Original Article

J Babol Univ Med Sci
Vol 18, Issu 4; Apr 2016. P:21-28

Effect of Temporary Inactivation of Nucleus Accumbens on Chronic Stress Induced by Electric Shock to the Sole of the Foot in Female NMRI Mice

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J Babol Univ Med Sci; 18(4); Apr 2016; PP: 21-8
Received: Mar 14th 2015, Revised: Jun 17th 2015, Accepted: Jan 4th 2016.

ABSTRACT

BACKGROUND AND OBJECTIVE: Activity changes in the neurons of nucleus accumbens during stress have been previously identified. However, the role of nucleus accumbens in diminishing stress-induced side-effects is not fully understood. In this study, we aimed to evaluate the effects of temporary inactivation of nucleus accumbens on stress-induced metabolic changes in female mice.

METHODS: This experimental study was performed on 48 female NMRI mice with an average 27±3 g. The nucleus accumbens was unilaterally and bilaterally cannulated. After one week of recovery, 2% lidocaine or saline was administered in mice for four consecutive days (5 min per day) before inducing electric shock to the sole of the foot. Plasma corticosterone level, food and water intake, and delay in eating were assessed as stress-induced metabolic parameters.

FINDINGS: Stress lonely, caused an increase in plasma corticosterone (17±0.8) compared with the control group (4.5±0.3) ($p<0.001$). It also, caused an increase delay in eating (%218±9.8, $p<0.01$) and, decrease water (%80±4.5) and food (%84±5.5) intake ($p<0.05$). Temporary inactivation of nucleus accumbens did not affect the stress-induced changes in plasma corticosterone, and it suppressed the effect of stress on the amount of water intake; inactivation of the left nucleus accumbens was more effective (%195±7.6, $p<0.01$). Temporary inactivation of nucleus accumbens neutralized the effect of stress on the amount of food intake. Temporary inactivation of the right nucleus accumbens augmented the effect of stress on delay in eating (%264±10.8, $p<0.01$), and inactivation of the left nucleus accumbens could suppress this effect.

CONCLUSION: It seems that temporary inactivation of nucleus accumbens can be effective in diminishing stress-induced metabolic changes. However, this influence is indicative of asymmetry in the function of right and left nucleus accumbens.

KEY WORDS: *Lidocaine, Mice, Nucleus accumbens, Stress, Temporary inactivation*.

Please cite this article as follows:

Nicaeili F, Sahraei H, Khosravi M, Rezaeian J, Eftekhari F, Sarahian N, Ghamari F. Effect of Temporary Inactivation of Nucleus Accumbens on Chronic Stress Induced by Electric Shock to the Sole of the Foot in Female NMRI Mice. J Babol Univ Med Sci. 2016;18(4):21-8.

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Introduction

Brain is the most important part of the human body influenced by stress. Also, the nervous system is believed to show the first reaction to stress (1). Various studies have been conducted on the role of different parts of the brain in stress management and occurrence of stress-induced side-effects. In animal studies, stress has been shown to disturb hormonal and metabolic parameters in small and large laboratory mice. Inactivation of NMDA glutamate receptors in nucleus accumbens or temporary inactivation of this region can diminish hormonal and metabolic effects of stress (2, 3). Also, in human studies, conducted by functional magnetic resonance imaging, stress-induced changes have been reported in the activity of different parts of the brain, including the hippocampus, amygdala, and prefrontal cortex (4, 5). However, further extensive research is required to determine the role of different parts of the brain in exerting diverse stress-induced effects, e.g., metabolic changes.

Stress leads to the activation of hypothalamic-pituitary-adrenal axis (HPA), which in turn results in the secretion of releasing factor in the paraventricular nucleus of the hypothalamus and adrenocorticotropic hormones (ACTHs) from the anterior pituitary. In response to ACTH secretion, glucocorticoids (cortisol and corticosterone) are secreted from the adrenal cortex (6). The function of nucleus accumbens during stress induction has been neglected, so far. Consequently, different pathways in the brain, which are involved in stress and the associated responses, are not fully determined. Although an increasing number of anatomical and electrophysiological studies have been conducted on nucleus accumbens over the past two decades, the role of this region in response to stress is not fully understood.

In previous research, the function of dopamine system of nucleus accumbens in neutralizing stress has been noted (7). Moreover, previous studies have revealed the function of nucleus accumbens in response to stress. Evidently, dopamine and GABAergic transmissions in the nucleus accumbens change in response to acute or chronic stress. Considering the relationship between nucleus accumbens and central amygdala, and consequently, the involvement of nucleus accumbens responses in central amygdala (8), in this study, through temporary inactivation of nucleus accumbens, we aimed to determine the role of this region in metabolic changes, induced by chronic stress.

