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Effects of Memantine, an NMDA Antagonist, on Metabolic Syndromes in Female NMRI Mice

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ABSTRACT

Introduction: The brain glutamate neurotransmitter system and its NMDA (N-methyl-D-aspartate) receptors in the nucleus accumbens play an important role in the incidence of sensitivity and addiction. The present study examined the inhibitory effect of glutamate NMDA receptors in the nucleus accumbens in response to chronic stress.

Methods: After the unilateral and bilateral placement of cannula(e) in the nucleus accumbens, one group of the animals received different doses of intra-accumbens memantine (0.1, 0.5 and 1 µg/mouse) 5 minutes before receiving the electric shock stress at their soles (using a Communication Box) and the other group received intraperitoneal memantine (doses of 0.1, 0.5 and 1mg/kg) 30 minutes before receiving the same shock. Chronic stress increased the animals' weight, plasma corticosterone, food and water intake, but reduced their defecation rates and eating latency.

Results: The intraperitoneal administration of memantine increased plasma corticosterone, water intake, fecal weight, and eating latency, but had no effect on food intake or weight. The dose and site-dependent intra-accumbens administration of memantine either exacerbated the effects of stress on plasma corticosterone levels, water and food intake, or had no effect on these parameters. Furthermore, the administration of memantine had no effect on animal's weight and inhibited the effects of stress on fecal weight and eating latency.

Discussion: The inhibition of glutamate NMDA receptors in the nucleus accumbens can inhibit and/or exacerbate the dose and site-dependent effects of chronic stress, and gender plays a significant role in producing this effect too.

1. Introduction

Stress is known as a state of mind that involves the responses and interactions brain and body. Stress varies from one person to another and can be induced by important life events, such as the loss of relatives and serious car crashes; cheerful events, like marriage and the birth of children; or routine events, such as traffic jam, weather change, and pollution. These events change physiological stress systems and consequently the brain and body functions. One of the main responsive neuroendocrine systems in humans and animals in to stress is the Hypothalamus-Pituitary-Adrenal (HPA) axis (McEwen, 2012). Studies have shown that during physiological or psychological stress, a cascade of neurotransmitters and hormones are released from the nervous or the endocrine system, which help maintain the internal balance of living creature. First, stressors cause the release of Corticotropin-Releasing-Factors (CRF) and vasopressin from paraventricular nucleus of hypothalamus into the pituitary portal blood flow. They

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stimulate the secretion of Adrenocorticotropin Hormone (ACTH) from the anterior pituitary into the blood flow. By affecting its own receptors in the cortical part of the adrenal gland, ACTH increases the synthesis and secretion of glucocorticoids (cortisol in humans and corticosterone in animals). While HPA axis activity can be considered an essential coping mechanism in response to stress, its prolonged activity can be a threat to living creatures. Glucocorticoids increase blood pressure and the likelihood of diabetes mellitus and arterial diseases as well as impairing tissue growth and repair. In addition, HPA axis activity suppresses the immune system, which can be harmful in chronic cases, as it increases the risk of infection (McEwen, 2012).

Reduced nucleus accumbens dopaminergic activity might have a key role in increasing glutamate NMDA (N-methyl-D-aspartate) receptor mediation in cerebral hippocampus (Wood, 2004). Glutamate is a neural and often excitatory transmitter in the brain of mammals and has a major role in synaptic plasticity, learning, and memory (Castellano et al., 2001). Chronic stress certainly affects the synthesis, diffusion, and reabsorption of essential transmitters such as glutamate and gamma-amino butyric acid (GABA) (Grønli et al., 2007). Stress or corticosterone reduces the expression of glutamate transmitters and increase extracellular glutamates (Tse et al., 2012). Because of their small size and lipid structure, natural corticosteroids, particularly corticosterone, can easily pass through the Blood-Brain Barrier (BBB) by passive diffusion (Joëls et al., 2012). High concentration of corticosterone leads to histological and functional impairments in the hippocampus, including the reversible disintegration of CA3 pyramidal dendrites in impaired long-term potentiation (LTP) and also in hippocampus-dependent memory and learning. Thus, glutamate and NMDA receptors contribute a great part to this effect (Feng et al., 2005).

The nucleus accumbens (NAc) is a large part of the ventral striatum that is located at the front of the hypothalamus. According to neurochemical and histological differences, the accumbens is divided into two parts –a core and a shell. Its shell is part of the extended amygdala. The nucleus accumbens shell is the most sensitive part of the dopamine reward system (Wise, 2008). Studies have shown that stress increases dopamine in the prefrontal cortex and the nucleus accumbens. NMDA receptors of the nucleus accumbens release stress-induced dopamine in the nucleus accumbens and the ventral tegmental area (VTA). The dopaminergic input from the VTA to the nucleus accumbens appears to play an important role in motor and reward processes (Wise, 2008). Evidence suggests that stress can increase motivation and vulnerability to drug use (Wise, 2008).

Memantine is a non-competitive antagonist of NMDA receptors with moderate affinity. It has been approved for treating memory loss in patients with Alzheimer's disease. Compared to other antagonists, this drug acts much faster and therefore has fewer effects on physiological mechanisms. Since the mechanism of memantine action is voltage-dependent, depolarization of the membrane inhibits its function (Bresink et al., 1996). Animal studies have shown that neuronal loss can be reduced by blocking NMDA receptors (Garibova et al., 2008). Studies have also shown that memantine affects both dopaminergic parameters and the transmission of noradrenergic and serotonergic neurotransmitters (Babic et al., 2012). The present study investigates the inhibitory effect of glutamate NMDA receptors in the nucleus accumbens by the chronic administration of memantine as an antagonist of this type of receptors in response to the metabolic symptoms of stress, such as food and water intake, weight change rates, defecation rates, duration of anorexia (delay to eating time), and plasma corticosterone levels.

2. Methods

2.1. Animals

Female NMRI mice with a mean weight of 30±5 g were examined in the present study. Each 6 animals were kept in a cage (Hunter et al., 2014) with 12:12 h light dark cycle at 22-24°C and were fed sufficient food and water. In each series of experiments 6 animals were tested. The animals were randomly divided into the trial and control group. Before the experiments, vaginal smears were taken from all the animals and their sexual cycles were examined. All tested animals were in their proestrus phase. Animals were housed under standard laboratory conditions in agreement with Baqiyatallah University of Medical Sciences, Community for Laboratory Animal Care and Use.

