



ORIGINAL ARTICLE

# Temporary amygdala inhibition reduces stress effects in female mice



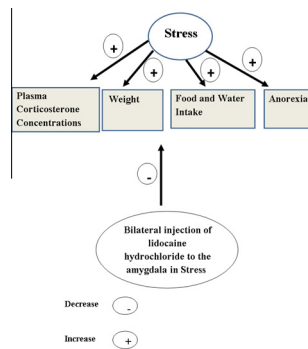
Jila Rezaeian Dalooei <sup>a</sup>, Hedayat Sahraei <sup>b</sup>, Gholam Hossein Meftahi <sup>b,\*</sup>,  
Maryam Khosravi <sup>a</sup>, Zahra Bahari <sup>c</sup>, Boshra Hatf <sup>b</sup>, Alireza Mohammadi <sup>b</sup>,  
Fateme Nicaeili <sup>a</sup>, Fateme Eftekhari <sup>a</sup>, Fateme Ghamari <sup>a</sup>,  
Mohadmehdi Hadipour <sup>b</sup>, Gholamreza Kaka <sup>b</sup>

<sup>a</sup> Department of Biology, Islamic Azad University, North Tehran Branch, Tehran, Iran

<sup>b</sup> Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

<sup>c</sup> Department of Physiology and Biophysics, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

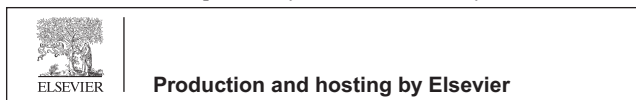
GRAPHICAL ABSTRACT



\* Corresponding author. Fax: +98 21 26127286.

E-mail addresses: [hossein.meftahi@bmsu.ac.ir](mailto:hossein.meftahi@bmsu.ac.ir), [meftahi208@yahoo.com](mailto:meftahi208@yahoo.com) (G.H. Meftahi).

Peer review under responsibility of Cairo University.



## ARTICLE INFO

*Article history:*

Received 6 April 2016

Received in revised form 21 June 2016

Accepted 26 June 2016

Available online 1 July 2016

*Keywords:*

Anorexia

Communication box

Corticosterone

Food intake

Hypothalamic–pituitary–adrenal axis

Lidocaine hydrochloride

## ABSTRACT

The current study investigated the effect of temporary inhibition of amygdala in response to metabolic changes caused by stress in female mice. Unilateral and bilateral amygdala cannulation was carried out, and after a week of recovery, 2% lidocaine hydrochloride was injected into the mice amygdalae five minutes before the induction of stress. A communication box was employed to induce stress for four consecutive days and plasma corticosterone, food and water intake, weight changes, and anorexia were measured as stress-induced metabolic changes. Results demonstrated that stress, increases stress, increased plasma corticosterone concentrations, weight, food, and water intake. Temporary inhibition of the amygdala slightly decreased plasma corticosterone concentrations, but did not fully reduce the effect of stress. The bilateral injection of lidocaine hydrochloride to the amygdala reduced the effect of stress and reduced water intake and weight. Unilateral injection of lidocaine hydrochloride into the left and right amygdala reduced food intake. In conclusion, the present study demonstrated that the left side and right side of amygdala nuclei play a different role in metabolic responses in stress.

© 2016 Production and hosting by Elsevier B.V. on behalf of Cairo University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

**Introduction**

Stress is an inevitable part of the modern world, and a factor in the expression of disease. Maintaining stability of the body's internal environment (homeostasis) in the presence of stressors needs several complex responses, such as the endocrine, the nervous, and the immune system activities known as the stress response. In other words, several behavioral and physiological changes were initiated in response to stress in order to increase survival when stability of homeostasis is threatened. Poor regulation of the internal environment in response to stress leads to pathological responses such as high blood pressure, mood disorders, and depression [1,2]. Although the central nervous system is involved in maintaining stability of homeostasis and organization of stress responses, certain cerebral areas also play a major role in this regulatory mechanism, such that mood disorders may result to a dysfunctional limbic system or hypothalamus–pituitary–adrenal (HPA) axis [3–7].

