Research article

Effects of exposure to enriched environment during adolescence on passive avoidance memory, nociception, and prefrontal BDNF level in adult male and female rats

Farshid Sadegzadeh\textsuperscript{a}, Nona Sakhale\textsuperscript{a}, Khatereh Isazadehfar\textsuperscript{a}, Hakimeh Saadati\textsuperscript{b, c, *}

\textsuperscript{a} Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran
\textsuperscript{b} Department of Physiology, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran
\textsuperscript{c} Pharmaceutical Sciences Research Center, Ardabil University of Medical Sciences, Ardabil, Iran

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ABSTRACT

Previous studies demonstrated that an enriched environment (EE) exposure improves cognitive functions, synaptic plasticity, neurogenesis, and induction of brain-derived neurotrophic factor (BDNF) in multiple brain regions of laboratory animal models. Also, studies on the sex-dependent effects of exposure to EE during adolescence on adult cognitive functions are less. Therefore, the present experiment was aimed to assess the effects of EE during adolescence on passive avoidance learning and memory, nociception, and prefrontal cortex (PFC) BDNF mRNA levels in the adult male and female rats. Our results indicated that housing in the EE during adolescence improves passive avoidance memory and increases nociceptive response against thermal stimulus in both sexes. Findings of our study also showed an increased BDNF level in the PFC of female animals. As a result, sex differences can affect the expression of BDNF mRNA in the PFC. Further research concerning the precise mechanisms underlying sex hormone-dependent production of BDNF in PFC is critical.

1. Introduction

Adolescence is the critical stage of postnatal neurodevelopment in which social and cognitive skills mature. Additionally, behavioral and adaptive changes occur during this period to allow the transition from an immature person to an independent adult [1]. Also, different neurobiological and hormonal changes are detected in many different species during this stage [1, 2]. Therefore, significant alterations in brain structure and function can be occurred by the manipulation of the physical and social environment during the adolescence stage [3]. The enriched environment is an enriched animal habitat, first Donald Hebb in early 1947 described in a neuroscientific context [4]. It refers to special housing situations that can facilitate sensory, cognitive, and motor stimulation in experimental animals compared to the standard housing conditions. Additionally, EE contains different objects for visual and sensory stimulation, different tunnels to guide spatial navigation, ladders and running wheels to enhance their motor activity. EE- housed animals also have a chance for gathering in social groups [3]. Therefore, enriched housing is a non-pharmacological intervention that can stimulate the mechanisms of brain plasticity in normal and brain-damaged animals. The beneficial changes such as improving dendritic spine density [6, 7], synaptogenesis [8], and neurogenesis [9] can induce in the hippocampus and PFC by EE exposure. These changes relate to learning and memory improving [10]. Exposure to EE can cause neuronal signaling levels alteration in the different areas of the central nervous system. For example, BDNF is an essential trophic factor responsible for mediating the synaptic plasticity facilitation and increasing sensory, motor, and cognitive abilities [11].

Generally, pain is a warning phenomenon in physiological situations that influences emotional aspects, cognitive functions, and quality of life. In addition, pain signals are transmitted to different areas of the brain that control the sensory and affective modality [12]. The prefrontal cortex is the region of the cerebral cortex which is important for learning, memory [13], and pain processing via a relationship with pain pathways [14]. Previously, it has been indicated that environmental quality can affect injury-related pain development and recovery in animals [15]; but, modulation of pain sensitivity differently depends on EE protocol used [16].

Also, data from several studies in humans and animals indicated that there are sex-dependent functional and structural brain variations such as the number of neural cells, synaptic plasticity, cognitive functions, etc [17, 18]. These structural and functional alterations cause sex

\* Corresponding author at: Department of Physiology, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
E-mail address: h.saadati@arums.ac.ir (H. Saadati).

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differences in vulnerability to psychiatric and cognitive impairments during the adolescence period [19,20]. Consequently, information about the sex-dependent effects of EE during adolescence on cognitive functions is less. Besides, PFC is one of the important regions of the brain that involves cognition and pain perception. Moreover, increased BDNF level in the PFC as a main neurotrophic factor might mediate the beneficial effects of EE. Therefore, the present study is aimed at testing the hypothesis that exposure to an enriched environment during adolescence changes passive avoidance learning and memory, nociceptive response against thermal stimulus, and prefrontal BDNF mRNA expression in male and female rats.

