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Prenatal exposure to diesel exhaust particles causes anxiety, spatial memory disorders with alters expression of hippocampal pro-inflammatory cytokines and NMDA receptor subunits in adult male mice offspring

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ABSTRACT

Air pollution by Diesel exhaust (DE) consists of gaseous compounds and diesel exhaust particles (DEPs). Previous studies show associations between prenatal exposure to diesel exhaust affects the central nervous system (CNS). However, there was not reported that these effects were caused by gaseous compounds, diesel exhaust particles, or both. A limited number of studies in rodent models have shown that exposure to DEPs can result in CNS. Here, we explored the effects of prenatal exposure to DEPs on anxiety and learning and memory in NMRI mice male offspring.

Three groups of pregnant mice were exposed to 350–400 μg DEPs/m^3 for 2, 4 and 6 h daily in a closed system room. We examined anxiety and learning and memory in 8-to-9-week-old male offspring using the Elevated plus maze and Morris water maze (MWM) test. Hippocampi were isolated after the behavioral tests and measured pro-inflammatory cytokines and N-methyl-D-aspartate (NMDA) receptor expression by quantitative RT-PCR analysis. Mice exposed to DEPs in utero showed deficits in the Elevated plus maze and Morris water maze test. In addition, DEPs exposed mice exhibited decreased hippocampal NR2A and NR3B expression. Taken together, our data suggest that maternal DEP exposure is associated with anxiety, disrupts learning and memory and reduction hippocampal NR2A and NR3B expression in male offspring.

1. Introduction

Air pollution is considered one of the most related and universal environmental toxins in the mega cities (Bolton et al., 2012).

Prenatal exposure to air pollution is associated with mood dysregulation and cognitive difficulties in early childhood (Perera et al., 2012), Also Exposure to Air pollution by particulate matter (PM) causes blood–brain barrier damage, increased oxidative stress response and amyloid-b deposition in brain tissue, which suggests a causal link between PM exposure and acceleration of the pathogenesis of neurodegenerative diseases (Block and Calderón-Garcidueñas, 2009; Ehsanifar et al., 2019). PM exposure is associated with impaired cognitive function (Calderón-Garcidueñas et al., 2008). It has been estimated that up to 85% of PM in cities is related to traffic (Jonidi Jafari and Ehsanifar, 2016). Diesel exhaust (DE) is a complex mixture of gaseous-phase compounds and diesel exhaust particles (DEPs). The PM in DE contains more than 1000 soluble organic fraction compounds such as heavy metals and variety of polycyclic aromatic hydrocarbons. Diesel combustion can produce nano-sized PM. DEPs, particularly nanoscale PM (< 100 nm in aerodynamic diameter), may penetrate into brain tissue by passed through the blood–brain barrier. These particles can also carry large amounts of toxic and hazardous compounds on their surface, such as heavy metals and hydrocarbons, which suggests that nanoscale DEPs may cause neurotoxic effects (Hesterberg et al., 2010).

With regard to the abundant documents on DE exposure, by a section of the World Health Organization recognized as the International Agency for Research on Cancer, DE was introduced as carcinogenic to humans (Group 1) (Claxton, 2015). Maternal exposure to DE increases heart failure and lung inflammation and Developmental toxicity following DE exposure has been also reported (Weldy et al., 2013). For

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example, prenatal exposure to DE in mice exacerbates weight gain in offspring following exposure to a high-fat diet in adulthood (Bolton et al., 2012). Prenatal exposures to environmental chemicals may be able to have significant consequences for adult physiology and behavior (Heindel and vom Saal, 2009). Prenatal exposure to diesel exhaust, could affect monoaminergic systems of male offspring in several brain regions in mice (Yokota et al., 2013).

Indeed, the primary life environment condition can also affect brain development (Welberg and Seckl, 2001). Also maternal exposure to DE, affected the morphology of hippocampus and cerebral cortex, where accumulation of nanoscale DEPs was observed (Sugamata et al., 2006). The hippocampus transmission is mainly mediated by glutamate receptors, and this is crucial for spatial learning and memory (Ishii et al., 1993; Tombaugh et al., 2002).

Many reports indicate that sub-chronic inhaled DEPs exposure may instantly affect the hippocampus and cerebral cortex in male mice offspring. In the present study, we hypothesized that maternal DEPs exposure would cause neurotoxic effects and neuroinflammation by inducing pro-inflammatory cytokines in mice. Thus, this work aimed to define whether maternal exposure to DEPs affected cognitive functions in adult male mice offspring. To study the effects of DEPs prenatal exposure on cognitive function, procedure such as prenatal exposure are useful to DEPs inhalation. Therefore, in this study, we focused on the effects of maternal DEPs exposure on anxiety and learning and memory in adult male offspring using behavioral tests, followed by measurement of pro-inflammatory cytokines and NMDA receptor gene expression in the hippocampus.

2. Materials and methods

2.1. Diesel exhausts particles (DEPs) collection and extraction

Diesel exhaust particulate matters were collected with a constant volume sampler system joined to the end of a dilution tunnel that was in turn attached to a pickup (Iran Khodro Diesel Co., Tehran, Iran), light-duty (2776 -cc), 4-cylinder, diesel engine. At the speed of 1500 rpm and under 10 load torque (kg/m), a standard diesel fuel was exploited to connect the engine to the dynamometer and operate it. The exhaust was installed into a stainless steel dilution tunnel (300 × 5800 mm). The sampling point had a temperature of below 50 °C. DEPs were gathered on samples of filter and suspended at 1 mg/mL in sodium chloride and were sonicated for approximately 30 min immediately before exposure to animals. In addition, subjecting of the DEPs to dynamic light scattering (DLS) measurements was carried out by a Zetasizer Nano-ZS system (Malvern Instruments Ltd., UK) so that the level of distribution of DEPs in the suspension could be determined (Fig. 1) (Organization and UNAIDS, 2006; Yoshizaki et al., 2010).

