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The effect of basolateral amygdala nucleus lesion on memory under acute, mid and chronic stress in male rats

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Background/aim: The basolateral amygdala (BLA) modulates memory for emotional events and is involved in both stress and memory. This study investigated different durations of stress and the role of BLA on serum corticosterone level and spatial and cognitive memory.

Materials and methods: Different durations of stress (acute, mid, and chronic stress), with and without BLA lesion were induced in rats by 6 h/day restraint stress for 1, 7, and 21 days. Memory functions were evaluated by novel object recognition (NOR) and object location test (OLT).

Results: The OLT findings showed locomotor activity and spatial memory slightly decreased with different durations of stress. The NOR findings significantly showed locomotor activity impairment in different durations of stress. Cognitive memory deficit was observed in mid stress. The corticosterone level significantly increased in the mid and chronic stress groups. Moreover, the mid stress was the strongest stress condition. There is a possibility that different stress durations act by different mechanisms. The recognition of a novel location decreased in all lesion groups. It was more severe in the NOR. The BLA lesion significantly decreased corticosterone level in the mid and chronic stress groups compared to similar groups without lesion.

Conclusion: The BLA lesion caused more damage to cognitive than spatial memory in stressed groups.

Key words: Basolateral amygdala, corticosterone, memory, novel object recognition, object location test, stress, rat

1. Introduction

Stress is defined as any endogenous or exogenous environmental changes that disturb brain homeostasis (1). It is demonstrated that stress plays a significant role in the acceleration of psychological disorders (2). Stress has different complex effects (e.g., facilitating, impairing, and neutral) on memory (3–5) related to various responses of stress-related hormones involved in different stress durations (6). It is well established that stress leads to activation of the hypothalamic–pituitary–adrenocortical axis (HPA), the autonomic nervous system (ANS), and the amygdala (7–9). Studies showed that different kinds of stress are associated with different levels of amygdala activation (10,11). Previous reports proposed that the amygdaloid complex, especially the basolateral amygdala (BLA), plays a crucial role in regulating emotion-related memories (12,13). Furthermore, the BLA is connected to the hippocampus, which is the main region for memory functions (10,14–17). It seems that the BLA controls the working memory and consolidation and retention of memories (7,18) to integrate modulatory effects on memory processing (19–21). In addition, it has an important role in

mediating stress and its effects on memory (10,22) through stress hormones and noradrenergic activation (23–26).

Accordingly, the BLA is involved in both stress and memory function to a great extent. It is not clear how the BLA interacts with the duration of stress since different durations of stress are associated with the various hormonal systems. Hence, the aim of the present investigation was to assess the effects of stress duration (acute, mid, and chronic) and the role of BLA lesion on serum corticosterone level and behavioral (object location and novel object recognition) tests for evaluating spatial and cognitive memory.

2. Materials and methods

2.1. Experimental animals

Experiments were performed on 63 male Wistar rats with an initial weight of 250–300 g obtained from the Pasteur Institute, Tehran, Iran. All experimental protocols were approved by the Ethical Committee of Isfahan University of Medical Science (Isfahan, Iran) in compliance with the “Principles of Laboratory Animal Care” and the European Community Council Directive of 24 November 1986

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(86/609/EEC). The rats were maintained under light-controlled conditions (12-h light/dark; lights on 0700–1900) in a room with a temperature of 22 ± 2 °C. Food and water were available ad libitum, except during the stress condition. At the end of the experiments, all behavioral experiments were carried out between 1400 and 1600 in all groups (Figure 1). The animals were randomly assigned to nine groups (n = 7 in each group) as follows:

1- Control group (Co): the rats were transported to the laboratory room where they had no special treatment and were handled the same as the experimental animals throughout the study period.

2- Sham-operated group (Sh): the rats underwent stereotaxic surgery without lesion in the BLA.

3- Acute stress group (AS): the rats were under restraint stress 6 h/day for 1 day.

4- Mid stress group (MS): the rats were under restraint stress 6 h/day for 7 days.

5- Chronic stress group (CS): the rats were under restraint stress 6 h/day for 21 days.

6- BLA lesion group (Le); the rats had stereotaxic surgery so as to develop a lesion in the BLA and were not exposed to restraint stress.

7- Acute stress with BLA lesion group (ASL): the rats had stereotaxic surgery for electrical lesion in the BLA and were under restraint stress 6 h/day for 1 day.

8- Mid stress with BLA lesion group (MSL): the rats underwent stereotaxic surgery for electrical lesion in the BLA and were under restraint stress 6 h/day for 7 days.

9- Chronic stress with BLA lesion group (CSL): the rats had stereotaxic surgery for electrical lesion in the BLA and were under restraint stress 6 h/day for 21 days.

