Correlation of fear memory in a PTSD animal model and hippocampal BDNF in response to β-estradiol treatment

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ABSTRACT

The most important characteristics of PTSD, as an anxiety disorder, are memory disorders and hippocampus is one of the essential structures which plays a critical role in PTSD memory disorders. Traumatic events cause apoptosis and alter the expression of neurotrophic factors in hippocampus. The aim of this study is to evaluate the effects of β-Estradiol on behavioral responses in PTSD and to study its biochemical and histological mechanisms.

We used single prolonged stress (SPS) to develop PTSD in rats. The day after, the rats received electrical foot shock within shock chamber. One week later, in order to test the conditioned fear responses, the freezing behavior of rats were examined for 5 continuous days, as they were placed back in the chamber without any shock. Animals received multiple injections of β-estradiol or sesame oil, immediately after shock and also on a daily basis through the seven days prior to the test. Hippocampal cell count was implemented after cresyl violet staining. We measured BDNF protein levels by ELISA kit.

Main findings of this study confirmed that exaggerated fear response is observed in PTSD group as compared with control group and β-estradiol administration reduced these exaggerated behavioral responses. We found out that SPS decreases the density of cells in hippocampus and this effect is partly corrected by β-estradiol; β-estradiol increased BDNF protein level in hippocampus as compared with PTSD group; BDNF protein level was negatively correlated with freezing response in both SPS+β-estradiol and SPS+sesame group.

The results of this study is consistent with the hypothesis that decreased expression of BDNF contributes to memory impairment in PTSD and up regulation of BDNF by β-estradiol plays a role in memory treatment.

Key words: Post-traumatic stress disorder; β-estradiol; BDNF; Hippocampus.

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a stress-related mental disorder caused by exposure to severe traumatic events, such as war or natural disasters, and characterized by re-experiencing symptoms as intrusive memories (flashback), hyper-arousal, and avoidance symptoms. Biological alterations in this disorder include adrenergic hyper-responsiveness [1], increased thyroid activity, low cortisol levels, and increased negative feedback sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis [2]. Three brain regions are involved in the pathophysiology of PTSD: Amygdala, Medial Prefrontal Cortex, and Hippocampus. Most importantly, Hippocampus appears to interact with Amygdala during the encoding of emotional memories, a process which is highly relevant to the study of trauma and PTSD [3].

One neurocircuitry model of PTSD posits that Amygdala is hyper-responsive, medial prefrontal cortex is hypo-responsive, and medial prefrontal cortex and Hippocampus fail to inhibit Amygdala. Furthermore, hippocampal volumes have been inversely associated with verbal memory deficits, combat exposure severity, dissociative symptom severity, depression severity and PTSD symptom severity [4].

The rat model involving single-prolonged stress (SPS) is a common animal model for PTSD research [5]. This paradigm is thought to be a good model for PTSD because fear conditioning resembles the occurrence of a traumatic event

and fear extinction forms the basis of exposure therapy, which is a commonly used treatment for anxiety [7]. Fear extinction is the process of replacing a fearful response to a stimulus with a non-fearful response. Impairment of fear extinction or reconsolidation plays an important role in the development of clinical symptoms, such as re-experiencing of trauma, in PTSD [11, 13]. Hippocampus is the brain region in which estrogen synthesis as well as the expression of estrogen receptors is taking place. Estradiol induces formation and breakdown of new dendritic spines and excitatory synapses in the dorsal hippocampus of adult female animals [5]. Estrogens also influence neuroplasticity in Hippocampus. As for adult females, 17β-estradiol influences apical spine density inCA1 region of Hippocampus [7] and alters neurogenesis in Hippocampus [9]. Adult hippocampal neurogenesisis modulated by many factors including hippocampus-dependent learning and gonadal hormones, such as estradiol [9].

In recent years, it has become increasingly clear that estradiolal so plays important non-reproductive roles in the brain, such as enhancing synaptic plasticity and exerting neurotrophic and neuroprotective actions [10].Estrogens have been established as potent neuroprotective and neurotrophic factors [11]. Specifically, clinical studies have demonstrated that estrogens enhance mood and cognition and delay cognitive decline [12].

Furthermore, many studies suggest that estrogens are able to protect against neurodegenerative diseases, such as Alzheimer's disease [13]. Women are twice as likely to develop PTSD as men, which may be related to psychosocial factors (gender differences in types of trauma exposure) or biological factors (differences in gonadal hormone influences). Some evidences point to the protective role of estrogen in anxiety regulation [14]. Several studies have found that women are more likely to report symptoms of depression and anxiety during premenstrual, postpartum, and perimenopausal periods when estrogen levels are low. Studies using rodent models of anxiety have shown that females in the proestrus phase of their cycle (marked by high estrogen levels) show less fear and anxiety related behaviors than females in the metestrus or diestrus phases (lower estrogen levels). Changes in endogenous levels of E2can alter anxiety and depressive behavior of rodents [14].

PTSD women with low estradiol exhibited significantly elevated conditioned responses during extinction training in comparison to control groups and they had greater symptom severity in comparison to those with high estradiol [15]. Infusions of estradiol into Hippocampus facilitate extinction of contextually conditioned fear in ovariectomized rats and also that estradiol may facilitate consolidation of extinction learning. Increased estradiol levels in women are associated with increased activations of the neural circuitry associated with fear extinction (ventromedial prefrontal cortex, hippocampus, and amygdala) during extinction retention [16].