Amygdala, the major part of the limbic system, plays a central role in processing emotional states and organizing the response to stress [8]. Stimulation of the amygdala neurons produces corticotropin-releasing factor (CRF) and releases them into the blood. The amygdala is known as the center of cardiovascular and behavioral response to stress and also engages in emotional responses, especially in the case of fear and dread. These responses result in the release of stress hormones and changes in blood pressure and heart rate [9,10]. Amygdala reduces stress-induced changes in swallowing behavior and has a more prominent role in psychological stress. Moreover, there are structural differences between the left and right amygdalae, and the stimulation of the right amygdala induces negative emotions, especially fear, while the stimulation of the left amygdala induces good (happiness) and bad (stress) feelings simultaneously [8].

Gender differences in the incidence and prevalence of psychological disorders related to stress have been offered; for instance, when women and men experience the same stressors, women may be more prone than men to develop depression [11]. Previous studies have demonstrated that amygdala is

one of the best known cerebral areas associated with gender differences. It is known that the amygdala is larger in adult males than in females [12,13]. Furthermore, amygdala has a central role in remembering emotional experiences, and it has been shown that, on average, women have a stronger memory for recollecting emotional events when compared to men [14]. Thus, there is a difference between the male and female amygdala and left amygdalae and right amygdalae have different roles in the responses to stress. Therefore, examining this bias in the response to stress is important. Hence, the present study was carried out to investigate the effect of the temporary inhibition of the left and right amygdala in response to the hormonal and metabolic changes caused by stress in female mice.

**Material and methods***Animals*

Female NMRI mice weighing  $25 \pm 5$  g were kept in groups of six per cage in 12/12 light/dark conditions at 22–24 °C, with food and water provided *ad libitum*. The animals were randomly divided into control and experimental groups ( $n = 6$ /group). The food and water intake was recorded for each animal at specific hours every day. Vaginal smears were taken from all the animals in the control and stress group in order to determine their phase of sexual maturity before commencing the tests; the tests started in the proestrus phase. *Animal experiments were conducted in accordance with the Guidelines of the National Institute of Health (NIH) for the Care and Use of Laboratory Animals, and were approved by the local ethical committee (The Baqiyatallah University of Medical Sciences Committee on the Use and Care of Animals, 87/381).*

*Animal group*

Animals were randomly divided into eight groups ( $n = 6$ ). Group 1 (control) received no treatment and group 2 (stress) received 4 days stress. Three groups of animals were injected

with lidocaine hydrochloride (2%) 0.25  $\mu\text{L}/\text{mouse}$ , 5 min before the stress, in the left (L.L), right (L.R) or both sides (L. Bi) of amygdala. The last three groups were injected saline, 5 min before the stress, in the left (S.L), right (S.R) or both sides (S. Bi) of amygdala. The plasma corticosterone concentrations, food and water intake, weight changes, and delay to eating (anorexia) were measured as metabolic criteria in all groups. The mean changes from four days were studied.

### *Surgical procedures*

For the amygdala cannulation, the animals were first anesthetized with ketamine (Sigma–Aldrich, CA, USA, 50–75 mg/kg) and diazepam (Sigma–Aldrich, CA, USA, 5–7 mg/kg), and the surgical area was shaved. The animals were placed in a stereotaxic apparatus. Thereafter, a small incision was made in the scalp to expose the skull. Using bregma and lambda as landmarks, the skull was leveled in the coronal and sagittal planes with one or two guide cannulas (gauge No. 23, World Precision Instruments) implanted into the skull 500 $\mu$  above the amygdala utilizing the Paxinos atlas [15] (for the amygdala nucleus, 0.8 mm posterior to the bregma,  $\pm 2.5$  mm from the midline and 4.5 mm below the skull surface), and fixed with dental acrylic cement. The animals were given seven days to recover after the surgery. Dental needles heads No. 30 (Alibaba; INTR), polyethylene tubes and 10  $\mu\text{L}$  Hamilton syringes were used for injection. A bilateral intra-amygdala administration of lidocaine hydrochloride 2% with an injection volume of 25  $\mu\text{L}$  was conducted daily for five minutes prior to the stress induction. The brain injection was gradual and lasted 30 s, and the animals were free to move during this time.