Lidocaine is a local anesthetic agent, which can control the passing of sodium through the cell membrane by competing with calcium in resting on neuronal membrane receptors; also, the depolarization phase diminishes the activity potential (9). Since lidocaine can temporarily inhibit the neural activity for more than 30 min, in this study, we used this agent to temporarily inactivate the nucleus accumbens (10). Regarding the diversity of neurotransmitters in the nucleus accumbens and the importance of dopamine and glutamate, which seem to be involved in stress responses and asymmetry of nucleus accumbens, the question may arise as to whether the mechanism of lidocaine (similar to the ventral tegmental area) is related to the inactivation of dopamine receptors. In response to this question, as noted in the mechanism of memantine activity, all voltage-gated sodium channels in regions free of myelinated neurons are inhibited by lidocaine (9); therefore, receptors or neurotransmitters play no specific role.

Chronic stress can lead to the occurrence of some complications such as blood pressure, heart attack, stroke, damage to the digestive system, reduced activity of the immune system, and behavioural disorders such as depression, anxiety, and even addiction. A substantial amount of global health funding is allocated to these conditions each year (11, 12). Therefore, management of stress, as well as the induced side-effects, is the most important health program throughout the world. For this purpose, we need to identify brain areas involved in stress. One of the proposed methods is temporary inactivation of various nuclei of the brain in order to determine the role of each nucleus. It should be mentioned that part of the nucleus accumbens is related to amygdala, which helps modify the performance of amygdala. In previous research, two points remain unanswered. First, with respect to the asymmetric distribution of

dopamine receptors and cholecystokinins in the left and right nucleus accumbens and asymmetry of nucleus accumbens in response to morphine (13), this question arises as to whether this asymmetry is also observed in the response of nucleus accumbens to stress. Second, we need to determine if this asymmetry applies to the female sex, considering the sex differences. Therefore, in this study, the response of small laboratory female mice to chronic stress was evaluated by temporary inactivation of nucleus accumbens via lidocaine.

Methods

In this interventional, experimental study, 48 small laboratory female NMRI mice with the mean weight of 27±3 g were used. The animals were kept in cages (six mice each) at controlled temperature of 22-24°C with free access to water and food (12 hours of light and 12 hours of darkness). In each experiment, six mice were used, and the amount of water and food intake was recorded during the experiment. Vaginal smears were obtained from all the animals, and their sexual cycle was evaluated before the start of the experiment; all the animals were tested in the proestrous phase. The experiments were conducted, based on the protocol of working with laboratory animals by the medical ethics committee of Baqiyatallah University.

The animals were randomly divided into eight groups of six mice. Stress was not induced in the control group and no medications were prescribed. On the other hand, animals in the stress group were exposed to stress for only four days. The other six groups were either unilaterally (right or left side) or bilaterally cannulated in the nucleus accumbens. Lidocaine and/or saline (alongside stress induction) was administered for the animals.

The used drug in this experiment was 2% lidocaine hydrochloride, which was injected into the nucleus accumbens, using Hamilton syringe. Another group received the same amount of saline instead of the drug. The animals were anesthetized using a mixture of ketamine hydrochloride (50-75 mg/kg) and diazepam (5-7 mg/kg). One or two stainless steel guide cannulae (No. 23) were inserted in the animals' heads by the stereotaxic device, according to the stereotaxic coordinates in Paxinos Atlas for nucleus accumbens (AP=1, ML=±1.5, DV=4.5) (14) (fig 1). The animals were allowed one week after the surgery to recover. The injection cannula (30G dental injection needle) with the length of 500 µ (longer than the guide cannula) was inserted, and 0.25 µl of the drug was slowly injected in each side within 30 sec, using the Hamilton syringe. Stress was induced, using the Communication Box device (Industrial Tower Co., Tehran, Iran). This device consisted of nine separate Plexiglas sections (50×16×16 cm) (length × width × height), and the bottom of the device contained stainless steel bars in a 1.3 cm distance from each other. The bars were attached to a generator, which was connected to a computer. The voltage and duration of shock induction (voltage of 60 mV and frequency of 10 Hz for 60 sec) were determined by the user. In this study, electric shock was randomly induced to the soles of animals' feet between 9 a.m. and 13 p.m. for four consecutive days.

