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Original Article

Enriched environment restores passive avoidance memory impairment in a rat model of neuroinflammation

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ABSTRACT

Introduction: Neuroinflammation is a primary pathophysiological condition that is associated with cognitive impairment and neurodegenerative diseases. The present study was designed to evaluate the effects of enriched environment (EE) on passive avoidance (PA) memory impairment caused by lipopolysaccharide (LPS) induced neuroinflammation.

Methods: Twenty-eight male Wistar rats were assigned into the following groups: 1, control; 2, control+ EE; 3, LPS and 4, LPS+ EE. LPS injection (1mg/kg/i.p.) was done on days 1, 3, 5 and 7 of experiment. Two different housing conditions were used in this experiment, including a standard environment house and an enriched environment house. The passive avoidance task was used to examine animals learning and memory performance. The hippocampal level of interleukin-6 (IL-6) and brain-derived neurotrophic factor (BDNF) was also measured using sandwich-ELISA method.

Results: Obtained data indicated that LPS significantly impaired passive avoidance memory, decreased the step-through latency and increased the time spent in the dark compartment of the LPS treated group compared to the control group. On the other hand, EE housing could significantly ameliorate memory impairment. Hippocampal IL-6 level was increased and BDNF was decreased in the LPS group, whereas EE could decrease and increase IL-6 and BDNF levels in the LPS+EE group, respectively.

Conclusion: EE should probably be considered as an alternative strategy in neuro-inflammatory diseases to minimize the memory impairment.

Keywords:
Inflammation
Enriched environment
Memory
Passive avoidance

Introduction

Neuro-inflammation is a primary pathophysiological condition that is associated with cognitive impairment and neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's Disease, multiple sclerosis, Huntington's disease along with other forms such as amyotrophic lateral sclerosis, fronto-temporal dementia and tauopathies (Zhao et al., 2019; Manickavasagam et

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al., 2020). Many reports have indicated that neuro-inflammation is related to the activation of brain-resident immune cells, microglia. It is also accompanied by the excessive release of pro-inflammatory and neurotoxic mediators such as nitric oxide, reactive oxygen intermediates and cytokines like interleukins (IL-6 and IL-1β) and tumor necrosis factor-α (TNF-α) (Glass et al., 2010; Wang et al., 2015).

Neurotrophins are a family of growth factors which are expressed in the mammalian central nervous system (CNS) and play critical roles in the formation of neural networks. This family includes similar proteins such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin-4 (Meldolesi, 2017). Among these neurotrophic factors, BDNF and its receptor, tyrosine kinase B, are essential for hippocampal-dependent learning and memory (Boehme et al., 2011). Inflammatory agents, such as IL-6, have the ability to cross the blood-brain-barrier (BBB) (Phillips et al., 2014) and can inhibit the expression of BDNF genes, thereby reducing neuronal survival and neurogenesis (Patanella et al., 2010).

Besides the convincing evidence that proved the etiologic role of immune function in AD, a growing number of epidemiological and translational research studies have identified that systemic inflammation may promote neurodegenerative diseases, such as AD (Giridharan et al., 2019). It is interesting to note that people with AD and mild cognitive impairment tend to have higher levels of proinflammatory cytokines, such as IL-6 and C-reactive protein in their blood (Walker et al., 2019b, 2019a). Also, it has been indicated that many metabolic disorders are associated with chronic systemic inflammation and a higher risk of developing neurodegenerative diseases such as AD (Keymoradzadeh et al., 2020). Both *in vivo* and *in vitro* studies have demonstrated that lipopolysaccharide (LPS) can induce systemic inflammation and detrimental effects on the brain via the up-regulation of different pro-inflammatory mediators including nitric oxide species, prostaglandin E2, cyclooxygenase-2 and pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α (Chao et al., 1992; Font-Nieves et al., 2012; Badshah et al., 2016). It is known that LPS injection is associated with neuro-inflammation in hippocampus (Sheppard et al., 2019) and memory impairment in different animal models of neuro-inflammatory diseases (Lee et al., 2018; Zhan et al., 2018). Some of the studies have shown that humans' Gram-negative bacterial periodontal disease and consequent neuro-inflammation is associated with AD (Noble et al., 2014; Kamer et al., 2015; Olsen and Singhrao, 2015). Besides, the evidence that blood LPS level in AD patients is 3-times higher than the control people, indicates the significant role of LPS in AD development (Zhang et al., 2009).