### *Communication box*

After intra-amygdala lidocaine hydrochloride injection, the animals were transferred to a communication box (Borje Sanat Co., Tehran, Iran), which comprise nine separate parts with plexiglass walls and tiny holes with a diameter of 2 mm that enables communication between the mice. The floor of the box had stainless steel bars connected to a generator, which was linked to a computer for controlling voltage and duration of the shock (10 mV voltage, 10 Hz frequency and 60 s long). The animals in the control group were also placed in a switched off communication box for 30 min (the animals were randomly divided into control and stress groups). Stress induction continued for four consecutive days. On the last day of the test, blood samples were collected from all the animals in all groups from their retro-orbital sinus. Thereafter, the blood was centrifuged at 3000 rpm for 5 min at 4  $^{\circ}\text{C}$  and serum was collected for corticosterone detection. The serum was collected and frozen at  $-20$   $^{\circ}\text{C}$  and corticosterone concentrations were determined by ELISA kit (Rat Corticosterone ELISA kit; EIA-4164; DRG Instruments GmbH, Germany). Briefly, serum samples were added to 96-well plates containing biotinylated primary antibody and then incubated at 37  $^{\circ}\text{C}$  for 45 min. Thereafter, plates were washed and horseradish peroxidase-conjugated streptavidin solution was added to the wells and incubated for an additional 30 min at 37  $^{\circ}\text{C}$ . The 3,3',5,5'-tetramethylbenzidine substrate was added and the plates were incubated for an additional 15 min at 37  $^{\circ}\text{C}$  and then, stop solution was added to the wells to terminate the



**Fig. 1** Location of the cannula tips in the amygdala.

reaction. The corticosterone concentration was determined using a standard curve.

### *Histology*

After completing the test, all animals were anesthetized and transcardially perfused with 0.9% normal saline followed by 10% buffered formalin. The brains were removed, blocked, and cut coronally into 40- $\mu\text{m}$ -thick sections via the cannula placements. The tissues were stained with cresyl violet and examined by light microscopy by an unknown observer. Only the animals with correct cannula placements were included in the analysis (Fig. 1).

### *Data analysis*

Data were expressed as Mean  $\pm$  standard error (Mean  $\pm$  SEM). Two-way analysis of variance (Two-Way-ANOVA) was applied using lidocaine hydrochloride and stress as factors followed by Tukey post HOC. The differences of  $P < 0.05$  were considered as statistical significance.

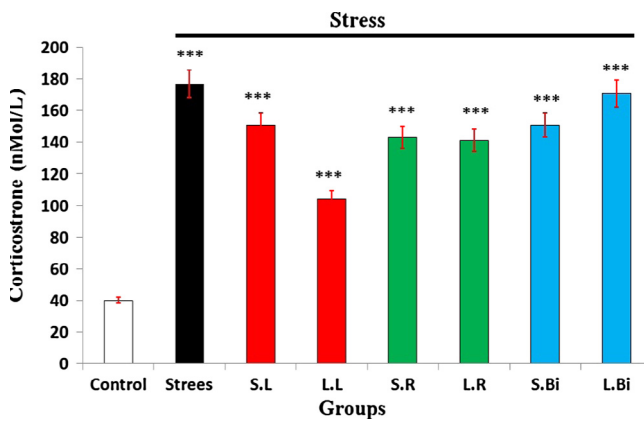
## **Results**

### *Changes in corticosterone concentrations caused by the administration of lidocaine hydrochloride to the amygdala and electric shock*

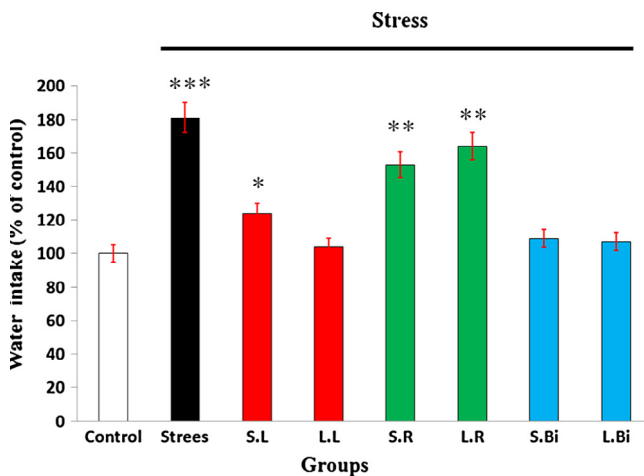
The mice received lidocaine hydrochloride 2%, 5 min before induction of stress for four consecutive days and the mean changes were studied. Results demonstrated that stress led to increase of concentration in serum corticosterone levels. Both unilateral and bilateral administrations of lidocaine hydrochloride to some extent reduced corticosterone concentrations, but could not fully inhibit the effect of stress (Fig. 2). It seems that inhibition of left amygdala reduced more serum corticosterone in stressed mice than the inhibition of the right amygdala.