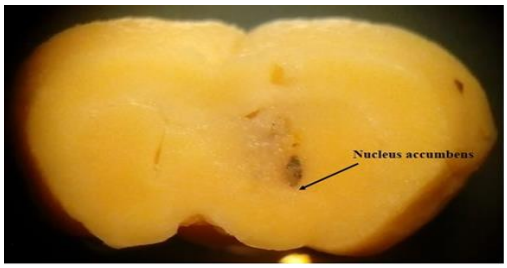

Figure 1. The position of the guide cannula in the nucleus accumbens of mice

The animals were transferred to the experiment room one hour before the intervention so that they could adapt to the environment. Afterwards, they were placed inside the device for 20 min and received 60 sec of electric shock in the sole of the foot. The animals in the control group were also placed inside the device for 30 min with no electric shock. A blood sample was obtained from the retro-orbital sinus, located in the corner of animals' eyes in the control and test groups on the first and last days of the experiment between 9 and 11 am. Then, the samples were centrifuged at 3,000 rpm for 5 min. The surface plasma was collected

in order to evaluate corticosterone level. Afterwards, the samples were assessed, using the Corticosterone Parameter Assay Kit (Corticosterone ELISA Kit, DRG, Germany) via ELISA method at a wavelength of 450 nm. In addition, the amount of water and food intake, weight changes, and delay in eating were assessed as metabolic indices.

On each day of the experiment at specific time intervals, the animals were given 10 g of food and 30 cc of water inside the cages, and the amount of water and food intake was measured within 24 hours. Also, the weight of animals was measured every day at specific intervals for four consecutive days. By the end of stress induction, the animals were returned to their cages.

The period of time since the animals were placed inside the cages until they started to eat was recorded (in sec), using a timer. This interval, which indicated the delay in eating, was recorded as a criterion for stress assessment. The obtained data were expressed as mean±SD. Three-way analysis of variance (three factors including asymmetry, stress, and lidocaine), followed by Tukey's test was performed for data analysis. $p<0.05$ was considered statistically significant.

Results

Changes in plasma corticosterone concentration due to chronic lidocaine administration and electric shock induction to the sole of the foot: As the findings indicated, stress significantly increased the amount of plasma corticosterone ($p<0.001$). On the other hand, temporary inactivation of the right and left nucleus accumbens did not affect stress-induced changes in the amount of plasma corticosterone (fig 2).

Effect of lidocaine injection inside the nucleus accumbens on the amount of water intake in mice exposed to chronic stress: Based on the findings, stress caused a decline in water intake ($p<0.05$). On the other hand, administration of lidocaine in the right and left nucleus accumbens caused an increase in water intake. The response was more prominent in the left nucleus accumbens, and inactivation of the left nucleus accumbens resulted in a significant increase in water intake ($p<0.05$) (fig 3).

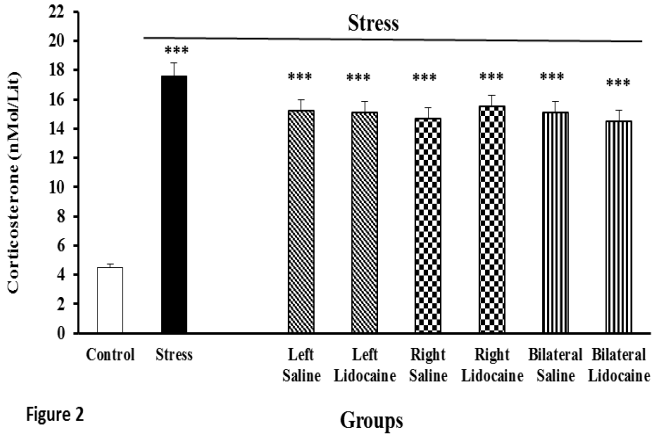

Figure 2. Changes in plasma corticosterone level as a result of chronic lidocaine administration and electric shock induction to the sole of the foot

Mean±SD values are related to six mice. ***p<0.001 shows a significant difference with the control group.

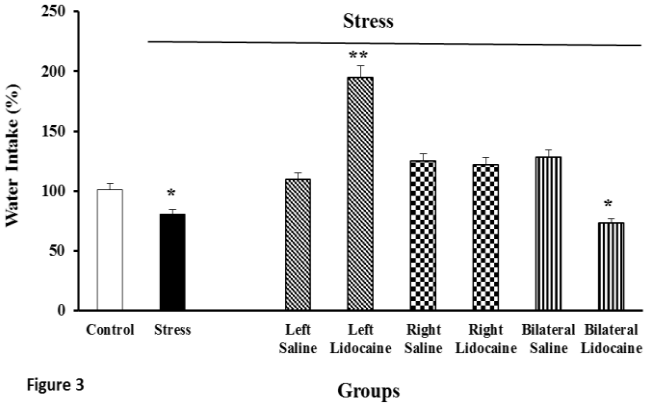

Figure 3. Effect of lidocaine injection inside the nucleus accumbens on the amount of water intake in animals after chronic stress induction.