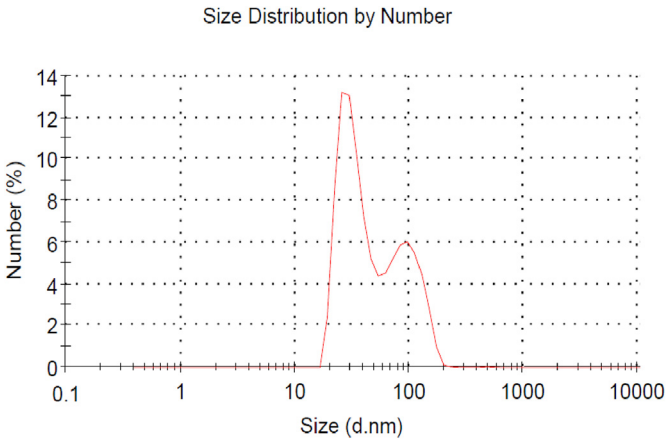


Fig. 1. Size distribution of diesel exhaust particles (DEPs) in suspension as determined by dynamic light scattering.

2.2. Pregnant and nonpregnant animals

Thirty female NMRI mice obtained from Kashan University of Medical Sciences (Kaums) Laboratories of animal facilities (Kashan, Iran) and then were pregnanted. All pregnant mice (except controls) at during of gestation were exposed daily to DEPs. Three groups of pregnant mice were DEPs exposed inhalation the 2, 4 and 6 h per day. Another pregnant group as control pregnant mice was exposed inhalation to saline solution alone for the same period of time. The values from the latter group were also compared with a group of nonpregnant female mice. After DEP exposure, mothers and male pups were maintained in the same clean room.

2.3. Offspring

After weaning on postnatal day 21, the male mice offspring were maintained in groups in their home cages (5 mice/cage) at 22 °C, with a 12-h light/dark cycle (lights on from 7:00 to 19:00), in a humidity-controlled environment (40–45% humidity). Food and water were provided ad libitum. Body weights of male mice were recorded. The offspring maternally exposed to 2, 4, and 6 h DEPs exposure assigned to T2, T4 and T6, respectively. Pups born from the pregnant control mice were determined as control (CONTROL). The litters at 7–8 week of postnatal age were entered the experiments. In general, one to Three pups were taken from each mother. The T2, T4, T6, and CONTROL mice (one group of each, n = 10 in each group) were introduced to the behavioral (Morris water maze and elevated plus maze) testing. After the behavioral (Morris water maze and elevated plus maze) testing, all animals experiments were performed in accordance with National Institutes of Health (NIH, USA) guidelines. All animals anesthesia were obtained under sodium pentobarbital (50 mg/kg), and to minimize suffering, all efforts were made.

2.4. Behavioral testing

Behavioral tests were performed at 7 and 8 weeks of age. In each group ten male offspring were used for behavioral tests. All behavioral exams were conducted between 12:00 and 16:00. Whit in the test time, we counterbalanced the task by controlling the order of animals tested among the control and DEPs exposed groups. Behavioral tests performed included the elevated plus maze and Morris water maze test. Each mouse was used alone in each behavioral test to avoid carryover effects between the elevated plus maze and Morris water maze test. This plan does not change the explanation of the results in the current set of tests.

2.4.1. Morris water maze

Morris water maze test were measured using a spatial learning and memory in adult male offspring (7–8 weeks of age). All mice were allowed to swim freely in four 90-s tests to allow them to adapt to moisture. Then, starting from the second day after the last exposure, the mice received MWM tasks for five consecutive days: the acquisition or training phase was four days and the exploration trial was one day. The labyrinth is a white circular pool 100 cm in diameter and 30 cm high, filled with water (fixed at 22 ± 1 °C). This labyrinth is located in a laboratory containing super-maze trails, including posters on the walls. The maze is divided into four quadrants. The starting point of each training experiment is the mid-arc in each quadrant. During the collection phase, mice were tested four times a day for four consecutive days to find a hidden platform (10 cm in diameter) 1.0 cm below the water surface in the pool. During each trial, a single mouse was placed in the water facing the pool wall at one of the four designated starting points and left to find a hidden platform. A fixed overhead camera and tracking system was used to measure escape latency, the time it takes

for the mouse to find and climb onto the platform. Whit in each trial, each mouse was given 90 s to find the hidden platform. If they find the platform, they can stay on the platform for 10 s and then move from the maze to their home cage. If it failed, the animal manually guided to the platform and then returns to its cage after 30 s for the about 10 min intertrial interval. The position of the platform remains unchanged for four consecutive days. Swimming speed is also recorded throughout the acquisition phase. On day 5, the platform was removed from the pool and a probe test was performed to evaluate memory retention. Each mouse was allowed to swim in the maze for 90 s. Record and average the duration of the mouse in the target quadrant.

2.4.2. Elevated plus maze

In mice, spatial stress was assessed using the elevated plus maze (Carobrez and Bertoglio, 2005; Haller and Alicki, 2012). In short, the apparatus, which had four 50 × 10 cm arms and a 10 × 10 cm center platform, was raised up to 60 cm above the ground. Two arms were remained open, whereas two opposite arms were circled by 40-cm walls. In addition, moderate brightening of the test room was carried out. Facing an open arm, each animal was situated in the platform at the center. The mice were allowed to walk in the maze for 5 min, during which we observed the animals' behavior. It was agreed to define an entry as having all four paws in the arm. The time spent in open arms (OAT) and the number of entries into open arms (OAE) was the measured factors for the plus maze navigation (Ehsanifar et al., 2019).