Brain-derived neurotrophic factor (BDNF) is a member of the neutrophin family, and plays a role in neuronal birth, maturation, differentiation, migration and survival. It is necessary for dendritic growth, synaptic plasticity and long-term potentiation. Inactivation of BDNF in Hippocampus impairs consolidation of fear extinction in a cued fear conditioning paradigm [17]. Structural neuroimaging studies showed that hippocampal volumes were relatively low in PTSD patients. Several evidences have demonstrated a strong relationship between atrophy of Hippocampus and PTSD. Apoptosis is a genetically controlled and complex process, central to the development, homeostasis and disease, turned on in response to environmental signals or triggered by intrinsic factors, and designed to kill errant cells in an orderly and clean way [18].

One of the hippocampal dependent behaviors that increases in PTSD patients is Conditioned Fear Response. Accordingly, this study is an attempt to examine this response after SPS procedure and then to evaluate the effects of administration of subcutaneous β-estradiol (multiple injections) in order to prevent the hippocampal dependent response .It was possible that β-estradiol would do this by decreasing hippocampal cell death or by affecting the most important factors which interfere with memory, such as BDNF. The present study investigates the neuroprotective effect of 17β-estradiol against SPS-induced hippocampal changes, using behavioral studies as well as biochemical and histological analyses in order to achieve a conclusion.

MATERIALS AND METHODS

Animals

A total of 80 wistar rats (200–250 g) were housed into a cage, maintained on a 12-h light/dark cycle (light on from 08:00 to 20:00), and fed and watered ad libitum. The rats were obtained from the breeding colony of University of Damghan, Iran. All procedures were conducted in agreement with the National Institutes of Health's guide for care and use of laboratory animals. Every effort was made to minimize the number of animals being used per group and to minimize the suffering of the animals throughout all experimental procedures.

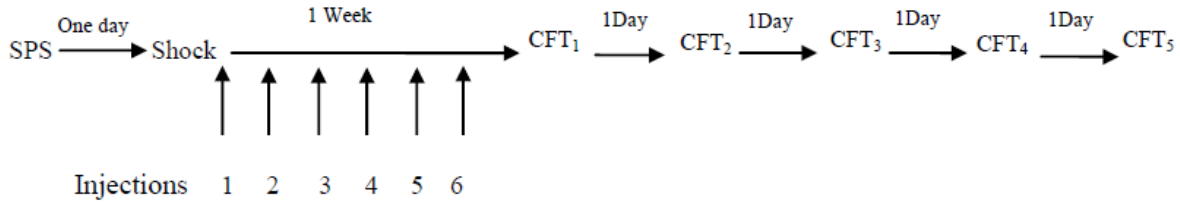
Experimental groups

Rats were randomly assigned to two SPS and Shock groups(10 animals in each group).

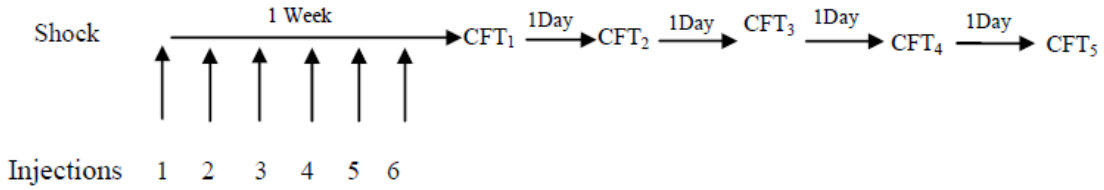
SPS group: the rats were treated through the procedures described as Single prolonged stress procedure.

Shock group: the rats received a shock conducted through the procedure described in Section shock application and test of conditioned fear response. Then, the exposed animals received multiple injections of vehicle or β-estradiol (45 µg/kg). The first injection was implemented immediately after the shock and the other injections were as below.

SPS Group



Shock Group



Single prolonged stress procedure

Detailed SPS procedure, as an animal model of PTSD, has been described in previous studies [5]. Briefly, the rats were restrained for 2 h and immediately after were forced to swim for 20 min in 24 ^{0}C water contained in a clear acrylic cylinder (24 cm in diameter and 50 cm in height). After 15 min of recuperation, the animals were exposed to diethyl ether until they lost consciousness (for1–2 min).

Shock application and test of conditioned fear response

Apparatus

An automated rodent fear conditioning system (TSE, Bad Homburg, Germany) was used to study contextual fear conditioning of each rat. Contextual fear conditioning took place in a conditioning box. The walls and the ceiling of the box were made of clear Plexiglass. The box was in an isolation cubicle (45 cm × 45 cm × 47 cm) containing a loud speaker and a light bulb which provided dim illumination. The floor of the box was made of 28 stainless steel rods (6 mm in diameter, 12 mm apart) through which foot shock could be accomplished by a constant current source. The chamber was illuminated by a single house light, and was cleaned before and after utilization. A software program was used to control the test in the box, and to collect, display and store all experimental data for ''off-line'' analysis. One day after SPS, the stressed rats received electrical foot shock within the shock chamber, after 180s they received 4s, 1 mA shock, administered via the metal grid. The stressed rats were held in the shock chamber for another 60 s before being returned to the home cages.