2.2. Experimental Drugs

The drug used in the present study was memantine hydrochloride (a non-competitive NMDA receptor antagonist; Sigma, USA), which was dissolved in normal saline (0.9%) and administered at different doses intraperitoneally (0.1, 0.5, and 1mg/kg) and through intra-accumbens (0.1, 0.5, and 1µg/mouse). About 50-75mg/kg of ketamine hydrochloride (Sigma, US) and 5-7mg/kg of

diazepam hydrochloride (Sigma, US) were administered intraperitoneally for anesthesia.

2.3. Surgical procedure

The animals were then placed in a Stereotaxic instrument and one or two stainless steel (23-gauge thin-walled) guide cannulas (Razipakhsh, Iran) were inserted above the nucleus accumbens in the animal's head according to Paxinos and Watson atlas Coordinates (2001) (4.5 mm from skull, 1.5 mm from middle line, and 0.5 mm anterior to bregma point) and fixed in place using a lens screw and dental cement (Pars Acryl, Iran). Different doses of memantine (0.1, 0.5, and 1µg/mouse) were administered on either side at a 0.25µL volume using 10µL Hamilton syringe with an injection needle (30-gauge thin-walled; Razipakhsh co., Iran). The intracerebral administration of memantine was performed slowly over 60 seconds, and then the needle was left in place for another 60 seconds for the drug to be fully diffused. The intraperitoneal administration of memantine (0.1, 0.5, and 1mg/kg) was performed 30 minutes before inducing stress, and in its intra-accumbens administration, 5 minutes before the stress induction.

Then, the animals were placed inside a Communication Box (Borj-Sanat Co., Tehran, Iran). This device consists of 9 separate parts (16×16×50cm for length x width x height) made of Plexiglas with transparent walls containing small holes for visual, olfactory, and audio communication. Steel bars with a 4 mm diameter are placed on the floor of the instrument at 1.3 cm distances apart, through which electric shock is transmitted to the animal's soles. The intensity and duration of the induced shock is monitored by a computer connected to the instrument (40 mV, 10Hz, for 100 s). To adapt themselves to the environment, the animals were transferred to the test room one hour before the induction of the stress, and remained there 30 minutes before and 30 minutes after the induction of the stress. The control group also remained inside the instrument for 60 minutes without receiving any shock.

2.4. Blood sampling

One day before the experiment and on the seventh day following the induction of the stress, blood samples were taken from all the animals through their retro-orbital sinus (0.5mL blood in 0.5mL of EDTA 3%) and were centrifuged at 3000 rpm for 5 minutes at 4°C. Then, the animals' supernatant plasma was collected for measuring corticosterone levels using a corticosterone measurement kit (Rat Corticosterone ELISA kit; EIA-4164; DRG Instruments GmbH, Germany) at 450 nm.

2.5. Metabolic parameters recording

In this study, the animals' food and water intake, weight, fecal weight, eating latency (duration of anorexia) and plasma corticosterone levels were measured as metabolic criteria in both trial and control groups.

2.6. Statistical analysis

Data were expressed as mean and standard error of mean (Mean±S.E.M). The 1-way ANOVA and Tukey test were used to analyze the differences (SPSS 16.0 for Windows). To determine the normality of the data, the Kolmogorov-Smirnov (KS) test was performed. The level of significance for the differences was set at $P<0.05$.

3. Results

3.1. The effect of intraperitoneal and intra-accumbens administrations of memantine on plasma corticosterone levels in animals exposed to chronic stress

After placement of the unilateral and bilateral cannula(e) in the nucleus accumbens, one group of mice received different intraperitoneal doses of memantine (0.1, 0.5, and 1mg/kg) 30 minutes before the induction of the stress and the other group received intra-accumbens doses of memantine (0.1, 0.5, and 1 µg/mouse) 5 minutes before it. The results showed that stress increases plasma corticosterone levels. In addition, the intraperitoneal administration of memantine exacerbated the effect of stress (Figure 2a). The intra-accumbens administra-

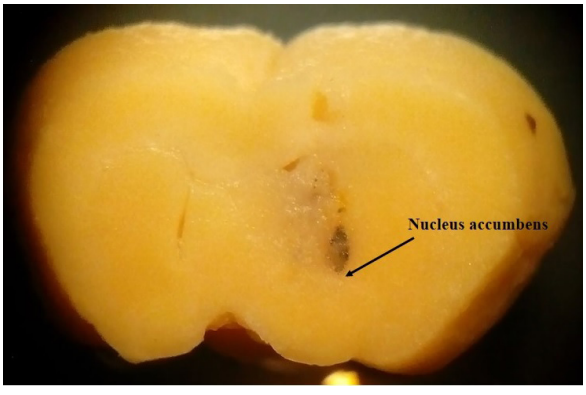

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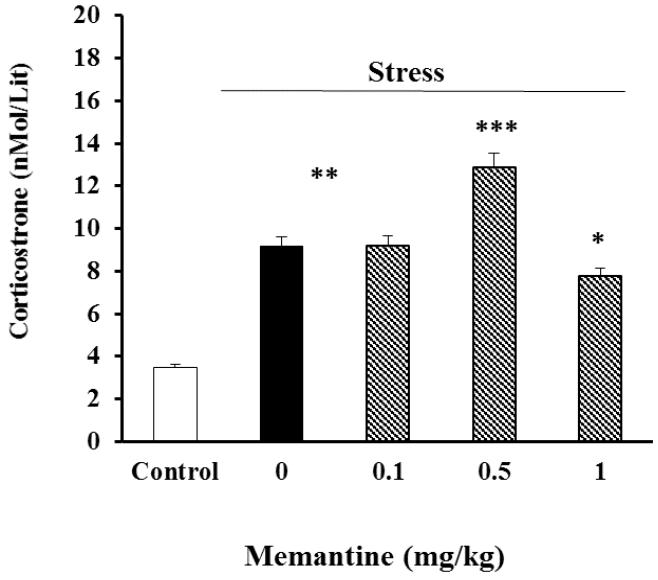
Figure 1. Cannula location in the nucleus accumbens of animals used in this study.