2.2. Experimental procedures

2.2.1. Stress paradigms

In the current study, rats were placed in Plexiglas cylindrical restrainers for 6 h/day (0800–1400) and fitted tightly for the aforementioned days (27). The rats were not able to move or turn around. Therefore, restraint was a strong stressor in rats (28) and evoked unconditioned and unavoidable neuroendocrine responses (29).

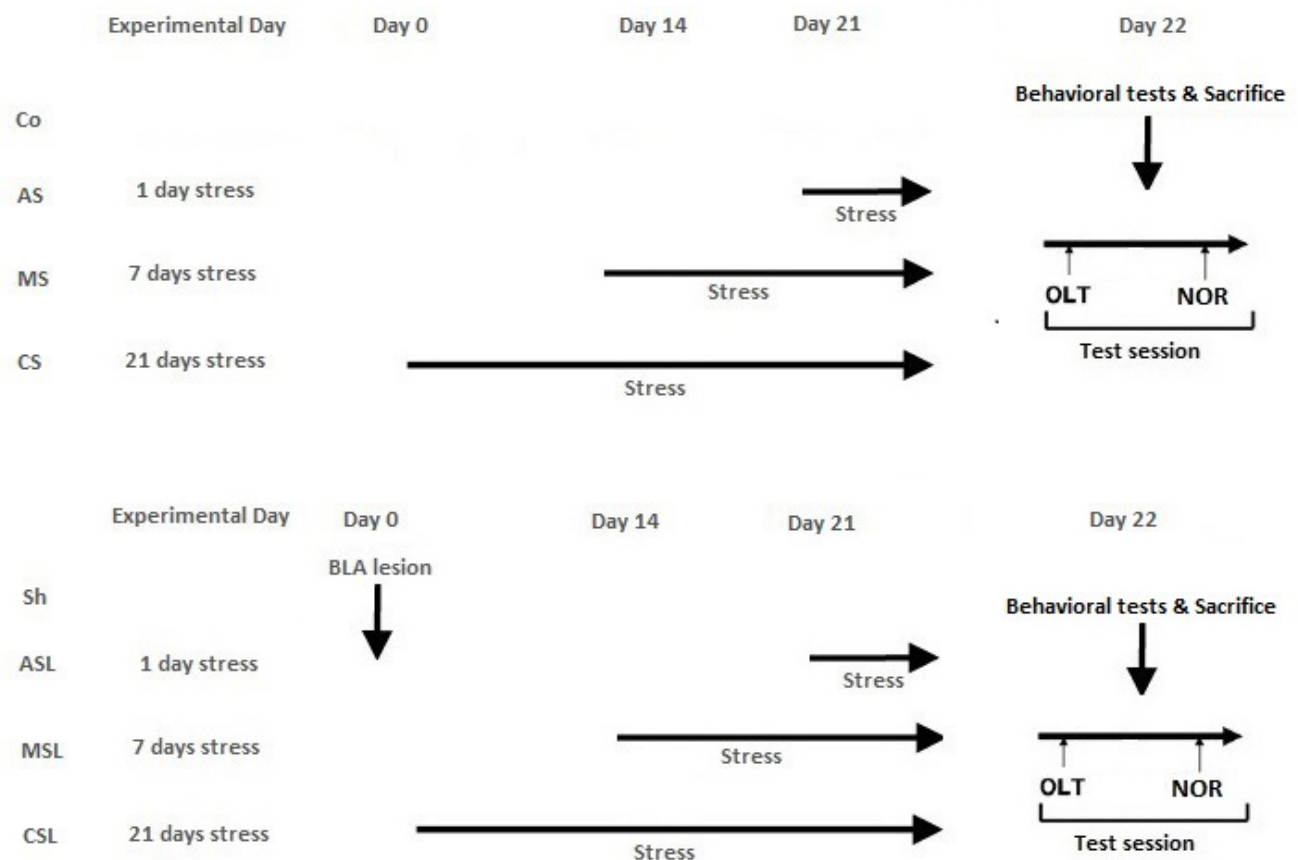


Figure 1. Experimental schedule for all groups, stereotaxic surgeries, and the days on which behavioral tests were tested. Animals were exposed to restraint stress. After the stress session, the animals were submitted to a test session composed of two different behavioral tests: object location test (OLT) and novel object recognition (NOR). Then the rats were sacrificed immediately after a behavioral session for hormonal analysis.

Co: Control group; AS: Acute stress group; MS: Mid stress group, CS: Chronic stress group; Le: Lesion group; ASL: Acute stress + lesion group; MSL: Mid stress + lesion group, CSL: Chronic stress + lesion group.