In order to test the conditioned fear response (CFR), a week after shock application, over 5 continuous days the rats were placed back in the chamber for 5 min periods without further shock application. The duration of freezing (Absence of all visible movement expect respiration) was evaluated.

Iwamoto et al. (2007) demonstrated that 24 h after contextual fear conditioning (FC),SPS rats exhibited a significant increase in contextual freezing as compared with sham rats [19].

Histological Methods

After the final behavior test the rats were anesthetized intrapertoneally with a mixture of ketamine (100 mg/kg) and xylazine (4 mg/kg) and perfused intracardially with 0.1 M phosphate buffer for 10 minutes followed by phosphate-buffered 4% paraformaldehyde for 15 minutes.

Infiltration and embedding

The brains were removed and Hippocampus was dehydrated through a graded series of alcohols (50%, 60%, 70%, 80%, 90%for 1 hour each, and 100% twice for 1.5 hours) prior to infiltration. After dehydration, clearing (xylene for twice 1.5 hour) and impregnation, the hippocampal blocks were embedded in disposable tissue molds[20].

Staining

Three animals of each group and 9 coronal sections (5 μm) per animal were cut at the level of the dorsal hippocampus and stained by cresyl violet. The staining solution contained 0.5g cresyl violet dissolved in100ml distilled water. The mounted sections were placed in the staining solution for 20-30 minutes at room temperature, differentiated in 0.25% acetic acid until most of the stain had been removed (4-8seconds) and then were briefly passed through absolute alcohol into xylene and checked microscopically. The differentiation was repeated in case of a necessity. Sections were then cleared with xylene and the coverslip was bonded with Entellan. The number of pyramidal cells in2mm^{2}segments of each hippocampal CA1 and CA3 fields and 800-μm^{2}segments of granule cells in the dentate gyrus were counted in a light microscope at × 400 magnification [20].

Definitions of hippocampal cell layers

The principal neurons in the different subdivisions of Hippocampus were clearly differentiated from each other. Neurons were counted based on identification of a clear and distinct nuclear membrane. The cell bodies of CA3 are large, elongated and tightly packed in a four to five cells deep layer. The cell bodies and nuclei of the pyramidal cells of CA1 are smaller than those of CA3.The granular layer of the DG contains the smallest and most densely packed cell bodies in Hippocampus. The cell bodies are packed 8-15 cells deep and have well defined borders[20].

Measurement of BDNF protein levels by enzyme-linked immune sorbent assay (ELISA)

To examine the protein levels of BDNF, we measured BDNF protein levels by ELISA. Two hour after fear conditioning tests one (day 9) and five (day 13), the rats were sacrificed by decapitation. ELISA was performed using the BDNF Emax Immuno Assay System kit (Promega, Madison, Wis., USA) according to the manufacturer's instructions[21].Briefly, 96-well ELISA plates (Iwaki, Chiba, Japan) were coated with monoclonal antibody and incubated overnight at 4 °C. The plates containing sample and blocking buffers were incubated together at room temperature for 1 hour, followed by incubation of the immobilized monoclonal antibody to BDNF, according to the standards. Duplicate samples were maintained at room temperature, being shaken for 2 hours. The plates were incubated with polyclonal antibody for 2 hours at room temperature, and then washed, and incubated at room temperature with a secondary anti-IgY antibody conjugated to horseradish peroxidase for 1 hour. The plates were incubated in peroxidase substrate and tetramethylbenzidine solution to produce color reaction. The reaction was stopped with 1 M hydrochloric acid and the absorbance at 450 nm was measured with an automated microplate reader (SH-1000, Corona, Ibaraki, Japan). Standard curves were plotted for each plate. Values were corrected for the total amount of protein in the sample. The BDNF assay sensitivity ranged from 7.8 to 500pg/ml, respectively, according to the standards provided by Promega. Values of sections were above 16pg/ml for each plate.

Drug treatment

β-estradiol was purchased from Sigma (USA). β-estradiol was prepared in sesame oil (vehicle, Sigma-USA), and was injected in 2 ml/kg volume (S.C., 45mg/kg). Control animals were given vehicle only. The drug dose were mainly derived from our previous studies and a survey of reports on these drugs [22].

Statistical analysis

Data are presented as mean ± SEM. Data were analyzed by 1- and 2-way ANOVA for repeated

measurements by means of SPSS 16.0. Tukey post hoc test was performed to determine the source of the detected significant differences. Values of $P < 0.05$ were considered significant. Relationships between behavior and BDNF protein levels were assessed by two-tailed Pearson correlations.

RESULTS

Behavioral results

The aim of this experiment was to investigate the conditioned fear response in rats after post-traumatic stress disorder induction by SPS. The rats were randomly divided into three groups: Shock+Sesame oil, SPS+Sesame oil and SPS+β-estradiol(45 mg/kg) as described in section 2.2. Conditioned fear responses of all rats were tested in five following days, according to the procedures described in section 2.4, (Test 1-5).

