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ORIGINAL ARTICLE

Ventral tegmental area inactivation alters hormonal, metabolic, and locomotor responses to inescapable stress

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ABSTRACT

Context: The involvement of unilateral and bilateral inhibition of the ventral tegmental area (VTA) in response to stress is not well understood.
Objective: In the present study, the effect of unilateral and bilateral inhibition of the VTA on hormonal, metabolic, and locomotor responses to stress was assessed.
Material and methods: Male rats seven days after cannulation into the VTA received electro foot-shock stress for seven consecutive days. Twenty minutes before induction of stress, 2% lidocaine hydrochloride or sterile saline (control) was injected either uni- or bi-laterally into the VTA.
Results: Results showed that stress significantly increased serum corticosterone level, adrenal gland weight and anorexia, reduced weight gain, food-intake, and locomotor activity. However, bilateral inactivation of VTA prevented stress-induced these parameters changes.
Conclusion: The present study demonstrated that the bilateral VTA blockade effectively relieves the symptoms of stress, while the unilateral VTA blockade does not significantly improve the changes caused by stress.

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Corticosterone; lidocaine; locomotor activity; stress; ventral tegmental area

Introduction

It is now clear that stress and its consequences are one of the most important causes of chronic diseases, metabolic disorders, and social problems such as anxiety, depression, and addiction (Ellenbroek *et al*. 2005, Ghobadi *et al*. 2016, Ehteram *et al*. 2017). Therefore, this issue has led to extensive research in recognizing the neurobiology of stress which aids in preventing the deleterious effect of stress (Der-Avakian *et al*. 2007). Many studies have shown that exposure to stressful events activates several neural and endocrine systems to increase animal survival in a dangerous environment. Activation of the hypothalamic-pituitary-adrenal (HPA) axis and increase secretion of corticotropin-releasing factor system (CRF) is a primary initiator of response to stress (McCormick and Mathews 2007, Stephens and Wand 2012, Asalgoo *et al*. 2015). Several evidences have been shown that stressors can increase CRF secretion, which can, in turn, modulate dopamine (DA) neurons in the ventral tegmental area (VTA) (Saal *et al*. 2003, Ungless *et al*. 2003). VTA is one of the main brain areas that play an important role in responding to stress (Michaels and Holtzman 2008). Neurons of VTA project to the nucleus accumbens, prefrontal, and limbic regions. All these areas are critical parts of the brain reward system (Ikemoto 2007, Chalabi-Yani *et al*. 2015, Motahari *et al*. 2016). Stressful events can change, feeding behavior, energy balance, and locomotor activity (Hassantash *et al*. 2017). However, the exact physiologic mechanisms that regulate appetite and feeding behavior in stress conditions are far from clear. It has been shown that DA is involved in the control of feeding behavior (Volkow et al. 2011) and stress has been shown to alter normal dopaminergic neurotransmission (Pani *et al*. 2000, Dalooei *et al*. 2016, Sadeghi-Gharajehdaghi *et al*. 2017). The release of DA from the VTA in the nucleus accumbens is necessary for the rewarding properties of natural stimuli required for survival, such as food. Stressful events are also capable of enhancing dopaminergic function within the mesolimbic system. For example, VTA dopaminergic neurons are physically excited by foot-shock and acute restraint stress. Moreover, both excitatory and inhibitory synapses in the VTA dopaminergic neurons express long-term potentiation that is altered by exposure to acute stress (Brischoux *et al*. 2009). Several stresses signaling molecules such as glucocorticoids can regulate VTA function. Activation of glucocorticoids can induce potentiation of NMDA receptors. The local block of both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate (NMDA) receptors in the VTA also prevents stress-induced dopamine efflux in the prefrontal cortex (Polter and Kauer 2014).

There is mounting evidence demonstrating heterogeneity of cellular phenotypes in the VTA, which this midbrain

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Supplemental data for this article can be accessed here.

nucleus receive and integrate information from a large number of brain areas and organize appropriate behavioral responses based on this information and damage to different parts of this nucleus are involved in aberrant processes such as addiction and mental illness (Luo *et al*. 2008, Good and Lupica 2009). However, to our knowledge, there is no evidence about the effects of uni- or bi-lateral inhibition of the VTA neurons on feeding behavior, hormonal and locomotor activity in response to stress. Therefore, the present study was designed to evaluate the effects of uni- and bilateral inhibition of the VTA by lidocaine on metabolic behaviors such as food and water intake, weight changes, anorexia time, and the corticosterone level and locomotor activity after foot-shock stress induction in male rats.

Material and methods

Animals

Adult male Wistar rats (250–300 g) were used in all experiments. All animals were housed under standard conditions and maintained at a temperature controlled colony room under 12 h light-dark cycle with free access to food and water. The stress exposures were carried out in a separate room. All efforts were made to minimize the number of animals used and their suffering.