2.2.2. Stereotaxic surgery

In the surgery groups, initially, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) (30,31) and then placed in a stereotaxic frame (Stoelting Co., USA). Next, the skull was exposed and two small holes were drilled over the BLA (AP = -2.28, ML = \pm 5, and DV = 8.6 mm from bregma). A bipolar electrode (Teflon-coated stainless steel, 0.125 mm diameter, Advent Co., UK) was positioned in the BLA. All animals were allowed 3 days to recover from surgery and the remnant effect of anesthetic agents. Electric lesions were produced by passing 1.5 mA current for 7 s (26). Signals were passed through an analogue to digital interface (Data Acquisition ScienceBeam-D3111) to a computer.

2.2.3. Behavioral apparatus and method

Learning and memory tasks in animal experiments often entail certain degrees of stress (7,32), whereas OLT and NOR behavioral tests require no external motivation, reward, or punishment (33) and they are not aversive memory tests (34). These tasks are widely used to assess memory in rodents and humans (35–39). Rats often respond to environmental changes by preferential exploration of moved or novel objects over the familiar one. Such preferences indicate the formation of memories regarding the location and identity of objects (39). On the other hand, the OLT and the NOR were carried out to investigate hippocampal-dependent spatial and cognitive memory, respectively (40). The NOR can also be configured to measure the working memory (33). Hence, the OLT and NOR behavioral tests was performed in the current study.

The open field apparatus (60 × 65 × 50 cm) was used for OLT and NOR tests. On day 21 of the experiment, each rat was placed in the center of the apparatus for 5 min to habituate it to the apparatus. On a later day (24 h after exposure to the last stress, on day 22), sample and test phases were performed. In sample phase trial (training), two identical objects (A1, A2; cuboid with height 5) were placed on the adjacent corner for each arena, leaving 8 cm from the walls. The rat was placed between the two objects in order to start 5-min object exploration training. Furthermore, the test phase trial (retention) was conducted after a delay of 7 min. In this trial, the objects were replaced by their identical copies; one of the two objects was placed in the same position as it was in the sample phase trial (familiar location, F), whereas the other one was moved to the opposite side (new location, N). In the test phase trials, both objects were equally familiar to the animals, yet one had a changed location (Figure 2A). These objects were heavy enough for the rats not to be able to move them. Finally, the animal's performance was recorded by video for later analysis.

The NOR tests are divided into two trials of acquisition and retention. In the NOR sample phase trial the two foregoing trials were performed in the same way as those of the OLT. Two familiar objects (A1, A2) were located at the adjacent corner of the chamber for each arena. The rats were then placed between the two objects and were allowed to explore the objects for 5 min. The test phase trial was performed 7 min after the sample phase trial. In the test trial, one of the two objects was the identical object (familiar object, F) while the other was replaced by a new one with a black-and-white pattern cylinder (new object, N) (Figure 3A).

N and F represent the time spent to explore novel and familiar locations in the OLT and/or novel and familiar objects in NOR during a 5-min observation period (41). In the OLT and NOR tests, the locomotor activity in the sample phase (training) and test phase (retention) trials was measured using the total amount of time spent exploring each of the two objects ($T_1 = A_1 + A_2$ in the sample phase; $T_2 = F + N$ in the test phase) (41,42).

Finally, a discrimination index (DI) was calculated according to the following equation: $DI = N/(N + F) \times 100$ (42,44). In both the OLT and NOR, object exploration was defined as directing the nose to the object at a distance of 2 cm and touching it with the nose, in accordance with a previous report (42). Additionally, turning around, climbing over, and sitting on the object were not recorded as exploration. Between each trial, the open field and the objects were cleaned with 70% ethanol after each individual trial to prevent a build-up of olfactory cues (41).

2.2.4. Assessment of serum corticosterone levels

At the end of the experiments, 24 h after exposure to the last stress (on day 22), the animals were sacrificed at 1600–1700 by decapitation. Their blood samples were obtained from the trunk blood; serum was separated by centrifugation (6000 rpm, 20 min) and stored at -80 °C until analysis. Commercial enzyme-linked immunosorbent assay (ELISA) kits (DRG Co., Marburg, Germany) were used to assess the serum corticosterone levels.

2.3. Histology

Histological verification of the electrode tip localization was performed on the animals. After decapitation, the brain was removed and stored in 10% formalin for at least 3 days. In addition, frozen serial transverse sections (60 μ m) of the forebrain were cut and the lesion sites were determined according to a rat brain atlas (45) using a light microscope.