Two-way ANOVA with repeated measurements on freezing data showed a significant effect of groups ($F2, 35=20.401$, $P=0.000$), days ($F4, 140=4.405$, $P=0.002$), and no significant effect of interaction between groups and days ($F8, 140 =0.308$, $P=0.962$). Post-hoc comparisons indicated that SPS+Sesame group showed significant increase in CFR as compared with Shock+Sesame group in all tests ($P=0.00$). Animals in this group were likely to "freeze" for a longer time during the entire period of observation as compared with shock group, and this result remained the same in five Tests. Post-hoc comparison indicated that there is a significant difference between SPS+Sesame oil and SPS+β-estradiol group (45 mg/kg) and also that the result remained the same in five Tests. Rats given β-estradiol showed decreased percentage of freezing as compared with SPS group which received sesame (Figure 1). Comparison of conditioned fear responses between tests 1 and 5, in each group, with paired T-test showed that there was significant decrease in freezing in shock group and also SPS+β estradiol group, but the response remained constant in SPS+sesame oil group (Figure 2).

Histological results

The animals were trained as described in experiment 1 and Hippocampus was stained with crysil violet as described in section 2.5. The principal neurons in the different subdivisions of Hippocampus were clearly differentiated from each other. Neurons were counted based on identification of a clear and distinct nuclear membrane.

Number of CA_1 pyramidal cells

One-way ANOVA showed a significant effect of groups ($F2, 23=9.275$, $P=0.001$). Post-hoc comparisons indicated that the number of CA_1 pyramidal cells in SPS+β-estradiol group was significantly more than SPS+sesame oil group ($P=0.045$) and SPS+sesame oil group showed significant decrease in the number of cells as compared with Shock+Sesame group ($P=0.001$). The average number of neurons in all sections of each group is shown in table 1 (Figure 3).

CA_3 pyramidal cells

One-way ANOVA showed a significant effect of groups ($F2, 23=9.275$, $P=0.001$). Post-hoc comparisons indicated that the number of CA_3 pyramidal cells in SPS+β-estradiol group was significantly more than SPS+sesame oil group ($P=0.02$) and SPS+sesame oil group showed significant decrease in the number of cells as compared with Shock+Sesame group ($P=0.001$). The average number of neurons in all sections of each of group is shown in table 1(Figure 3).

DG granule cells

The total number of DG neurons was significantly different among groups ($F2, 21=2.826$, $P=0.082$). There was no significant difference in the number of cells between SPS+sesame oil and SPS+β-estradiol groups. SPS+sesame oil group showed significant decrease in the number of cells as compared with Shock+Sesame group ($P=0.001$). The average number of DG neurons is shown in table 1(Figure 3).

Biochemical results: measurement of BDNF protein levels

We measured total protein in right and left hippocampus, BDNF protein levels and the ratio of these two in SPS+sesame oil and SPS+β-estradiol groups by ELISA. These measurements were done after the first (day 9) and fifth (day 13)tests.

T-test showed a significant difference between the SPS+sesame oil and SPS+β-estradiol groups9 days after SPS induction($p < 0.01$). But this difference was not found to be statistically significant 13 days after SPS(Figure 4).

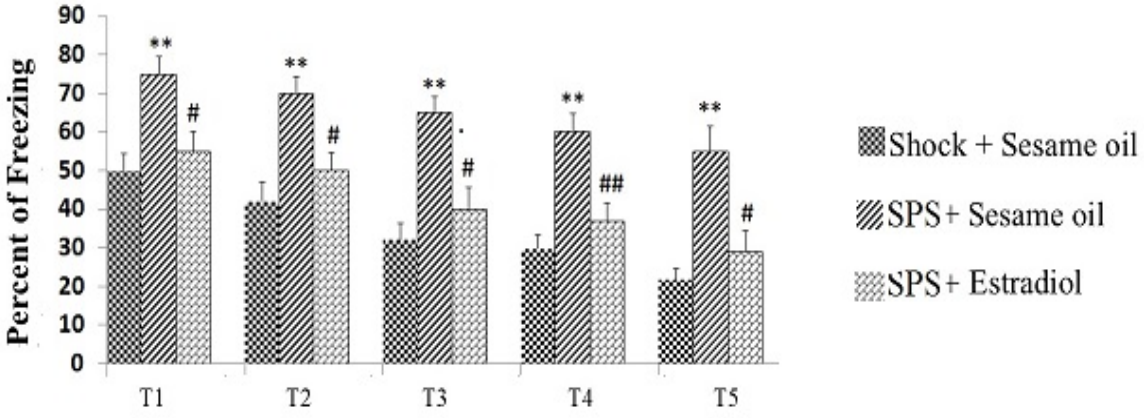


Figure 1. Conditioned fear response which was measured as percentage of time spent freezing during tests. Experimental groups were described in section 2.2. And rats were tested in five following weeks, according to the procedures described in section 2.4.
** P< 0.01 as compared with Shock group; # P< 0.05 as compared with SPS+Sesame oil group; ## P< 0.01 as compared with SPS+Sesame oil group.SPS: Single prolonged stress. Data were presented as Mean ± SEM (n= 8-10 in each group).

