Front. Biol. 2017, 12(6): 430–441
DOI 10.1007/s11515-017-1470-2

RESEARCH ARTICLE

Effect of testosterone replacement on feeding behaviors after acute and chronic stress in gonadectomized male NMRI mice

Sara Salehi Shemiran[1], Gholam Hossein Meftahi (✉)[2], Hedayat Sahraei[2], Negin Ghobadi[1]

[1] *Department of Biology, School of Biological Sciences, Islamic Azad University, North Tehran Branch, Tehran, Iran*
[2] *Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

BACKGROUND: We study the role of gonadectomy on the response to unavoidable stress and the role of testosterone replacement on gonadectomy in the male Naval Medical Research Institute mice (30±5 g) were studied. For this purpose, the hormonal and metabolic changes were investigated.
METHODS: In the experimental group, the gonads were surgically removed, and a cannula was inserted into the left lateral ventricle. For acute and chronic stress induction, animals were placed in the communication box for 30 min for one day and four consecutive days, respectively. The animals received different doses of intraventricular (ICV) testosterone (0.01, 0.05, 0.1 μg/mouse) 5 minutes or intraperitoneal (IP) testosterone (0.05, 0.01, 0.1 mg/kg) 30 minutes before the stress induction.
RESULTS: The results showed that acute and chronic stress increases plasma cortisol concentration. IP testosterone injections of testosterone did not decrease cortisol concentrations in response to acute stress, whereas ICV injections did reduce cortisol concentrations. The stress reduced anorexia time, while the administration of testosterone increased anorexia time. In addition, acute stress reduced food intake in the gonadectomized mice. IP testosterone at 0.01 and 0.05 mg/kg increased food intake. Additionally, stress in gonadectomized mice reduced water intake, while the IP injection of testosterone in chronic stress further reduced water intake. Also, stress reduced the animals' brain/adrenal volumes, while the IP and ICV injection of testosterone at 0.01 mg/kg inhibited this effect.
CONCLUSION: The results showed that the IP (0.05, 0.01, 0.1 mg/kg) and ICV (0.01, 0.05, 0.1 μg/mouse) administration of testosterone in the gonadectomized mice can modulate hormonal and metabolic changes induced by stress.

Keywords anorexia, cortisol, food intake, gonadectomy, water intake

Introduction

Stress is a physical, mental, and emotional response to an external stimulus that could cause adaptation to the changes. Today, the study of stress and its complications is important because acute and chronic stresses are among the most important mental health problems in human society (Derijk et al., 2008; Ehteram et al., 2017; Asalgoo et al., 2015). Stress also plays an important role in many health problems, including asthma, cancer, diabetes, and endocrine disorders. These diseases place large financial burdens on healthcare systems every year (Segerstrom and Miller, 2004; Salleh, 2008).

Stress responses, the coordinated responses that increase one's chances of survival, include changes in behavior, autonomic system performance, and the secretion of several hormones such as adrenocorticotropin, cortisol, corticosterone, and catecholamine. One of the important neuroendocrine system responses to stress is activation of the hypothalamic–pituitary–adrenal (HPA) axis (Derijk et al., 2008; Pourhashemi et al., 2016). A stressful stimulus causes the release of corticotropin-releasing hormone (CRH) and vasopressin (VP) from the hypothalamus paraventricular nucleus (PVN). CRH and VP stimulate the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary to the blood, which results in the production and secretion of glucocorticoids from the adrenal cortex (Ghobadi et al., 2016; McEwen et al., 2016; Hassantash et al., 2017). Through their receptors located in the peripheral target organs, glucocorticoids cause metabolic changes such as mobilizing energy to maintain muscle and brain function, increasing blood flow and cerebral glucose utilization, increasing cardiac output, increasing the

Received July 29, 2017; accepted November 27, 2017
Correspondence: Gholam Hossein Meftahi
E-mail: meftahi208@yahoo.com; hossein.meftahi@bmsu.ac.ir

respiratory rate, and distributing the blood flow to increase substrate and energy transmission to the brain and muscles (McEwen, 2007; McEwen et al., 2016; Erfani et al., 2017). CRH and ACTH act on the hypothalamus neurons to play a major role in the development of anorexia nervosa in stress (Connan et al., 2007). Thus, stress can affect the hormonal and neurotransmitter systems. Many studies have shown that psychological and physiologic stress can induce sexual dysfunction and infertility (Kennedy et al., 1999; Baldwin, 2001). There is a functional interaction between the gonads and the adrenal axes that is in large part due to the interactive effects of sex hormones and glucocorticoids, demonstrating that several disease states connected to stress are sex-dependent. Testosterone can act and interact with different aspects of basal and stress HPA function. The basal ACTH release is regulated by testosterone-dependent effects on arginine vasopressin synthesis and corticosterone-dependent effects on CRH synthesis in the PVN of the hypothalamus. In contrast, testosterone and corticosterone interact on stress-induced ACTH release and drive to the PVN motor neurons. In males, stress significantly reduces levels of gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (Arun et al., 2016; Rai et al., 2004).