2.4. Data analysis

All data are reported as the mean \pm SEM. The behavioral data of various groups (between group comparisons) were compared using ANOVA followed by LSD post-hoc test for multiple comparison. The comparisons of the total times

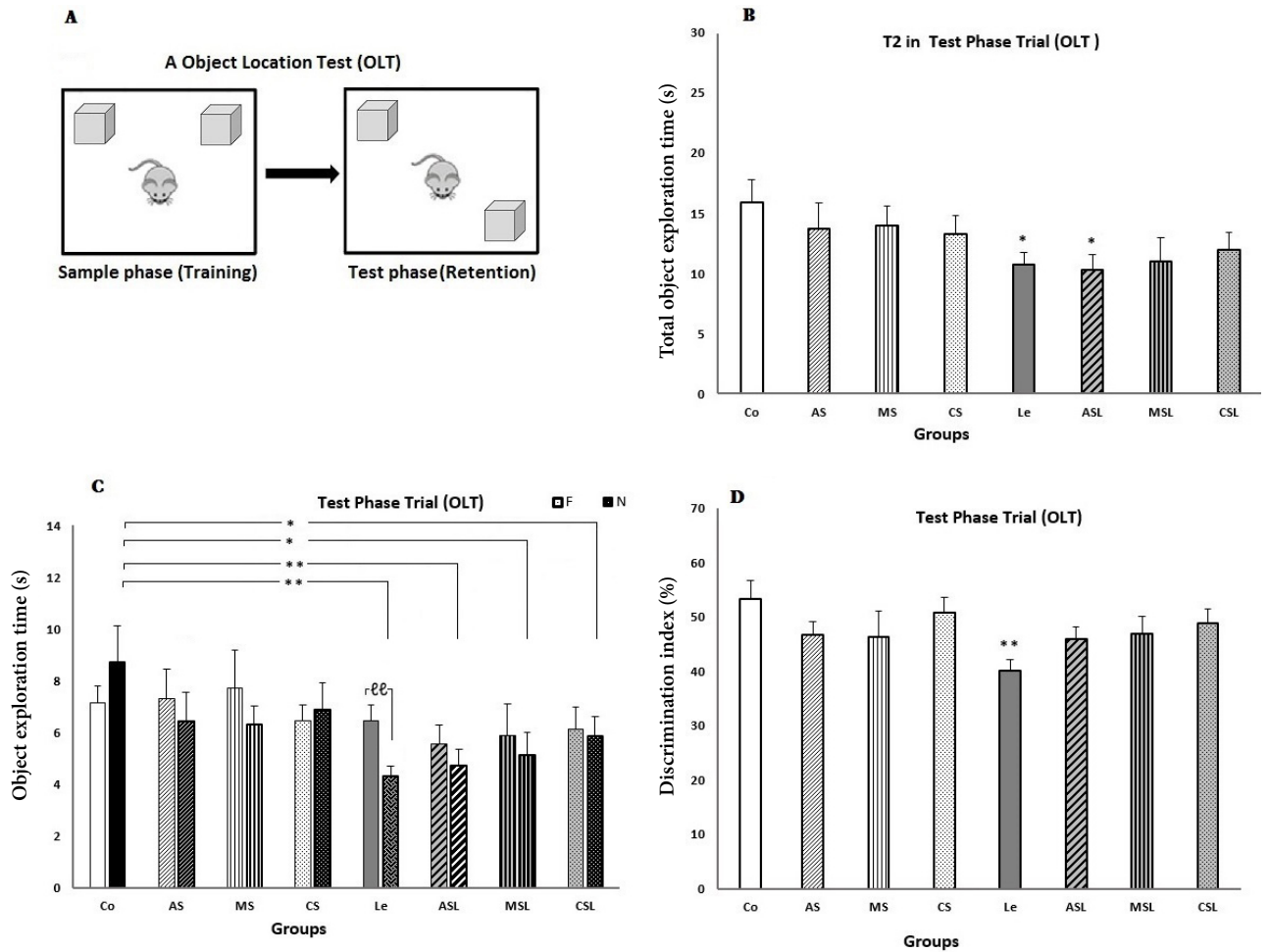


Figure 2. Effects of different duration of stress and BLA lesion in object location test (OLT) that was carried out 24 h after the last exposure to restraint stress. (A) Representation of sample and test phase trial conditions in OLT. (B) The total time of object exploration (s) for test phase trial (T2) in the OLT. (C) Object exploration time (s) of familiar and new locations (F and N, respectively) in the test phase trial of the OLT. (D) Percent of discrimination index (DI) for test phase trials of the OLT (n = 7). Results are expressed as mean ± SEM (ANOVA test, Tukey's post-hoc test for comparisons between groups and paired t-test for comparisons within groups; *P < 0.05 and **P < 0.01 compared to Co group; ^{†††}P < 0.01 when the exploration time of N compared to the F in each group.

Co: Control group; AS: Acute stress group; MS: Mid stress group, CS: Chronic stress group; Le: Lesion group; ASL: Acute stress + lesion group; MSL: Mid stress + lesion group, CSL: Chronic stress + lesion group.

of object exploration (within groups) were analyzed using paired Student's t-tests. The corticosterone levels of various groups were compared using ANOVA followed by Tukey's post-hoc test for multiple comparison. A P-value of less than 0.05 was considered statistically significant. Ultimately, the calculations were performed using SPSS 21 (SPSS Inc., Chicago, IL, USA).