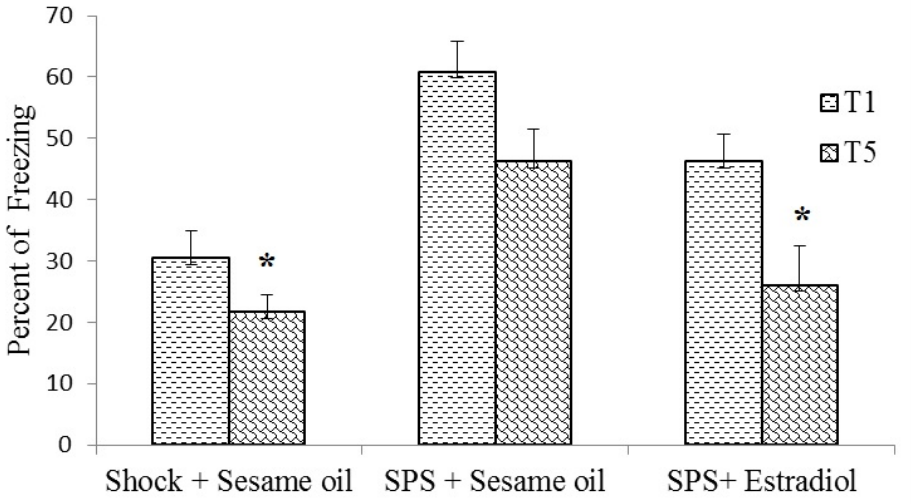


Figure 2. Comparison of conditioned fear responses between tests 1 and 5, in each group.
* P< 0.05 as compared withTest one.Data were presented as Mean ± SEM (n= 8-10 in each group).

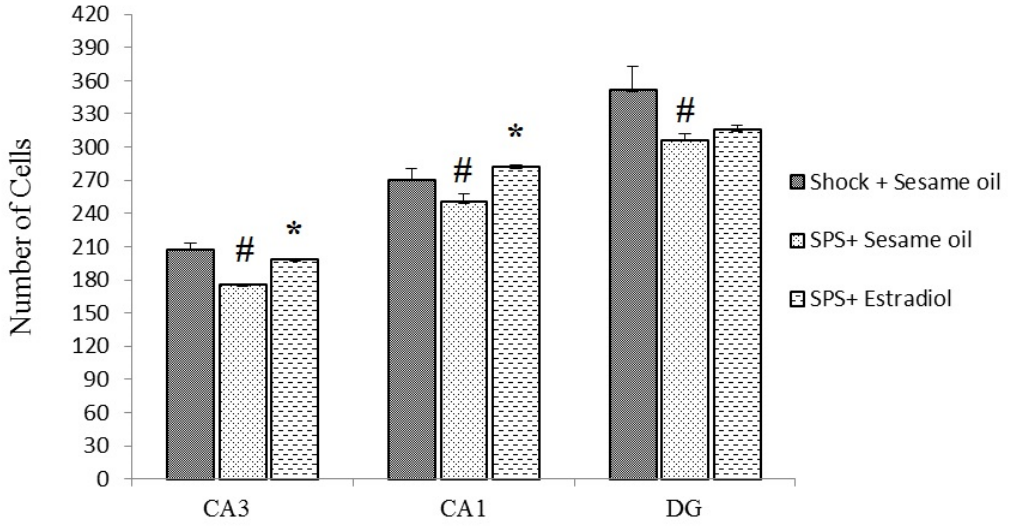


Figure 3. The number of principal cells in different regions of Hippocampus.Cell count was done according to the procedures described in section 2.5.2.* P< 0.05 as compared with SPS+Sesame oil group; # P< 0.05 as compared withShock group. Data are expressed as means ± SEM.

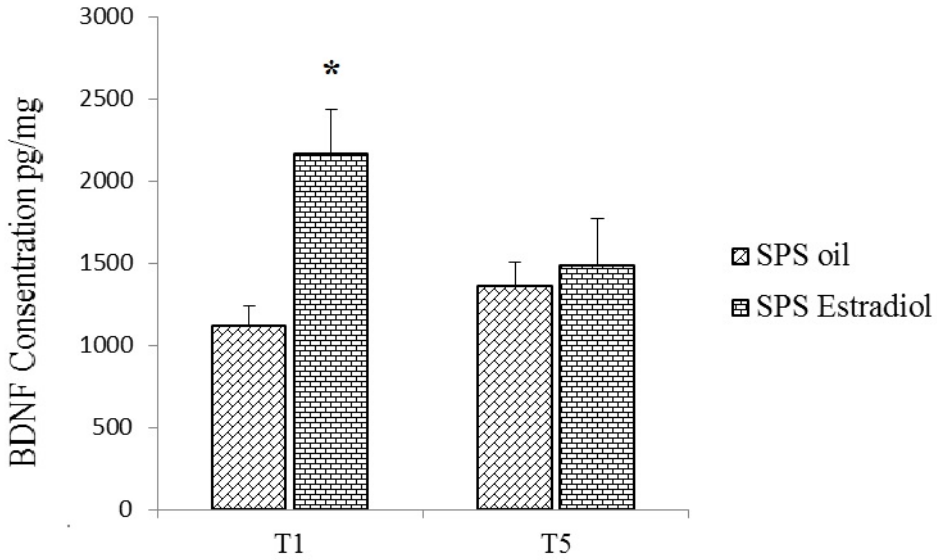


Figure 4. BDNF protein levelsin right and left hippocampusby ELISA.Measurements were done after the first and fifth tests. * P< 0.01 as compared with SPS+Sesame oil group;