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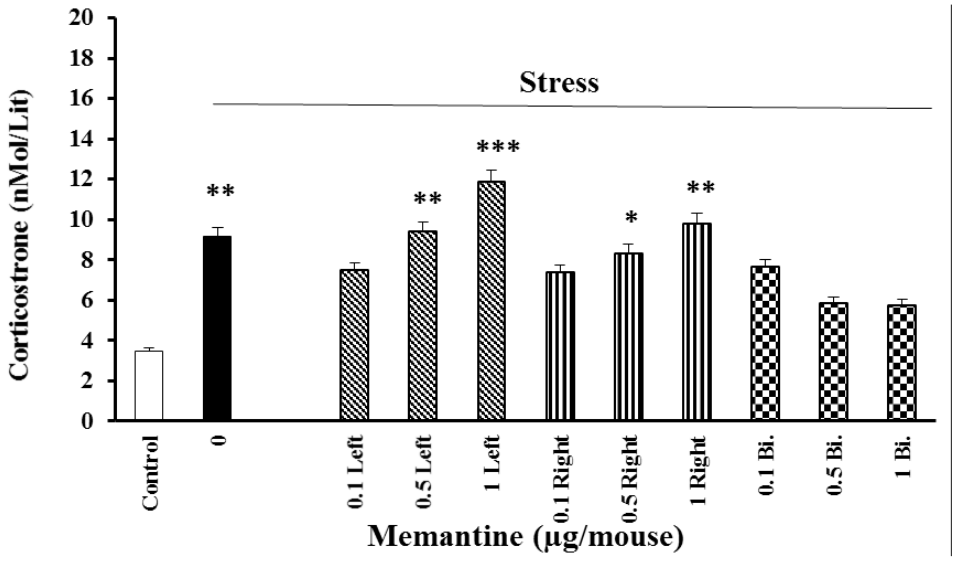

2b

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Figures 2a and 2b: Plasma corticosterone levels in animals receiving different doses of memantine (0.1, 0.5, and 1mg/kg, ip) or (0.1, 0.5, and 1 µg/mouse; i-NAc) before stress application. Each point is mean±SEM for 6 animals. *P<0.05, **P<0.01, ***P<0.001 different from control group.

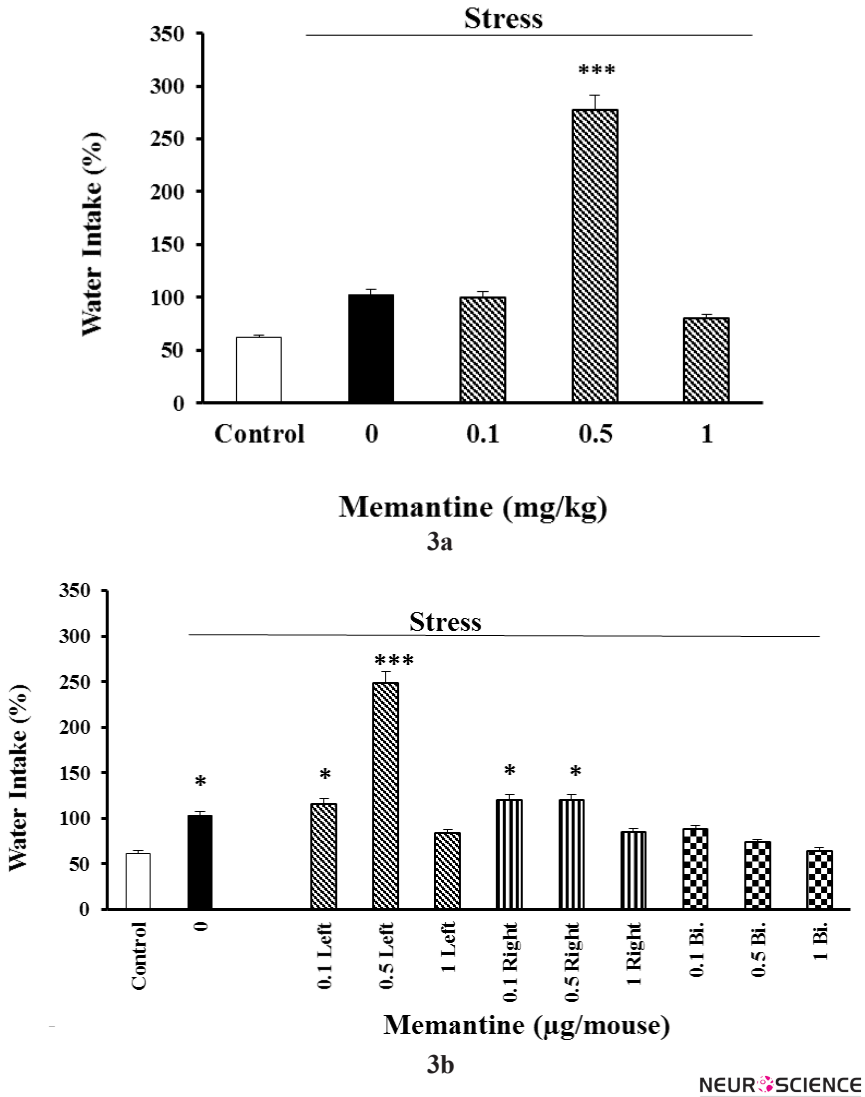
tion of memantine also exacerbated the effect of stress instead of inhibiting it (Figure 2b).

3.2. Relationship between intraperitoneal and intra-accumbens accumbens administrations of memantine and electro foot shock on changes in water intake in female mice

The results showed that chronic stress can increase the level of water intake. In addition, the intraperitoneal administration of memantine either exacerbated the effect of stress or had no effect on it (Figure 3a). Moreover, the intra-accumbens administration of memantine had the same effect (Figure 3b).