Surgical procedures and lidocaine injection

Animals were anesthetized with ketamine (75 mg/kg) and diazepam (5 mg/kg) and were placed in a stereotaxic frame (Stoelting, Wood Dale, IL), later 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. Bilateral cannula were implanted in the skull at the anteroposterior (in reference to bregma) and the mediolateral coordinates that correspond to the VTA (4.5 mm anteroposterior [AP], ± 0.8–1 mm mediolateral [ML], and 7.7–8-1 mm [DV] based on the Paxinos and Watson's atlas (2007). The rats were prepared with one or two stainless steel 23 gauge cannulas placed above the VTA. At the end of the surgery, removable wire styles (30 gauge) were inserted in the cannula to maintain patency. The cannula were permanently secured to the skull by using dental acrylic cement. The animals were given seven days to recover after the surgery. Dental needles head No. 30 (Alibaba; INTR), polyethylene tubes, and 10 μL Hamilton syringes were used for injection. Lidocaine hydrochloride (2%; dissolved in sterile saline) was administered uni- (0.5 μl/rat) or bilaterally (0.25 μl/side) into the VTA by 30-gauge blunts tapered needle at a rate of 0.5 μl/min, 20 min prior to the stress induced. The injection needle was left in place for five min after injection to allow diffusion from the injector tip and the animals were free to move during this time.

After the completion of testing the animals were anesthetized. For histological verification of the location of injection cannulas, eosin was injected into the VTA. Later the animals received a transcardiac perfusion of 0.9% normal saline followed by 10% buffered formalin. The brains were removed, blocked, and cut coronally through the cannula placement (Figure 1).

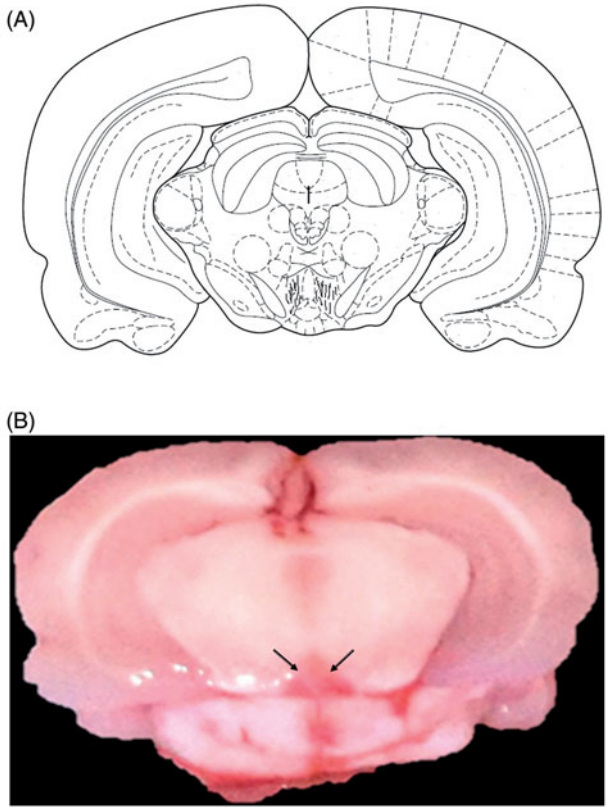

Figure 1. (A) Location of the cannula tips in the VTA according to the atlas of Paxinos and Watson (2007). The lines indicate where the cannula tips were placed. (B) Real histological approval of the cannula placement.

Stress procedure

During a one week acclimatization period before the start of the experiments, all rats were weighed daily. After intra-VTA lidocaine hydrochloride injection, the rats were transferred to a communication box. The communication box was equipped with a grid floor composed of 0.5 cm diameter stainless steel rods placed 1.3 cm apart. The box was divided into nine smaller compartments (16 × 16 × 50 cm). Stress induction continued for seven consecutive days. During the session in the foot-shock box rats received five 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 (10 mV voltage, 10 Hz frequency, and 60 s long). This session lasted for an hour . This kind of stress was induced randomly, so animals do not get used to the habit.

Experimental groups

Animals were randomly divided into eight groups ($n = 7$ for each group). In the negative control group (CNTL − rats were put into a communication box, but do not receive any foot-shock stress. The positive control group (CNTL+) rats received foot-shock stress without surgery. In control left group (CL) rats received saline (0.5 μl/rat) 20 min before stress

in the left VTA. In the control right group (CR) rats received saline (0.5 μl/rat) 20 min before stress in the right VTA. In the control bilateral group (CB) rats received saline (0.5 μl/rat or 0.25 μl/side) 20 min before stress on both sides of the VTA. In experimental left group (EXP L) rats received 2% lidocaine hydrochloride (0.5 μl/rat) 20 min before stress on the left side of the VTA. In the experimental right group (EXP R); rats received 2% lidocaine hydrochloride (0.5 μl/rat) 20 min before stress on the right side of the VTA. In the experimental bilaterally group (EXP B) rats received 2% lidocaine hydrochloride (0.5 μl/rat or 0.25 μl/side) 20 min before stress on both sides of the VTA.