Environmental risk factors such as poverty, stressful urban environments and negative social interactions such as bullying and abuse during childhood or adolescence can act synergistically to increase susceptibility to progressive neurodevelopmental disorders (Arango et al., 2018). Nowadays, it is generally accepted that no effective treatment has been developed to treat cognitive impairment and the research focus has further shifted toward more promising preventive approaches (Huang et al., 2020). A number of works have shown that cognitive function can be influenced by changes in lifestyle. One way to improve cognitive impairments is cognitive stimulation which is one of the suggested strategies to aid the neuroprotective processes (Nelson et al., 2019). In experimental studies, the environmental enrichment (EE) method is being used to induce cognitive stimulation. There are increasing evidences that show the benefits of EE on the synaptic transmission and consequent improvements in cognitive function (Sampedro-Piquero and Begega, 2017).

The establishment of an appropriate animal model is very important for researching neuro-inflammation associated with cognitive impairment and neurodegenerative diseases (Zhao et al., 2019). The passive avoidance (PA) task is believed to be based on contextual memory which is linked with the place and the event of "being given the electric shock in the dark box". Since the hippocampus plays an important role in contextual memory, injuries of the hippocampal region decrease the performance of PA (Nikkhah et al., 2014). Thus, the aim of the present study was to investigate the effect of EE on PA impairment following LPS induced neuro-inflammation.

Material and methods

Animals

In this study we used twenty-eight seven-week-old male Wistar rats (weight: 200-220g, School of Medicine, Rasht, Iran). The room conditions were a 12-hour light/dark cycle (lights on at 7:00 and off at 19:00) with

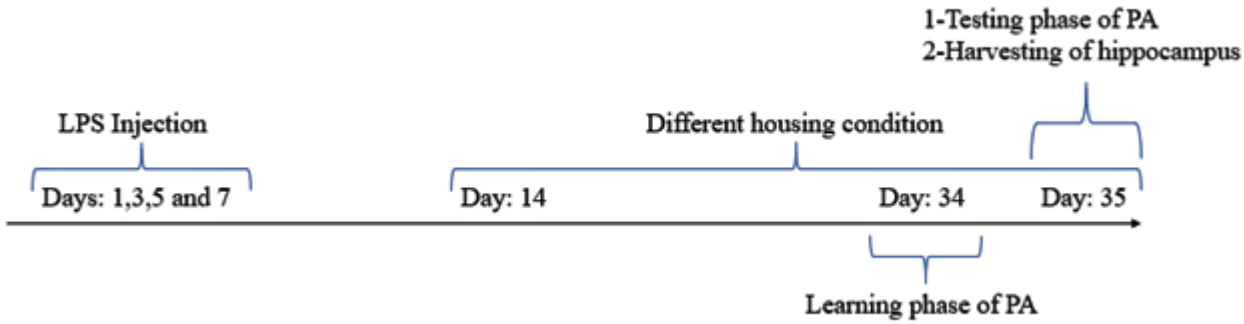

FIGURE 1. Study procedures time-line. For 3 weeks, the rats in the control and LPS groups were placed in enriched environment and standard environment house.

FIGURE 2. A: Standard environment house, and B: Enriched environment house

a relative humidity of 55 percent and a temperature of 22±2 degrees Celsius. The Animal Care and Use Committee of Guilan University of Medical Sciences accepted all methods and procedures with the code IR.GUMS.REC.1396.459. Experiments were also carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

Experimental groups

The rats were randomly divided into four groups (7 rats in each): 1, control group which received saline injections; 2, control group which was kept under conditions of enriched environment; 3, LPS group which received LPS injections and 4, group of animals which received LPS and were kept under the conditions of enriched environment.