We calculated the OAE and OAT percentages, as presented below:

$$OAE\% = \text{number of entries into open arms/total number of entries} \times 100$$

$$OAT\% = \text{duration spent in open arms(s)/300(s)} \times 100$$

2.5. Total RNA isolation

Immediately after completing the social behavioral tests, the hippocampus was isolated, frozen in liquid nitrogen, and stored at −80 °C. The RNA was totally isolated using RNX- Plus kits (Sina Clon) according to the manufacturer's protocol. Afterwards, the quantity and purity of the total RNA applying the Nano Drop RNA Assay protocol (Thermo scientific NanoDrop One C, USA) were calculated and assessed. Extracted RNA from each sample was used for quantitative RT-PCR analysis.

2.6. Quantitative real-time RT-PCR

Applying the Takara Kit reverse transcriptase (Takara Bio USA, Inc.) from the RNA of each sample, a template based on the company directions of the mentioned kit, was exploited for synthesizing cDNA. After that, the mRNA expressions of IL6, TNFα, IL1β, TNFα, NR2A, NR2B and NR3B in the hippocampus were assessed. One research tool was quantitative real-time RT-PCR with SYBR Green Real-Time PCR Master Mix (Toyobo Co., Ltd., Osaka Japan) in a Bio-Rad system (Agilent Technologies Inc., Santa Clara, CA, USA) with an initial hold step (95 °C for 60 s). The other tool was 40 cycles of a two-step PCR (60 and 15 s at the temperatures of 60 °C and 95 °C, respectively). Amplification of the target gene was monitored by measuring each sample's fluorescence intensity during each cycle. After normalization against the housekeeping gene, Hypoxanthine phosphoribosyl transferase (HPRT), comparative levels of expression of target genes were estimated for each sample. According to the results, no significant difference was observed between the groups in terms of HPRT expression (data not shown). It is notable that the target primers were custom-made. The obtained sequences are shown in Table 1.

2.7. Measurement of sserum corticosterone concentration

For measuring the serum corticosterone levels, the blood samples were taken from all mice groups entered the exam and mothers either exposed to DEPs. Mice were deeply anesthetized and blood was collected from heart and the samples were centrifuged at 2500 rpm for 10 min. The Serum samples were handled and stored at −80 °C until assayed and the serum corticosterone levels measurement was implemented by an enzyme-linked immunosorbent assay kit (DRG, Germany) and the manufacturer supplied protocol.

2.8. Nissl staining

To detect changes in HI tissue, histological examination was performed using Nissl staining after exposure to DEP. For Nissl staining 5-μm, coronal sections were first prepared. These sections were deparaffinized in xylene and then hydrated. After rinsing with tap water and distilled water, staining with 0.1% Cresyl violet for 3 min, dehydration, and finally placed under a glass cover slip. The histological changes were observed using an optical microscope (Nikon, Japan). The Nissl body was dyed purple blue.

2.9. Statistical analysis

One-way ANOVA with learning the water maze task, remembering the learned task and the anxiety evaluation as between subject's factors and DEPs exposure as within subjects factor was carried out on the behavioral procedures. Also one-way analysis of variance (ANOVA) with corticosterone as between subjects factor and DEPs exposure was performed on the corticosterone level of serum. Data were reported in the form of mean ± standard deviation. Quantitative RT-PCR analysis was carried out via Prism ver.7.3 software using One-way analysis of variance (ANOVA) followed by Neumann Keul's multiple comparison tests. The difference was considered significant Alpha level at $P < 0.05$.

3. Results

3.1. Effects of prenatal DEPs exposure on body weight of male offspring

The body weight of male offspring was not significant effects by prenatal DEPs exposure during the youngster to adult age (postnatal day 1: Control, 2.3 ± 0.2 g; DEPs, 2.2 ± 0.3 g. 7 weeks: Control, 36.8 + 1.0 g; DEP, 37.3 + 0.8 g. 9 weeks: Control, 39.4 + 0.9 g; DEP, 40.7 + 0.7 g).

3.2. Effects of prenatal exposure to DEPs on serum corticosterone levels

We measured serum corticosterone concentration of both DEPs exposed and control mice, to check for confounding effects of stress on behavioral endpoints. The serum level of corticosterone in DEPs exposed mice showed no significant change compared with the control group (data not shown).

3.3. The effect of prenatal DEPs exposure on learning the spatial maze task

The spatial learning and memory of the mice prenatally exposed to DEPs were evaluated via two strategies of maze navigation; training and probe trial. We performed a Morris water maze test, to examine hippocampus dependent spatial learning and memory. Analysis of variance show that the 4 groups of mice introduced to the training phase indicated a different performance in learning the maze task ($P < 0.0001$). All groups improved their maze steering over 4 days of experiment. Although, the post hoc test appeared that the T4 ($P = 0.03$) and T6 ($P < 0.0001$) mice, behaved significantly lower compared with their control group.

Two hour exposure to the DEPs, not significantly affected the maze performance. Fig. 2A indicate how the different DEPs exposure groups of mice learned the maze task. In the probe test, DEPs exposed mice

Table 1
Primer design for quantitative RT-PCR.