3.3. Food intake changes by chronic stress and intraperitoneal and intra-accumbens administrations of memantine in animals

The third part of the study examined the animals' food intake after the induced stress and the memantine impact. The results showed that chronic stress slightly



Figures 3a and 3b. Effect of intraperitoneal (0.1, 0.5, and 1mg/kg, ip) and intra-accumbens administrations (0.1, 0.5, and 1 µg/mouse; i-NAc) of memantine on water intake changes. The results for the groups were considered 100, in the first day, and for the other days it is evaluated according to first day (percentage). Each point is mean±SEM for 6 animals. *P<0.05, ***P<0.001 different from control group.

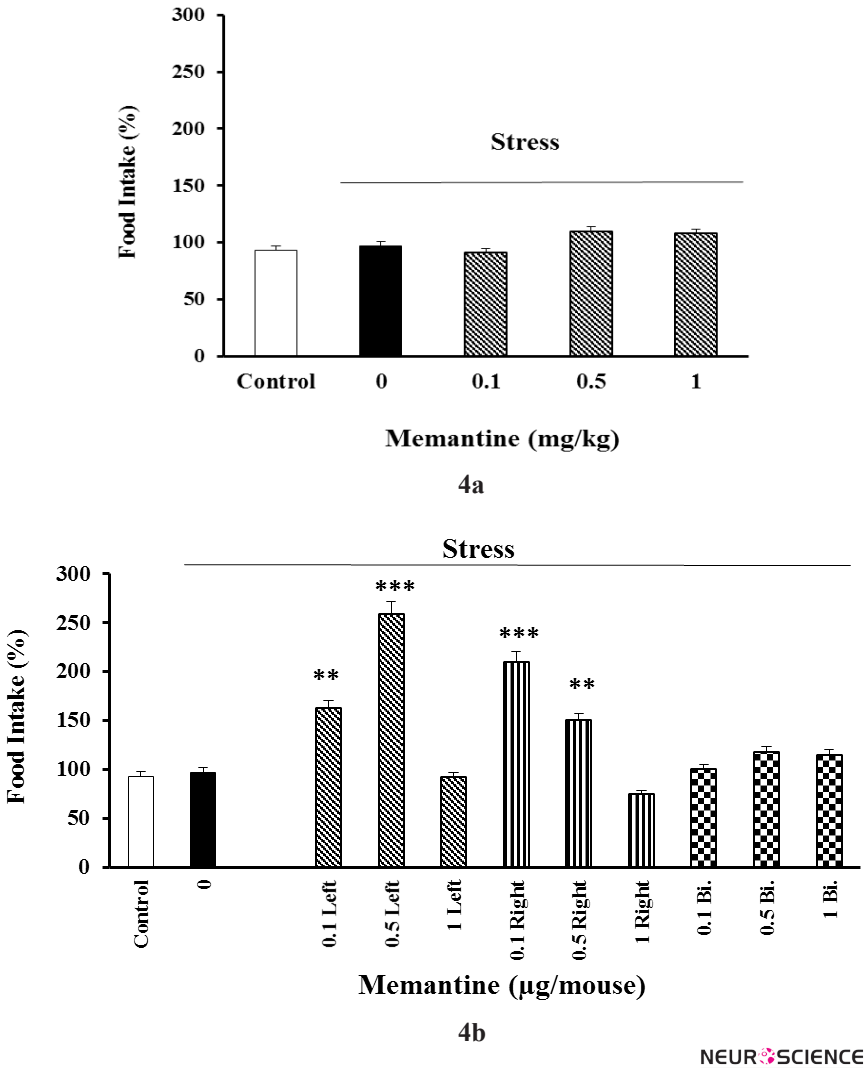
increased food intake, which was not, however, statistically significant. In addition, the intraperitoneal administration of memantine had no effect on food intake (Figure 4a). Moreover, the intra-accumbens administration of memantine at 0.1 and 0.5µg/mouse, especially when administered unilaterally to the right and left of the nucleus, increased food intake and exacerbated the effect of stress (Figure 4b).

3.4. The effect of intraperitoneal and intra-accumbens administrations of memantine on weight following the induction of chronic stress

The results showed that chronic stress slightly increased the animals' weight, which was significant. The intraperitoneal administration of memantine had no effect on weight (Figure 5a). In addition, the intra-accumbens administration of memantine inhibited the effect of stress and led to weight loss in moderate doses, but only in unilateral administration (right and left) (Figure 5b).

3.5. Memantine administration and chronic stress induction on fecal weight during electro foot shock stress

The next part of the study examined the animals' fecal weight with the induction of electric shock at their soles. The results showed that stress reduced fecal weight. In addition, the intraperitoneal administration of memantine increased fecal weight (Figure 6a). Moreover, the



Figures 4a and 4b. Memantine administration (0.1, 0.5, and 1mg/kg, ip and, 0.1, 0.5, and 1 µg/mouse; i-NAc) and chronic stress induction on food intake changes. The results for the groups were considered 100, in the first day, and for the other days it is evaluated according to first day (percentage). Each point is mean±SEM for 6 animals. **P<0.01, ***P<0.001 different from control group.