3. Results

No significant difference was observed between the control (Co) and sham-operated (Sh) groups, indicating that the surgery in the initial phase of the experiments had no effect on the behavioral data or the CORT level (not presented as a graph here).

The total time of object exploration in the sample phase ($T_1 = A_1 + A_2$) showed no significant differences in all groups compared to the Co group and each other in both the OLT and NOR tests (not presented as a graph here).

3.1. The time of object exploration and discrimination index in the OLT

The total time of object exploration in the test phase ($T_2 = F + N$) of the OLT revealed significant ($P < 0.05$) decreases in the BLA lesion (Le) and acute stress-BLA lesion (ASL) groups compared to the Co group. It indicated the reduction of locomotor activity in these groups. Moreover, T_2 did not show significant decreases in the acute, mid, or chronic stress groups with BLA lesion (ASL, MSL, and

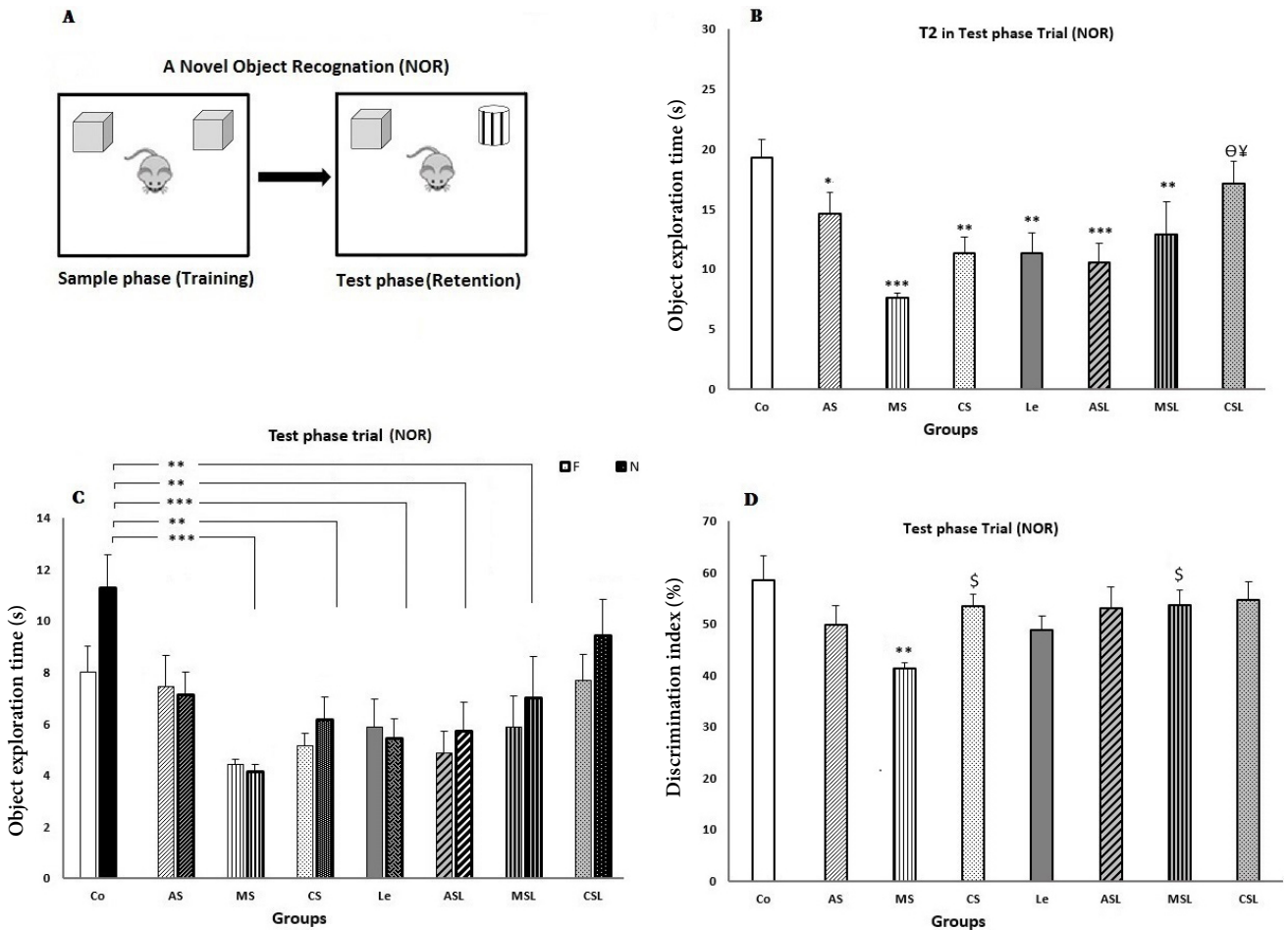


Figure 3. Effects of different durations of stress and BLA lesion in the novel object recognition (NOR) test that was carried out 24 h after the last exposure to restraint stress. (A) Representation of sample and test phase trial conditions in NOR. (B) The total time of object exploration (s) for test phase trials (T2) in NOR. (C) Object exploration time (s) of familiar and new objects (F and N, respectively) in the test phase trial of NOR. (D) Percent of discrimination index (DI) for test phase trials of NOR (n = 7). Results are expressed as mean ± SEM (ANOVA test, Tukey's post-hoc test for comparisons between groups and paired t-test for comparisons within groups; *P < 0.05, **P < 0.01, and ***P < 0.001 when compared to the Co group; [⊙]P < 0.05; [†]P < 0.05, and [§]P < 0.01 when respectively compared to the CS, Le, AS, and MS groups. Co: Control group; AS: Acute stress group; MS: Mid stress group, CS: Chronic stress group; Le: Lesion group; ASL: Acute stress + lesion group; MSL: Mid stress + lesion group, CSL: Chronic stress + lesion group.

CSL) compared to their similar stressed groups without BLA lesion (AS, MS, and CS, respectively). It indicated that locomotor activity partially decreased with the BLA lesion in the OLT (Figure 2B).

As shown in Figure 2C, the exploration time of the new location (N) showed a significant decrease (P < 0.01) compared to its familiar location (F) only in the BLA-lesion group (Le) group. It suggested that the lesion of the BLA decreased the recognition of the novel location with respect to the familiar location in this group

The exploration time of N showed significant decreases in the Le and ASL groups (P < 0.01 in both) and also the MSL and CSL (P < 0.05 in both) groups compared to the Co

group. It indicated that the recognition of a novel location decreased in all lesion groups with respect to the control group (Figure 2C).

The discrimination index (DI) showed a significant decrease (P < 0.01) in the Le group compared to the Co group (Figure 2D). Therefore, the spatial memory significantly decreased in the BLA lesion group.