Measuring the concentration of corticosterone hormone

On day three and day seven after following the induction of the stress, blood samples were taken from all the animals from their retro-orbital sinus (0.5 ml blood was mixed with 0.5 ml of 3%EDTA) and were centrifuged at 3000 rpm for 5 min at 4 °C. Later, the animals supernatant plasma was collected and frozen at −20 °C and corticosterone concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) kit for measuring cortisol levels using a cortisol measurement 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 were incubated at 37 °C for 45 min. Later, the plates were washed and horseradish peroxidase-conjugated streptavidin solution was added to the wells and were incubated for an additional 30 min at 37 °C. The 3, 3′,5,5′-tetramethylbenzidine substrate was added and the plates were incubated for an additional 15 min at 37 °C and then, stop solution was added to the wells to terminate the reaction. The corticosterone concentration was determined using a standard curve.

Measurement of food intake, anorexia time, body weight, and locomotor activity

The animals returned to their cages 30 min after the end of stress induction and their anorexia time (the time between the return of the animal in the cage and starts feeding) was measured daily. Moreover, each day after the end of stress induction, animals returned to their cages and 25 g of food per animal were fed in each group. The next day, the amount of food consumed by each rat was measured before induction of stress. Moreover, animals were weighed daily and their weight changes were also evaluated.

Locomotor activity were also measured for all the animals which were recorded with a video camera for 10 min on day 1, 3, and 7 before and after the foot shock stress induction.

Measurement of adrenal gland weight

On day 8 post induction of stress, the animals were deeply anesthetized with high doses of ketamine and their adrenal glands were removed and were stored in 4% formalin solution for fixation. The weight of the adrenal glands was assessed by mercury immersion.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* (see Supplementary Materials). Statistical analyses were performed using SPSS software version 20 (SPSS, Chicago, IL). The differences of $p < .05$ were considered as statistically significant.

Results

Effects of lidocaine intra-VTA administration on serum corticosterone level with or without stress

The results demonstrated that foot shock stress significantly increased serum corticosterone level in the CNTL+, CL, CR, and CB groups when compared to the CNTL− ($p < .01$). Administration of saline in the left, right, or both sides of VTA did not show a significant change compared to the CNTL+. Bilateral administration of lidocaine (EXP B), 20 min before induction of stress for seven consecutive days in experimental group results in a lower serum corticosterone level than in the CNTL+. It seems that inhibition only on the left (EXP L) or the right (EXP R) side of the VTA didn't significantly change serum corticosterone concentration as against the CNTL+ (Figure 2) [$F(7, 48) = 15.83$, $p < .01$ and $F(7, 48) = 23.05$, $p < .01$ on days 3 and 7, respectively].

The effects of inactivation of the VTA on adrenal gland weight in stress

Stress causes a significant increase in adrenal gland weight in the CNTL+, CL, CR, and CB groups when compared to the CNTL− ($p < .01$). There was no significant difference between CL, CR, and CB groups with the CNTL+. The right (EXP R) or left side (EXP L) administration of lidocaine into VTA did not show a significant difference in comparison with the CNTL+ (Figure 3). But bilateral injection of lidocaine into VTA showed that foot shock stress could reduce the weight of the adrenal gland compared to the CNTL+ [$F(7, 48) = 4.462$, $p < .01$].

Effects of intra-VTA lidocaine administration on stress-induced alteration in food and water intake

Results revealed that the food intake in the CNTL + reduced when compared to the CNTL − CL, CR, and CB groups. The administration of 2% lidocaine hydrochloride and the inhibition on both sides of the VTA led to the increase in food intake. Nevertheless, unilateral administration of lidocaine hydrochloride to the VTA didn't increase food intake relative to the CNTL + group.

