



Original Article

Inactivation of β 1-adrenergic receptor in the basolateral amygdala nucleus attenuated anxiety-like behaviour in response to foot-shock stress in the male rat

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Abstract

Introduction: The basolateral amygdala (BLA) is implicated in stress-related disorders such as anxiety-like behavior. Substantial data exist demonstrating a close relationship between anxiety and adrenergic receptor function in patients with anxiety disorders; however, little is known about the effects of the β 1 adrenergic receptor in the BLA on anxiety. This experiment examined the effects of the β 1 adrenergic receptor in the BLA on anxiety-like behavior.

Methods: Male Wistar rats were exposed to foot-shock stress four consecutive days that were uncontrollable. The β 1-adrenoreceptor agonist (dobutamine; 0.5 μ l/side) or antagonist (atenolol; 0.25 μ l/side) bilaterally infused into the BLA five minutes before foot-shock stress. Anxiety-like behaviors were assessed 24h after four consecutive day's uncontrollable stress using elevated plus-maze (EPM) and open field test (OFT).

Results: Findings of EPM revealed that foot-shock stress leads to anxiogenic effect with reduction the time spent and the number of entries into the open arms and increased head-dipping. Intra-BLA infusions of atenolol before stress affected animal behavior differently, such that it significantly increased the time spent and the number of entries into the open arms and decreased head-dipping. Also, OFT results showed the intra-BLA infusion of atenolol increased the time periods spent in the center, number of center entries and reduced the number of rearing as compared with the stress group.

Conclusion: These results suggest that the anxiety-like behavior observed after the foot-shock stress is mediated, in part, by exaggerated β 1 adrenergic receptor acting at the BLA.

Keywords:

Anxiety-like behaviour;
Basolateral amygdala;
 β 1-Adrenergic receptor;
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Introduction

Repeated exposure to stress causes cognitive

dysfunction and depression. Chronic stress additionally leads to increased anxiety (Conrad et al., 1999; Wood et al., 2003). The basolateral amygdala (BLA), the prefrontal cortex and hippocampus are key

regions involved in the modulation of stress effects. These stress-responsive regions mediate anxiety and affective responses. BLA modulates the stress responses through its efferent to the prefrontal cortex (Wei et al., 2017) and hippocampus (Rei et al., 2015). Also, chronic stress and its effects on the amygdala, both contribute to the formation of affective and anxiety disorders (McEwen, 2003). Studies have demonstrated that amygdala activity is increased in patients suffering from anxiety and affective disorders (Davidson, 2002; Etkin and Wager, 2007), which represent some of the foremost causes of disability worldwide (Lopez et al., 1998). Therefore, BLA is central in the modulation of the neural circuitry underlying stress and the formation of affective and anxiety disorders. On the other hand, this brain region is important to characterize the effects of chronic stress and is a key structure in the mediation of anxiety.

Several lines of evidence showed that the adrenergic/noradrenergic system plays a pivotal role in anxiety-like behaviors and these effects are mediated by α and β adrenoceptors in different regions of the brain. On the other hand, evidence showed that the BLA receives a dense norepinephrine innervation originating from the locus coeruleus (Siuda et al., 2016; McCall et al., 2017) and following a stressful stimuli, release of norepinephrine is increased in the BLA (Onur et al., 2009). Activation of endogenous adrenergic tone from the locus coeruleus revealed that both necessary and sufficient for stress-induced anxiety, and optogenetic-induction of locus coeruleus-mediated anxiety-like behavior is sensitive to systemic inhibition of β -adrenergic receptors (McCall et al., 2015). In humans, the BLA exhibits hyperactivity in most forms of anxiety disorders (Rauch et al., 2003) and in rodents BLA hyperexcitability and hypertrophy is associated with enduring facilitation of anxiety-like behaviors (Rozen daal et al., 2009). Therefore, the norepinephrine is thought to be critical in providing information about stressful stimuli to the BLA and allowing for proper behavioral and neuroendocrine responses to stressors.

Among the different types of adrenoceptors, the regulatory role of β adrenoceptors, especially β 1-adrenoceptors, in the central nervous system have effects on the anxiety-like behaviors (Bremner et al.,

1996; Fu et al., 2008). β -1 and β -2 adrenoceptors elevation in the amygdala, hippocampus and other parts of the limbic system has been shown in anxiety complications (Buffalari and Grace, 2007). It is believed that the β -adrenergic receptor function changed in patients with anxiety disorders. For example, reduced β -adrenergic receptor responsiveness were reported in patients with panic disorder (Kang et al., 2005). Also, it has been shown that individuals with extremely stressful lives have lower β -adrenergic receptor sensitivity (Dimsdale et al., 1994). Furthermore, Rudoy et al. (2007) showed that selective β -1 adrenoceptors antagonists are effective in treating acute anxiety, but they don't have any effects on treating chronic anxiety.

Despite evidence confirming the norepinephrine in the BLA involved in stress-related behaviors and anxiety, there is a lack of information about how norepinephrine affects the BLA *in vivo* via the β 1-adrenergic receptor on anxiety-like behavior and locomotor activity in the stress. Hence, the present study examined the effects of infusion of β 1-adrenergic receptor agonist and antagonist in the BLA with and without stress experience on anxiety-like behavior and locomotor activity.

Materials and methods

Animals

Adult (60–70 days old and weighing 180–220g at the time of testing) male Wistar rats were used in all experiments. The animals were housed two rats per plastic cage and allowed to acclimatize under standard conditions (12 h light/dark cycles and the room temperature was maintained at $25\pm 2^\circ\text{C}$) for one week. The animals had free access to food and water except during the time of behavioral test session. The foot-shock stress exposures were done in a separate room.

Behavioral testing was performed during the light cycle between 10:00 am and 2:00 pm. The rats were randomly divided into seven groups with 8 rats in each group. In the control group rats without surgery and do not receive any foot-shock stress. In the sham group rats received saline (0.5 μ l/rat or 0.25 μ l/side) in both sides of the BLA and put into communication box, but do not receive any foot-shock stress. In the stress group, rats received foot-shock stress without surgery.