Gene		Sequence	T_m
HPRT	Hypoxanthine phosphor ribosyl transferase	Forward: 5'- GCT GGT GAA AAG GAC CTC T-3' Reverse: 5'- CAC AGG ACT AGA ACA CCT GC-3'	60
NR2A	N-methyl-$_D$-aspartate receptor subunit 2A	Forward: 5'- AGCCCCCTTCGTCATCGTAGA-3' Reverse: 5'- ACCCCTTGCAGCACTTCTTCAC-3'	60
NR2B	N-methyl-$_D$-aspartate receptor subunit 2B	Forward: 5'- GCCATGAACGAGACTGACCC -3' Reverse: 5'- GCTTCCTGGTCCGTGTCATC -3'	60
NR3B	N-methyl-$_D$-aspartate receptor subunit 3B	Forward: 5'- TCCTACTCCTCCGCTCTCAA-3' Reverse: 5'- TGGATTCCAGACAGCTCCTC-3'	60
IL-6	Interleukin 6	Forward: 5′- TTGCCTTCTTGGGACTGATG-3′ Reverse: 5′- AGGTCTGTTGGGAGTGGTAT-3′	60
TNF-α	tumor necrosis factor alpha	Forward: 5'- GGCCTTCCTACCTTCAGACC-3' Reverse: 5'- AGCAAAAGAGGAGGCAACAA-3'	58
IL-1β	Interleukin 1β	Forward: 5'- CAG CTC ATA TGG GTC CGA CA -3' Reverse: 5'-CTG TGT CTT TCC CGT GGA CC -3'	60

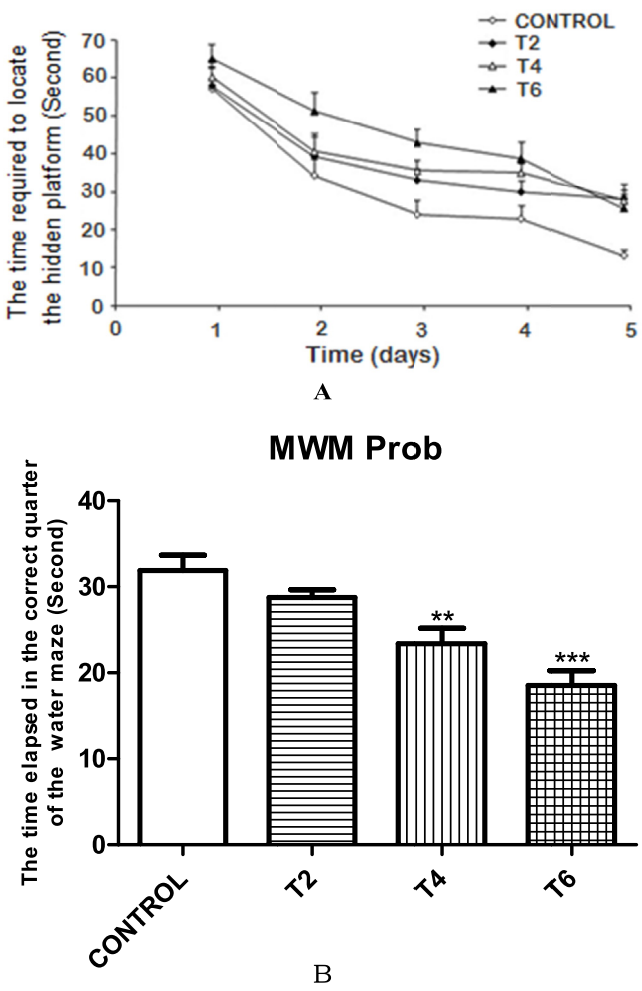


Fig. 2. Performance of the different groups of mice in the Morris water maze test. A) The time required to locate the hidden platform in the water maze during the learning stage. The mice exposed to the DEPs for 4 h ($P = 0.03$) and 6 h ($P < 0.0001$) during the fetal life performed significantly lower than the control group. B) The time elapsed in the correct quarter of the water maze during the retrieval test. The mice exposed to the DEPs for 4 h (**$P = 0.002$) and 6 h (***$P < 0.0001$) during the prenatal life displayed a weaker performance than the control group. The data is shown as an average of 10 SEMs.

indicated considerably deficits in reference memory compared to control mice (Fig. 2B). Remembering the learned task was assessed by removing the platform and measuring elapsing time in the correct quadrant of maze. According to the findings in the learning phase, the T4 ($P = 0.002$) and T6 ($P < 0.0001$) groups had a shorter navigation in the correct quadrant when compared with the Control mice. Statistical analysis showed a general variation between testing groups in performing the retrieval task ($P < 0.0001$). The values are 17.3 + 1.63 s and 21.6 + 1.23 s for the T4 and T6 groups, respectively. Behavior of the mice in T2 group resembled that in the Control one so that a marked difference was evident between the T2 group with the T4 ($P < 0.013$) and T6 ($P < 0.0001$) groups.

3.4. The effect of prenatal DEPs exposure on anxiety

The mice exposed to DEPs were introduced to plus maze task for evaluate the degree of anxiety.

Entry to the open arms and the elapsing time in the open arms were two factors by which the level of anxiety was measured. One way ANOVA indicated a variation between the efficiency of the different groups of mice. The offspring born from mothers exposed to 2, 4 and 6 h DEPs, displayed a significant reduction in entering the open arms in comparison to their control counterparts (Fig. 3A). Moreover, the mice in the T2, T4 and T6 groups passed a shorter time in the open arms (Fig. 3B).

3.5. Effect of prenatal DEPs exposure on cytokines mRNA expression in the hippocampus

We used RT-PCR to examine the mRNA expressions of pro-inflammatory cytokines, such as IL6, TNFα, and IL1β, in the hippocampus of mice prenatally exposed to DEPs. The mRNA level of IL6 was significantly higher in the T4 and T6 exposed groups compared with the Control group ($P < 0.05$) (Fig. 4 A), and the mRNA level of TNFα, and IL1β, were significantly higher in the T6 exposed groups compared with the Control group. However we did not observe significant change in the mRNA expression level among the T2, T4 and T6 groups (Fig. 4 B, C).