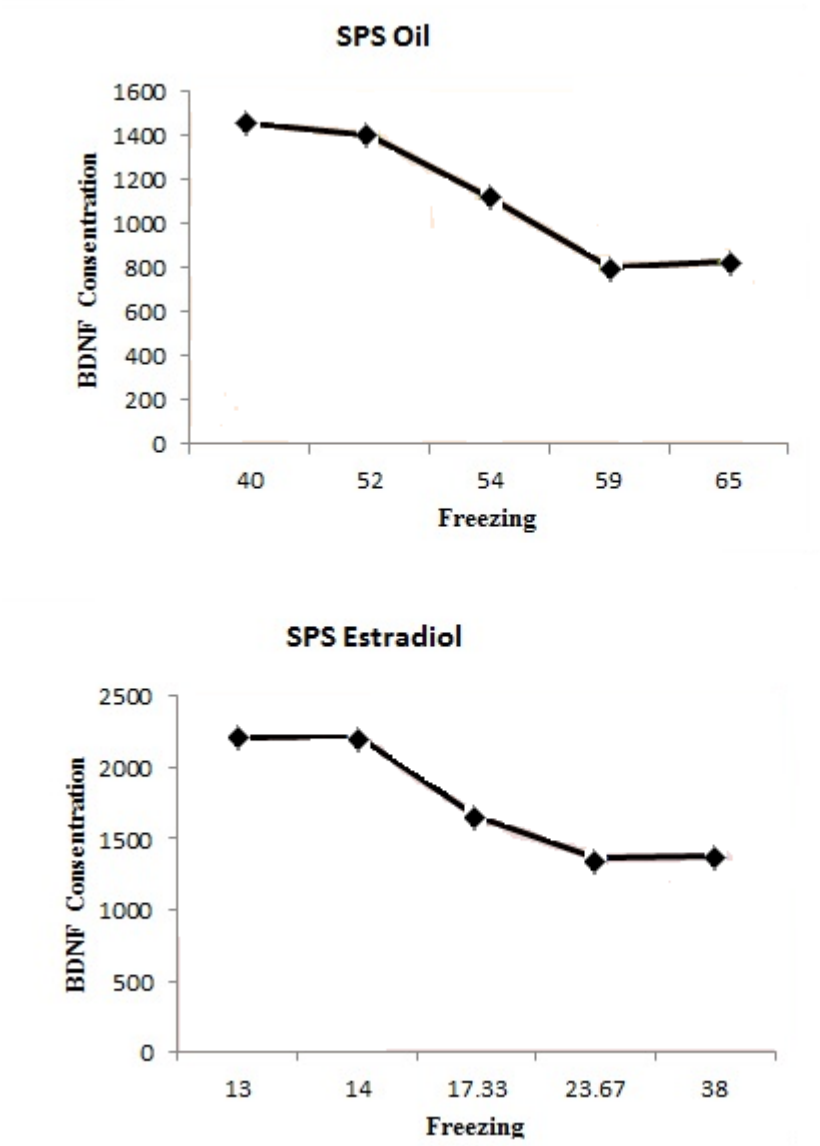


Figure 5. Correlations between behavior and BDNF protein levels. Up:in SPS+sesame group; down: in SPS+β-estradiolgroup. BDNF protein level wasnegatively correlated with freezing responseeither in SPS+β-estradiol (r = -0.87,p = 0.02) or SPS+Sesame group (r = -0.82; p = -0.04).

Correlations between behavior and BDNF protein levels

As we showed in the previous session, there was a significant increase in BDNF protein level in SPS+β-estradiol group as compared with SPS+sesame group after the first test. We also reported that the freezing response in SPS+β-estradiol group is significantly lower than SPS+sesame group. In order to examine the correlation between these two factors, we two-tailed Pearson correlations. BDNF protein level was negatively correlated with freezing response either in SPS+β-estradiol (Pearson correlation coefficient, two-tailed, r=-0.87,p= 0.02) or in SPS+sesame group (Pearson correlation coefficient, two-tailed, r=-0.82; p = -0.04)(Figure 5).

DISCUSSION

The main findings in the present study were: (1) SPS rats exhibited enhancement of contextual fear in comparison to Shock rats, and this effect was permanent in five fear tests. Rats given β-estradiol (45 mg/kg) showed decreased percent of freezing as compared with SPS group that received sesame (2) SPS procedure reduced the number of CA_3 and CA_1 pyramidal cells, and β-estradiol treatment elevated the number of cells. (3) BDNF protein in

SPS+ β-estradiol rats was significantly higher than that in SPS+sesame oil rats. (4)BDNF protein level was negatively correlated with freezing response in both SPS+β-estradiol and SPS+sesame group.