The results on the first day were considered as the reference point (100) for the groups, and the following days were compared accordingly (in percentage). Mean±SD values are related to six mice. *p<0.05 and **p<0.01 shows a significant difference with the control group.

Effects of chronic stress induction and lidocaine administration inside the nucleus accumbens on the amount of food intake: Based on the findings, stress caused a decline in the amount of food intake ($p<0.05$). Also, bilateral administration of lidocaine caused a decrease in food intake ($p<0.05$). However, administration

of lidocaine in the left nucleus accumbens induced a slight increase in the amount of food intake, which was not statistically significant (fig 4).

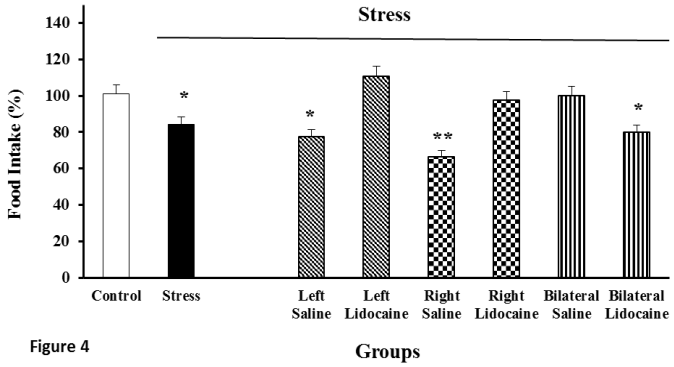

Figure 4. Effect of lidocaine injection inside the nucleus accumbens on the amount of food intake after chronic stress induction.

Mean±SD values are attributed to six mice. *p<0.05 and **p<0.01 show significant differences with the control group.

Effects of chronic stress induction and lidocaine administration inside the nucleus accumbens on delay in eating: Stress caused an increase in delay before eating (p<0.01). Also, bilateral inactivation of the nucleus accumbens and inactivation of the left nucleus accumbens suppressed the effect of stress and reduced the delay before eating (fig 5).

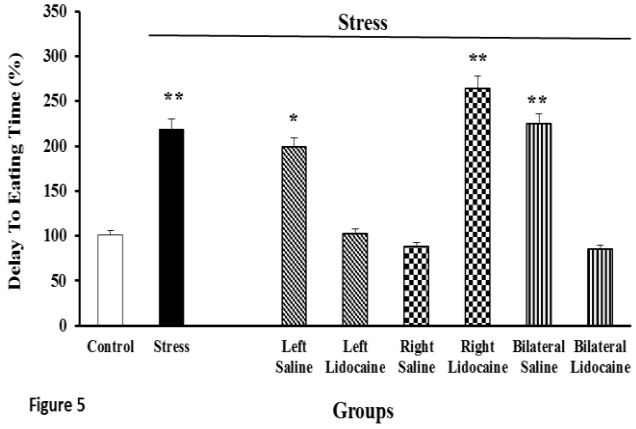

Figure 5. The effect of chronic lidocaine administration inside the nucleus accumbens on delay in eating after electric shock induction to the sole of the foot in mice

Mean±SD values are attributed to six mice. *p<0.05 and **p<0.01 show significant differences with the control group

Discussion

In this study, the nucleus accumbens showed asymmetry in stress-induced metabolic responses. The results of this study could clearly help identify the function of nucleus accumbens and its responses to chronic stress. These findings may be useful in designing stress management methods, based on the role of different parts of the brain in response to stress, and aid with developing drugs to control the nucleus accumbens during stress induction.

Based on previous studies, bilateral inactivation of the nucleus accumbens or stria terminalis (nerve fibers which are connected to the nucleus accumbens from amygdala), inhibits neural modifications caused by systemic injection of glucocorticoids. Information, which is probably transferred from the lateral amygdaloid nucleus and hippocampus to the nucleus accumbens, is converged, thus contributing to memory preservation. The results of the present study showed that stress causes a significant increase in the amount of plasma corticosterone; this finding was consistent with the results reported by other researchers (3, 15). As explained in the introduction section, since stress causes a decline in the activity of HPA axis, which in turn results in an increase in glucocorticoids (16), the rise in plasma corticosterone level could be predicted. On the other hand, inactivation of nucleus accumbens by lidocaine administration (whether unilaterally or bilaterally) could not suppress the effect of stress; as a result, the amount of plasma corticosterone was not reduced. Although few studies have been conducted on temporary inactivation of nucleus accumbens and its impact on plasma corticosterone, we showed that temporary inactivation of the shell of right nucleus accumbens led to a decline in the amount of plasma corticosterone in laboratory mice.