Experimental procedure

LPS (Escherichia coli; O127: B8, Sigma-Aldrich Co., St. Louis, MO, USA) was used to induce memory impairment (1mg/kg/i.p). Injections were carried out at days 1, 3, 5 and 7 of the study (Keymoradzadeh et al., 2020). Then at day 14, groups 2 and 4 were moved to EE and groups 1 and 3 were assigned to the non-enriched standard environment house. All groups stayed in their own environments for three weeks. Afterwards, the PA test was employed to assess the animals' aversive memory and sandwich-ELISA method was used to measure hippocampal IL-6 and BDNF levels (Figure 2).

Housing conditions

Two different housing conditions were used in this study, including a standard environment house and an enriched environment house (Figure 2). Standard environment house was a standard cage (42×34×15cm) containing only food and water. While enriched environment house consisted of a larger cage (96×49×38cm) that contained many different objects in addition to food and water such as running wheel, pipe, a small compartment, stairs, a mirror and many other colorful objects (e.g., colorful stones, plastic things of varied colors and shapes, plastic cup, and hanging cubes). The objects

in the EE cage were changed every day (Hammami Abrandabadi et al., 2016).

Passive avoidance task

Apparatus

Evaluation of associative memory in rats was conducted using inhibitory avoidance task by a shuttle box (Borg Sanat Azma Company, Tehran, Iran). The apparatus consisted of two equally sized light and dark compartments (20×40×20cm) with walls made of opaque plastic. The floor of each chamber was embedded with parallel stainless-steel bars (3mm diameter, spaced 1cm apart), connected to an electric shock generator. A rectangular opening was located between the two chambers which could be closed by an opaque guillotine door (Khakpour-Taleghani et al., 2008).

Adaptation

Twenty-eight days after the last LPS injection, all experimental groups were given two trials to habituate them to the apparatus. Rats were placed in the lighted compartment of apparatus facing away from the door and 5s later, the guillotine door was raised. After the rats entered the dark compartment, the door was closed and animals were removed from the dark compartment and placed in their home cage. The habituation trial was repeated after 30min and followed after the same interval by the first acquisition trial. In the first adaptation trial, the entrance latency to the dark compartment (step-through latency, STLa) was recorded when the animal placed all four paws in the dark compartment (Rezvani-Kamran et al., 2017).

Learning phase

In the learning phase, the rat had received a constant current foot shock 50Hz square wave, 1mA for 1s when entered the dark compartment with all four paws. Training procedure was repeated and terminated when the rat remained in the light compartment for 120 consecutive seconds. At the end of the learning phase, the rat was removed and placed in its home cage. All of the trials were performed from 9:00 to 12:00 (Khakpour-Taleghani et al., 2008).

Retention test

The retention test was performed 24h after the learning phase. Rats were placed in the lighted chamber and 5s later the guillotine door was raised and step-through latency in the retention test (STLr), the time spent in the dark compartment (TDC) and the number of entries to dark room, were recorded up to 600s. Cut-off point was considered 600s and if the animal did not enter the dark compartment within it, the retention test was finished and a ceiling score of 600s was recorded. These trials were performed from 9:00 to 12:00 (Khakpour-Taleghani et al., 2008).

Hippocampal tissue preparation

At the end of the experiments, the rats were decapitated, their hippocampus dissected and frozen at -80°C. The brain tissue was homogenized in a lysis buffer (Tris-HCl, pH 8.0, NaCl, sodium deoxycholate, SDS, EDTA, Triton x-100, protease inhibitor). The lysate was then centrifuged for 10min at 3000g to collect the supernatant (Aminyavari et al., 2019).

Determination of hippocampal IL-6 and BDNF levels

Anti-IL-6 and anti-BDNF antibodies were used for sandwich-ELISA method assaying hippocampal IL-6 and BDNF level using e-Bioscience company (San Diego, CA, USA) and (Hangzhou Eastbiopharm Co., LTP) rat ELISA kits, respectively, according to the manufacturer's instructions.