3.6. Effect of DEPs exposure on the mRNA expressions of NMDA receptor subunits in the hippocampus

In addition to observing the effect of prenatal mice DEPs exposure on spatial learning ability, to test the reason of the spatial learning and memory deficits, we also examined the effect of DEPs on receptor subunit expression in the hippocampus, and show that the expression level of NR2A and NR3B mRNA was significantly lower in the T4 and T6 exposed group than in the control group ($P < 0.05$) (Fig. 5A, C). We don't observed significant different in the expression levels of NR2B between the control and the DEPs exposed group (Fig. 5B). Also, we did not observe significant change in the mRNA expression level among the groups.

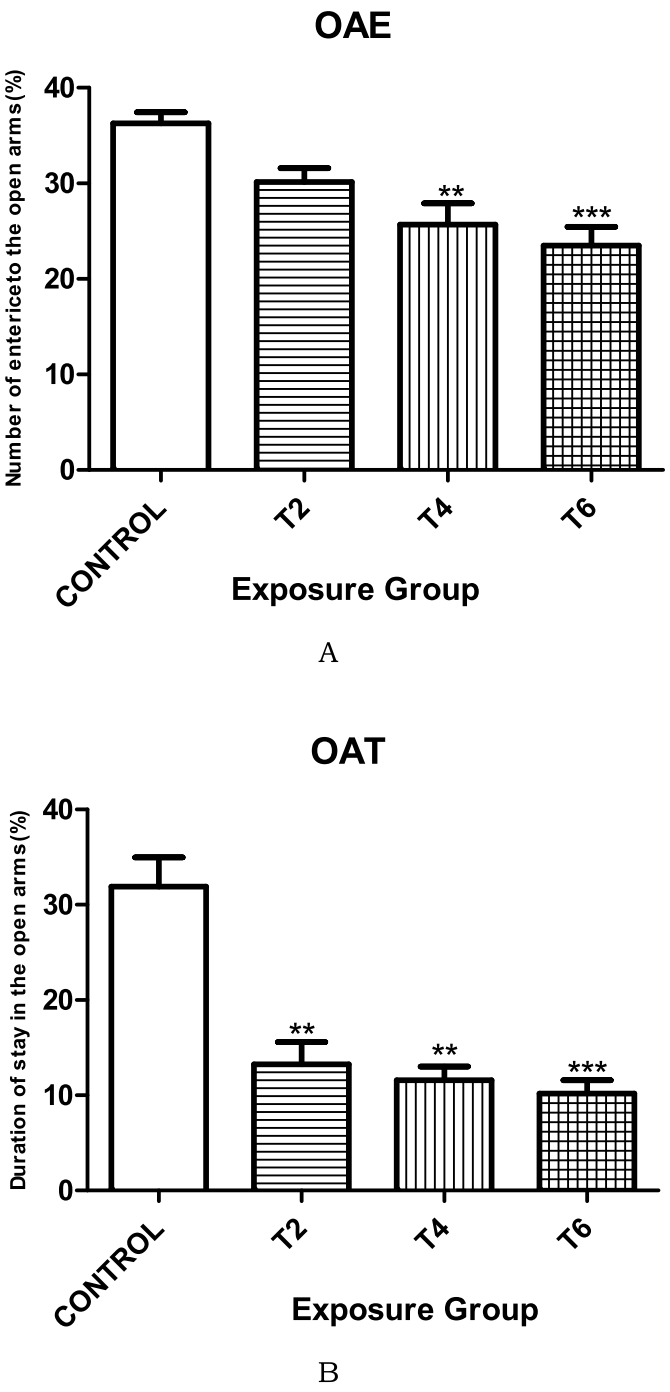


Fig. 3. Elevated plus maze navigation for different groups of animals. A) Percentage of the entered open arms by the different groups of mice during the plus maze searching. While the DEPs exposure led a marked decrease in entering the open arms in all exposure groups. B) Percentage of the duration of stay in the entered open arms during the plus maze navigation. The all exposure animals displayed a considerable decrease in the time of the open arm steering. (*P < 0.05, **P < 0.01, ***P < 0.001) when compared with control group. The data is shown as an average of 10 SEMs.

3.7. Hippocampal morphological analysis

Nissl staining in the HI of the control and T2, T4 and T6 group exposure to DEPs for 5 days per week in 12 weeks, showed that above 2 h/d prolonged exposure is associated with changes in neuronal morphology in the CA1 regions of the hippocampus (Fig. 6).