On the other hand, temporary inactivation of the left nucleus accumbens caused an increase in the amount of plasma corticosterone (2). Owing to the small size of the brain in small mice, it should be noted that in the present study, lidocaine was inevitably injected to all brain nuclei. In fact, separate inactivation of the shell and nucleus accumbens in small mice can induce different effects. The results of

this study indicated that chronic stress in small laboratory female mice caused a decline in water intake. However, previous research has shown that stress increases water intake through simultaneous stimulation of corticotropin-releasing factor (CRF) and vasopressin secretion (17). This finding was in contrast with the present study, which may be an indicator of the effect of sex on stress.

Considering the scarcity of information in this area, our only explanation for the discrepancy between the mentioned findings is the involvement of sex parameter. It should be noted that sex hormones, whether in males or females, can be effective in the induction of stress. However, the specific role of these hormones in the occurrence of stress-associated effects (e.g., water and food intake) is still undetermined. It should be noted that cellular organization in the paraventricular nucleus of hypothalamus varies in male and female mice. In fact, CRF-containing neurons account for the highest number of neurons in the nucleus accumbens in female mice, while vasopressin-containing neurons have the highest number in male mice (18). There is a difference between the responses of right and left nucleus accumbens. Also, the right and left nucleus accumbens may be morphologically or structurally different. The morphological difference may be attributed to the diversity in the number of neurons in each region or the relationship between neurons. This difference may be also related to the lack of uniformity in neurotransmitter receptors. In fact, it has been shown that the distribution and density of dopamine and cholecystokinin receptors are not similar in the right and left nucleus accumbens.

Moreover, the noted difference may be a result of evaluating all nuclei in the brain; therefore, responses may be attributed to each of these regions. Inactivation of the left nucleus accumbens led to a significant increase in water intake in chronic stress induction, whereas a trivial increase was reported by inhibiting the right nucleus accumbens. In addition, chronic stress induction in female mice led to a decline in food intake. In previous studies on rodents, stress was shown to reduce food intake in animals, which is in accordance with the results of the present study (19). One of the most important effects of stress, in addition to changing the psychosocial behavior, is the shift in food intake patterns, which can appear in form of overeating or reduced food intake. Various studies have suggested that chronic stress causes an increase in food intake in humans and a decline in food intake in large and small laboratory mice (20). The results revealed that bilateral administration of lidocaine caused a decline in food intake, while injection in the left nucleus accumbens led to an increase in food intake; however, this increase in food intake was not statistically significant. The left nucleus accumbens plays a more important role than its right counterpart in stress inhibition since inactivation of this region induces a more prominent response to stress and leads to an increase in food intake; as a result, asymmetry can be seen in the nucleus accumbens.

On the final day of the experiment, the effect of stress was reversed, which could be the main cause of anorexia. In the present study, the mice were in the estrogen phase on the first day of stress induction, therefore, estrogen hindered the effects of stress; however, on the fourth day, the mice were in the progesterone phase. Also, chronic stress may be a contributing factor. Based on the findings, asymmetry was observed in the left and right nucleus accumbens, and the effects of stress were less prominent in the left nucleus accumbens. Chronic stress led to increased delay in eating, which was consistent with the results of previous studies (21).

The delay in eating may be increased due to changes in the hormonal phase and chronic stress. Inactivation of the right and left nucleus accumbens increased the delay in eating, while inactivation of the left nucleus accumbens had a more prominent effect. This might be related to the more important role of the left nucleus accumbens in motor activities, compared to the right nucleus accumbens (13). Although the left nucleus accumbens inhibited the effects of stress, inactivation of the right nucleus accumbens had a more significant impact, which can be indicative of asymmetry. In the present study, it was concluded that temporary inactivation of nucleus accumbens can be effective in diminishing the metabolic effects of stress;

this in fact shows asymmetry in the performance of right and left nucleus accumbens. Moreover, the greater efficiency of the left nucleus accumbens compared to the right side has been demonstrated in previous studies (3). It should be noted that brain laterality and lack of equal performance in different regions of the right and left parts of the brain (brain asymmetry) have been demonstrated in various cases, as well as cortical and subcortical regions of the brain such as amygdala, hippocampus, and cerebral cortex (22). As mentioned earlier, considering the importance of stress management, further research by using more drugs and electrophysiological tests is required to more accurately determine the function of nucleus accumbens in response to stress.

Acknowledgments

Hereby, we would like to thank the Neuroscience Research Center of Baqiyatallah University.

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