Only when lidocaine was bilaterally administrated in the VTA there was no significant difference in food intake compared to the CNTL− [$F(7, 48) = 10.153$, $p < .01$;

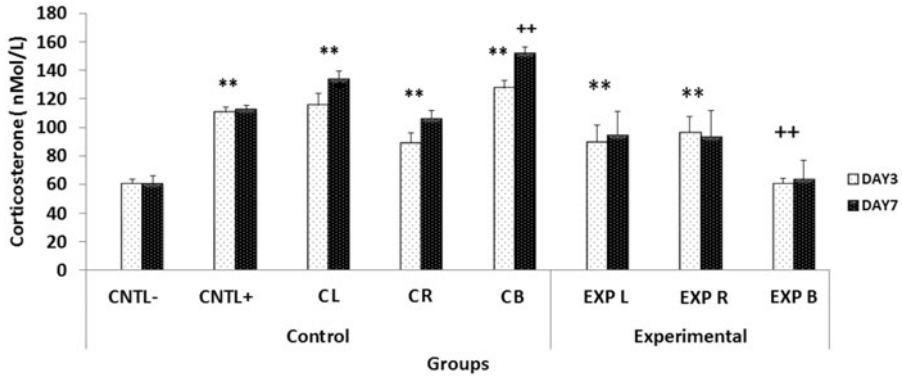

Figure 2. Foot-shock stress and administration of lidocaine hydrochloride (2%) into VTA induced changes in serum corticosterone level. To measure the serum corticosterone level, blood samples were taken from all rats in the control and foot shock stress groups from the corner of their eyes after the experiment. The Mean ± SEM was presented for seven rats. $^{**}p < .01$ shows a significant difference compared to the CNTL − group. $++p < .01$ shows a significant difference compared to the CNTL + group.

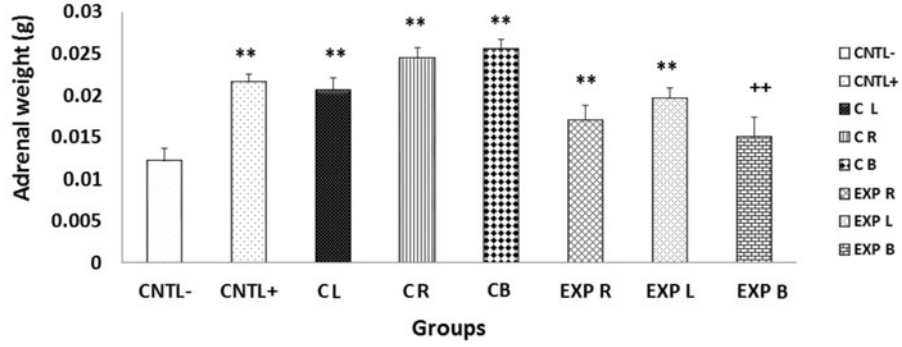

Figure 3. The effect of unilateral or bilateral inactivation of VTA on the changes of adrenal gland weight following foot-shock stress. Lidocaine hydrochloride (2%) was injected into the left (EXP L), right (EXP R), or both sides (EXP B) of VTA. Saline was injected in the left (CL), right (CR), or both sides (CB) of VTA. Each bar represents the mean ± SEM for seven rats. $^{**}p < .01$ shows a significant difference compared to the CNTL − group. $++p < .01$ shows a significant difference compared to the CNTL + group.

F (7, 48) = 10.732, $p < .01$; F (7, 48) = 12.858, $p < .01$; F (7, 48) = 13.790, $p < .01$; F (7, 48) = 13.470, $p < 0.01$; and F (7, 48) = 16.896, $p < 0.01$ on days 2–7, respectively for food intake] (Figure 4(A)).

Results indicated that stress slightly increased the water intake but, there was no statistically significant ($p < .05$) differences between the CNTL − and the CNTL + groups in water intake and the inhibition of the left and/or right or both sides of VTA did not significantly change water intake in the CNTL − and the CNTL + groups [F (7, 48) = 1.423, $p < .218$; F (7, 48) = 1.751, $p < .120$; F (7, 48) = 1.200, $p < .321$; F (7, 48) = 1.422, $p < .219$; F (7, 48) = 1.413, $p < .222$; and F (7, 48) = 0.903, $p < .512$ on days 2–7, respectively for water intake] (Figure 4 (B)).

The effects of inactivation of the VTA on weight changes with or without stress

As illustrated in Figure 5, in the CNTL + group there was a reduction in the weight gain when compared to the CNTL − group.

Moreover, the CNTL − group showed a higher mean weight gain than the CL, CR, and CB groups.

Inhibition of left/right VTA did not prevent weight gain reduction after the stress. Bilaterally VTA inhibition could prevent the effect of stress on weight changes with no significant difference relative to the CNTL − group [F (7, 48) = 9.339, $p < .01$; F (7, 48) = 12.202, $p < .01$; F (7, 48) = 8.935, $p < .01$; F (7, 48) = 8.421, $p < .01$; F (7, 48) = 7.720, $p < .01$; and F (7, 48) = 10.544, $p < .01$ on days 2–7 , respectively].