β-estradiol and conditioned fear response

In the present study, we examined the effects of SPS model for PTSD-like symptoms. One day after SPS, the rats were placed in a conditioning chamber with a grid floor. During a conditioning session, the rats were exposed to foot shock. One week after conditioning, the animals were placed again in the same chamber and freezing was examined. The maintenance of the response was measured for five following days. Findings indicated that after one week conditioned fear response was enhanced in the SPS rats. This finding confirms the results of other studies showing that SPS model enhances fear conditioning response after one week [5]. The freezing time in shock group was around 30% of the initial time and this was half of that in SPS+Sesame oil group.

Comparison of conditioned fear responses between tests 1 and 5 in each group showed that there was a significant decrease in the freezing time in shock group, but this response remained constant in SPS+sesame oil group. It means that the enhancement of fear response in this model of PTSD induction lasted even after several tests, as previous studies have shown [23].

Extinction is a process whereby a learned fear response is reduced via repeated presentation of the conditioned stimulus. Patients suffering from PTSD are impaired in extinction of learned fear [24].

Research in basic science and functional neuroimaging has helped to identify three brain regions that may be involved in the pathophysiology of PTSD: Amygdala, Medial Prefrontal Cortex, and Hippocampus. Numerous molecular and clinical studies have implicated estrogen in modulating brain function including that related to mood [25].

The brain is widely responsive to gonadal hormones. Not only is the hypothalamus regulated by these hormones in relation to reproductive behavior and neuroendocrine physiology, but also structures like Hippocampus and midbrain serotonin system undergo sexual differentiation. Estrogens regulate dorsal hippocampus during the estrous cycle of the female rat, and the functional consequences include changes in neurotransmission and memory. This finding was surprising because, until recently, Hippocampus was known as a brain region in which cells' nuclear estrogen receptors are present in scattered inhibitory interneurons but not in principal neurons where spine formation occurs. Yet the effects of ovarian hormones on synaptic turnover were as impressive in Hippocampus as those in the ventromedial hypothalamus, a classic estrogen target area of the brain for female sexual behavior [26].

Female rats undergoing extinction learning during the proestrous phase of the estrus cycle (when estradiol and progesterone levels are elevated) exhibited better extinction memory during subsequent extinction recall (i.e. retention) testing. Moreover, exogenously administered estradiol and progesterone facilitated extinction recall, whereas estradiol receptor antagonists impaired it. Estradiol administration into Hippocampus in female rats facilitated fear extinction [27].Collectively, these data suggest that gonadal hormones influence the consolidation of extinction memory.

Our results indicate that rats given β-estradiol, immediately after shock presentation (fear conditioning), showed decreased percentage of freezing time as compared with SPS group that received sesame. Comparison of conditioned fear responses between tests 1 and 5, in each group, showed that there was significant decrease in fear in SPS+β estradiol group, but this response remained constant in SPS+sesame oil group. In other words, multiple injection of β estradiol reduced the fear response, the same as what happened in shock group; it alleviates the enhanced fear induced by SPS. Accordingly, the recuperative effect of β estradiol not only abided, but also invigorated after five tests. These findings support our previous data and indicate that prescribing β estradiol immediately after traumatic events could prevent memory impairment in PTSD patients.

β-estradiol and cell density

At the end of the behavioral tests, the animals were perfused and the density of cells in hippocampus was measured after removal of the brains and processing the tissues. Although we didn't measure total volume of hippocampus, our results indicated that SPS decreases the density of cells in right and left hippocampus and that this decrease was improved by β-estradiol. The number of CA_1 and CA_3 pyramidal cells in SPS+β-estradiol group was significantly more than SPS+sesame oil group. SPS+sesame oil

group showed significant decrease in the number of cells as compared with Shock+Sesame group. There was no difference between the two groups regarding the number of cells in DG region of hippocampus. Decreased hippocampal volumes in PTSD is confirmed by the present findings.

Estradiol is a neuroprotective factor that decreases cell death in a variety of in vitro and in vivo paradigms of brain injury [28].

Previous reports implicate E2 as a modulator of Ca2+ concentration in mitochondrial matrix, and ultimately in the cytosol. Given the vital role of Ca2+ in regulation of total nerve cells activity, especially energy metabolism, neurotransmission and directing the cells toward survival or cell death, the effects on mitochondrial Ca2+ transport could be one of the important modes of E2 neuromodulatory action independent of the genome [28].

Apoptosis is tightly regulated by anti apoptotic and pro apoptotic effector molecules and may be one of the inducing reasons for hippocampus decrease in cell density or atrophy in PTSD. Mitochondrial pathway participated in SPS-induced apoptosis, including Bcl-2 family, cytochrome C caspase-9 and caspase-3. However, more researches are needed in order to achieve a better understanding of the molecular mechanism underlying SPS induced apoptosis, which may provide important information for the treatment of PTSD. Probably, β-estradiol affects the cell density in hippocampus by its anti-apoptotic effect.

β-estradiol and BDNF

BDNF has been postulated to be an important signaling molecule in regulating synaptic strength and overall circuit activity in the adult. BDNF plays a critical role in activity-dependent neuroplasticity underlying learning and memory in Hippocampus. Previous studies suggest that animals with reduced BDNF have diminished extinction of fear. Furthermore, diminished BDNF in Hippocampus is thought to possibly be related to the decreased hippocampal volume found in patients with major depressive disorder and posttraumatic stress disorder (PTSD).These disorders have also been hypothesized to result in part from a deficit in extinction of fear [29].