### *The effect of the induction of stress and the intra-amygdala administration of lidocaine hydrochloride on water intake*

Results indicated that stress increased water intake while the inhibition of the left amygdala slightly decreased it;



**Fig. 2** Stress and long-term administration of lidocaine hydrochloride induced changes in serum corticosterone concentrations. To measure the serum corticosterone concentration, blood samples were taken from all mice in the control and stress groups from the corner of their eyes after the experiment. Five minutes before the stress, lidocaine hydrochloride (2%) 0.25  $\mu$ L/mouse was injected in the left (L.L), right (L.R) or both sides (L. Bi) of amygdala. Five minutes before the stress, saline was injected in the left (S.L), right (S.R) or both sides (S. Bi) of amygdala. The Mean  $\pm$  SEM was presented for 6 mice. \*\*\* $P < 0.001$  shows a significant difference compared to the control group.



**Fig. 3** The effect of the intra-amygdala administration of lidocaine hydrochloride and induced stress on water intake. Every day, after the induction of stress, the mice were returned to their cages, and their water intake was then measured over the next 24 h for 4 consecutive days at a specific time. The results obtained on the first day were taken as 100 and as a point of reference for measurements made in subsequent days (percentage). Injection of the lidocaine hydrochloride in the left (L.L), right (L.R) or both sides (L. Bi) of amygdala. Injection of saline in the left (S.L), right (S.R) or both sides (S. Bi) of amygdala. \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$  show a significant difference compared to the control group.

nevertheless, it did not fully inhibit the effect of stress. The temporary inhibition of the left and bilateral amygdala decreased the effect of stress. However, administration of

lidocaine hydrochloride to the right amygdala did not reduce water intake (Fig. 3).

#### *The effect of the intra-amygdala administration of lidocaine hydrochloride on food intake in stress*

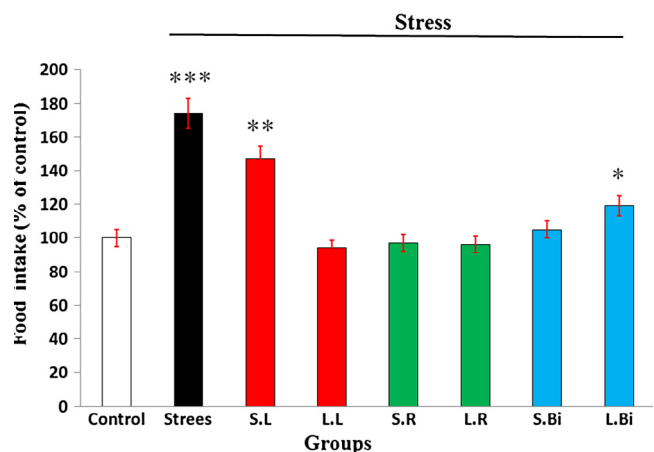
The food intake of the stress group increased when compared to the control group. The administration of lidocaine hydrochloride and the inhibition of the amygdala led to the reduction in food intake. Nevertheless, the unilateral administration of lidocaine hydrochloride to the left or right amygdala was more effective in reducing food intake than bilateral inhibition (Fig. 4).