Atenolol is a well-known β_1 -selective β -blocker (Nuttall et al., 2003) and dobutamine is a well-known as a selective β_1 -adrenoceptor agonist (Schiffelers et al., 1999), which has been used very extensively worldwide. Atenolol and dobutamine were dissolved in saline. In the control of atenolol (Darou Pakhsh, Tehran, Iran) group, β_1 receptor antagonist, rats received atenolol (0.5 μ l/rat or 0.25 μ l/side) in both sides of the BLA but without the stress. In the atenolol+stress group rats received atenolol (0.5 μ l/rat) five minutes before foot-shock stress in both sides of the BLA. In the control of dobutamine (Darou Pakhsh, Tehran, Iran), β_1 receptor agonist, rats received dobutamine (1 μ l/rat or 0.5 μ l/side) on both sides of the BLA but without the stress. In the dobutamine+stress group animals received dobutamine (0.5 μ l/rat) five minutes before stress on both sides of the BLA. All procedures were conducted in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and were approved by the Baqiyatallah University of Medical Committee on the Use and Care of Animals. All efforts were made to reduce the number of animals used and their pain and suffering.

Surgical preparation and cannulation in the BLA

Rats were initially anesthetized with chloral hydrate (Sigma-Aldrich, USA; 350mg/kg, ip, with supplemental doses if needed) then, were placed in a stereotaxic instrument (Stoelting, Wood Dale, IL). An incision was made along the scalp and the underlying skull was exposed. Burr holes were drilled in the skull and the dura under the burr holes was removed. Using Bregma and lambda as landmarks, the skull was leveled in the coronal and sagittal planes. Two sterile stainless steel guide cannulas (23-gauge) were placed bilaterally one mm above the BLA. The locations of the BLA AP= -2.8mm, ML= \pm 4.8mm and DV= 7.5mm were calculated using a stereotaxic coordinate according to the Paxinos and Watson atlas (2006). At the end of the surgery, removable wire styles (30 gauge) were inserted in the cannula to maintain patency. The cannula was permanently affixed on the surface of the skull using dental acrylic cement. After surgery, animals were kept warm and returned to a clean cage (single-housed). All the rats were allowed seven days to recover after the surgery. Dental needles head No. 30 (Alibaba; INTR), polyethylene tubes, and 2 μ l Hamilton syringes were

used for the microinjection of the atenolol or dobutamine.

Stress apparatus and procedure

Foot-shock stress testing was conducted as described previously (Ehteram et al., 2017). Briefly, to adapt to the new environment, animals were transferred to the experimental room one hour before tests. After that, the rats were placed inside a communication box. The communication box was equipped with a grid floor composed of 0.5cm diameter stainless steel rods placed 1.3cm apart. The box was divided into nine smaller compartments (16 \times 16 \times 50cm). Stress induction continued for four consecutive days. During the session in the foot-shock box, rats received six uncontrollable and inescapable foot shocks, in which the duration and intensity of the induced shock were controlled by a computer connected to the communication box (10mV voltage, 10Hz frequency, and 60s long). This kind of stress was induced randomly, so animals do not get used to the habit. Injections were administered using an injection cannula (33 gauge cannula), which extended 1mm beyond the tip of the guide cannula. Drugs were delivered manually with a 2 μ l Hamilton microsyringe attached to the injection cannula via polyethylene tubing and administration of a volume of 0.5 μ l/side was delivered over a period of 60s. The confirmation of successful infusion was obtained by monitoring the movement of a small air bubble in the tubing. After each infusion, the injection cannula remained in place for an additional two min after injection to allow diffusion from the injector tip and the animals were free to move during this time. No other subjects were present in the experimental room during stress exposure, and at the end of the stress session, the animals were returned to the colony room.

Elevated plus maze (EPM) test

The EPM is an unconditional anxiety model which is used for measuring the anxiety like parameters (Matuszewich et al., 2007; Walf et al., 2007). The EPM apparatus consisted of two open arms (30 \times 5cm) and two enclosed arms (30 \times 5 \times 30cm) extending from a central intersection platform (5 \times 5 cm). The arms were attached to a central square (10cm²) and the whole apparatus was elevated 75cm above the floor.

The testing room was quiet and dimly lit, and animals were habituated to this room for at least one hour before starting the tests. On the test day, rats were placed in the center of the maze facing an enclosed arm and allowed 5 minutes of free exploration and the number of entries and the time spent on the open and closed arms were recorded and analyzed with video-based Ethovision System (Borjasma, Tehran, Iran). An entry was defined as when all four paws of the rat were inside an arm. Also, we calculated and analyzed some other anxiety behavior, such as, head-dipping (dipping the head below the open arm of the EPM, with all four paws on an open arm) (Walf et al., 2007).

The maze was cleaned with a 70% ethanol solution both before and after each trial. The time spent in open arms relative to the total time spent in both open and closed arms was used as an index of anxiety. All animals were tested in the EPM 24h after the four consecutive day's foot-shock experience.

Open field test (OFT)

To evaluate the anxiety-like behavior and general locomotion the OFT also used. The apparatus consists of an opaque 60×60×40cm Plexiglas square arena. The whole arena was subdivided into 8 "corner" and one "center" zone each 20×20cm. At the start of the test, the rat was placed in the center and

allowed to explore the arena for 5min. Total distance moved in the apparatus and the time that spends in the central zone were recorded and analyzed with video-based Ethovision System (Borjasma, Tehran, Iran). Locomotor behavior was assessed by measuring the total distance traveled during this time, while anxiety-like behavior was assessed as the number of central entries, the time spent in the center of the open field, number of rearing (vertical standing of rodent on two hind-legs), grooming times, freezing times (absence of any movement), the percentage of time spent in the center of the chamber relative to the perimeter, line crossing, the frequency with which the animal crossed one of the black lines with all four paws and total locomotor activity. Greater scores of freezing behaviour, fewer entries, as well as lower numbers and shorter lengths of time spent in the central area or in the inner area reflected decreased exploratory activity and increased anxiety. The center was defined as a square comprised of 25% the total area of the OFT (i.e., each length was 50% that of the total OFT) (Nagata et al., 2009; Silberman et al., 2010). Behavioral variables were analyzed by an experienced experimenter blind to the treatment condition. The test was performed in the light phase of the light/dark cycle, and after each test session, the arena was cleaned with 70% ethanolic alcohol to remove olfactory cues. Figure 1 shows a timeline of