It has been shown that response to stress involves sexual dimorphism, which means that men and women respond to stress differently. On the other hand, studies in humans and animals have indicated sexual differences in terms of sensitivity to the stress response. These sexual differences may be related to environmental, cultural, social, genetic, and sex hormone factors (Curtis et al., 2006; McEwen and Milner, 2007; McEwen et al., 2016). Prolonged stress reduces the male sex hormone level, which can cause a rapid response to new situations. Chronic stress, which keeps the body in a state of constant threat, is a major factor in the decline of testosterone levels. It has been shown that testosterone and estrogen exert reliable inhibitory and stimulatory effects, respectively, on HPA axis activity (Williamson et al., 2005). Lund et al. (2006) showed that different metabolites of testosterone, such as 5α-dihydrotestosterone and its 3β-diol metabolite, are capable of acting locally to suppress stress-induced levels of PVN Fos mRNA and plasma ACTH and corticosterone. Studies also showed that different kinds of stress, like restraint, electrical shock, cold, and sleep deprivation, could decrease testosterone levels (Hardy et al., 2005; Hari Priya and Reddy, 2012). However, studies of the relationship between stress and sex hormones in rodents showed conflicting results. Although several studies have investigated the effects of testosterone during stress, studies that compare the effect of the intraventricular (ICV) and intraperitoneal (IP) administration of testosterone on metabolic changes during electric foot shock stress are lacking. Therefore, the aim of this study was to investigate the effects of the ICV and IP injection of testosterone on plasma cortisol concentration and eating behaviors such as anorexia, water and food intake, weight change, and brain/adrenal gland volume during acute and chronic stress in male gonadectomized Naval Medical Research Institute (NMRI) mice.

Material and methods

Animals

Male NMRI mice weighing 30±5 g each were kept in groups of four per cage in 12-h light/dark conditions at 22–24°C with food and water provided *ad libitum* except during the experimental time. The animals were housed in the standard animal room for 1 week for adaptation before the start of the study. The animals were randomly divided into control and experimental groups (n = 8/each). Food and water intake was recorded for each animal at specific hours every day using the amount of food and water left in each cage. Animal experiments were conducted in accordance with the Guidelines of the National Institute of Health for the Care and Use of Laboratory Animals and approved by the local ethical committee (The Baqiyatallah University of Medical Sciences Committee on the Use and Care of Animals, 87/381).

Drugs

The following drugs were used throughout the experiments: testosterone (Abu-Rayhan-Iran), ketamine hydrochloride (Sigma–Aldrich, USA), and diazepam (Sigma–Aldrich). Testosterone was dissolved in sesame oil and injected ICV (0.01, 0.1, 0.05 μg/mouse) 5 min or IP (0.01, 0.1, 0.05 mg/kg) 30 min before the stress induction. The ketamine hydrochloride and diazepam hydrochloride were dissolved in sterile saline.

Cannulation

For cannulation, the animals were first anesthetized with ketamine 50–75 mg/kg and diazepam 5–7 mg/kg and the surgical area was shaved. The animals were placed in a stereotaxic apparatus and a small incision was made in the scalp to expose the skull. Using bregma and lambda as landmarks, the skull was leveled in the coronal and sagittal planes with one or two guide cannulas (gauge no. 23; World Precision Instruments) implanted into the skull above the lateral ventricles utilizing the Paxinos and Franklin (2001) atlas (for the lateral ventricles, AP = + 0.9 mm; ML = ±2 mm; DV = 3 mm) and fixed with dental acrylic cement. The animals were given 7 days to recover after the surgery. A dental needle head no. 30 (Alibaba; INTR), polyethylene tubes, and 10-mL Hamilton syringes were used for the injections. The unilateral (left lateral ventricle) administration of different doses of testosterone (0.1, 0.01, and 0.05 μg/mouse) was conducted daily for 5 min prior to the stress induction. The brain injection was gradual and lasted 60 s, during which time the animals were free to move around.