Data analysis

The Shapiro-Wilk test was used to ensure the normality of the data. One-way ANOVA followed by post-hoc Tuckey-Kramer test was conducted to determine significant differences between all groups. All of the data expressed as the mean±SEM. A statistically significant value of $P<0.05$ was considered. SPSS, version 16.0, was used for all statistical analysis (IBM, Somers, NY, USA).

Results

Passive avoidance task

At first, our results indicated that there were no significant differences among the experimental groups in the STLa in the first adaptation trial (before receiving the electrical shock). Thus, it means that the LPS or EE had no effect on the animals' locomotor ability and differences of STL and TDC between groups (Data not shown). Based on the results, in LPS group, STL was significantly lower than control and control+ EE groups

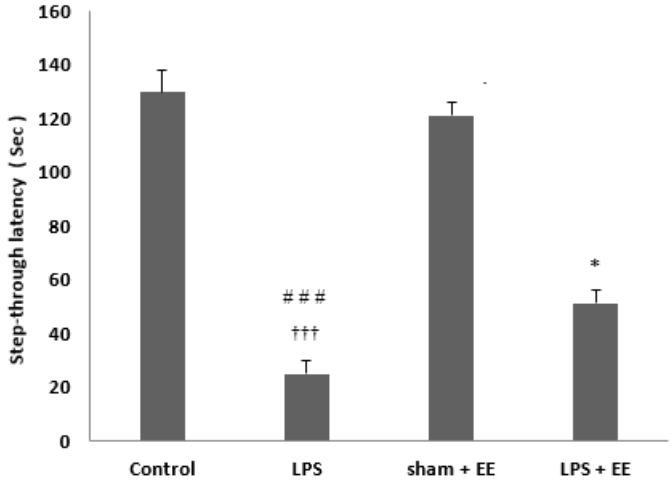

FIGURE 3. Step-through latency measurements. Step-through latency was measured in all groups. The values are the mean±SEM. $^{*}P<0.05$ compared to the LPS group, $^{†††}P<0.001$ compared to the control group and $^{###}P<0.001$ compared to the control+ EE.

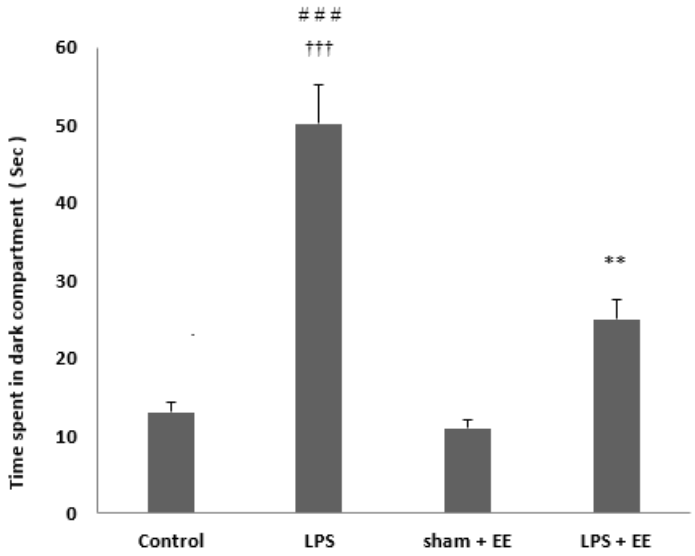

FIGURE 4. Measurements of time that rats spent in dark compartment. Values represent the mean±SEM. $^{**}P<0.01$ versus LPS group, $^{†††}P<0.001$ compared to the control group and $^{###}P<0.001$ compared to the control+ EE.

($P<0.001$). STL in LPS+ EE group, was significantly higher than LPS group ($P<0.05$) and lower than control and control+ EE groups ($P<0.001$, Figure 3).

Furthermore, as shown in Figure 4, TDC was substantially higher in LPS group than control and control+ EE groups ($P<0.001$). TDC in LPS+ EE group was significantly ($P< 0.01$) lower than LPS group. Figure 5 shows the number of entries to the dark compartment of control, LPS, control+ EE and LPS+ EE groups. Number of entries in LPS group was significantly more than the control group ($P<0.01$). Also, the number of entries in LPS+ EE was significantly less than the LPS group ($P<0.05$).