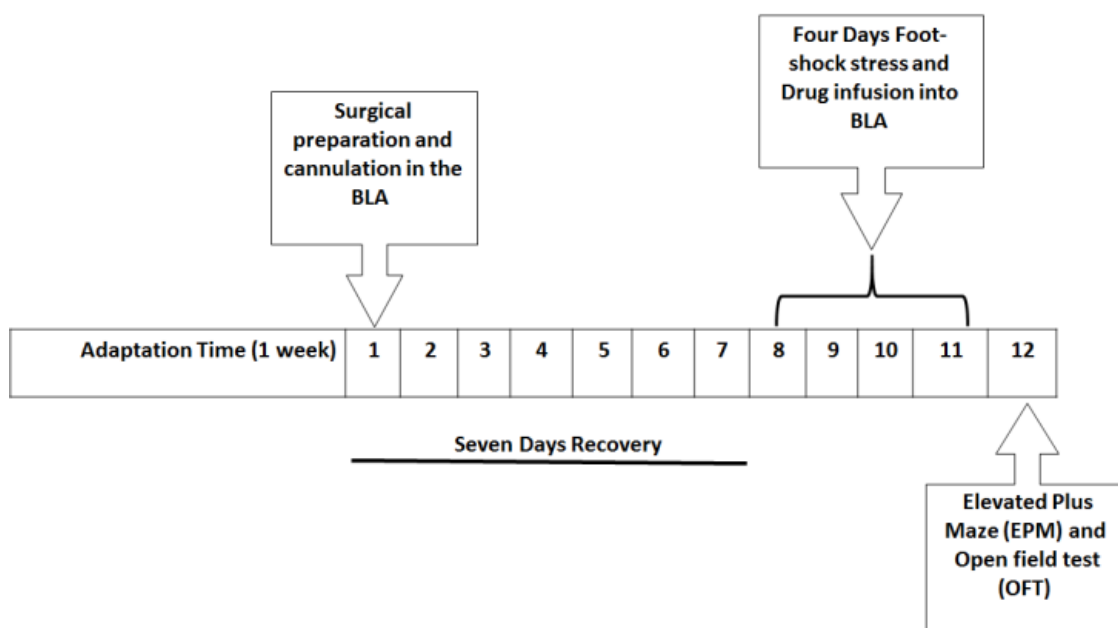


Fig.1. Schematic timeline of drug administration and behavioral test order in animals. After 1 week of adaptation in the animal room, rats were subjected to surgical preparation and cannulation in the BLA. All the animals were allowed seven days to recover after the surgery. Then, stress induction continued for four consecutive days. Twenty-four after four consecutive days stress the EPM and OFT were used.

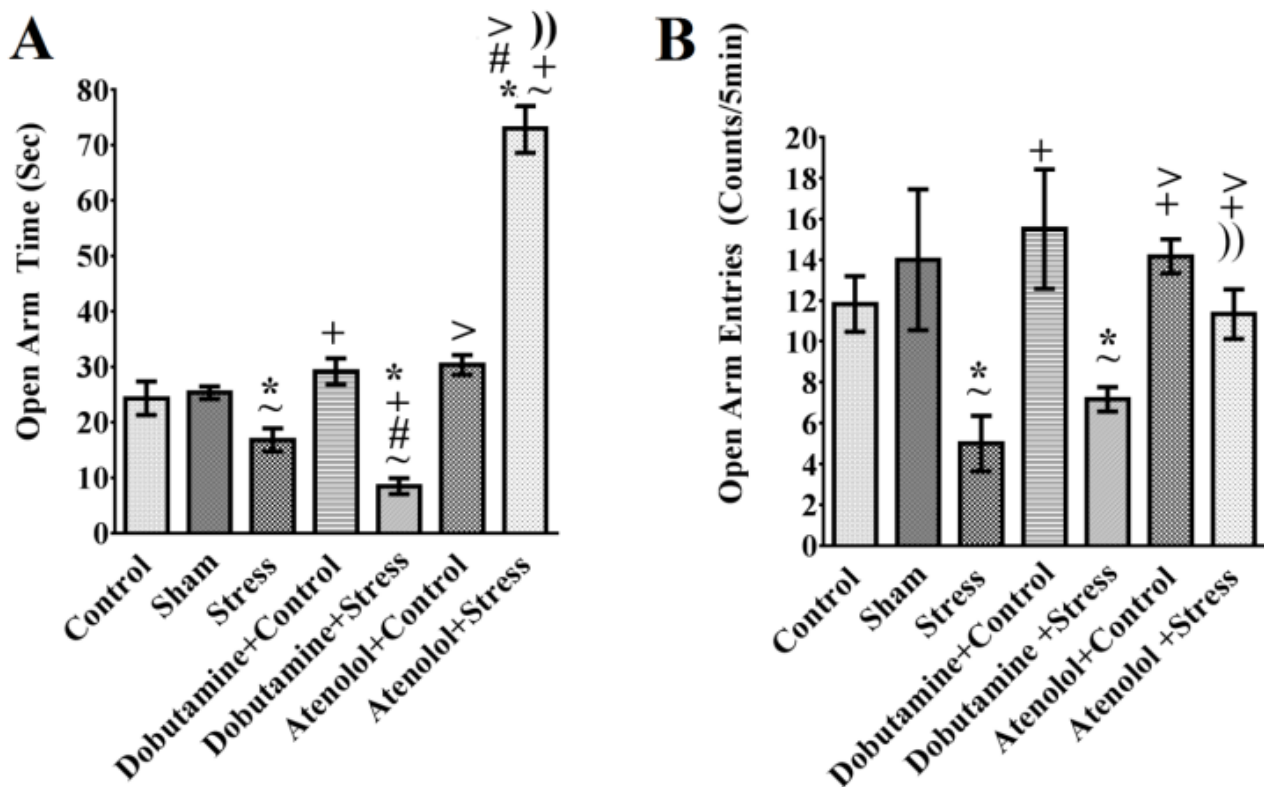


Fig.2. Effect of intra-BLA infusion of β_1 -adrenergic receptor agonist and antagonist prior to foot-shock stress in the anxiety-like behavior on the EPM. A) The time spent in the open arms. B) The number of entries by the rats in the open arms within five minutes. The data represent the mean \pm SEM. * P <0.05 as compared to the control group, ~ P <0.05 as compared to the sham group, + P <0.05 as compared to the stress group,)) P <0.05 as compared to the atenolol+control, # P <0.05 as compared to the dobutamine+control and > P <0.05 as compared to the dobutamine+stress.

the experiment indicating the pre-surgery adaptation time, foot-shock stress and drug infusion into BLA and behavioural testing.