The effects of stress , unilateral, and bilateral inhibition of the VTA on anorexia time (delay in eating) in rats

Foot-shock stress statistically increased the anorexia in the CNTL + group when compared to CNTL − group. Also, the CL, CR, and CB groups showed a higher delay in eating than the CNTL − group. Inhibition of R/L VTA did not prevent delay in eating after the stress.

However, bilaterally VTA inhibition could prevent the effect of foot-shock stress on delay in eating, but there was no significant difference in the delay to eat between bilaterally VTA inhibition and the CNTL− [F (7, 48) = 56.759, $p < .01$; F (7, 48) = 45.389, $p < .01$; F (7, 48) = 39.183, $p < .01$; F (7, 48) = 39.025, $p < .01$; F (7, 48) = 74.656, $p < .01$; F (7, 48) = 56.311, $p < .01$; and F (7, 48) = 57.486, $p < .01$ on days 1–7, respectively for delay to eating] (Figure 6).

Inhibition of left/right VTA did not prevent any delay to eat after the stress.

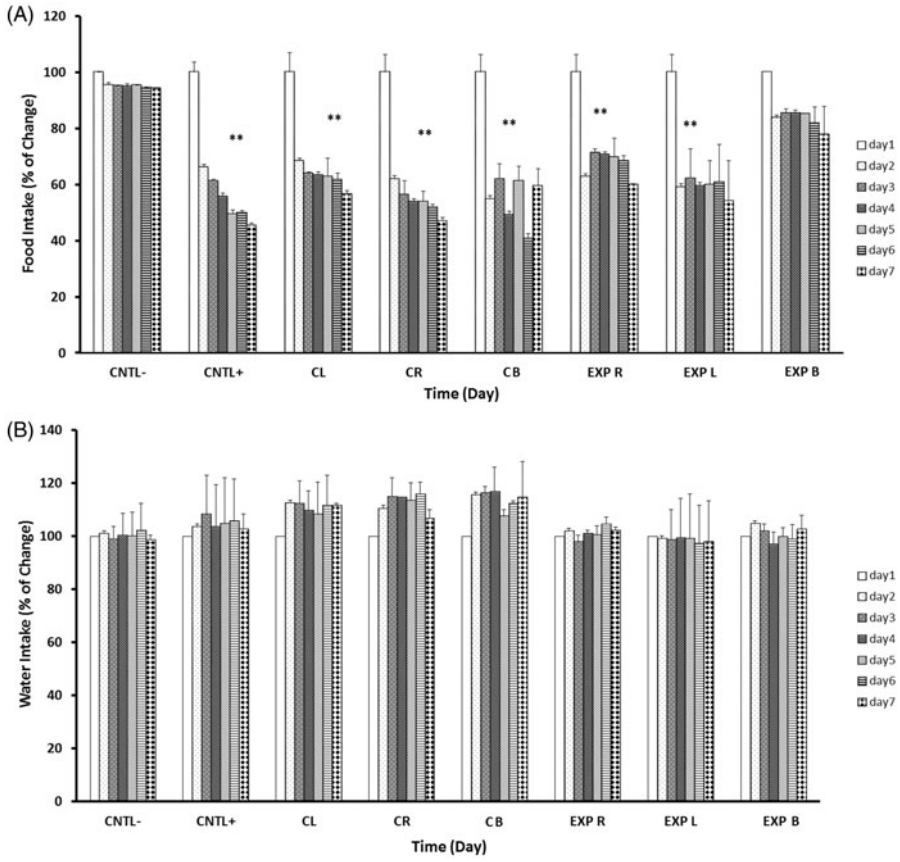

Figure 4. (A) The effect of the intra-VTA administration of lidocaine hydrochloride (2%) and induced foot-shock stress on water intake. Every day, after the induction of foot-shock stress, the rats were returned to their cages and their water intake was then measured over the next 24 h for seven consecutive 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). (B) The effect of foot shock-stress and inactivation of the VTA on water intake. The amount of water intake was measured at a specific time every day. Each bar represents the mean ± SEM for seven rats. $^{**}p < .01$ shows a significant difference compared to the CNTL − group.