2. Material and methods

2.1. Animals

In the present study, 32 male and female Wistar rats were weaned on a postnatal day 21 (PND = 21) and housed in the enriched or standard cages in groups. Experimental animals had free access to food and water ad libitum. They were maintained under controlled situation [temperature (23 ± 1 °C) and 12-h light-dark cycle (light illuminated 07:00am–19:00)]. All intact male and female rats were randomly allocated into the following subgroups (n = 8): two male and female groups were exposed to enriched environment (enriched environment groups), two remaining male and female rats were chosen as controls. The control male rats kept in standard cages (45.5 × 26.6 × 18 cm) (4 rats per cage), but the control female rats were housed in the same big cage (61.28 × 43.5 × 21.6 cm) for synchronization of estrous cycles (8 rats per cage). Based on the findings of our previous study, most of the rats in each group were similar in estrous cycles by this method [21]. Vaginal smear to evaluate the estrous cycle was not conducted because this procedure is a stressful activity. Following post-weaning exposure to an enriched environment, behavioral tasks were accomplished on PND 61 to PND70. After the finishing of behavioral tasks, the sacrificing of rats was performed by using CO2 under deep anesthesia. The present experimental procedure and animal management were done according to the Ethics Committee of Ardabil University of Medical Sciences. We tried to diminish the suffering for the animals at all stages of the study. (Ethics code: IR.ARUMS.REC.1398.037). The experimental timeline is shown in Fig. 1.

2.2. Enriched environment

Sixteen male and female rats (EE groups) were housed in the enriched environment for 40 successive days instantaneously after weaning. As shown in Fig. 2, in the enriched cages 8 rats housed in a transparent plastic chamber (90 × 60 × 50 cm) which set with different things (climbing ladders, running wheel, nest box, plastic tubes and balls, and various types of decoration) to motivate exploration. To keep their innovation, the position of these objects were exchanged commonly once a week. Also, several 0.5 cm diameter holes were built in the doors and walls to keep air exchange.

2.3. Passive avoidance apparatus (PA)

To evaluate passive avoidance learning and memory, a shuttle box was used. This device consists of a light and a dark chamber of similar size (20 × 20 × 30 cm) divided by a guillotine door. Stainless steel rods (3 mm diameter and separated by a 1 cm apart) were used to make the floor of both compartments. Electrical shocks by an isolated stimulator were designed on the floor of the dark chamber.

At first, two trials were given to all experimental groups to habituate them to the shuttle box. For conducting these habituation trials, each rat was located in the lightened chamber of the shuttle box. Meanwhile, the mentioned rat was facing away from the door of the compartment and after 10 s the dividing guillotine door was elevated. The animal prefers the dark compartment naturally. After the entrance of the rat to the dark compartment, the door was closed. Entrance latency into the dark compartment (step-through latency, STL) was recorded when the animal had placed all four paws into the dark compartment. Thirty seconds later, the rat was placed in the home cage from the dark compartment. The second habituation trial was repeated after 30 min and the same interval by the primary acquisition trial was followed. In the acquisition trial, the closure of the guillotine door and application of an electrical shock (50-Hz square wave, 1 mA for 3 s) was conducted after the spontaneous pass of the animal in the dark compartment. The rat was taken and located in its home cage after the 30 s. The repeated trial was conducted after 2 min. In each reentrance of the rat to the dark compartment, it received a foot-shock. The end of the trial was the time when the rat stayed in the light compartment for 120 successive seconds. The task acquisition index and short term memory were recorded by the use of the trial numbers or entries into the dark compartment.

In order to determine long term memory, the retention test was done 24 h after the finishing of the acquisition trial. The experiment was carried out similarly to the acquisition trial. Each rat was placed in the lighted section and after 10 s, the guillotine door was elevated. The latency of entrance to the dark chamber or STL, the time spent and the number of entries to the dark compartment were evaluated as a measurement of memory. The latency of entrance to the dark chamber was documented for up to 600 s. In the condition that the rat didn’t enter the dark compartment during 600 s, the retention test was finished and the assignment of the maximum score of 600 s was confirmed [22].

2.4. Hot-plate test

The hot plate test was performed according to a procedure as previously described [23] with modification. The reaction time to the thermal stimulus was examined by using the hot plate apparatus (Borj Sanat Azma, M.H9500) that contains a plate that was pre-heated and adjusted at a temperature of 51 ± 1°C. The diameter of the plate and the height Plexiglas wall of this apparatus are 19 cm and 30 cm respectively. Reaction time to the thermal stimulus was examined as the time between test onset and whipping front paw or jumping (maximum cut off was considered 40 s to avoid tissue damage).