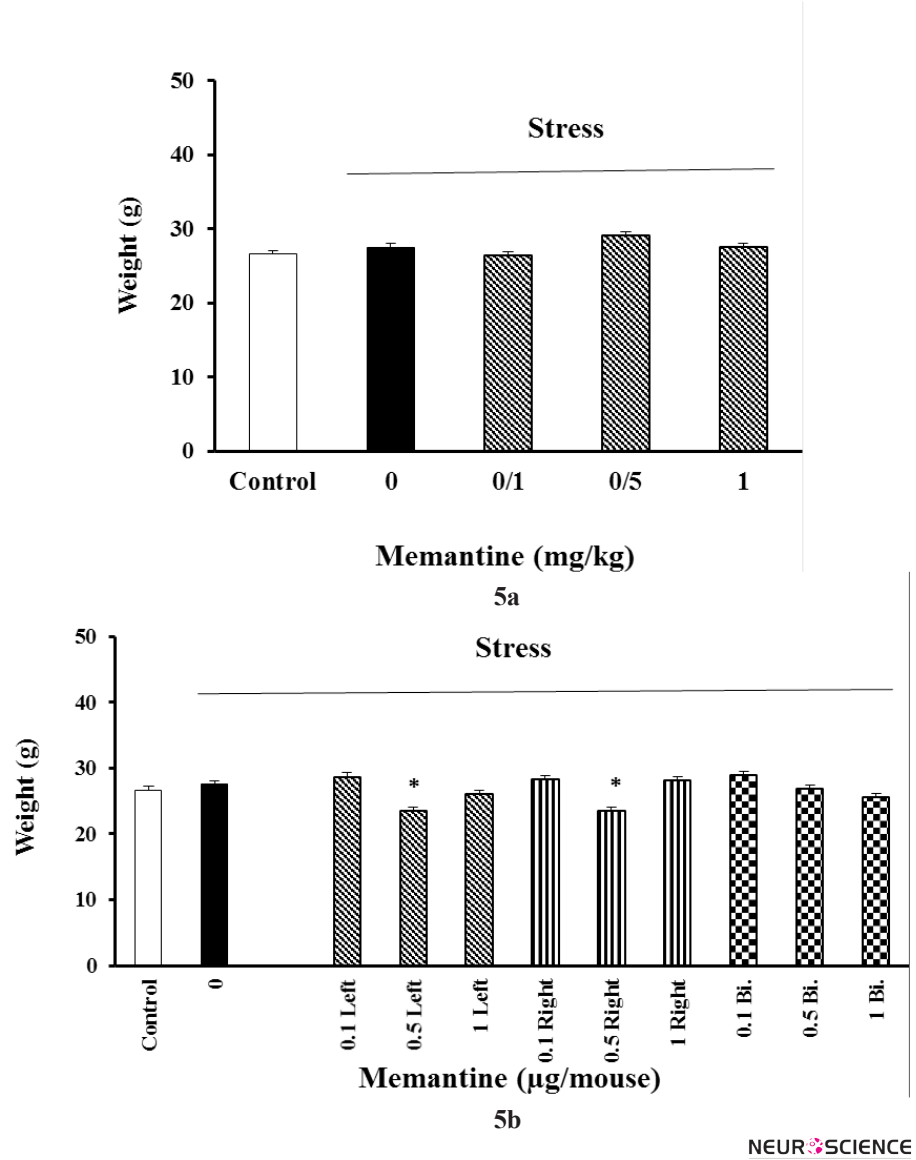
unilateral and bilateral intra-accumbens administration of memantine increased fecal weight at all three doses (Figure 6b).

3.6. Intraperitoneal and intra-accumbens administrations of memantine and stress induction on delay to eating time in female mice

For the final part of the study, the animals returned to their cages after the inducing of stress and the time taken to start eating was recorded for 7 consecutive days every day. The obtained results showed that chronic stress reduced the animals' eating latency. In addition, both intraperitoneal and intra-accumbens administration of memantine increased the duration of eating latency, which was greater in the groups that received all three doses of memantine bilaterally (Figures 7a and 7b).

4. Discussion

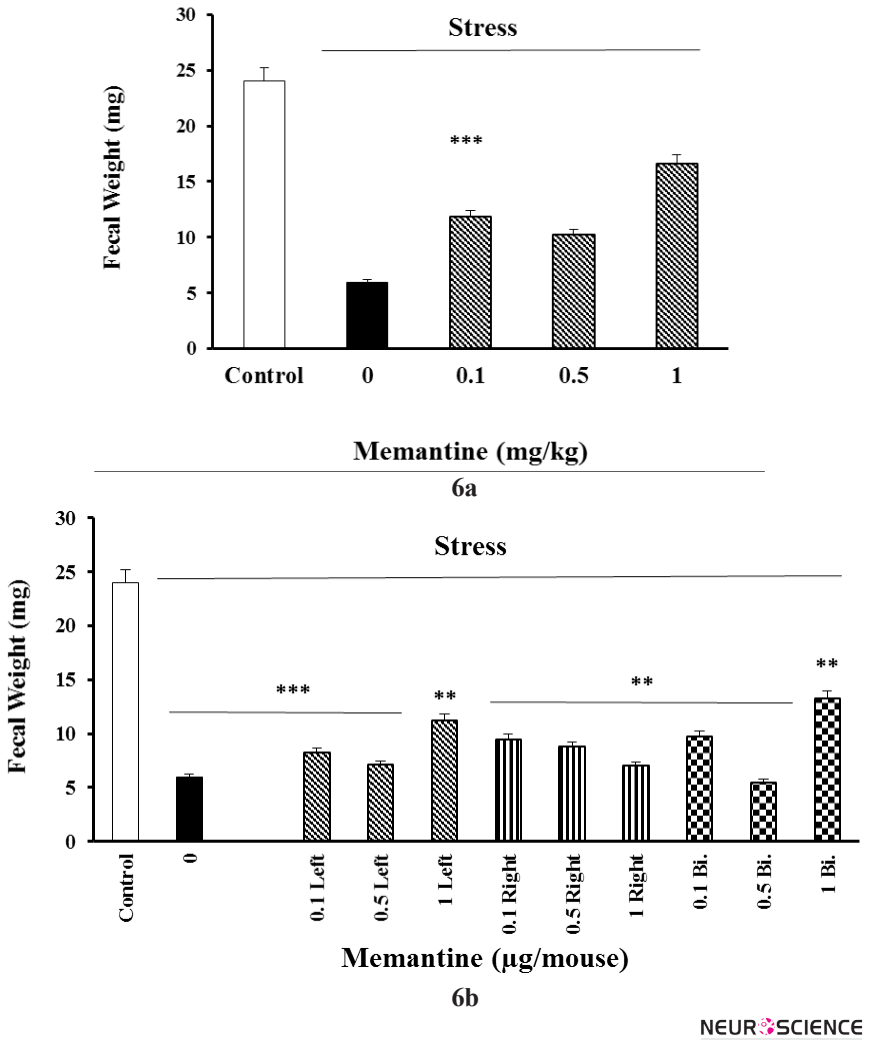
Through a series of coordinated and complex reactions and activities involving endocrine and central nervous system, stress leads to behavioral and physiological changes in the animals under stress. These behavioral and physiological changes will result in the adaptation of the living creature to the conditions of its environment. Chronic stress, especially its uncontrollable and unpredictable type, increases humans' vulnerability to a variety of diseases, including nervous and psychological diseases (McEwen, 2012).