Hippocampal level of IL-6 and BDNF

According to data analysis, the LPS group had significantly higher hippocampal IL-6 level than the control, control+ EE ($P<0.01$) and LPS+ EE ($P<0.05$) groups. Compared with LPS group, EE reduced IL-6 in the LPS+ EE group ($P<0.05$). Between the control and control+ EE groups, there was no substantial difference in hippocampal IL-6 level (Figure 6). As shown in Figure 7, the hippocampal BDNF level of the LPS group was significantly lower than the control group ($P<0.01$). EE increased hippocampal BDNF level of the LPS+ EE group compared to the LPS group ($P<0.05$). Also, there wasn't any significant difference between hippocampal BDNF level of control and control+ EE group.

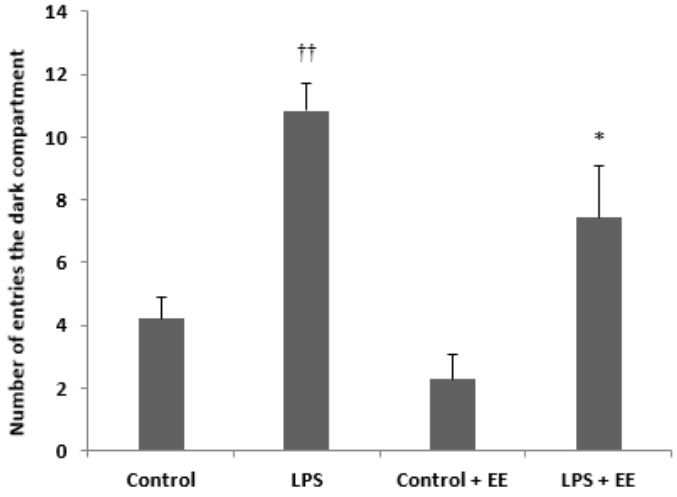

FIGURE 5. Number of entries the dark compartment in all groups. Values represent the mean±SEM. $^{*}P<0.05$ versus LPS group and $^{\dagger\dagger}P<0.001$ compared to the control group.

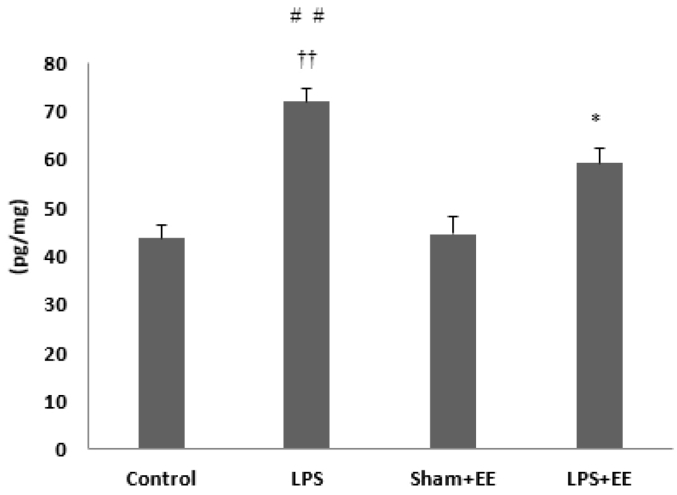

FIGURE 6. IL-6 cytokine measurements. Hippocampal level of IL-6 in all groups. Values represent the mean±SEM. $^{*}P<0.05$ versus LPS group, $^{\dagger\dagger}P<0.01$ compared to the control group and $^{\#\#}P<0.01$ compared to the control+ EE.