2.5. Molecular experiments

For molecular experiments, all animals anesthetized with atmospheric carbon dioxide (CO2) in desiccators jar with the low-pressure flow. After decapitation, the prefrontal cortex of both hemispheres was separated on an ice-cold surface from the coronal brain slice that prolonged from posteriorly to the optic chiasm (about 0.48 mm relative to...
2.5.1. Semi-quantitative PCR and cDNA synthesis

At first, PFC samples were weighed and 100 mg of each sample was homogenized for RNA extraction. A modification of the guanidine thiocyanate-phenol-chloroform method using RNase + reagent to separate prefrontal RNA according to the manufacturer’s protocol and our previous study methods [26] was used. In brief, the reverse transcription reaction was accomplished with a first-strand cDNA synthesis kit (SinaClonBioScience Cat. No: RT5201). A semi-quantitative PCR process was done in the GeneAmp PCR system (Bio-Rad). The reaction was performed using selective forward and reverse primers for BDNF and GAPDH (as an internal standard) genes. The subsequent primer sequences were used for PCR: BDNF-forward: 5′-GATGTCCTCCCTGCTGAAAG-3′; BDNF-reverse: 5′-AGCTCACATTAGCTCCTCACA-3′; and GAPDH-forward: 5′-CCATGTATCCGTTGTGGAT-3′; GAPDH-reverse: 5′-CATCAAAGGTGAAGAATGG-3′. All sequences of primers were synthesized by Sinaclon.

2.6. Statistical analysis

All acquired data were shown as the mean ± standard error. Two-way analysis of variance (ANOVA) was used to perform statistical analysis and the expression of the differences among the groups was done by Tukey’s HSD post-hoc tests. SPSS software was used to perform all of the analysis and calculations. P < 0.05 was considered statistically significant.

3. Results

3.1. Effects of adolescent exposure to EE on passive avoidance learning and memory

Two-way analysis showed a significant interaction between sex and group on the STL [F (1, 28) = 21.56, p < 0.001], time spent [F (1, 28) = 6.7, p = 0.001] and number of entries to the dark compartment [F (1, 28) = 18.65, p < 0.001] in the passive avoidance test.

The results of the present examination showed that before the acquisition trial, there was no significant difference in the time of first entrance among groups representing their matching. Also, there were no statistically significant differences among groups during the acquisition phase and short term memory in the passive avoidance apparatus.

The influence of EE exposure during adolescence on passive avoidance retention in the test trial is shown in Fig. 3A-C in male and female rats. Our results indicated that exposure to EE significantly increased STL in male and female animals [F (3,28) = 21.56, p < 0.01 and p < 0.001 respectively; Fig. 3A] but significantly decreased the time spent [F (3,28) = 6.7, p = 0.021 and p = 0.001 respectively; Fig. 3B] and number of entries [F (3,28) = 18.65, p = 0.026 and p < 0.001; respectively; Fig. 3C] to the dark compartment in EE exposed male and female rats compared to the control groups.

3.2. Effects of exposure to EE during adolescence on nociceptive response against the thermal stimulus

Response to nociception was examined via the hot-plate test. The high nociceptive response was observed in EE exposed groups [F (3, 28) = 8.35, p < 0.001]. Therefore, EE exposure during adolescence significantly increased nociceptive response in hot-plate in males (p = 0.021) and female (p = 0.002) animals compared to control groups (Fig. 4); meanwhile, the level of significance was higher in female rats.

Also, the interaction of sex was statistically significant (p < 0.001).

3.3. Effects of EE exposure on mRNA expression of BDNF in the PFC of male and female rats

The two-way analysis showed a main effect of sex on the expression of the BDNF mRNA [F (1,16) = 9.7, p = 0.002]. Only female animals exposed to EE showed a significant increase in expression of BDNF compared to control [F (3,16) = 9.7, p = 0.005] and EE exposed male groups (p = 0.004) (Fig. 5).

4. Discussion

According to the data of the present study, adolescent EE exposure improves passive avoidance memory and increases nociceptive response against thermal stimulus in both sexes (meanwhile, a significance level of pain sensitivity is higher in female rats), but BDNF only increases in the prefrontal cortex of females. Besides, we kept all female rats in the big cage for synchronization of the estrous cycle. The results in behavioral and molecular experiments were similar in both bregma) and anteriorly toward the olfactory bulbs (approximately 2.0 mm from bregma) [24,25]. The samples freeze in liquid nitrogen and stored at –80 °C until being prepared for RT-PCR assays.
control groups; therefore, social living alone could not affect in results of our study. Furthermore, the running wheel and other objects in the EE may mediate the positive effects of adolescent EE exposure. Previous experimental studies showed that enriched situations and running exercise may be a potential non-pharmaceutical intervention at the peak of neural flexibility and plasticity observed in the adolescence period. Long term exposure to EE during the adolescence stage of development can influence cognitive functions and neural development [2,27].