Figures 5a and 5b. Effect of memantine administration on the weight of animals after induction of chronic stress. Each point is mean±SEM for 6 animals. *P<0.05 different from control group.

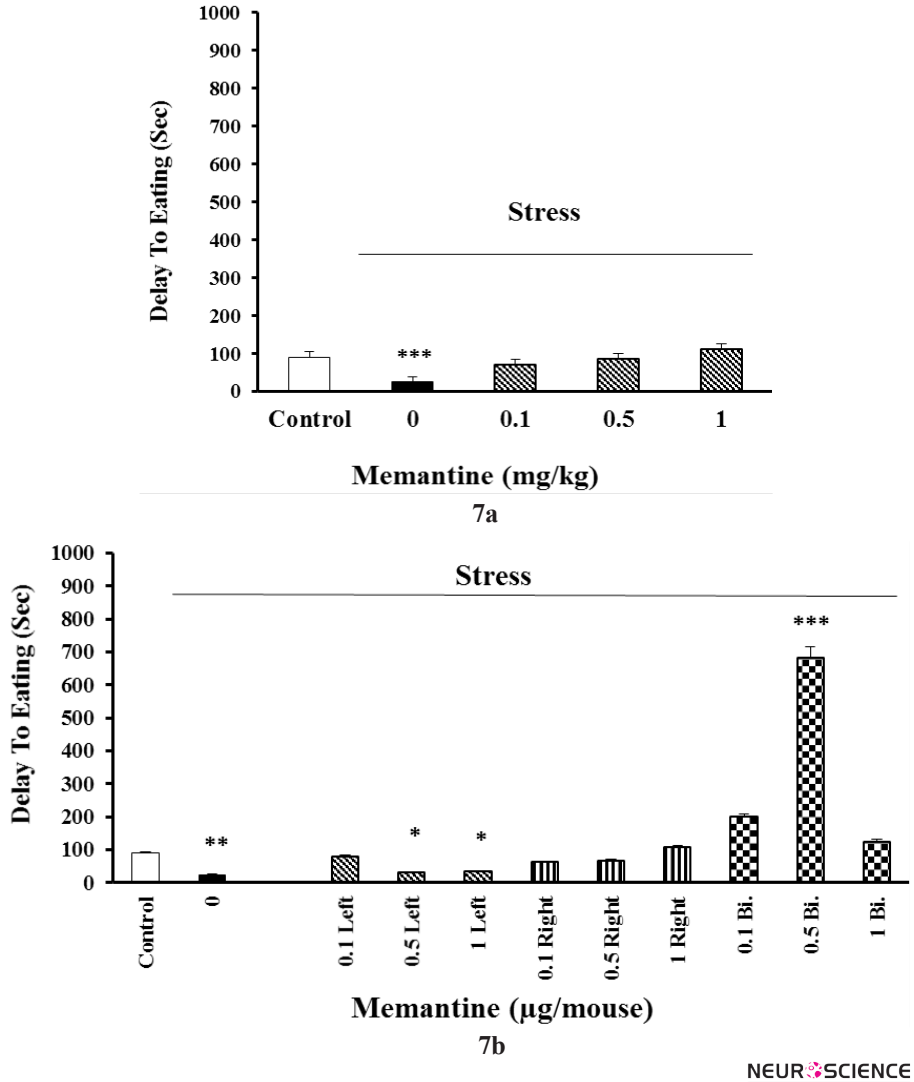
The present study examined the effect of chronic stress (designed as uncontrollable or inescapable) on the changes in plasma corticosterone levels, weight, fecal weight, eating latency, food and water intake in female mice. In addition, the inhibitory effect of glutamate NMDA receptors in the nucleus accumbens was examined. According to the results of the present study, chronic stress increased plasma corticosterone levels as well as weight and water and food intake, which was inconsistent with the results of previous studies conducted on the effect of inescapable chronic stress in male rats. Chronic stress also reduced fecal weight and eating latency. In addition, in the present study, the chronic administration of memantine as an antagonist of glutamate NMDA receptors (Tse et al., 2012) in the nucleus accumbens prior to the inducing of stress led to the inhibition of some of the destructive effects of chronic stress, which suggests that glutamate NMDA receptors in the nucleus accumbens are likely to have a significant inhibitory effect on the inhibition of the destructive effects of chronic stress.

Chronic stress increased plasma corticosterone levels in female mice. Stress, especially psychological stress, has many biological indicators; yet, increased plasma levels of glucocorticoids, such as cortisol in humans and corticosterone in rodents, is known as a potential indicator for the diagnosis of stress (Tse et al., 2012). Studies have shown that stress can increase the HPA axis activities, thereby increasing glucocorticoids as the significant markers of stress (McEwen, 2012). The



Figures 6a and 6b. Effects of stress and memantine administration (0.1, 0.5, and 1mg/kg, ip and, 0.1, 0.5, and 1 µg/mouse; i-NAc) on fecal weight in female mice. Each point is mean±SEM for 6 animals. **P<0.01, ***P<0.001 different from control group.

same mechanism appears to increase plasma corticosterone concentrations in the animals under chronic stress, which is consistent with the results of previous studies conducted on male and female rats (Halataei et al., 2011; Hooshmandi et al., 2011). Brain cells are constantly exposed to varying levels of corticosteroids, during the course of the day. Estrogen appears to affect the response to stress through several mechanisms, such as increased inhibitory effects of glucocorticoids on the HPA axis, reduced corticosterone metabolism, increased corticosterone synthesis, increased sensitivity and reduced destruction of glucocorticoid receptors (Lo et al., 2000). Researchers have found that, while chronic stress impairs the spatial memory function in male rats, it has the opposite effect on female rats (Tse et al., 2012). The intraperitoneal administration of memantine is unable to inhibit the effects of stress and consequently reduce plasma corticosterone levels. Moreover, the intra-accumbens administration of memantine exacerbated the effects of stress and increased plasma corticosterone levels. Studies have shown that the inhibition of glutamate NMDA receptors in the adrenal zona fasciculata cell membrane increases corticosterone secretion in rats (Iyengar et al., 1990). No such findings have been reported for mice; however, the same receptors might exist in these animals. That explains why the intraperitoneal administration of memantine exacerbates the effects of stress in inducing corticosterone release from these cells as a result of inhibiting the receptors. The intra-accumbens administration of memantine was also unable to inhibit the effect of stress in triggering corticosterone secretion. Apparently, memantine affects various types of NMDA glutamate receptors within nucleus accumbens and thus different effects of this drug are seen. Given the close