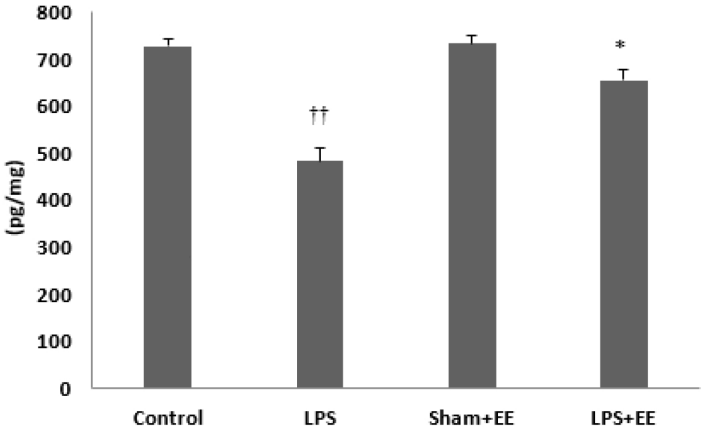

FIGURE 7. BDNF cytokine measurements. Hippocampal level of BDNF in all groups. Values represent the mean±SEM. $^{*}P<0.05$ versus LPS group, $^{\dagger\dagger}P<0.01$ compared to the control group and $^{\#\#}P<0.01$ compared to the control+ EE.

Discussion

In the present study, we investigated hippocampal BDNF and IL-6 levels and also analyzed PA memory performance (a hippocampus dependent animal model of aversive memory) after neuro-inflammation induced by systemic LPS administration. One important finding was that LPS administration decreased hippocampal BDNF and increased IL-6 level and at the same time it had memory impairing effect. However, enriched environment housing increased the level of BDNF and reduced the level of IL-6 in the hippocampus of LPS-treated rats and finally, it led to memory improvement. In the PA task, animals learn to withhold contacting a stimulus, based on their aversive experience in contacting it, previously (Abdel-Mouttalib, 2015). In this test, the latency that it takes the animal to perform the frightful activity, serves as a measure for its memory of the aversive consequences of that action (Kassa et al., 2015). The PA task is a hippocampal-dependent test and hippocampal lesions can impair memory in this task (Xu et al., 2020). Based on our results, LPS application could impair PA memory. This finding confirms the results of the previous study indicating that LPS injection is associated with PA memory impairment (Noorbakhshnia et al., 2015).

On the other hand, EE could improve PA memory in LPS treated animals. Based on our study design we selected an aversive test for evaluating the EE effects on LPS-induced neuro-inflammation. Data from previous studies have demonstrated that EE can be stressful and it can even have negative impact upon laboratory animals (McQuaid et al., 2012), thus one of our top priorities was to find the best cage density or EE strategy (Laber et al., 2008). At the present study, EE was made up of a larger cage than normal with a running wheel, a pipe, a small compartment, a mirror, stairs and a variety of other colorful items. The animals kept in EE experienced proper combination of multi-sensory/cognitive stimulation, thus they had more physical activity, social interactions and normal explorative behaviors consequently it enhanced the improvement of cognitive disorder in them (Ball et al., 2019).

Based on our results, housing in EE for 21 days, could increase STLr, decrease TDC and also the number of entries to the dark room in LPS+ EE group, which indicates an improvement in PA memory. The interactions between an organism and its environment are known to influence and evoke neuro-behavioral changes. Researchers have been trying to identify the use of EE to induce changes in both intact and injured CNS (Kumar et al., 2018). It seems that EE can even reverse the detrimental action of early inconsistent stimulation and increase the advantageous effects of postnatal handling on shuttle box learning in adult rats (Ahmadalipour et al., 2017). It has been reported that EE can increase the expression of pre- and post-synaptic proteins involved in the synaptogenesis process (Sampedro-Piquero and Begega, 2017). EE can also increase the expression of other types of molecules such as vascular endothelial growth factor (Ahmadalipour et al., 2017) and BDNF (De Vincenti et al., 2019). Furthermore, we know that EE can increase the survival of the new granular neurons which proliferate in the hippocampus of the mice (You et al., 2020) and aged rats (Segovia et al., 2006). On the other hand, EE produces changes in the morphology of nerve cells by increasing the number of dendritic branches and spines (Sampedro-Piquero and Begega, 2017).