The DI index of OLT revealed that the performances were not significantly lower in all stressed groups with BLA lesion (ASL, MSL, and CSL) compared to the similar nonlesion stressed groups (AS, MS, and CS). It showed that probably the BLA lesion slightly impaired the spatial memory in the stressed groups (Figure 2D).

3.2. The time of object exploration and discrimination index in the NOR

In the test phase of NOR, T_2 showed significant decreases in all the stressed groups without BLA lesion (AS, MS, CS) and Le, ASL, and MSL groups compared to the Co group ($P < 0.05$ in the AS group; $P < 0.01$ in the CS, Le, and MSL groups; $P < 0.001$ in the MS and ASL groups). It indicated that locomotor activity was significantly impaired in the retention trial of NOR. Furthermore, T_2 was significantly ($P < 0.05$) different in the CSL group compared to the CS group (Figure 3B). It suggested that locomotor activity was increased in the CS group by BLA lesion in the NOR test.

As shown in Figure 3C, there were significant decreases in the exploration time of the new objects (N) of the CS, ASL, and MSL groups ($P < 0.01$ in all), and the AS and Le groups ($P < 0.001$ in both) compared to the Co group.

The discrimination index (DI) of the NOR showed a significant decline ($P < 0.01$) in the MS group compared to the Co group (Figure 3D). Therefore, cognitive memory significantly decreased only in the mid stress (MS) group, suggesting that different mechanisms may be involved in this group.

The DI of NOR showed significant enhancements ($P < 0.01$ in both) in the CS and MSL groups compared to the MS group (Figure 3D). Therefore, cognitive memory increased with duration of stress in chronic stress and the BLA lesion in the mid stress group.

Finally, the effects of different durations of stress in groups with and without the BLA lesion reflected more changes in the identity of the novel object than the moved object (Figures 2C and 3C). It suggested complex mechanisms involved in the interaction of the BLA function and duration of stress.

3.3. Assessment of serum corticosterone levels

As shown in Figure 4, the serum CORT levels significantly increased ($P < 0.01$ and $P < 0.05$, respectively) in the MS and CS groups compared to the Co group. It indicated that mid stress (7 days stress) is the strongest stress model. It may also show an impaired feedback regulation in the HPA axis after exposure to mid stress.

In the MSL and CSL groups, the serum CORT level was significantly ($P < 0.001$ in both them) lower than in the MS and CS group, respectively (Figure 4). It demonstrated that there was a connection between the BLA and involved corticosterone secretion in mid and chronic stress with respect to acute stress. Furthermore, the serum CORT level showed no significant enhancement in the acute stress (AS) group compared to the Co group (Figure 4), indicating that different durations of stress can cause differences in the level of serum CORT.

