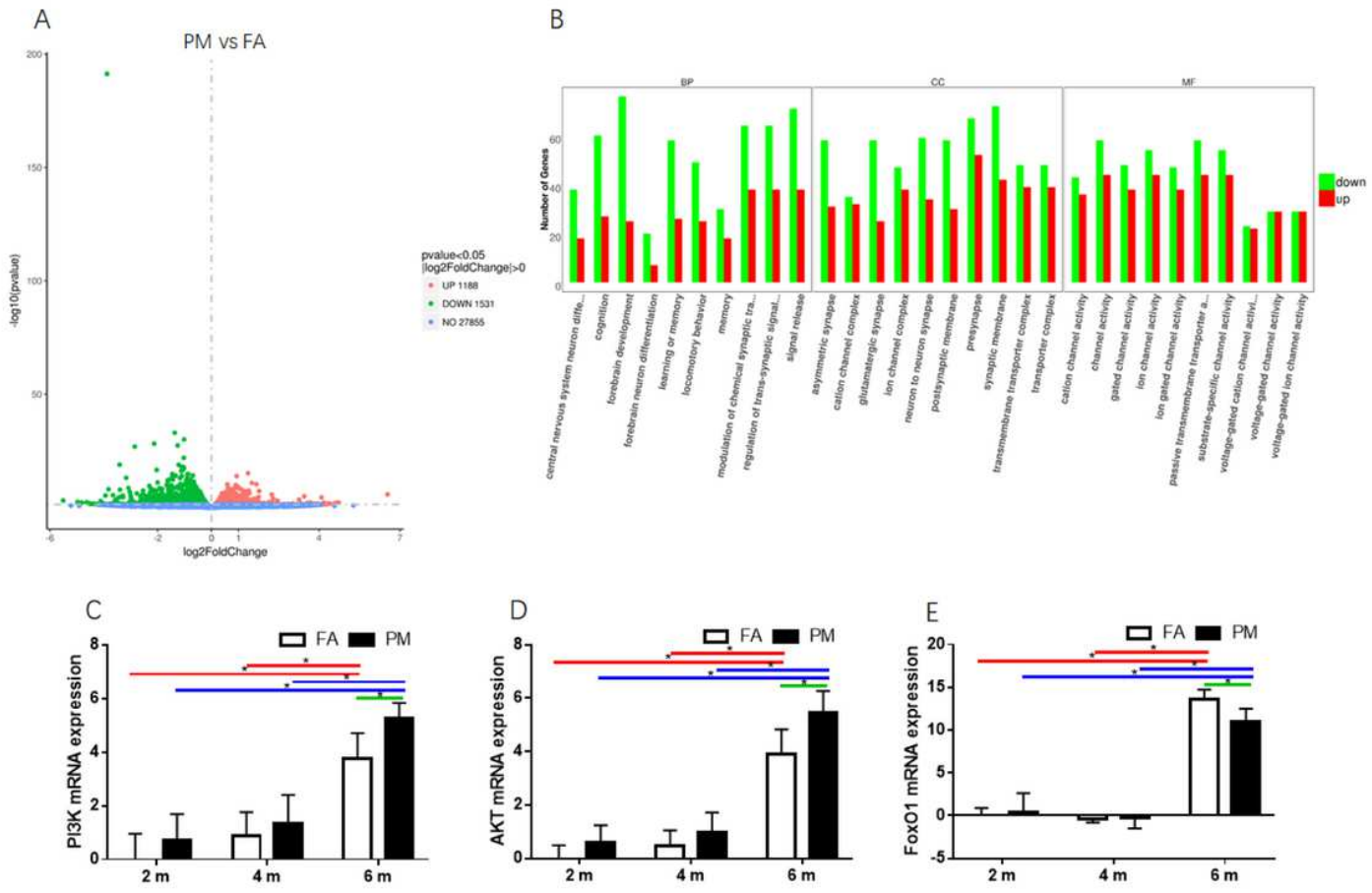

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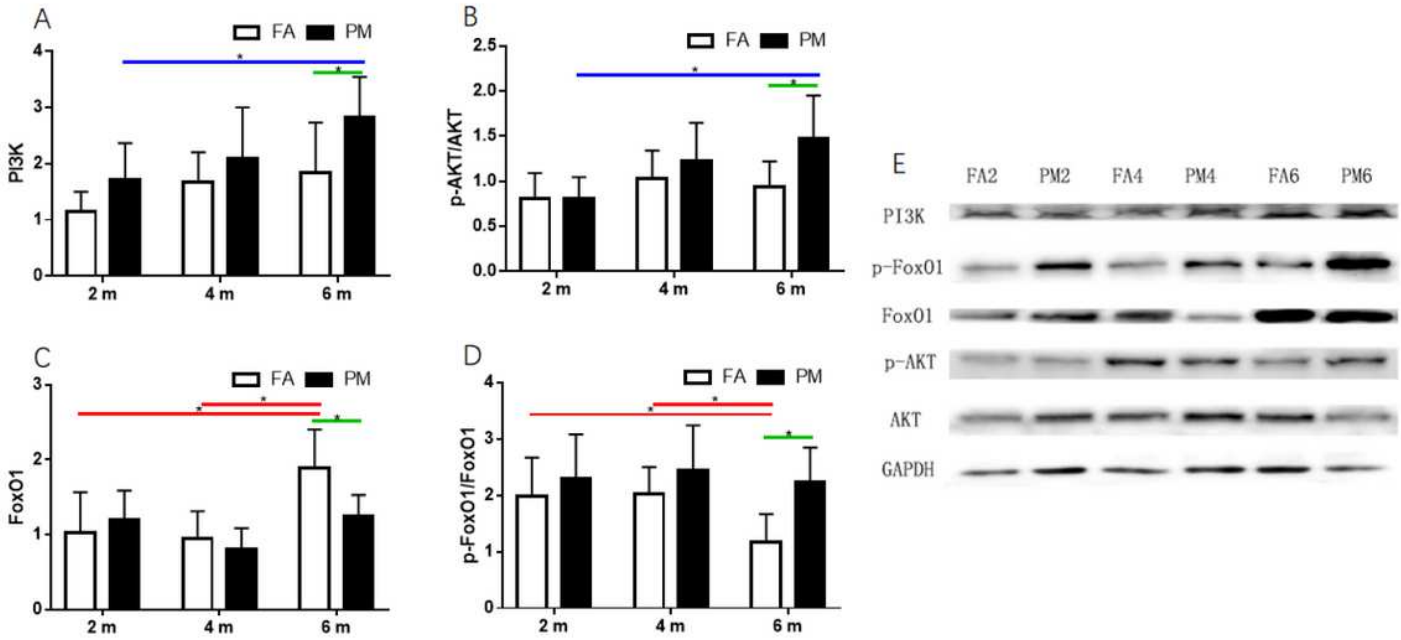

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