Statistical analysis

Statistical analyses were performed using SPSS software (SPSS-PC, version 22) and Prism 6, GraphPad Software. Data are shown as the mean \pm SEM for 8 animals. One-way analysis of variance (ANOVA) followed by Tukey test was performed to assess specific group comparisons. Differences with P <0.05 were considered statistically significant.

Results

The effects of the β_1 -adrenergic receptor in the BLA on anxiety-like behavior induced by stress

Twenty four hours after the four consecutive day's foot-shock stress experience, the rats were undergoing EPM to evaluate anxiety-like behaviors. The results revealed that four consecutive day's foot-

shock stress reduced the time rat spent in the open arms (16.83 ± 2.07 s, $n=8$; P <0.05) than the control (24.33 ± 3.02 s, $n=8$) and sham (25.33 ± 1.11 s, $n=8$) animals indicating an anxiogenic effect of foot-shock stress. As shown in Figure 2A., intra-BLA infusions of dobutamine, β_1 receptor agonist, five minutes before stress significantly decreased open arm time (8.51 ± 1.43 s, $n=8$; P <0.05) when compared to the control, sham and stress groups, while injection of β_1 receptor antagonist, atenolol, into BLA before stress significantly increased open arm time (73.01 ± 4.14 s, $n=8$; P <0.001) than the control, sham and stress animals. Also, the number of entries into the open arms (counts/5min) in the control (11.83 ± 1.36 , P <0.05) and sham (14.01 ± 3.44 , P <0.05) groups were greater than those of the stress group (5.02 ± 1.36). Intra- BLA infusion of dobutamine before stress couldn't increase the number of entries into the open arms (7.16 ± 0.62 , P <0.05), whereas atenolol increased the number of entries into the open arms (11.33 ± 1.21 , P <0.05) when compared to stress animals (Fig. 2B).

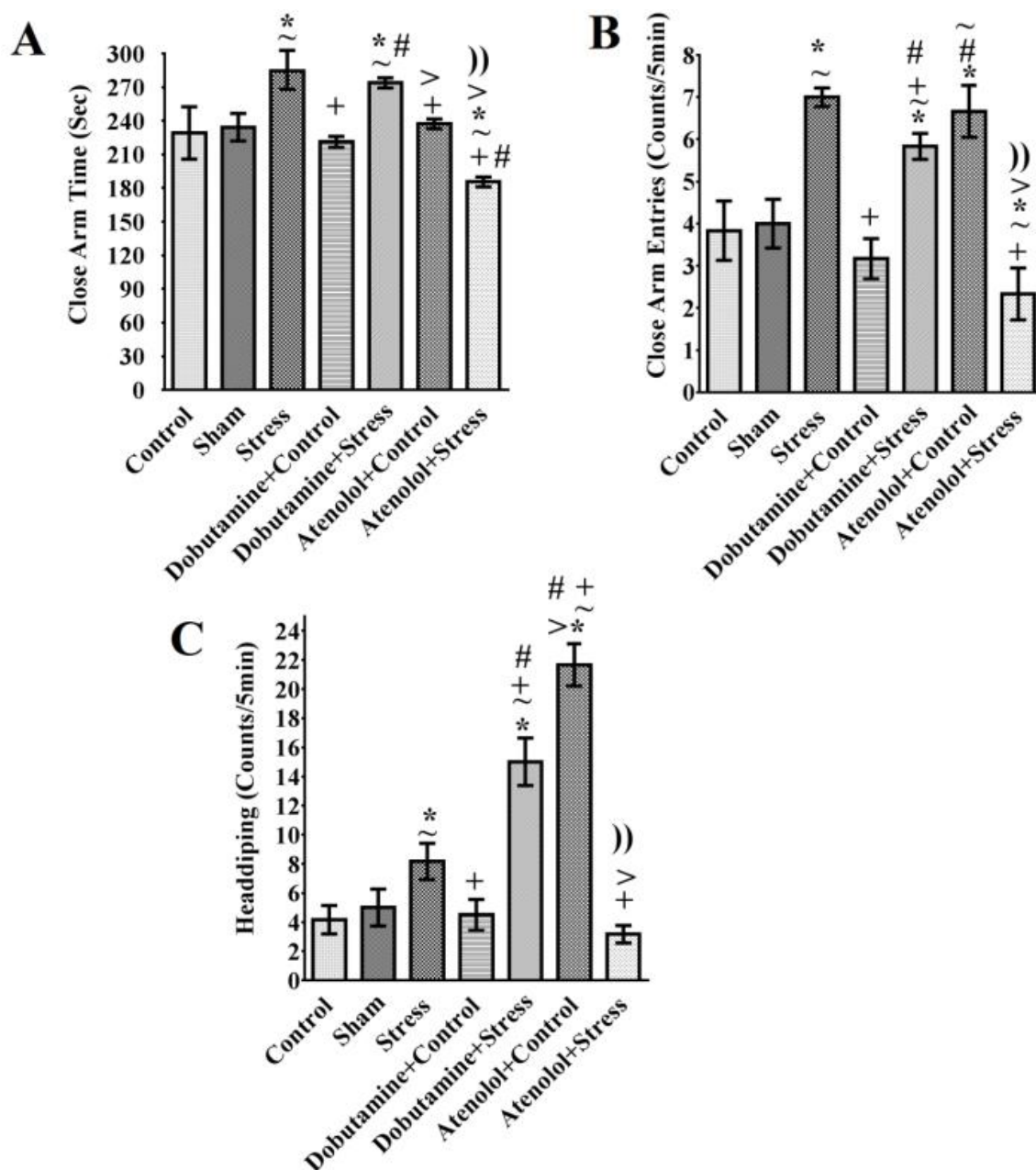


Fig.3. Foot-shock stress-induced anxiety-like behavior in the EPM, which was prevented by intra-BLA infusion of β1-adrenergic receptor antagonist. A) The time spent in the closed arms. B) The number of entries in the closed arms. C) Number of head dipping. Data expressed as mean±SEM. **P*<0.05 as compared to the control group, ~*P*<0.05 as compared to the sham group, +*P*<0.05 as compared to the stress group, >>*P*<0.05 as compared to the atenolol+control, #*P*<0.05 as compared to the dobutamine+control and >*P*<0.05 as compared to the dobutamine+stress.