4. Discussion

The present OLT findings showed that different durations of stress (acute, mid, and chronic) slightly decreased

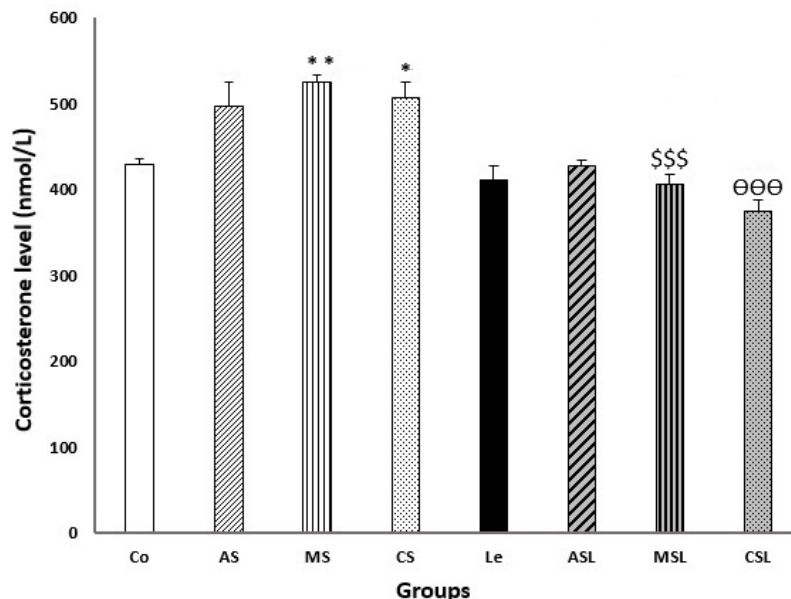


Figure 4. Effects of different durations of stress and BLA lesion on serum corticosterone (CORT) levels (nmol/L) in different groups ($n = 7$). Results are expressed as mean \pm SEM (ANOVA test, Tukey's post-hoc test; * $P < 0.05$, ** $P < 0.01$ when compared to the Co group; $^{000}P < 0.001$ and $^{555}P < 0.001$ when respectively compared to the CS and MS groups).

Co: Control group; AS: Acute stress group; MS: Mid stress group; CS: Chronic stress group; Le: Lesion group; ASL: Acute stress + lesion group; MSL: Mid stress + lesion group, CSL: Chronic stress + lesion group.

locomotor activity and spatial memory (Figure 2). Therefore, duration of stress had no relevant effect on spatial memory. These results were in agreement with a previous study (43). Moreover, the present NOR findings showed significant impairments in locomotor activity of the different durations of stress (acute, mid, and chronic) and cognitive memory of the mid stress group (Figure 3). Therefore, stress exposure had complex and diversity effects (improving, impairing, and even noneffective) on various types of memory in different behavioral tasks (3,29). Furthermore, previous studies demonstrated that the stressful conditions could induce or exacerbate cognitive deficits and emotional processes related to learning and memory (2,5,46–48).

According to the present results, the highest impairment of locomotor activity, recognition of novel object, and cognitive memory in the NOR test were observed in the mid stress condition. It is reported that stress hormones increase cognitive impairment (amnesia) (1,49) probably due to different mechanisms such as changes in glutamate, NMDA receptor, neuron apoptosis, neurotoxicity, TNF- α , and IL-1 β (32,50). The current hormonal results confirmed our cognitive data (Figures 3 and 4). Li et al. indicated the harmful role of CORT on learning and memory (51). Some reports have indicated that glucocorticoid actions impair cognition phases of memory more than memory storage (1,13,29,52,53). According to the present findings, it seems that in addition to the serum corticosterone level, various mechanisms may affect the responsiveness of cognitive and spatial memory such as the role of different nuclei and neuronal pathway, releasing of different neurotransmitters, changes in corticosteroid receptors, and types of behavioral task (40,48,54). Unfortunately, these parameters were not estimated in this study.

On the other hand, the enhancement of CORT levels was higher in chronic and particularly mid stress than in acute stress (Figure 4). A previous study indicated that memory impairments follow mid stress or mid glucocorticoid treatment (40). It is possibility related to stress hormones (e.g., epinephrine and glucocorticoids), which play a significant role in memory modulation (40). Therefore, the stress effects on the memory deficit were related to duration of stress. Some studies have shown that different CORT levels were induced by different durations of stress and it leads to various influences on memory (29,55). The present findings were in agreement with other experiments; Yoon et al. (2014) reported that an important interaction was observed between stress duration and behavioral changes (56).