#### *The effect of the intra-amygdala administration of lidocaine hydrochloride on weight changes in stress*

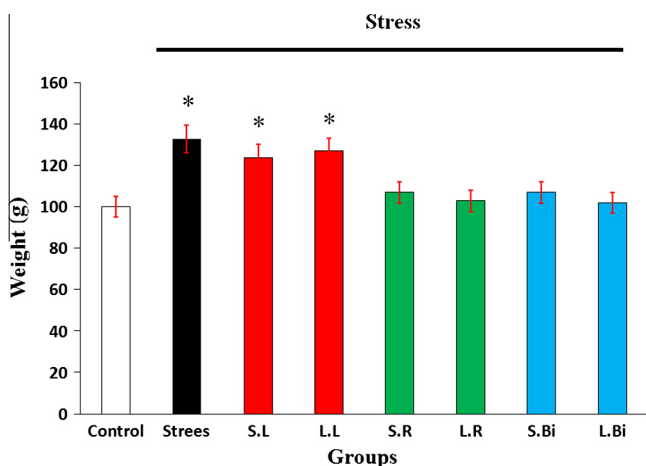
As illustrated in Fig. 5, stress led to weight gain in the female mice. The inhibition of the right and both sides of amygdala via the administration of lidocaine hydrochloride, reduced the effect of stress and resulted in slight and statistically significant weight loss in the control group animals than in stress group. However, inhibition of the left side of amygdala couldn't reduce weight gain when compared to stress group.

#### *The effect of stress and the inhibition of the amygdala on delay to eating (anorexia) in female mice*

Stress couldn't statistically increase delay to eating (anorexia) in the female mice when compared to control group. The inhibition of the unilateral and bilateral amygdala exacerbated the effect of stress and led to a significant increase in anorexia. The inhibition of the left amygdala further reinforced the effect of



**Fig. 4** The effect of stress and the inhibition of the amygdala on food intake. The amount of food intake was measured at a specific time every day. The results obtained on the first day were taken as 100 and as a point of reference for measurements made in the upcoming days (percentage). Injection of the lidocaine hydrochloride in the left (L.L), right (L.R) or both sides (L. Bi) of amygdala. Injection of saline in the left (S.L), right (S.R) or both sides (S. Bi) of amygdala. \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$  show a significant difference compared to the control group.



**Fig. 5** The effect of the intra-amygdala administration of lidocaine hydrochloride and induced stress on weight changes. The mice were weighed at a specific time every day for 4 consecutive days. The results obtained on the first day were taken as 100 and as a point of reference for measurements made in subsequent days (percentage). Injection of the lidocaine hydrochloride in the left (L.L), right (L.R) or both sides (L. Bi) of amygdala. Injection of saline in the left (S.L), right (S.R) or both sides (S. Bi) of amygdala. \* $P < 0.05$  shows a significant difference compared to the control group.

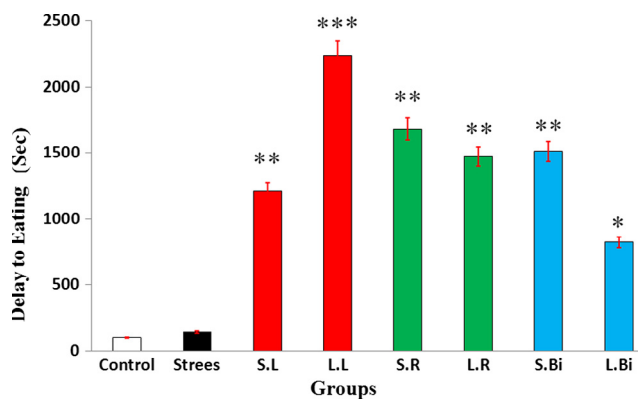
stress in anorexia when compared to the inhibition of the right and both sides of amygdala (Fig. 6).

## Discussion

Stress is known as one or a collection of highly challenging and uncontrollable emotional and physiological events that disrupt the body's balance [16]. Stress is followed with a greater activity of the HPA axis and secretion of ACTH and CRF and corticosteroids (corticosterone and cortisol) in the body for homeostasis despite, the metabolic changes. Glucocorticoids are important in several ways for physiological regulation and cognitive evaluation. They are involved in stress and fear responses, and energy metabolism, and regulate behavioral adaptation to the environment and internal conditions (via the regulation of several neurotransmitters and neural circuits). Glucocorticoids have different effects on several neurotransmitters and neuropeptide systems and thus, affect the brain functions [17,18].

In the present study, stress led to an increase in plasma corticosterone concentrations, which was confirmed by previous findings [19,20]. Increased glucocorticoids may have a major role in different areas of the brain, particularly in the reduction in hippocampal activity, increase in amygdala activity, the deformation of dendrites in hippocampal, amygdala, and ventral prefrontal cortex [17].