Gonadectomy

First, the animal was anesthetized by an IP injection of ketamine hydrochloride 70 mg/kg and xylazine 10 mg/kg (Mohammadian et al., 2017; Sadeghi-Gharajehdaghi et al., 2017). Each animal was placed in dorsal recumbency, the hair over the caudal abdomen was shaved, and the surgical area was disinfected with 70% alcohol followed by a 2% chlorhexidine solution. Next, a midline incision approximately 5–7 mm long was made in the scrotum to expose the tunica. One testis was pushed out of the tunica and the vas deferens and spermatic blood vessels were cauterized. Finally, the testes were removed and the incision was sutured.

Animal group

The animals were randomly divided into nine groups ($n = 8$ each). The control group animals received no treatment. The experimental group animals were gonadectomized and divided into two acute and chronic stress groups. Testosterone was injected IP or ICV (left side) into the experimental group animals. Testosterone was injected IP (0.01, 0.1, 0.5 mg/kg) 30 min or injected ICV (0.01, 0.1, 0.5 μg/mouse) 5 min before the induction of chronic or acute stress. After the testosterone administration, to induce stress, the animals were placed into a communication box consisting of nine separate parts (50 × 16 × 16 cm) with Plexiglas walls and tiny holes with a 2-mm diameter to allow olfactory and auditory communication between the mice. Steel bars (with a 4-mm diameter) were placed on the floor of the instrument at 1.3 cm apart through which the electric shock is transmitted to the animal's soles (Dalooei et al., 2016; Amouei et al., 2016). Shock duration and intensity were controlled by a computer connected to the communication box (60 mV, 10 Hz, for 60 s). The electric shock was induced randomly for 9–13 h. To allow the animals to adapt to the environment, they were transferred to the test room 60 min before the stress induction and remained there 30 min before and 30 min after the stress induction. In acute stress, the animals received foot shocks for 1 day; in chronic stress, the animals received foot shocks for 4 consecutive days. The control animals were placed in the device for 60 min without receiving any shocks. After the stress induction, the animals were returned to their cages. Anorexia (the elapsed time between mouse replacement in the home cage and the beginning of food intake was calculated as a delay of eating or anorexia), water and food intake, and weight were measured every day at a specific time. The control group received sesame oil (testosterone solvent). After completing the tests on day 4, the animals were anesthetized using high doses of ketamine and their brains and adrenal glands were removed and kept in 4% formalin solution for fixation. Sixty days later, the brains and adrenal glands were removed from the formalin. The weights and volumes of the brains and adrenal glands were measured by mercury immersion.

Cortisol concentration

One day before the start and on the last day of the experiment, blood samples were collected from all animals in all groups from their retro-orbital sinus (0.5 mL of blood in 0.5 mL of 3% ethylenediaminetetraacetic acid). The blood was then centrifuged at 3000 rpm for 5 min at 4°C and the serum was collected for cortisol detection. The serum was collected and frozen at −20°C and the cortisol concentrations were determined using an enzyme-linked immunosorbent assay kit (Cortisol ELISA kit 4164; DRG Instruments GmbH, Germany). Briefly, serum samples were added to 96-well plates containing biotinylated primary antibody and then incubated at 37°C for 45 min. Thereafter, the plates were washed and horseradish peroxidase–conjugated streptavidin solution was added to the wells and incubated for an additional 30 min at 37°C. The 3,30,5,50-tetramethylbenzidine substrate was added, the plates were incubated for an additional 15 min at 37°C, and stop solution was added to the wells to terminate the reaction. Cortisol concentrations were determined using a standard curve.

Data analysis

The data are expressed as mean±S.E.M. To analyze the data, one-way analysis of variance followed by Tukey test were used. Values of $p < 0.05$ were considered statistically significant.

Results

Effects of testosterone administration on plasma cortisol levels during acute and chronic stress induction

The results showed that acute and chronic stress significantly increased plasma cortisol concentrations compared to the control group. In both acute and chronic stress, the IP administration of testosterone has no significant effect on plasma cortisol concentrations. However, the ICV injection of testosterone in acute and chronic stress significantly decreased cortisol concentrations (Figs. 1A and 1B).