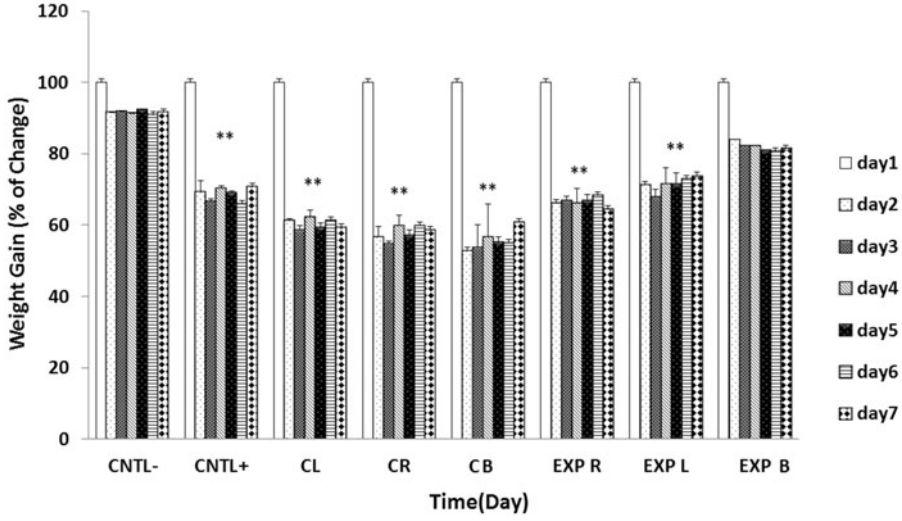

Figure 5. The effect of the intra-VTA administration of lidocaine hydrochloride and induced foot-shock stress on weight changes. The rats were weighed at a specific time every day for seven consecutive days. The results obtained on the first day were taken as 100 and as a point of reference for measurements made in subsequent days (percentage). $^{**}p < .01$ shows a significant difference compared to the CNTL − group.

The effects of unilateral and bilateral inhibition of the VTA on locomotion in inescapable stress

As shown in Figure 7, on days 1, 3, and 7 post foot-shock stress animals locomotion was significantly weaker than before the stress. The inactivation of left and/or right side of the VTA reduced stress-induced changes in locomotion after stress (which were observed in CNTL+, CL, CR, and CB groups; $p < .01$).

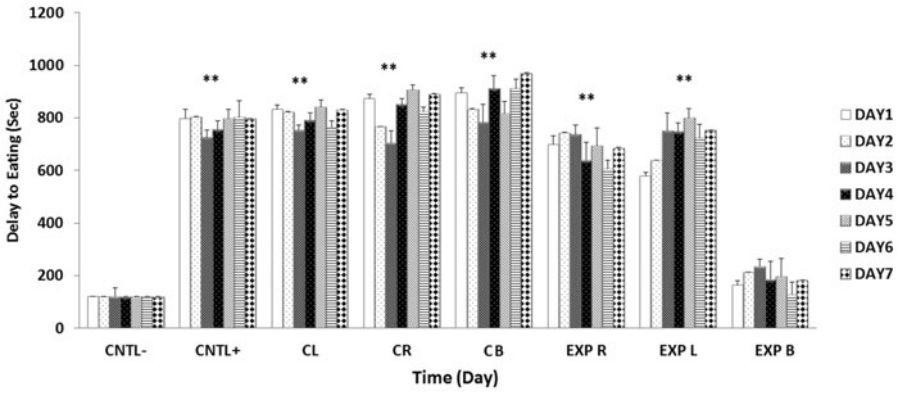

Figure 6. The effect of induced foot-shock stress and the intra-VTA administration of lidocaine hydrochloride on delay to eating. The animals were returned to their cages every day after the induction of foot shock stress and their eating latency was measured for seven consecutive days. The results obtained on the first day were taken as 100 and as a point of reference for measurements made in subsequent days (percentage). Each bar represents the mean ± SEM for seven rats. $^{**}p < .01$ show a significant difference compared to the CNTL − group.

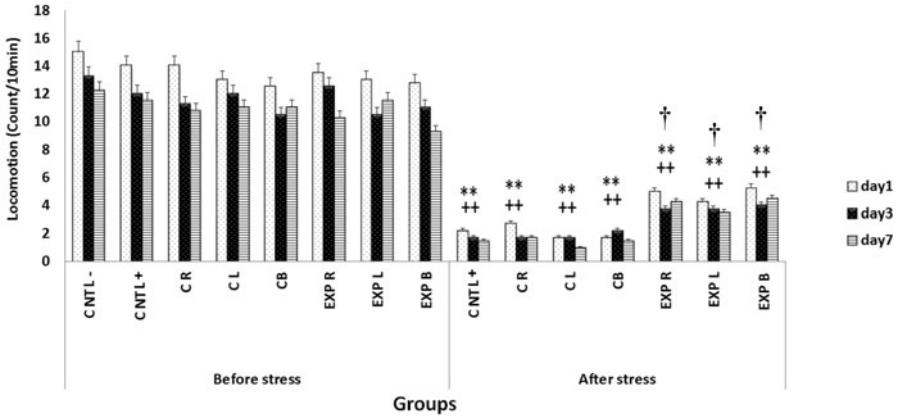

Figure 7. The effect of induced foot-shock stress and the intra-VTA administration of lidocaine hydrochloride to locomotor activity. Each bar represents the mean ± SEM for seven rats. ** showed a significant difference compare to the CNTL − group, ++ shows a significant difference compare to the CNTL + group before stress and † shows a significant difference compare to the CNTL + group after stress.