Lidocaine hydrochloride was utilized in this study for the temporary inhibition of the amygdala. Lidocaine hydrochloride is a local anesthetic that decreases depolarization by inhibiting inward sodium into the cell membrane [21–23]. In this study, the temporary unilateral and bilateral inhibition of the amygdala led to a slight decrease in corticosterone concentrations, but did not fully inhibit the effect of stress.



**Fig. 6** The effect of induced stress and the intra-amygdala administration of lidocaine hydrochloride on delay to eating. The animals were returned to their cages every day after the induction of stress and their eating latency was measured for 4 consecutive days. The results obtained on the first day were taken as 100 and as a point of reference for measurements made in subsequent days (percentage). Injection of the lidocaine hydrochloride in the left (L.L), right (L.R) or both sides (L. Bi) of amygdala. Injection of saline in the left (S.L), right (S.R) or both sides (S. Bi) of amygdala. \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$  show a significant difference compared to the control group.

According to the results, amygdala does not appear to have a major role in the inhibition of stress by increasing plasma corticosterone concentrations in female mice.

Results also demonstrated that stress, increases water intake in female mice. Similar results have also been reported in rodents. Stress increases water intake by means of concurrent stimulation of CRF and vasopressin secretions from hypothalamic paraventricular nuclei [24,25]. In addition, the inhibition of the amygdala, particularly the bilateral inhibition of the left amygdala, decreased water intake; nevertheless, the inhibition of the right amygdala did not decrease water intake, which showed that in stress the right amygdala has no inhibitory effects on water intake in female mice.

High levels of uncontrollable stress disrupt the expression of glucocorticoid genes in the HPA axis, which, in turn, affect the energy balance and nutritional behavior [26]. Stress increases food intake as well. The physiological response to stress might affect nutritional behavior, which might also affect the body weight and health [27]. Direct involvement of the CRF system in energy regulation and increasing CRF secretion in the hypothalamus leads to the stimulation or inhibition of the feeding paths in the stress [28,29]. It should be noted however, that gender differences play a major role in decreasing or increasing food intake in male and female, and according to previous studies, food intake in stress is often higher in female when compared to male [30].

Food intake increased in stress group. Temporary inhibition of the left or right amygdala reduced food intake. Several studies have demonstrated that the paraventricular nucleus, along with the lateral hypothalamus and the nucleus accumbens shell (part of the extended amygdala), plays an important role in controlling food intake, and the temporary inhibition of any of these parts could reduce the effect of stress [31,32]. Lidocaine hydrochloride can also, cause these responses by temporary inhibition of the amygdala. Long-term chronic

stress increases the risk of obesity, and stress is significantly involved in regulation of appetite and energy [33]. It has been shown that high levels of stress, result in excessive secretion of CRF and consequently glucocorticoids, change eating patterns and increase the appetite for food, especially sweet food, and therefore lead to weight gain [33,34]. In the present study, stress led to weight gain in female mice, which might be due to increased food intake. It should be noted that the effects of induced stress are different on small or large rats and in humans; that is, stress tends to cause weight loss in rats and weight gain in humans [35]. Furlan et al. revealed that high level secretion of cortisol in response to stress reduces bone minerals and abdominal obesity in humans [36]. In stress condition, women tend to choose fatty or sweet foods [30]. The increased secretion of ghrelin (an intestinal peptide) during stress might have a role in the pathophysiology of obesity and eating disorders. Some ghrelin axons innervate CRF cells in the hypothalamus paraventricular nucleus [37].

In the present study, only the temporary inhibition of the right amygdala reduced the effect of stress and thus slightly decreased the animal's weight; nevertheless, the reduction was not statistically significant. Considering that lidocaine hydrochloride had different effects on the right and left amygdalae, a bias seems to exist in the amygdala response to weight change, and the left amygdala seems to have an effective role in reducing the effect of stress.

Results demonstrated that stress causes prolonged delay in eating (anorexia) in female mice; however, the increase was not statistically significant. In line with this result, it has been demonstrated that stress induced anorexia in animal [38–40]. By activating the sympathetic nervous system, CRF prolongs delay to eating. It is believed that CRF inhibits the nuclei responsible for eating and induces anorexia by affecting its type 1 receptors in the hypothalamus [32]. It has been demonstrated that the release of norepinephrine in stress stimulates the release of neuropeptide Y, which induces anorexia in the hypothalamus [41]. The effect of stress severely exacerbated prolonged anorexia with the administration of lidocaine hydrochloride to the amygdala. On the other hand, anorexia was reinforced when the amygdala was inhibited in stress. The effect of stress was further reinforced with the inhibition of the left amygdala.