The current study's OLT data showed that the discrimination index (DI) was impaired in the BLA lesion group compared to the control group in the retention trial (Figure 2), whereas the NOR findings showed impairment

of the DI in the mid stress group. It indicated the critical role of the BLA and stress respectively on spatial and cognitive memory. Some reports have demonstrated that the basolateral amygdala mediates spatial learning and memory (26,57,58). However, a previous study reported that the BLA lesion did not impair spatial memory. The BLA is not an essential component of spatial memory, but BLA inactivation can influence spatial performance in rats (7). In addition, the BLA is connected with other regions in the nervous system for regulating memory (16,17). However, it is not a permanent storage site of such memory traces (59). Some studies have shown that GABA is involved in function of the BLA nucleus and impairs retention memory. Additionally, the administration of GABAergic antagonists enhances memory and administration of GABAergic agonists decreases it (53). Therefore, it is possible that GABAergic neurons are involved in spatial memory merely. These conflicting results could be related to various variables including the levels and duration of stress, sex, age, and nature of the subject and also the behavioral task used for experiences (1). Moreover, stress can cause different responses in different individuals with the same stimulus and rate (50).

Other data showed that in stress experiences the spatial (DI and T2 in OLT) and cognition (DI and T2 in NOR) memory slightly decreased and increased, respectively, with different durations of stress associated with BLA lesion groups (ASL, MSL, and CSL) compared to nonlesion stressed (AS, MS, CS) groups (Figures 2 and 3). It indicated that the electrical lesion of BLA partially improved cognition and impaired spatial memory in all stressed groups. Some previous studies reported the basolateral amygdala is an important region for mediating stress hormones on memory (40,60). Segev et al. demonstrated that the effects of stress on spatial memory are not probably mediated by the stress-related hormones in the BLA (40). Therefore, it seems that when BLA lesion is associated with stress, other mechanisms are involved too, including the activation of related neurotransmitter in memory storage in the BLA (1,13,29,53,54). Apparently, the BLA directly activates some regions in the nervous system (e.g., hippocampus) (17). Roozendaal et al. reported that the BLA lesion blocks the memory modulation due to the secretion of epinephrine or glucocorticoid (7). Moreover, although the BLA may be a global gateway in mediating the effects of stress hormone on memory (61), glucocorticoids require noradrenergic activation within the BLA to influence memory in stress conditions (26,62).

The comparison of DI between mid stress with and without BLA lesion showed a significant enhancement by BLA lesion (Figure 3). One of the suggestions is the effect of stress-related hormones on the amygdala through the interactions of noradrenergic mechanisms

within the amygdala (7,13,26), that is as a modulator of neurotransmitter and hormonal systems on memory storage (13,53). In addition, the BLA lesion may modify the balance of glutamate and GABA in memory processing (53,63). Therefore, it probably demonstrates the important role of the noradrenergic system within the basolateral amygdala via the BLA-GABAergic system and BLA-glutamatergic system on cognitive memory in the mid stress group.

The comparison of T_2 and DI in acute stress with and without BLA lesion in the NOR test showed slightly reverse differences with respect to mid and chronic stress. It indicated the role of the noradrenergic system of the locus coeruleus in acute stress (64). It is important to note that the changes in the behavioral test depended on the duration and/or the frequency of stimulus exposures (65).

The hormonal data of the present study confirmed our memory findings, indicating that the memory changes were probably due to the changes in serum CORT levels (Figure 4). The serum CORT levels significantly increased in chronic stress and particularly in mid stress, and there was no significant enhancement in the acute stress group. The serum CORT levels were assayed the day after the last stress session in all groups; therefore, the CORT levels probably were evaluated lower than the real amounts in all groups. In addition, the CORT levels lead to more negative feedback regulation in order to sustain the basal level of CORT after stress in chronic stress (66). It seems that this regulation can lead to decreased sensitivity to stress (67). Hence, they showed nearly adaptive behavior and habituation in chronic stress conditions. Previous other and our studies reported repeatedly stress exposure decreased behavioral and physiological consequences of

stress. Hence, the long-term duration of stress proposed adaptation to the stress, but not in normal conditions (5,68).

In the current study, an elevation in serum CORT levels was observed in mid and chronic stress, whereas the lesion of BLA significantly decreased serum corticosterone level in the mid and chronic stress groups. However, no reports were found addressing this context except one. There was an indirect pathway from BLA to paraventricular nucleus (PVN) in the hypothalamus (69). Therefore, it seems that the basolateral amygdala directly and indirectly stimulates corticotropin releasing hormone (CRH) secretion in normal conditions (69). Hence, the BLA lesion may inhibit and/or inactivate CRH secretion from the PVN, resulting in decreased release of CORT from adrenal glands.

In conclusion, our results of the behavioral tests emphasize that different durations of stress had different effects on memory deficit. Moreover, mid restraint stress (6 h/day for 7 days) was the most deleterious emotional stress. Furthermore, the lesion of the BLA caused more damage to cognitive memory compared to spatial memory in the stressed groups. It seems that the BLA lesion may inhibit and/or inactivate CRH secretion from the PVN. Further research needs to be conducted in order to identify these mechanisms in animals. Therefore, for a better understanding some neurotransmitters and other factors such as GABA and glutamate should be assayed.

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