Additionally, in the stress group rat spent more time in the closed arms (284.50±16.32s, *P*<0.05) as compared with the control (229.20±23.32s, *P*<0.05) and sham (234.32±12.32s, *P*<0.05) animals. Intra-BLA administration of β1 receptor agonist before stress, increased spending time in the closed arms (273.83±4.50s, *P*<0.05) of the EPM, and β1 receptor antagonist individuals had reduced spending time in the closed arms (185.50±4.43s, *P*<0.05) when compared to the stress animals (Fig. 3A). Moreover,

the number of entries into the closed arms (counts/5min) in the stress group (7±0.22, *P*<0.05) was greater than those of the control (3.83±0.70, *P*<0.05) and sham (4±0.57, *P*<0.05) groups, which intra-BLA infusion of β1 receptor agonist before stress, increased the number of entries into the closed arms (5.83±0.30, *P*<0.05) and β1 receptor antagonist reduced this parameter (2.33±0.61, *P*<0.05, Fig. 3B).

Stress rats exhibited a mean of 8.16±1.24 head-

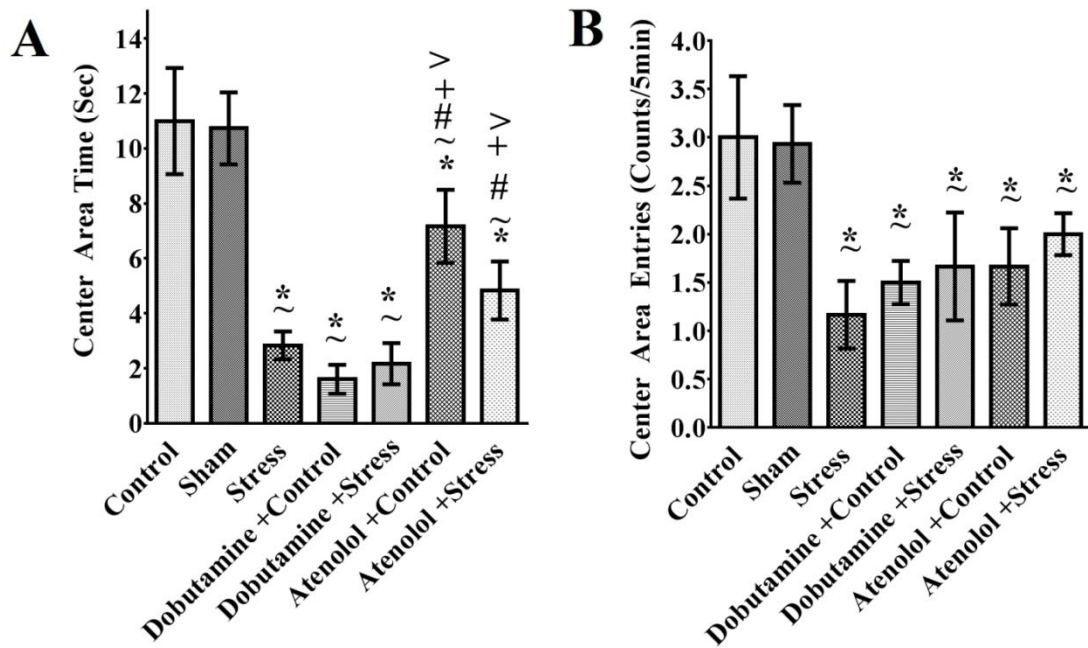


Fig.4. Effects of intra-BLA infusion of β_1 -adrenergic receptor agonist and antagonist prior to foot-shock stress in the anxiety-like behavior on the OFT. A) The time spent in the center area of an open field. B) The number of entries in the center area. Data expressed as mean \pm SEM. * P <0.05 as compared to the control group, ~ P <0.05 as compared to the sham group, + P <0.05 as compared to the stress group,)) P <0.05 as compared to the atenolol+control, # P <0.05 as compared to the dobutamine+control and > P <0.05 as compared to the dobutamine+stress.

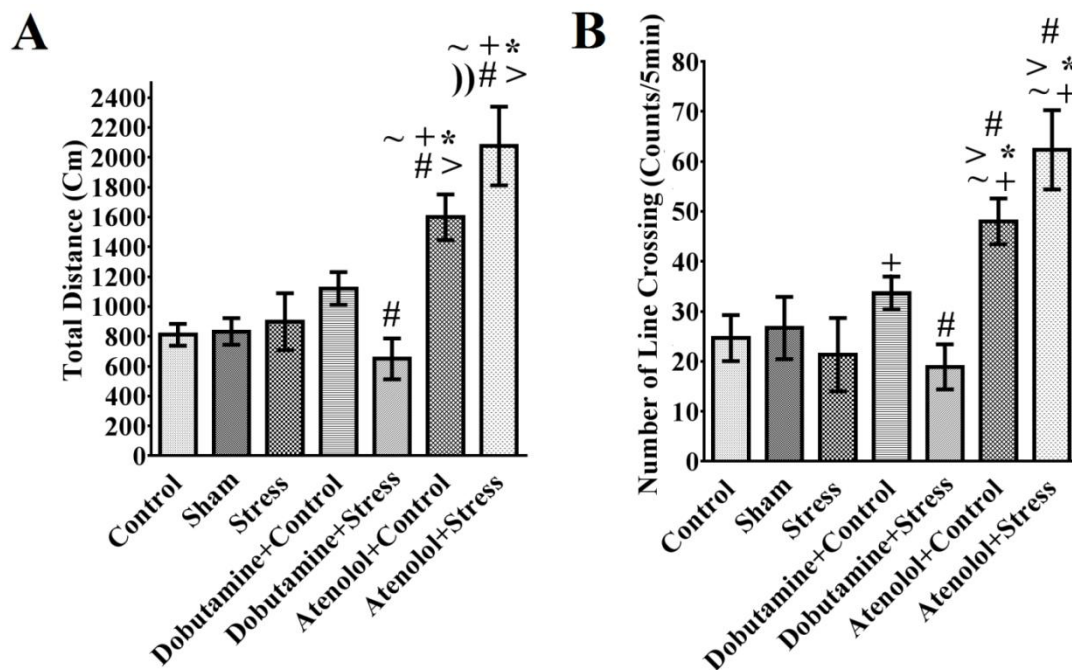


Fig.5. Effects of intra-BLA infusion of dobutamine and atenolol prior to foot-shock stress in the locomotor activity on the OFT. A) Total distance traveling in the open field. B) The number of line crossing in the open field. Data expressed as mean \pm SEM. * P <0.05 as compared to the control group, ~ P <0.05 as compared to the sham group, + P <0.05 as compared to the stress group,)) P <0.05 as compared to the atenolol+control, # P <0.05 as compared to the dobutamine+control and > P <0.05 as compared to the dobutamine+stress.