Discussion

Despite several studies have investigated the role of VTA on stress responses, there is a lack of studies on the differences between the left and right sides of VTA in stress responses. Thus, in the present study, we investigated the roles of the right and the left sides of VTA in hormonal, metabolic parameters, and behavioral changes in response to non-escapable stress.

Lidocaine hydrochloride is a widely used as a local anesthetic whose mechanism of action is use-dependent inhibiting inward sodium into the cell membrane (Mao and Chen 2000, Mohammadian *et al*. 2017). Lidocaine binds sodium channels with variable efficacy, depending on the channel states and bind at the pore of the channel (Bant *et al*. 2013). It is known that lidocaine have an onset of <2 min, a duration of 1–2 h (Fiorillo *et al*. 2003). Due to this, 2% lidocaine hydrochloride was used for local inactivation in the present study. To ensure the onset of the effect of 2% lidocaine hydrochloride 20 min after its administration to the VTA, foot-shock stress was induced.

The results showed that foot-shock stress increased serum corticosterone level on days 3 and 7 after stress. Also, serum corticosterone level in CNTL+ , CL, CR, and CB groups significantly increased than in CNTL − group. Several reports showed that the serum corticosterone level increases after stress, (Dhabhar and McEwen 1997, Ricart-Jan *et al*. 2002, Dalooei *et al*. 2016, Tekieh *et al*. 2017), which support and are consistent with the results of the present study that electro foot-shock stress induces plasma corticosterone elevation in these animals. The activity of the HPA-axis by stressful events can activate parvocellular neurons within the paraventricular nucleus in the hypothalamus and these neurons secrete CRF, that increasing the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH induces the cells located in the zona fasciculata of adrenal cortex to release the glucocorticoid into the blood (Xiong and Zhang 2013). Probably, such a mechanism could happen in the electro foot shock stress used in our study. However, temporary bilateral inhibition of VTA (EXP B) 20 min before induction of stress caused the reduction of serum corticosterone level than in the CNTL+, but temporary inactivation of the left (EXP L) or the right (EXP R) side of the VTA didn't significantly change serum corticosterone level when compared to the CNTL+ . This indicates that there is probably no prominent area on the left or right sides of the VTA in modulating the effect of stress in serum corticosterone level. Feenstra *et al*. (1992) showed that neonatal lesions of the VTA alter the normal hormonal response to stress, indicating

that the VTA may have an effect on the HPA axis. Corticosterone directly acts on the VTA, where glucocorticoid receptors are present in approximately 50–60% of dopamine neurons (Hensleigh and Pritchard 2013), also, CRF acts directly in the VTA, which via CRF1 and CRF2 receptors increase the firing frequency of most VTA cells (Wanat *et al.* 2008).

According to the results, both sides of the VTA appear to have a major role in stress by increasing plasma corticosterone concentrations in the rat.

The results also demonstrated that the weight of adrenal gland in stressed animals (CNTL+) was significantly increased in comparison with the negative control group. This increase in the weight of the adrenal gland is indicative of the fact that secretion of ACTH hormone from the pituitary gland following stress causes hypertrophy and hyperplasia of the adrenal gland cortex, thereby increase the weight of the adrenal gland. Ulrich-Lai *et al.* (2006) showed that chronic variable stress increased adrenal weight due to hyperplasia of the outer zona fasciculata and hypertrophy of the inner zona fasciculata and medulla that occur in specific adrenal sub-regions and is associated with the increased corticosterone in responses to ACTH. So, it is likely that the increase in adrenal weight is associated with increased size of the adrenal cortex. Several studies have shown that many depressed patients have exaggerated cortisol responses after ACTH administration (Heim *et al*. 1998, Mello *et al*. 2003). Furthermore, increased corticosterone levels are consistent with the studies that showed physiological responses to stress are correlated with the activation of the HPA axis (Oken *et al*. 2015, Pourhashemi *et al*. 2016, Shemiran *et al*. 2017). Bilateral inhibition of VTA prevent increase in adrenal gland weight after the stress.

However, to our knowledge, the exact neuroanatomical relationship between VTA and the adrenal gland is not understood. One possible mechanism is that inhibition of VTA by lidocaine probably through reduced corticosterone modulated the effect of stress on adrenal gland weight.