This study evaluated the effect of EE on IL-6 and BDNF levels in the hippocampus of LPS-treated animals. LPS injection significantly decreased hippocampal BDNF level and increased hippocampal IL-6 level. This finding is consistent with that of Bargi and colleagues (2017). It has been shown that systemic LPS injection can significantly reduce the level of BDNF in the hippocampus and cortex of a mice model of neuro-inflammation (Frühauf-Perez et al., 2018). There is a reciprocal interaction between IL-6 and BDNF. It has been reported that inflammatory cytokines such as IL-6 can affect BDNF receptor phosphorylation and thus interfere with BDNF signaling (Devasahayam et al., 2021). For example, in a study on inflamed hippocampus in a rat model of depression, increased IL-6 and decreased BDNF gene expression in the hippocampus were observed 6h after inflammation induction. They reported that cytokine expression returned to baseline after 48h, but BDNF mRNA level remained low in the hippocampus (Gibney et al., 2013). So, in the present study, the lower hippocampal BDNF level of the LPS-treated rats might be due to primary increase in hippocampal IL-6 level. Totally, the obtained results of our study showed that it is probable that systemic inflammation, followed by neuroinflammation, can reduce the level of BDNF in the hippocampus by increasing hippocampal IL-6 level.

It is interesting to note that nonheritable or environmental factors play a principal role in influencing the

inflammatory response of immune system (Brodin et al., 2015). Furthermore, there are evidences that circumstances such as psychological state (Vasile, 2020), living conditions (Furman et al., 2019) and socioeconomic status (Calixto and Anaya, 2014) may also play an important role in the pathogenesis of multiple inflammatory and autoimmune diseases. These results have led to the question of whether EE can influence immune system. It has been known that EE could exert immune-enhancing effect on inflammatory viral diseases (Brod et al., 2017). Overall, the results indicate that presence in EE decreased IL-6 and increased BDNF level in the hippocampus of LPS+ EE rats. Several reports have shown that EE reduces the severity of neuro-inflammation and improves laboratory animals neurological and cognitive problems by increasing sensory, motor, cognitive and social stimuli (Sampedro-Piquero and Begega, 2017). Our results are in agreement with those of Kazlauckas et al. (2011) who also found that 2 months presence of the animals in EE increased the hippocampal BDNF level. It has been reported that increased IL-6 level is associated with decreased brain BDNF level (Giacobbo et al., 2019), thus we can conclude that probably hippocampal BDNF increase is at least partly through decreased hippocampal IL-6 level, after 3 weeks housing of neuro-inflamed animals in EE. However, we must emphasize that some contradictions are found in the results of previous studies. For example, the results of a study on an animal model of ischemic stroke have proved that the presence in EE for 3 weeks, increased the production of IL-6 by astrocytes (Chen et al., 2017). There are evidences which have shown that the secretion of IL-6 by astrocytes is regulated by PI3K/AKT signaling pathway in the acute phase of CNS damage (Codeluppi et al., 2014). Therefore, considering inducing chronic and gradual injury in the present study, these discrepancies in the results may be due to differences in types of nerve damage.

Obtained results demonstrated that EE could not alter the level of IL-6 and BDNF in the hippocampus of the control+ EE group. Thus, it can be concluded that the positive effect of EE on the brain may require the neuro-inflammatory condition (Ziv and Schwartz, 2008; Vukovic et al., 2012) and we cannot expect that EE alters the level of normal BDNF and IL-6 in healthy rats.

The lack of evaluation of other pro-inflammatory and anti-inflammatory cytokines and neurotrophins such as IL-4, NGF and oxidative stress biomarkers, which would be useful to understand the exact impact of EE on neuro-inflammation and memory defect, is one of the limitations of the current study. In addition, further studies need to be carried out in order to find how EE affects the cytokines in other areas of the brain that may be involved in emotional and aversive cognitive processes.

Conclusion

This experimental procedure confirmed that EE significantly improved passive avoidance memory deficits, after LPS-induced neuro-inflammation. Enriched environment housing decreased and increased hippocampal level of IL-6 and BDNF respectively. An implication of this study is the possibility that EE as an alternative strategy in brain diseases can minimize memory impairment.

Acknowledgments

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Conflicts of Interest

The authors declare no potential conflicts of interest.

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