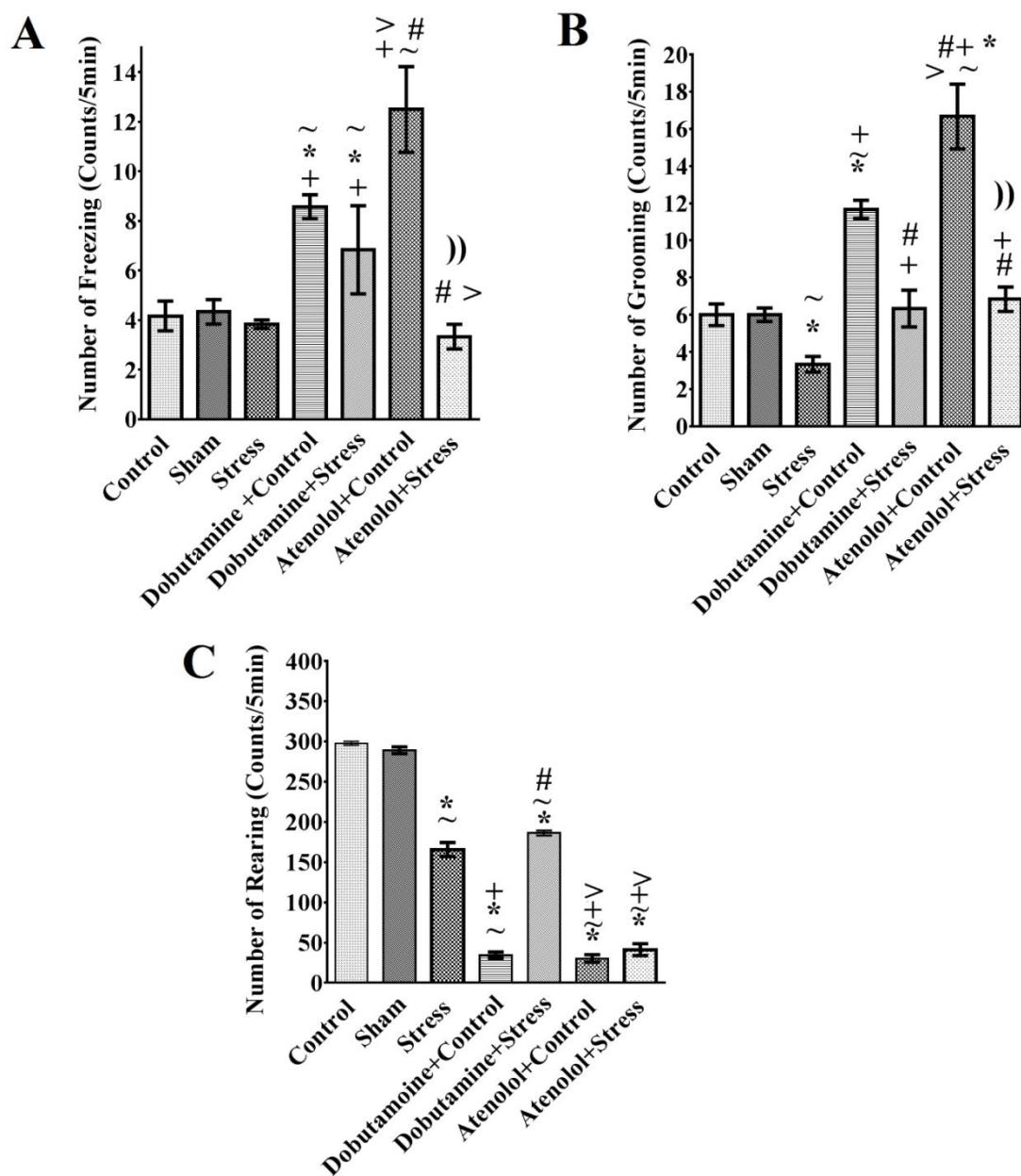


Fig.6. Effects of administration of β1-adrenergic receptor agonist and antagonist before stress into the BLA of rats on measures of the number of freezing (A), number of grooming (B) and number of rearing (C) behaviors in the OFT in the different groups (n=8). Data expressed as mean±SEM. **P*<0.05 as compared to the control group, ~*P*<0.05 as compared to the sham group, +*P*<0.05 as compared to the stress group,))*P*<0.05 as compared to the atenolol+control, #*P*<0.05 as compared to the dobutamine+control and >*P*<0.05 as compared to the dobutamine+stress.

dipping within 5min, whereas control and sham animals showed a mean of 4.16±0.9 and 5.01±1.26, respectively (*P*<0.05). Intra-BLA infusion of dobutamine before stress more significantly (*P*<0.05) increased the mean of 15.01±1.63 head-dipping. Intra-BLA infusion of atenolol without stress condition (atenolol+control group) significantly (*P*<0.05) enhanced means of a head-dipping (21.66±1.45), while atenolol injection before stress (atenolol+stress group) reduced mean of head-dipping (3.16±0.60) when compared to stress animals (Fig. 3C). These

findings indicate that intra-BLA injection of β1 receptor agonist before food-shock stress is sufficient to increase anxiety-related behavior, while β1 receptor antagonist reduces anxiety-like behavior in the EPM.

Changes anxiety behavior in effects β1-adrenergic drugs at BLA due to stress by OFT

To strengthen the above findings, we further evaluated anxiety-like behavior using the OFT 24h after the four consecutive day's foot-shock stress.