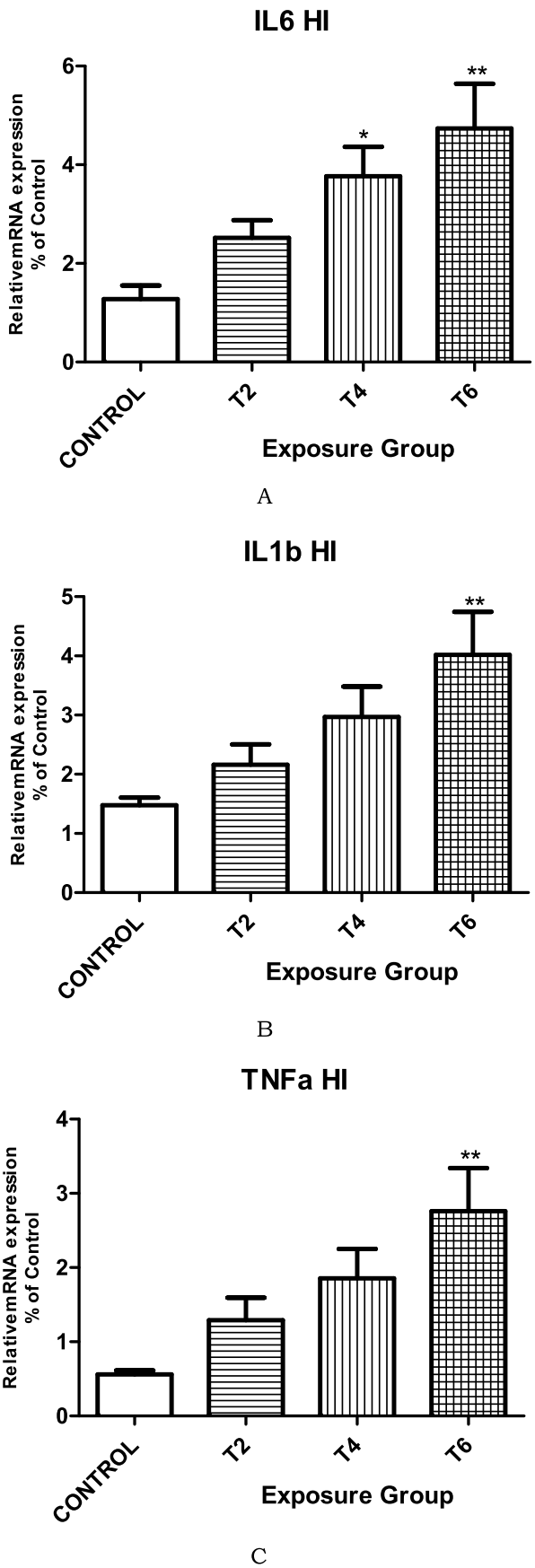


Fig. 4. Messenger RNA expressions of the proinflammatory cytokines (A) IL6 (B) IL1β and (C) TNFα in hippocampi of T2, T4 and T6 groups (*P < 0.05, **P < 0.01) when compared with control group. Each bar represents the mean ± SE (n = 6).

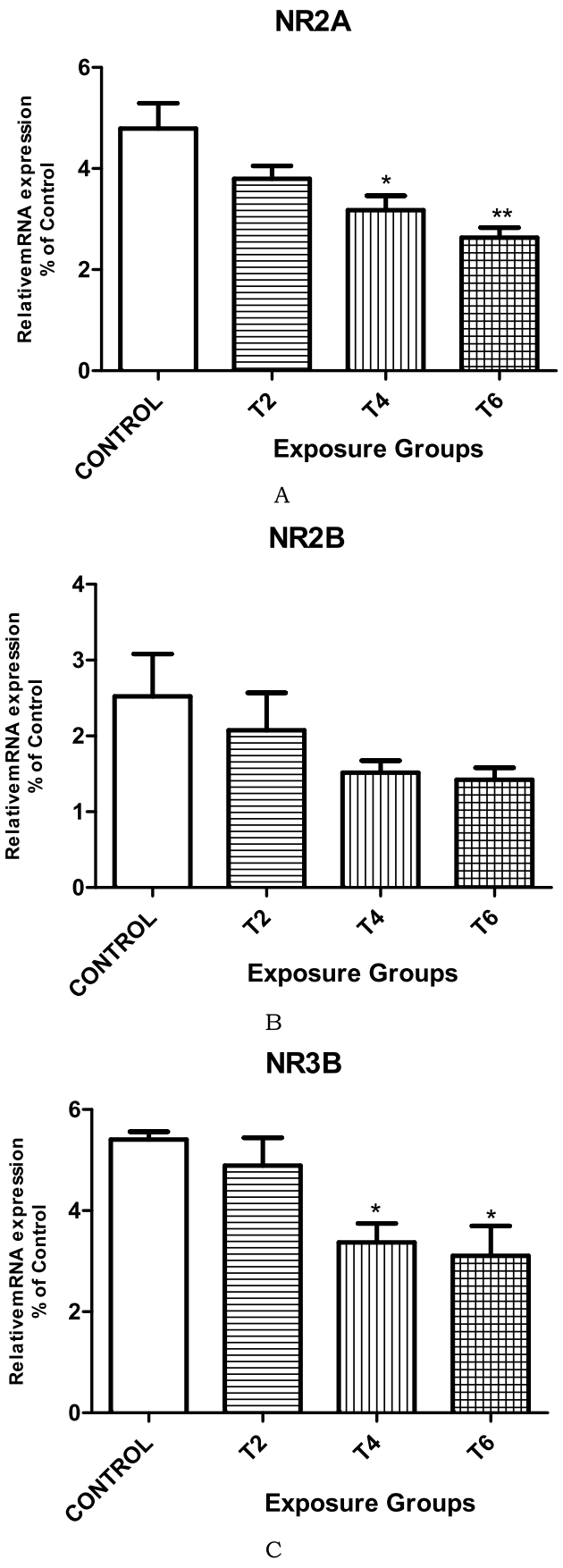
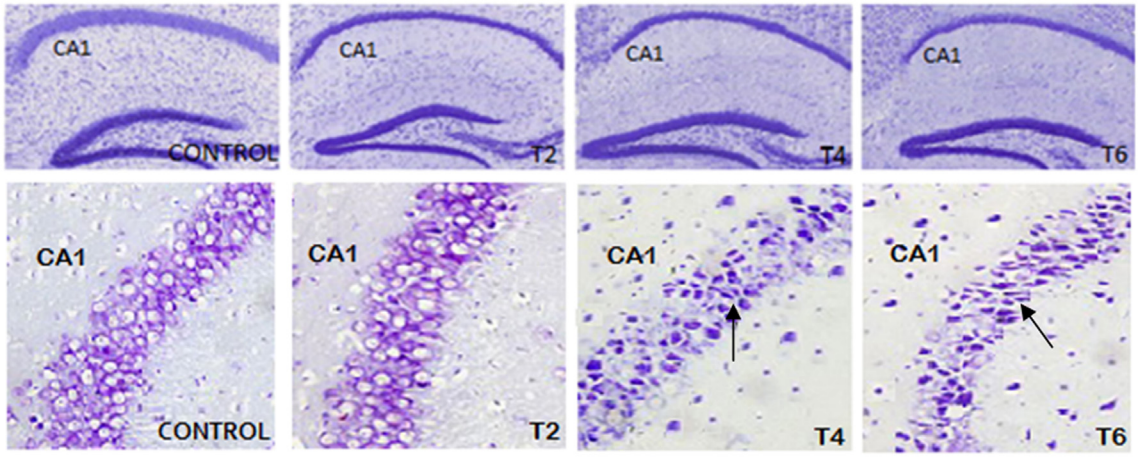