Figures 7a and 7b. Delay to eating time after electric foot shock and memantine administration (0.1, 0.5, and 1mg/kg, ip and, 0.1, 0.5 and 1 µg/mouse; i-NAc) in female mice. Each point is mean±SEM for 6 animals. *P<0.05, **P<0.01, ***P<0.001 different from control group.

functional relationship between the nucleus accumbens and the amygdala in mediating the effects of stress, memantine appears to increase the feelings of stress in the animal as a result of inhibiting glutamate transmission in this part of the brain, thus leading to an increased HPA stress response. Glutamate input into the hypothalamic paraventricular nucleus also appears to have a significant role in triggering CRF release from the neurons of this nucleus in response to stress (Tse et al., 2012). Nevertheless, given the site of administration in the nucleus accumbens, the drug might not have sufficiently diffused to the other side of the hypothalamus, which is why no effect is noticed of the drug in reducing CRF secretion. In addition, in its peritoneal administration, adrenal effects of memantine appear to have overtaken its central effects, which may have led to the increased secretion of corticosterone in the animals.

The results of the present study showed that chronic stress would lead to excessive drinking in mice, which was also observed in previous studies (Sadeghi et al., 2015). Researchers believe that, during stress, the mechanisms involved in thirst are also activated, causing a sensation of thirst due to their increased activity. Perhaps, it can be argued that the HPA axis, which plays an important role in the adaptation to environmental stressors through the secretion of CRF and vasopressin (VP) - two major hypothalamic peptides in the regulation of ACTH release from the anterior pituitary - is also involved in regulating the body's water intake. Stimulation of the simultaneous secretion of these two neurohor-

mones from the hypothalamic paraventricular neurons exacerbates the sensation of thirst and increases water intake in the aftermath of stress (McEwen, 2012). Arginine Vasopressin (AVP) is a neurohormonal regulator of water homeostasis. Cellular arrangement in the hypothalamic paraventricular nucleus in female rats differs from that in male rats. The largest number of neurons in this nucleus pertains to CRF in female rats and to VP in male rats (Mulder et al., 1970).

The present study showed that both dose-dependent intraperitoneal and intra-accumbens administrations of memantine either exacerbated the effect of stress or had no effect on its levels. NMDA glutamate receptor activation appears to play an important role in the regulation of water intake, particularly during stress. The stress-induced hypodipsia or polydipsia in female mice seems to partially occur through the NMDA receptors. Glutamate NMDA receptors are either pre-synaptic or post-synaptic, and the stimulation of each type might produce distinct effects (Tarazi et al., 1998). There is strong evidence on the presence of NMDA receptors in the nucleus accumbens (Wise, 2008). Through stimulating their target neurons, these receptors play an important role in transforming sensation into action in the nucleus accumbens.

Previous studies have shown that the inhibition of these receptors leads to the inhibition of morphine-induced euphoria in rats and also that the inhibition of NMDA receptors creates euphoria in rats. Some cases of abusing NMDA receptor antagonists such as ketamine and PCP have been reported in humans. Previous studies have also reported that the dopamine level in the nucleus accumbens does not decrease during times of chronic stress, which might be due to the adaptation of glutamate inputs to the nucleus accumbens. The nucleus accumbens shell was also found to be involved in the moderation of stress due to its extensive communication with the central amygdala nucleus. This part of the nucleus accumbens is controlled by glutamate inputs from the cortex and the amygdala, and the inhibition of glutamate receptors in this part can help moderate the effects of stress.

In the next part of the study, induced chronic stress was found to increase food intake; however, this increase was not statistically significant. These results were inconsistent with the results of previous studies conducted on the effect of the inducing of stress through electric shock at the soles of male mice, which found to significantly reduce food intake in male mice (Sadeghi et al., 2015). The disparity of results may be attributed to the gender differences in response to stress. Nevertheless, previous studies suggest that 30% of rats might develop overeating and gain weight as a result of chronic stress and this increase in food intake might be, however, due to the animals' adaptation to stress (Dalman et al., 2005). In our previous study, acute stress was shown to cause reduced food intake during 24 hours following induced stress in female mice (Sarahian et al., 2015).

The intraperitoneal administration of memantine had no significant inhibitory effect on food intake after the inducing of stress, while the intra-accumbens administration either exacerbated the effect of stress or had no effect on it. Memantine increased food intake at doses of 0.5 and 0.1µg/mouse, particularly when administered unilaterally to the nucleus accumbens. As memantine stimulates eating, the increased food intake might have been due to its administration. In both humans and animals, the HPA axis is one of the main neuroendocrine systems in response to stress. Scientists attribute a significant role to this axis in the endocrine regulation of appetite (Adam & Epel, 2007). The HPA axis constitutes at least one of the main causes of overeating (bulimia) or under-eating (anorexia) due to stress. CRF neurohormone released from the hypothalamic paraventricular nucleus is responsible for the loss of appetite during times of stress (Koob, 2008). Gender difference also contributes to this effect. Some studies have shown that food intake increases during stress, particularly in women (Dalman et al., 2005).

The hypothalamus-medulla oblongata regulatory pathways, particularly interrelated neurocerebral areas such as the arcuate nucleus, the Dorsal Vagal Complex (DVC) and the paraventricular nucleus play a role in the regulation of food intake and plasma osmatic regulation as well (Dalman et al., 2005). Researchers believe that, in stressed creatures, the paths associated with eating are unpredictably inhibited or stimulated, thereby causing either overeating or under-eating (Dalman et al., 2005). Chronic stress can have a double effect on nutritional priorities in laboratory animals and in humans (Dalman et al., 2005). Studies show that NMDA receptors have a role in the control of food intake through the vagal afferent neurons that innervate the upper digestive system (Czaja et al., 2006).