## Conclusions

It seems that temporary inhibition of the amygdala can increase the effect of metabolic stress in female mice. At the same time, there was lateralization in function of right and left amygdala and inhibition of the left amygdala was more effective.

## Conflict of Interest

*The authors have declared no conflict of interest.*

## Acknowledgment

This study was conducted as part of student thesis project, which was supported by the grant from the Neuroscience

Sciences Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

## References

- [1] Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 1992;267:1244–52.
- [2] Bahari Z, Manaheji H, Hosseinmardi N, Meftahi GH, Sadeghi M, Danyaly S, Noorbakhsh SM. Induction of spinal long-term synaptic potentiation is sensitive to inhibition of neuronal NOS in I5 spinal nerve-transected rats. *EXCLI* 2014;13:751–60.
- [3] Salehi M, Eimani H, Sahraei H, Meftahi GH. Stress can changes reward system function in second-generation (F2): a review. *Adv Biores* 2015;6(5):04–14.
- [4] Pacak K, Palkovits M. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. *Endocrine Rev* 2001;22(4):502–48.
- [5] Meftahi G, Ghotbedin Z, Eslamizade MJ, Hosseinmardi N, Janahmadi M. Suppressive effects of resveratrol treatment on the intrinsic evoked excitability of CA1 pyramidal neurons. *Cell J (Yakhteh)* 2015;17(3):532.
- [6] Evangelia C, Constantine T, George C. Endocrinology of the stress response. *Annu Rev Physiol* 2005;67:259–84.
- [7] Ghodrati M, Sahraei H, Razjouyan J, Meftahi GH. Effects of a saffron alcoholic extract on visual short-term memory in humans: a psychophysical study. *Neurophysiology* 2014;46(3):247–53.
- [8] Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry* 2005;29(8):1201–13.
- [9] Folkow B, Hallback-Nordlander M, Martner J, Nordborg C. Influence of amygdala lesions on cardiovascular responses to alerting stimuli, on behavior and on blood pressure development in spontaneously hypertensive rat. *Acta Physiol Scand* 1982;116(2):133–9.
- [10] Bang L, Rø Ø, Endestad T. Amygdala alterations during an emotional conflict task in women recovered from anorexia nervosa. *Psychiatry Res: Neuroimaging* 2016;248:126–33.
- [11] Nolen-Hoeksema S. Gender differences in depression. *Curr Dir Psychol* 2001;10:173–6.
- [12] Hines M, Allen LS, Gorski RA. Sex differences in subregions of the medial nucleus of the amygdala and the bed nucleus of the stria terminalis of the rat. *Brain Res* 1992;579(2):321–6.
- [13] Hamann S. Sex differences in the responses of the human amygdala. *Neuroscience* 2005;11(4):288–93.
- [14] Cahill L, Haier RJ, White NS, Fallon J, Kilpatrick L, Lawrence C, et al. Sex-related difference in amygdala activity during emotionally influenced memory storage. *Neurobiol Learn Mem* 2001;75(1):1–9.
- [15] Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. 2nd ed. San Diego: Academic Press; 2001.
- [16] McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev* 2007;87(3):873–904.
- [17] Erickson K, Drevets W, Schulkin J. Glucocorticoid regulation of diverse cognitive functions in normal and pathological emotional states. *Neurosci Biobehav Rev* 2003;27:233–46.
- [18] Hooshmandi Z, Rohani AH, Eidi A, Fatahi Z, Golmanesh L, Sahraei H. Reduction of metabolic and behavioral signs of acute stress on male Wistar rats by saffron water extract and its constituent safranal. *Pharm Biol* 2011;49:947–54.
- [19] Sahraei H, Fatahi Z, Rohani AH, Eidi A, Hooshmandi Z, Tavalaee SA. Ethanolic extract of saffron and its constituent crocin diminish stress-induced metabolic signs and alterations of dopamine-related behaviours in rats. *Int Res J Pharm Pharmacol* 2012;2(7):165–73.