Like EPM, the results of the OFT revealed no significant differences between the control and the sham groups, indicating that the stereotaxic surgery without intra-BLA β_1 receptor agonist or antagonist injection had no significant effects on any of the behavioral variables. The time period spent in the center exhibited significant ($P<0.05$) decreases in the foot-shock stress group compared to the control and sham groups. Significant ($P<0.05$) declines were observed in the number of center entries in the foot-shock stress group relative to the control and sham groups, thus explaining the reduced exploratory activity by the foot-shock stress group.

The time periods spent in the center (Fig. 4A) also were significant differences in the control and sham groups when compared to the intra-BLA infusion of β_1 receptor agonist as well as the intra-BLA infusion of the β_1 receptor antagonist ($P<0.05$). However, intra-BLA infusion of β_1 receptor antagonist before stress, increased the time periods spent in the center as compared with the stress group ($P<0.05$). There was no significant difference between the β_1 receptor agonist and the stress groups.

The number of center entries also made significant differences among the intra-BLA injection of dobutamine as well as the intra-BLA injection of atenolol groups as compared with the control and sham groups ($P<0.05$, Fig. 4B). Moreover, intra-BLA atenolol treatment before stress compared to stress group, lead to a significant ($P<0.05$) increase in the number of center entries.

The total distance traveled by the subjects recorded for all groups. There was no significant difference in the total distance traveled between the control and stress groups. The result showed that the total distance traveled by the subjects decreased significantly in the dobutamine+stress group ($P<0.05$) relative to that recorded for the dobutamine+control group. Intra-BLA infusion of atenolol in both stress and without stress condition significantly enhanced locomotor activity ($P<0.05$) as compared to the other groups, which this enhancement was greater in the dobutamine+stress group (Fig. 5A).

The number of line crosses used as measures of locomotor activity, but also measure of exploration and anxiety. A high frequency of this behaviour indicates enhanced locomotion and exploration and/or a lower level of anxiety. As shown in Figure 5B, the line crossing in the stress group did not

change significantly ($P<0.05$) when compared with the control and sham groups. Atenolol+control and atenolol+stress groups exhibited a significant ($P<0.05$) enhancement in its number of lines crossing compared to the control, sham, stress, dobutamine+control and dobutamine+stress groups.

The number of freezing exhibited no significant differences ($P<0.05$) in the stress group compared to the same parameters recorded in the control and sham groups, whereas administration of β_1 receptor agonist into BLA in both stress and without stress conditions significantly increased number of freezing compared to the control, sham and stress groups ($P<0.05$). Interestingly, administration of β_1 receptor antagonist into BLA without stress condition increased the number of freezing when compared to the control, sham, stress, dobutamine+control and dobutamine+stress groups, while injection of β_1 receptor antagonist into the BLA before foot-shock stress significantly ($P<0.05$) reduced the number of freezing relative to atenolol+control group (Fig. 6A).

The number of grooming by the subjects decreased significantly in the stress groups ($P<0.05$) relative to that recorded in the control and sham groups. Intra-BLA infusion of β_1 receptor agonist and antagonist before stress and without stress condition significantly increased the number of grooming as compared to stress group. Also, as shown in Figure 6B the number of grooming exhibited significant ($P<0.05$) increments in the dobutamine+control and atenolol+control groups when compared with those recorded in the control and sham groups.

Finally, foot-shock stress led to significant ($P<0.05$) reduction in the number of rearing events in the stress groups compared to the control and sham animals. Intra-BLA infusion of atenolol in both stress and without stress situation more significantly ($P<0.01$) reduced the number of rearing relative to the control, sham and stress animals. Moreover, as shown in Figure 6C intra-BLA infusion of dobutamine without stress condition significantly ($P<0.01$) decreased the number of rearing when compared to the control, sham and stress animals.

Discussion

This study demonstrates that four consecutive day's foot-shock stress reduced the time spent in the open arms, the number of entries into the open arms and

enhanced mean of head-dipping indicating an anxiogenic effect of foot-shock stress. Intra-BLA infusions of dobutamine, β 1 receptor agonist, five minutes before stress significantly decreased open arm time, while the intra-BLA infusion of atenolol five minutes before stress increased the time spent in the open arms, the number of entries into the open arms and reduced the mean of head-dipping. Moreover, our results showed that foot-shock stress leads to animals spent more time in the closed arms as well as increased the number of entries into the closed arms. Intra-BLA administration of β 1 receptor agonist before stress, increased spending time in the closed arms and the number of entries into the closed arms, and β 1 receptor antagonist individuals had reduced spending time in the closed arms, and β 1 receptor antagonist reduced this parameter. These findings indicate that intra-BLA injection of β 1 receptor agonist with and without foot-shock stress could increase the anxiety-related behavior, while β 1 receptor antagonist reduces anxiety-like behavior in the EPM.

In the other part of the present study, we further evaluated anxiety-like behavior using the OFT 24h after the four consecutive day's foot-shock stress. Compared to the control, foot-shock stress led to significant differences in the overall locomotor activity in the OFT. Furthermore, the animals spent less time in the central zone, but more in the peripheral one and the number of center entries reduced in stress animals. This suggests an increased anxiety-like behaviour under stress. It seems that stress might modify brain functions through such pathways as an increased adrenaline release. Intra-BLA infusion of β 1 receptor antagonist before stress, increased spending time in the central zone and the number of center entries. Grooming and the number of rearing exhibited significant differences under stress conditions, which intra-BLA infusion of the β 1 receptor antagonist (atenolol+stress group) before stress significantly increased the number of grooming and rearing relative to stress group.