The intravenous or intra-nucleus administration of some glutamate receptor antagonists into the median raphe nucleus or the accumbens nucleus increase food intake (Stanley et al., 1997). NMDA receptors regulate the gastric emptying rate and can therefore play an important role in controlling meal portions (Covasa et al., 2000). Chronic stress, which is identified with high

levels of plasma cortisol in humans, was shown to potentially lead to the choice of high-fat-content food and overeating (Dalman et al., 2005).

The present study showed that chronic stress caused only a slight weight gain, which is probably due to stress-induced overeating. The intraperitoneal administration of memantine had no effect on weight loss or weight gain, while its intra-accumbens administration to the right and left side of the nucleus accumbens and only at a dose of 0.5µg/mouse slightly inhibited the effects of stress. Researchers believe that stress-induced overeating is a widespread problem in human societies that may lead to metabolic diseases such as obesity and diabetes (Adam & Epel, 2007).

Studies have also shown that chronic stress can cause weight gain in humans, and weight loss in rats and mice (Foster, 2007). The widespread belief about the effect of the HPA axis on the nutritional activities of living creatures is that high concentrations of cortisol in the plasma and thus in the brain lead to an excessive sensitivity in the brain reward system, which is manifested through increased nutritional activities and tendency toward special types of food, such as those with a high fat content (Dalman et al., 2005). A major mediator in this phenomenon is a peptide called ghrelin, which has a similar function to the growth hormone. Ghrelin is an appetizing peptide that is mainly synthesized in the stomach and is also present in healthy individuals' blood circulation; however, it has a higher blood concentration in stressed individuals (Schellekens et al., 2013). Ghrelin was also shown to have the same effect on rodents which are increased food intake, weight gain and obesity (Schellekens et al., 2013). Stress can expose the brain and the body to high concentrations of cortisol, which has direct and indirect effects on the reward system. A combination of these factors (high cortisol levels and greater calorie intake) increase abdominal fat and obesity (Adam & Epel, 2007).

Chronic stress reduced fecal weight. Previous studies showed that stress can increase vagal activity and defecation (increased rectal discharge) (Rao et al., 1998). Stress is believed to activate the CRF-ergic pathway from the medial nucleus of the amygdala to the brain stem, thereby stimulating the parasympathetic motor nuclei or the vasomotor nucleus and induce parasympathetic or sympathetic responses (or both) in stressed creatures (Rao et al., 1998). As a result, parasympathetic responses such as rectal or bladder discharge, or sympathetic responses such as increased heart rate and blood pressure are caused by chronic stress (McEwen, 2007). However, since stress was induced chronically in the animals investigated in the present study, these stimulating effects might have gradually diminished. In fact, the increased activity of a neurotransmitter system will reduce the regulatory effect of its receptors on the target neurons, which in effect reduces the efficacy of the system in chronic activity (Brunton et al., 2007).

In the present study, the same effect is possible, as chronic stress and the stimulation of the aforementioned CRF-ergic pathway may reduce the regulatory effect on these neurotransmitter receptors, and cause them to lose their initial responsiveness in the stimulation of the parasympathetic nuclei. As glutamate inputs into the medial amygdala nucleus can inhibit CRF-ergic neuron activities, the inhibition of these inputs through memantine can inhibit the reducing effect of chronic stress on rectal discharge. The intraperitoneal administration of memantine at all three doses led to the inhibition of the effect of stress and increased defecation rates. Both unilateral and bilateral intra-accumbens administrations of the drug in the nucleus accumbens at all three doses also increased defecation rates. This result was easily predictable, as the nucleus accumbens shell functions in unity with the medial amygdala nuclei (the extended amygdala). Studies have shown that NMDA receptors have a role in controlling digestive motility through the enteric-nervous circuits and that the NMDA receptor antagonists can inhibit the transmission of pain signals from the intestines and also inhibit intestinal refluxes (Shafton et al., 2007). In addition, glutamate and NMDA increase noradrenaline and acetylcholine release from the enteric-nervous system (Shafton et al., 2007).

The last part of this study examined the effect of stress on delay to eating. Previous studies have shown that chronic stress delays eating and increases the delay to eating time (Dalman et al., 2005). The present study yielded opposing results; i.e. chronic stress reduced eating latency in stressed animals. The disparity of the findings might indicate the effect of gender on the response to chronic stress. Chronic stress in male mice was previously found to increase eating latency (Sadeghi et al., 2015). The CRF secreted from the hypothalamic paraventricular nucleus was also found to have a significant inhibiting effect on appetite, and is considered a strong appetite inhibiting neuropeptide. Injection of this neurohormone into rodents' brain induced strong loss of appetite responses in them (Dalman et al., 2005). During stress, CRF and ACTH play a major role in eating latency through affecting the hypothalamic neurons (Dalman et al., 2005). Intense stress-induced activity of glutamate inputs into the paraventricular nucleus stimulates CRF

release (Aubry et al., 1996). However, in females, these glutamate inputs are very limited and are controlled by the sex hormones, in particular estrogen (Zlotnik et al., 2011). Therefore, these inputs cannot have an important role in the incidence of anorexia in female mic. Moreover, during chronic stress a regulatory reduction or change might develop in the distribution plan of CRF receptors in this neurotransmitter's target nuclei in the hypothalamus, which may reduce the effect of CRF in inducing anorexia during chronic stress (McEwen, 2007). The disparity of results with the present study might be attributed to these effects. The intraperitoneal administration of memantine at all three doses increased delay to eating; also its intra-accumbens administration at all three doses, particularly if administered bilaterally, increased eating latency, which is consistent with the explanations provided.

To conclude, glutamate NMDA receptors appear to play an important role in the development of the symptoms of impaired metabolic disorders caused by chronic stress in the nucleus accumbens, which might also be gender-dependent. The present study also found that gender may be at play too, which can perhaps explain the disparity of results with previous studies on the effects of chronic stress on male animals in such behaviors as changes in plasma corticosterone levels, food and water intake, weight, fecal weight, and delay to eating.

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