There is a growing body of evidence demonstrating that stress decreases the expression of brain-derived neurotrophic factor (BDNF) in limbic structures that control the mood and also that antidepressant treatment reverses or blocks the effects of stress. Decreased levels of BDNF, as well as other neruotrophic factors, could contribute to the atrophy of certain limbic structures, including Hippocampus and prefrontal. Conversely, the neurotrophic actions of antidepressants could reverse neuronal atrophy and cell loss and thereby contribute to the therapeutic actions of these treatments [30].

In this study, we found out that hippocampal cell density was reduced in SPS groups as compared with shock group and this reduction was recovered by estradiol treatment. Neurotrophins, and particularly BDNF, may have a major protective role against neuronal damage by stimulation of sprouting and synaptic reorganization, promoting resilience of brain cells to cope with stressful challenges. Because BDNF expression in the adult hippocampus is required for precursor cell survival, exercise-induced cell proliferation, and dendritic development [31], we measured BDNF protein levels after the first (day 9) and fifth (day 13) tests in SPS+sesame oil and SPS+β-estradiol groups by ELISA. There was a significant difference between the SPS+sesame oil and SPS+β-estradiol groups 9 days after SPS induction. In this study, we found out that SPS rats treated with Estradiol showed an increase in the levels of BDNF protein on the first test. May be Estradiol up-regulates the induction of BDNF levels in Hippocampus. This difference was not found to be statistically significant 13 days after SPS, when the animals had not received Estradiol for 5 days.

We found out that BDNF protein level was negatively correlated with freezing response in SPS rats. This study suggests that the enhanced levels of BDNF during fear memory test is, at least in part, associated with decrease in fear memory in SPS rats which were treated with Estradiol. The rats with higher fear response possess lower level of BDNF in their hippocampus. The higher induction of BDNF response to Estradiol in SPS rats may contribute to the difference in freezing between the SPS groups which received Sesame oil and Estradiol.

Preclinical studies have demonstrated that exposure to footshock or maternal separation reduced hippocampal BDNF expression through a down-regulation of its mRNA levels. Dell'Osso et al. (2009) recently showed significantly lower plasma BDNF levels in PTSD patients as compared with matched. In contrast, a recent case report demonstrated elevated serum BDNF levels in 2 cases [32]. In another report they have shown that patients who were recently exposed to trauma

presented higher BDNF levels as compared with controls. They suggest that serum levels of brain-derived neurotrophic factor, in PTSD, are increased immediately after trauma. But this increase is not persistent and declines after several days [33]. Actually, it is also possible that an increase in BDNF, immediately after trauma, reflects an attempt to compensate for other neurobiological alterations that occur in PTSD. Further studies are required to elucidate whether BDNF is associated with the pathophysiology of PTSD.

Based on the findings of this study, it is conceivable that the pathophysiology of PTSD, leading to the declined extinction of fear memory, may be in part due to impairment of BDNF. Moreover, the enhanced BDNF also would participate in the facilitation of fear extinction. However, it cannot be ruled out that up-regulation in BDNF is coincident with the enhanced extinction of fear memory. Further studies are required to elucidate the involvement of BDNF in the enhanced extinction of fear memory and the effects of Estradiol on this function.

It is well established that estrogen is involved in the differentiation and plasticity of hippocampal neurons. Estrogen receptor is colocalized with BDNF in hippocampal pyramidal neurons and there is a direct action of estrogen receptor on the BDNF gene in pyramidal cells. It is possible that estrogen regulates the expression of BDNF indirectly through a step involving other transcription-regulating factors [34]. BDNF and its receptor trkB regulate both short-term synaptic functions and long-term potentiation (LTP). Estrogen has similarly been shown to enhance LTP and may regulate neural processes underlying learning and memory. The mechanisms by which estrogen modulates LTP are essentially unknown, but one possibility could be through interactions with the BDNF gene [34].

Several findings demonstrate that hormone replacement significantly affects relative levels of BDNF mRNA and protein within specific regions of the brain. These effects may, in turn, contribute to the effects of estrogen replacement on hippocampal connectivity and cognitive processes that have recently been reported. Estrogen replacement in young adult, ovariectomized, female rats increases BDNF expression in the olfactory bulb, hippocampus, cortex, amygdala, septum dorsolateral area of the bed nucleus terminalis and the lateral habenular nucleus. In ovariectomized, aged female rats, estrogen treatment increases BDNF, NGF and NT3 in the entorhinal cortex(Cavus and Duman, 2003). Estrogen also increases BDNF expression in Hippocampus of gonadectomized male mice. However, in some reports, estrogen has no effect on several cortical brain regions, Hippocampus or the nucleus/ventral pallidum. High endogenous estrogen levels during the estrus cycle are associated with decreased BDNF mRNA in Hippocampus and prefrontal cortex. Moreover, BDNF protein expression in the forebrain region is also down-regulated by exogenous estrogen replacement [35].

Some of these results are similar to ours, demonstrating an estrogen-induced increase in BDNF levels in hippocampal targets.

In summary, although there are some limitations, the results of these studies are consistent with the hypothesis that decreased expression of BDNF contributes to memory impairment in PTSD and that up regulation of BDNF by β-estradiol plays a role in memory treatment.

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