Exposure to EE also increases social, physical, somatosensory stimulation, and motor ability through larger group housing, running wheels, and other additional novel things [28]. Similarly, exposure to enriched housing has been shown to improve passive avoidance learning and memory of rats subjected to restraint stress [29]. Our finding also indicated that the positive correlation between frontal cortex BDNF levels and passive avoidance memory performance of rats exposed to EE in the adolescence period was shown only in female rats. Besides the frontal cortex, another critical brain region responsible for passive avoidance memory is the hippocampus [30] and the hippocampus is the brain region most affected by EE [8,9]. According to the results of our other study, adolescent EE exposure increases BDNF level in the hippocampus of both sexes [26]. Other investigations are in line with our findings indicated that housing in EE has a strong effect on neurotrophic gene expression and protein levels, neurogenesis and synaptic plasticity in the PFC and multiple areas of the brain in the rodents [31,32] and can mediate the cognitive effects of exercise in humans [33,34]. Additionally, the neurogenic and neurotrophic effects of BDNF can be inhibited by decreasing of tyrosine kinase receptors (TrkB) [35] and BDNF expression [36,37]. The helpful effects of running exercise on the creation of mature BDNF in the brain can be accomplished by activation of the NMDA glutamate receptors [38,39].

Fig. 3. Effects of exposure to EE on STL, time spent in the dark compartment, and the number of entries to the dark compartment in retention test of passive avoidance task in male and female rats (Fig. 3A-C respectively). Values are showed as mean ± SEM (n = 8). (*) p < 0.05, (**) p < 0.01 and (***) p < 0.001 compared to the control groups. (EE): enriched environment.

Fig. 4. Effects of EE exposure during adolescence on thermal sensitivity of male and female rats. There was a significant effect of EE on the nociceptive response against thermal stimulus in hot-plate tests in male and female rats. (*) p < 0.05, (**) p < 0.01 in comparison with control groups. Data are presented as mean ± SEM (n = 8). (EE): enriched environment.

Fig. 5. Effects of EE exposure during adolescence on gene expression of BDNF in the prefrontal cortex. Results are expressed as mean ± SEM (n = 4-6). (**) p < 0.01 compared to other groups. (EE): enriched environment.
effects of EE and running exercise on learning and memory [40–42].

Our findings also indicated that adolescent EE exposure increased the nociceptive response against the thermal stimulus in both sexes. Meanwhile, the response to the thermal stimulus was higher in female rats. According to scientific evidence, there is greater pain sensitivity among females compared to males. These differences are due to biological mechanisms, particularly sex hormones, that can influence pain sensitivity [43]. Another study also indicated that exposure to EE causes hypersensitivity to pain stimulus [28]; but in another investigation, EE has no significant effects on pain sensitivity [15]. Therefore, there are conflicting results regarding the effects of EE on pain sensitivity. These contrasting results are likely due to a variety of EE conditions (e.g. with or without wheels), age and strain of laboratory animals, and different protocols for pain assessment [16]. Additionally, PFC is one of the important parts of the brain that has a main role in executive functions and pain processing. PFC connects to other areas of the cerebral neocortex, hippocampus, periaqueductal gray matter, thalamus, and can manage the pain [14]. Thus, the PFC is involved in biopsychosocial pain management, and increasing the BDNF level may mediate the effects of PFC on pain perception in females.

As a result, the findings of our study demonstrate the sex-dependent effects of adolescent EE exposure on the gene expression of BDNF in the PFC. It has been stated that there is an obvious difference between male and female rats due to sex hormones that can affect brain functions. Gonadal hormones, especially estrogens, influence cognitive functions during development. Estrogen can regulate BDNF levels in the brain, and this trophic factor can increase dendritic spine and synaptic plasticity in the PFC and hippocampus. An increase in synaptic plasticity and spine density are parallel with memory improvement [32]; but, the exact mechanisms of BDNF and estrogen on cognitive functions are unclear. According to the findings of our previous study, adolescent exposure to the EE increases BDNF level in the hippocampus of male and female rats [26], while in the present study the mechanisms by which EE promotes the up-regulation of BDNF in the PFC are sex-dependent. In addition, other memory-related molecules other than BDNF can be investigated in the frontal cortex. For example, c-Fos, which is associated with avoidance and freezing. This factor plays an important role in pain perception and regulation of BDNF expression. NMDA receptors which are important on both memory and pain perception, also regulate BDNF mRNA expression. NMDA receptor activation causes c-fos expression following noxious stimulation [44]. Changes in synaptic plasticity and spine density in the PFC may also affect cognitive functions and pain processing, but this topic needs further study.

In conclusion, we have discovered that adolescent exposure to EE enhances performance in the passive avoidance test and increases the nociceptive response against thermal stimulus in both sexes. Also, exposure to EE results in increase prefrontal BDNF mRNA expression in female animals. In fact, it is clear that the role of BDNF, which is a very important mediating factor in the beneficial effects of EE, shows gender differences. Therefore, further studies are needed to examine underlying mechanisms of interaction between sex hormones and BDNF.

Authors statement
Farshid Sadegzadeh and Nona Sakhaihe performed the experiments and collected the data. Khatereh Isazadehfar helped to perform and analyze the data of experiments. Hakimeh Saadati designed the experiment, analyzed the data, and wrote the paper.

Declaration of Competing Interest
The authors declare that they have no conflict of interest.

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