The results showed that stress (CNTL+) reduced the food intake relative to the CNTL−. Bilateral inhibition of VTA prevent decrease in food intake after the stress. Also, stress rapidly decreased body weight on days 3 and 7 post the stress treatment that may be due to a reduction of food intake. Furthermore, there is no significant difference between different groups in water intake. However, bilateral inhibition of the VTA significantly decreased anorexia time as compared with CNTL+, CL, CR, and CB groups. The decrease in body weight may be due to an early decrease in food intake initially but then may be maintained by an increase in energy expenditure and body temperature during stress (Bhatnagar *et al*. 2006). It is likely that high levels of corticosterone have catabolic effects on muscle tissue (Menconi *et al*. 2007), therefore it is likely that direct effects of corticosterone on muscle contributed to the weight loss. Also, Harris *et al.* (1998) showed that chronically exposed to restraint stress caused rapid weight loss that did not recover even after removal of the stress.

Studies reported that high levels of inevitable stress disturb the expression of glucocorticoids genes in the HPA axis that, in turn, affect the energy balance and nutritional behavior (Lupien *et al*. 2009). It is known that the VTA receives direct information from the gastrointestinal tract. Ghrelin receptors (a stomach peptide) are seen to be expressed both on dopamine and non-dopamine neurons (GABAergic and glutamatergic) on the VTA (Abizaid *et al*. 2006). Egecioglu *et al.* (2010) showed that ghrelin signaling in the VTA enhances intake of palatable food. Skibicka *et al.* (2011) also revealed that ghrelin increases motivation for lever presses for palatable sucrose. Leptin, a mediator of regulation of energy balance, receptors (LepRs) inhibits hunger in part by acting on hypothalamic circuitry, were also seen to be also expressed on VTA dopaminergic and GABAergic neurons (Meye and Adan 2014). Trinko *et al.* (2011) showed that leptin directly hyperpolarized VTA dopamine neurons and reduces their neuronal firing frequency. Furthermore, Thompson and Borgland (2013) showed that leptin could suppress glutamatergic input onto VTA dopamine neurons. Thus, probably these mechanisms may increase food intake by bilateral inhibition of VTA in the present study.

Finally, we measured locomotor activity in response to stress to provide a general indicator of behavioral reactivity to foot-shock stress. We observed that stress significantly decreased the locomotor activity of the animals. Such lower locomotor activity following stress may reflect the increased fear or anxiety of animals. Indeed, exposure to stress stimuli produces freezing behaviors (Fanselow and Gale 2003, Maren and Holt 2004). Previous studies have identified increased freezing behavior following acute social defeat (Chung *et al*. 1999) and increased immobility in mice after prolonged aggressive confrontations (Kudryavtseva *et al*. 1991).

Locomotor activity in the CNTL+, CL, CR, and CB groups were more than after the administration of lidocaine into VTA, which is due to the VTA inhibition in the brain.

Even bilateral inhibition of VTA did not prevent locomotor activity reduction after the stress, although in all EXP groups this reduction was smaller than in control groups. This effect of inactivation of left and/or right side of the VTA identified the important role of the VTA in the spontaneous movements. Valenti *et al.* (2012) showed that chronic stress strongly reduced the number of dopamine neurons firing in the medial and central VTA and this VTA regions project to reward related ventral striatal regions. Stress also, may directly interact with brain reward areas and inhibit the enzyme tyrosine hydroxylase (which indirectly suppresses D1 dopamine receptors when activated) and alter dopamine-related behaviors such as locomotor activity (Czyrak *et al*. 2003). Therefore, according to these findings, it is acceptable to evaluate locomotor activity as part of an indirect profile for stress effects. However, in our study, temporary inactivation of both sides of VTA partly inhibited the stress effects on locomotor activity with no significant difference between the left and right sides of the VTA.

Moreover, the phenomenon of "contralateral facilitation of function" i.e. facilitation of behavioral responses (eating or exploration) induced by electrical stimulation of the VTA in one hemisphere in the conditions of unilateral electrolytic lesion or pharmacological blockade of the VTA in the other hemisphere (Trojniar and Klejbor 1999, Maliszewska-Scislo

and Trojniar 2000), could be the one possible reasons for the low efficiency of the unilateral VTA blockade on the stress parameters studied in this work. This "contralateral facilitation of function" manifests itself in the shortening of latency of stimulation-elicited feeding and exploratory locomotion. Majkutewicz *et al.* (2010) also showed that FOS expression was enhanced by VTA stimulation after contralateral VTA lesion. This phenomenon could be another reasons for the low efficiency of the unilateral VTA inhibition on the stress parameters.

Conclusion

In conclusion, this study shows that the bilateral inactivation of the VTA can attenuate the effects of stress. On the other hand, bilateral inhibition of VTA before stress moderating stress-induced behavioral, metabolic, and hormonal changes. According to these results, no laterality was observed in the activity of the VTA in responses to stress.

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Disclosure statement

The authors have no conflict of interest to declare.

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