- [20] Ghoshooni H, Mehr PP, Salimi SH, Golmanesh L, Dehpour A, Sahraei H. Milled stress reduces morphine-induced locomotion in F2 NMRI mice. *Iran J Pharm Res* 2011;10(2):347–54.
- [21] Sheets MF, Hanck DA. Molecular action of lidocaine on the voltage sensors of sodium channels. *J Gen Physiol* 2003;121(2):163–75.
- [22] Hosseini SB, Sahraei H, Mohammadi A, Hatf B, Meftahi GH, Chalabi-Yani D, et al. Inactivation of the nucl. accumbens core exerts no effect on nicotine-induced conditioned place preference. *Neurophysiol* 2015;47(4):295–301.
- [23] Chalabi-Yani D, Sahraei H, Meftahi GH, Hosseini SB, Sadeghi-Gharajehdagi S, Beig HA, et al. Effect of transient inactivation of ventral tegmental area on the expression and acquisition of nicotine-induced conditioned place preference in rats. *Iran Biomed J* 2015;19(4):214–9.
- [24] Aguilera G. HPA axis responsiveness to stress: implications for healthy aging. *Exp Gerontol* 2011;46:90–5.
- [25] Mulder AH, Geuze JJ, de Wied D. Studies on the subcellular localization of corticotrophin releasing factor (CRF) and vasopressin in the median eminence of the rat. *Endocrinology* 1970;87:61–79.
- [26] Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behavior and cognition. *Nat Rev Neurosci* 2009;10:434–45.
- [27] Adam TC, Epel ES. Stress, eating and the reward system. *Physiol Behav* 2007;91:449–58.
- [28] Stratford TR, Wirtshafter D. Injections of muscimol into the paraventricular thalamic nucleus, but not mediodorsal thalamic nuclei, induce feeding in rats. *Brain Res* 2013;1490:128–33.
- [29] Sominsky L, Spencer SJ. Eating behavior and stress: a pathway to obesity. *Front Psychol* 2014;5(434):1–8.
- [30] Habhab S, Sheldon JP, Loeb RC. The relationship between stress, dietary restraint, and food preferences in women. *Appetite* 2009;52:437–44.
- [31] Andrews ZB, Abizaid A. Neuroendocrine mechanisms that connect feeding behavior and stress. *Front Neurosci* 2014;8(312):1–2.
- [32] Stengel A, Taché Y. CRF and urocortin peptides as modulators of energy balance and feeding behavior during stress. *Front Neurosci* 2014;8(52):1–10.
- [33] Bose M, Oliván B, Laferrère B. Stress and obesity: the role of the hypothalamic–pituitary–adrenal axis in metabolic disease. *Curr Opin Endocrinol Diabetes Obes* 2009;16(5):340–6.
- [34] Meftahi GH, Janahmadi M, Eslamizade MJ. Effects of resveratrol on intrinsic neuronal properties of CA1 pyramidal neurons in rat hippocampal slices. *Physiol Pharmacol* 2014;18(2):144–55.
- [35] Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, et al. Chronic stress and obesity: a new view of “comfort food”. *PNAS* 2003;100(20):11696–701.
- [36] Furlan PM, Ten Have T, Cary M, Zemel B, Wehrli F, Katz IR, et al. The role of stress-induced cortisol in the relationship between depression and decreased bone mineral density. *Biol Psychiatry* 2005;1557(8):911–7.
- [37] Diz-Chaves Y. Ghrelin, appetite regulation, and food reward: interaction with chronic stress. *Int J Pept* 2011;898450:1–11.
- [38] Merali Z, Graitson S, MacKay JC, Kent P. Stress and eating: a dual role for bombesin-like peptides. *Front Neurosci* 2013;7(193):1–12.
- [39] Bahari Z, Manaheji H, Dargahi L, Daniali S, Norozian M, Meftahi GH, et al. Time profile of nNOS expression in the spinal dorsal horn after L5 spinal root transection in rats. *Neurophysiol* 2015;47(4):287–94.
- [40] Asalgoo G, Jahromi GP, Meftahi GH, Sahraei H. Posttraumatic Stress Disorder (PTSD): mechanisms and possible treatments. *Neurophysiol* 2015;47(6):482–9.
- [41] Rabasa C, Dickson SL. Impact of stress on metabolism and energy balance. *Curr Opin Behav Sci* 2016;9:71–7.