In line with the present study, a growing body of evidence has revealed that stress exposure such as foot-shock stress, social defeat stress and restraint stress often results in the enhancement of anxiety-like behavior in paradigms designed to assess anxiety (Calfa et al., 2006; Bignante et al., 2010; Bali and Jaggi, 2015) and the BLA is one key

region particularly associated with anxiety and mood (Feinstein et al., 2013). In the agreement, the present behavioral results showed excessive anxiety in the EPM in stressed animals with foot-shock stress. Several studies showed that repeated exposure of intense shock stimulation induces norepinephrine release within the amygdala (Galvez et al., 1996; Tanaka et al., 2000). Studies using *in vivo* microdialysis and HPLC have revealed that foot-shock stress induces the release of norepinephrine in the amygdala and that this increase in norepinephrine levels varies directly with stimulus intensity (Quirarte et al., 1998; Hatfield et al., 1999). Furthermore, glucocorticoids released from the adrenal cortex during the stressful condition may enhance norepinephrine levels in the amygdala during emotional arousal (Roosendaal et al., 2006). In the present study, inhibition of β 1-adrenergic receptors into BLA before stress using the selective antagonist atenolol produced anxiolysis effect in the EPM and OFT. It has been shown that lesion of BLA prior to stress prevented chronic immobilization stress-induced anxiety. Also, suppressing of BLA during stress blocks the stress-induced anxiety. However, lesion or inactivation of the BLA in the absence of stress did not affect the baseline anxiety as opposed to BLA lesion or inactivation (Tripathi et al., 2019). Several reports demonstrate that excitotoxic lesion or inactivation of BLA efferent in the PFC and hippocampus produces robust anxiolytic effects (Castro et al., 2010; Felix-Ortiz et al., 2013; Felix-Ortiz et al., 2016). Wei et al. (2017) demonstrated that chemogenetic inactivation of BLA blockage the stress-induced aggressive behavior, suggesting that amygdala inactivation during stress might promote emotional resilience. In addition, BLA hyperactivity is reported in clinical studies on depression and anxiety disorders (Van Eijndhoven et al., 2009). Furthermore, inhibition of BLA activity by over-expression of rectifying potassium channels reduces anxiety, stress-induced increase in corticosterone response and dendritic branching in the BLA (Mitra et al., 2009).

Our observations are in accordance with previous studies which demonstrated that stress exposure often enhancement of anxiety-like behavior and neuronal activation were prevented by propranolol, β -Adrenergic receptor antagonism, (Wohleb et al., 2011). As mention above, the results of the present

study revealed that infusion of β 1-adrenergic receptor antagonist in the BLA has anxiolytic effects induced by stress and also reduced locomotor activity. Clayton and Williams (2000) suggested that the amygdala receives severe noradrenergic innervations and down-regulation of the adrenergic system in the long or short term may occur after stressor cessation. Several potential mechanisms are suggested for the alteration in the effects of β 1-adrenergic receptors on BLA neuronal activity by chronic stress. The β 1-adrenergic receptors coupled to Gs (stimulatory G protein), such that stimulation of these receptors enhances cAMP levels through direct activation of adenylate cyclase. The enhanced cAMP activates the cAMP/PKA signal pathway, and the stimulation of PKA can elevate CREB phosphorylation, after that the phosphorylation of CREB regulates the downstream expression of cAMP-inducible genes (Johnson, 1998). The activation of the β 1-adrenergic receptor-mediated signal pathway can produce long-term anxiety symptoms. The β 1-adrenergic receptor antagonists can prevent the cAMP/PKA signal pathway, decrease the CREB phosphorylation and then facilitate the anxiolytic effect (Maj et al., 2003).

To consist with our results, Fu and et al. (2008) by western blot analyses showed that the β 1-adrenoceptors gene expression significantly increases in the BLA, which intra-BLA infusion of metoprolol, as a selective β 1-adrenoceptors antagonist, reduces the anxiety. Also, they showed that microinjection of metoprolol in the rats BLA prevents up-regulation of β 1-adrenergic receptors. Also, Siuda et al. (2016) found that optogenetic induction of β -adrenergic signaling in the BLA is sufficient to induce acute and social anxiety-like behavior. They suggested that activation of Gs signaling in the BLA via β -adrenergic receptors have a role in acute and social anxiety-like states.

It has been shown that stimulation of β 1-adrenergic receptors in prefrontal/frontal region seem to be relevant for mood improvement. Also, elevated activation of post-synaptic α ₂-receptors in other noradrenergic pathways in the frontal cortex may improve attention deficit disorders, and may be beneficial in the cognitive deficiency related with depression, schizophrenia and Alzheimer's disease (Stahl, 1998). Roszkowski et al. (2016) revealed that norepinephrine plays a predominant role in regulating the rapid transcriptional response to acute swim

stress in the hippocampus via β -adrenergic signaling. However, a decrease in density or down-regulation of β 1-adrenergic receptors have been used as a marker of antidepressant efficacy. In this regard, some *in vitro* and *in vivo* studies showed that various chronic antidepressant treatments cause β 1-adrenergic receptor decrease in density in rat forebrain (Sulser et al., 1978; Hosoda and Duman, 1993). However, it should be pointed out that there are remarkable procedural differences between these reports and the current study. In fact, in our behavioral procedure the intra-BLA administration of β 1-adrenergic receptor agonist or antagonist performed prior to foot-shock stress exposure and one day later such animals were exposed to the EPM and OFT testing.

Collectively, the above results confirm that the β 1-adrenergic receptors in the BLA play an important role in the emotional behavior following stress exposure. Consistent with this view, a large body of evidence has shown that the BLA is essential for emotional regulation and to assign emotional value to environmental information (LeDoux, 2000; Rodríguez Manzanares et al., 2005). According to the results of the present study, it is possible that the activity of β 1-adrenergic receptors in the BLA increases in the rats with anxiety-like behavior, and inhibition of the β 1-adrenergic receptors by the antagonist in the rats' BLA facilitated anxiolytic effect.

One limitation is that EPM parameters in rats is changed by sex, which for the male rat, the important factor was anxiety, with motor activity being relatively unimportant. For females, the situation was reversed with motor activity being the more important factor (Bourin et al., 2007). Thus, only male rats were used in the present study. This limitation is particularly important because a greater proportion of women than men suffer from anxiety and stress disorders (Kessler et al., 2005). Another limitation is that lack of the biological evidence for changes in β 1-adrenergic receptors within the BLA in mediating anxiety-like behaviour. Further experiments are required to determine how β 1-adrenergic receptors in the BLA affects the anxiety-like behavior during/after foot-shock stress.

Conclusion

In conclusion, here we showed the role of β 1-adrenergic receptors within the BLA in mediating

anxiety-like behavioral states. These results suggest that β 1-adrenergic receptors influence on signaling into the BLA may have important consequences for generating anxiogenic behaviors. On the other hand, these findings support the conclusion that activation of β 1-adrenergic receptor in the BLA has a role in anxiety-like behaviour, thus providing support that inhibition of this system may be an effective anxiolytic therapy and extend our understanding of the development of therapeutics for treating anxiety and stress disorders; however, further studies of these receptors, circuits and pathways are required.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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