Fig. 5. mRNA levels expression of the NMDA receptor subunits (A) NR2A, (B) NR2B, and (C) NR3B in the hippocampus of 8 week old male offspring exposed to DEPs and Control. Similar NR2B expression levels were observed in control and DEP-exposed animals. However, NR2A and NR3B mRNA expression in mice maternally exposed to DEPs (*P < 0.05, **P < 0.01) lower than compared with control group. Each barrepresents the mean ± SE (n = 6).

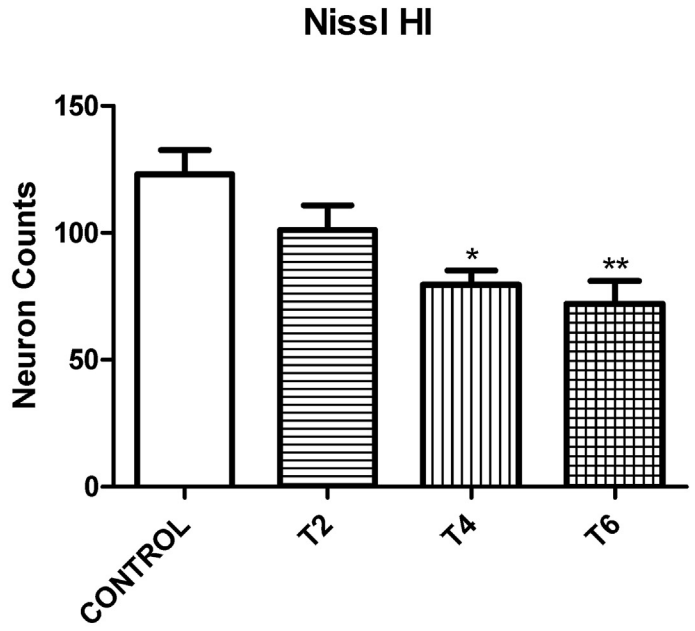
4. Discussion

Many reports have showed an altered behavioral performance in offspring born from mothers under exposed of DEPs during of pregnancy. The cognitive changes are proved to be associated with inflammation modifications in certain parts of brain. This study evaluates the effect of DEPs exposure in fetal life on anxiety, spatial memory and neuroinflammation in the postnatal period of life. The results of the this study indicate, the effects of maternal DEPs exposure inhalation on acquisition and spatial memory in male offspring in postnatal period of life, in addition to inflammation and reduced NR2A and NR3B expression in the hippocampus. The DEPs dose used in the present study did not affect body weight, body length, eye appearance, vibrissae, and sensorimotor responses. However, the reduced performance in the elevated plus maze and Morris water maze test was suggestive of anxiety and impaired spatial memory. Whiles prior studies eminent that DEPs exposure, during the adult period resulted in behavioral and/or molecular effects on the CNS in vitro and in vivo (Oppenheim et al., 2013; Tobwala et al., 2013; Yamagishi et al., 2012). The present study addressed potential long-term effects of prenatal exposure inhalation to DEPs on the CNS of adult male mice offspring. This model of prenatal DEPs exposure is unique because it enables to better clarify environmental factors involved in DEPs intercede neurodegeneration, the effects of exposure during pregnancy clarification. The hippocampus for encoding spatial information in rodents, has long been recognized as a critical structure (Milner et al., 1998). The Morris water maze test in adult male offspring of mice prenatally exposed to DEPs, show an impairment of spatial learning and memory, which requires a fully functional hippocampus. Also, NR2A and NR3B expression in the hippocampus of mice prenatally exposed inhalation to DEPs were significantly lower than that of control group (Sugamata et al., 2006). It has been reported hippocampal NMDA receptor activity are critical for spatial learning and memory (Bannerman et al., 1995). Another study showed that mice exposed to lipoteichoic acid (LTA) and DEPs took longer to reach the hidden plateau in the MWM task, and the mRNA expression of pro-inflammatory cytokines and NMDA receptor subunits was up regulated (Win-Shwe et al., 2009). Exposure of aerosols and nanoscale carbon black particles caused of lung and brain inflammation, and that such particles have enhanced ability to produce reactive oxygen species and therefore have extensive toxicity (Yamamoto et al., 2006). Another study showed that mice exposed to DEPs took longer to reach the hidden plateau in the MWM task, and the relative mRNA expression of NMDA receptor subunits and pro-inflammatory cytokines was upregulated. It was also shown that exposure of DEPs affects changes in the expression of NMDA receptor subunits accompanied by neurotoxicity (Win-Shwe et al., 2009). The CA1 neurons are important for spatial learning and volume of dorsal hippocampal lesions correlates with the degree of spatial learning impairment in rats, and dorsal hippocampal lesions result in more deep impairment than ventral hippocampal tissue damage (Morris et al., 1982; Moser et al., 1993). These results are similar to those of another study in which in mice exposed to maternal DE inhalation, was found a significant pathological impairment of the CA1 region of the hippocampus (Sugamata et al., 2006).

The other study, show that DEPs affect spatial learning and memory, but in the passive avoidance test were no differences between the control and exposure groups, indicating that not effect on non-spatial learning and memory (Yokota et al., 2015). In the hippocampus of male mice treated with high-dose DEPs, the mRNA level of the pro-



A



B

Fig. 6. Prenatal Exposure to Diesel Exhaust Particles effects on neuronal injury in the hippocampal CA1 of adult male mice offspring (Nissl staining). (A) Nissl staining was used to identify Nissl bodies and the extent of neuronal damage in the CA1 subfield (× 400). Control, T2, T4, T6. (Control, T2) Neurons were neatly arranged and displayed sharp edges. Nissl bodies showed visible cytoplasm. (T4, T6) Hippocampal CA1 neurons were loose and absent; nucleoli were missing or indistinct (arrows). (B) Neuronal counts. In the control group, neurons were fairly well preserved and sparse in the CA1 subregion. By 4 h/d exposure, CA1 region neurons are clearly lost in the mouse (T4) but minimal neuronal dropout is evident in the 2 h/day exposure mouse at this exposure (T2). By 6 h/d exposure, the majority of CA1 neurons are lost in mice (T6) (*P < 0.05, **P < 0.01). Data are expressed as the mean ± SEM (n = 4).

inflammatory cytokine TNF-α was elevated for one month (Win-Shwe et al., 2008).

We report that prolonged exposure to DEP leads to up-regulation of inflammatory gene expression associated with neuronal morphological changes in hippocampal CA1 regions, suggesting that prenatal low-grade inflammation caused by exposure to DEP may contribute to changes in CA1 and hippocampus Reduction of cell complexity in this region (Fonken et al., 2011). In this study, we did observe a significant change in the level of IL6, TNFα, and IL1α expression, although the expression of proinflammatory cytokines involved in nanoparticle exposure induced neuroinflammation was significantly increased in the T4 and T6 DEP exposure groups. This finding suggests that prenatal exposure to DEPs may induce inflammation of the mouse hippocampus. Recently, many investigation has focused on relationships between behavior and neurochemistry researches show that excitatory transmission is intercede by glutamate through metabotropic (mGluR) in the brain and inotropic (AMPA and NRs) receptors and the expression of NRs has received special share In this regard (Monyer et al., 1994). Generally recognized that functional NMDA receptors required at least one NR1 subunit that contains a glycine binding site and other NR2 subunits that each contains a glutamate binding site (Dingledine et al., 1999). NRs are heteromeric assemblies of a core NR1 subunit and various modulatory NR2 subunits. NR2A and NR2B subunits serve as the major NR2 components in association with NR1 subunits in the hippocampus (Monyer et al., 1994). Recently, the search for novel NMDA receptor subunits discovered two types of NR3 subunits: NR3A and NR3B (Chatterton et al., 2002; Nishi et al., 2001). Given that NRs are involved in long-term depression and long-term potentiation (LTP), this receptor type is important for spatial memory (Morris et al., 1986). For example, the transgenic mice lacking the NR2A subunit, as well as

to impaired hidden-platform acquisition and probe trial performance in the water maze test indicate defects in hippocampal LTP (Sakimura et al., 1995). In this study, prenatal DEPs exposure led to the observed deficits in spatial learning and memory which may be Due to significantly decrease hippocampal NR2A and NR3B expression. The NR2B subunit is needed for neuronal pattern formation during the maternal period and for fetal viability, however prenatal exposure to DEPs did not affect NR2B expression (Kutsuwada et al., 1996), whiles NR2A subunit expression and synaptic incorporation increasingly increase throughout development (Tovar and Westbrook, 1999). Generally, synaptic NRs play critical roles in brain development, pathology, and plasticity (Dingledine et al., 1999; Zoghbi et al., 2000). Incorporation of NRs into synaptic sites dependent upon receptor subunit compound follows various mechanisms. Synaptic insertion of NR2B including receptors does not increase with promoted levels of NR2B gene expression, whiles synaptic insertion of NR2A including receptors need synaptic activity, which is elevated by increased levels of NR2A gene expression (Barria, 2007). Epidemiological studies have shown that DEPs exposure, may contribute to the start of Alzheimer's disease. Thus, maternal exposure to DEPs might affect NR2A insertion into synapses in the hippocampus because NR2A expression was reduced in the hippocampus (Calderón-Garcidueñas et al., 2008, 2015). This result highlights the requirement for controlling the developmental effects and identifying means of preventing of maternal exposure to DEPs on cognitive function. During the perinatal term, the living environment is of interest for prohibiting the developmental effects of DEPs. Certainly, environmental enrichment also prevents impairment of hippocampal function (Beauquis et al., 2013; Hutchinson et al., 2012; Xie et al., 2012). Major study is essential to recognize further preventive action against the effects of DEPs exposure on recognition.

5. Conclusion

Maternal exposure to DEPs, the same time to increasing Pro-inflammatory Cytokines expression and reduced in NR2A, NR3B expression in the hippocampus, led to anxiety and impairment of spatial learning and memory in adult male mice offspring.

Competing financial interests

None.

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