Effect of testosterone administration on anorexia during acute and chronic stress

After stress induction, the experimental groups were returned to their holding cages and the duration of anorexia (delay of eating) was measured. The results obtained on the first day were taken as 100 and as a point of reference for measurements made on subsequent days (percentage). As shown in Fig. 2A, acute stress significantly decreased anorexia time compared to the control group. The IP (0.1 and 0.05 mg/kg) and ICV (all doses) injections of testosterone in acute stress significantly increased anorexia time compared to the stress group. This increase was higher when

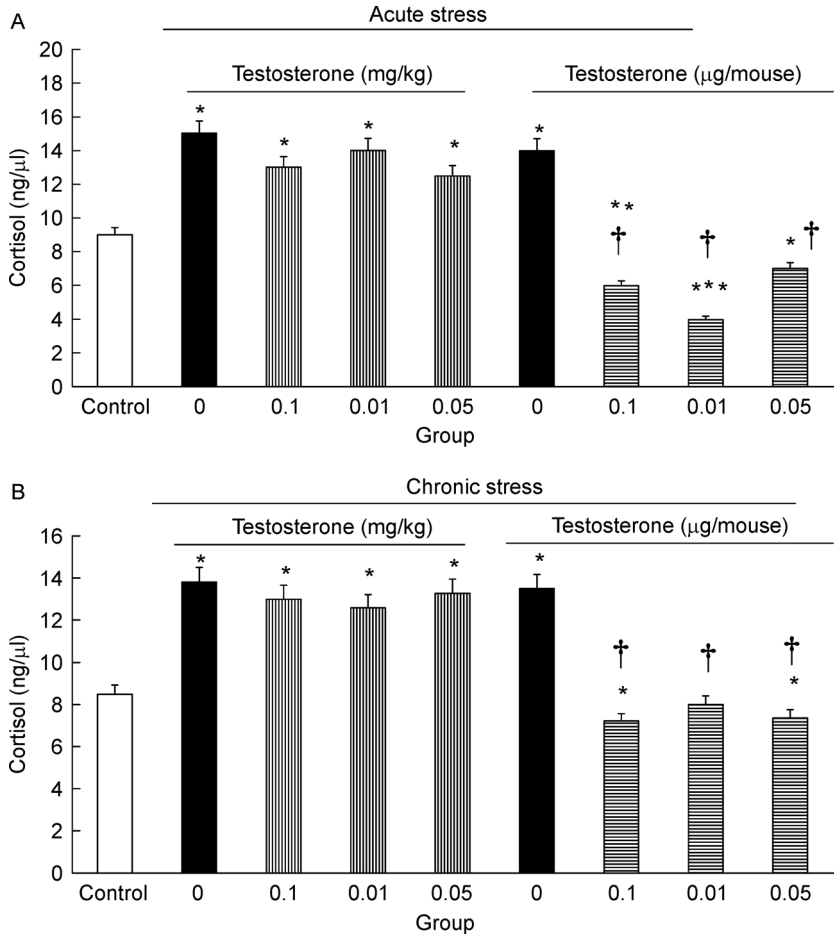

Figure 1 Effect of intraperitoneal and intraventricular injection of testosterone on plasma cortisol concentrations during the induction of acute (A) and chronic (B) stress. Mean±S.E.M. for 8 animals. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences compared to the control group. †$P < 0.05$ indicates a significant difference compared to the stress group.

testosterone was injected ICV. Anorexia time in chronic stress also decreased compared to the control group. Only the IP administration of testosterone 0.05 mg/kg significantly increased the anorexia time compared to the chronic stress group, whereas the ICV administration of testosterone at all doses significantly increased anorexia time compared to the chronic stress group (Fig. 2B).

Effect of testosterone administration on food intake after acute and chronic stress induction

The animals were returned to their cages after stress induction and the food intake was measured during 24 h for 4 consecutive days. The results obtained on the first day were taken as 100 and as a point of reference for measurements made on subsequent days (percentage). The results showed that both acute and chronic stress significantly reduced food intake in gonadectomized mice. However, the effect of chronic stress on food intake reduction was lower than that of acute stress. In both acute and chronic stress, IP injections of testosterone (0.01 and 0.05 mg/kg in acute stress and 0.1 and 0.05 mg/kg in chronic stress) increased the food intake compared to the stress group. However, in the chronic stress experiments, the IP injection of testosterone at 0.01 mg/kg significantly decreased the food intake compared to the stress group (Figs. 3A, 3B). In the acute stress experiments, the ICV injection of testosterone 0.01 and 0.05 μg/mouse increased the food intake compared to the stress group, but the injection of 0.1 μg/mouse decreased the food intake compared to the stress group. In chronic stress, the ICV injection of testosterone at 0.1 and 0.01 μg/mouse reduced the food intake, while no significant changes were observed at 0.05 μg/mouse compared to the stress group.

Effect of testosterone administration on water intake after acute and chronic stress induction

As shown in Figs. 4A and 4B, both acute and chronic stress reduced the water intake compared to the control group. The IP administration of testosterone at 0.05 and 0.1 mg/kg in acute stress significantly reduced water intake compared to the stress group. However, the ICV administration of

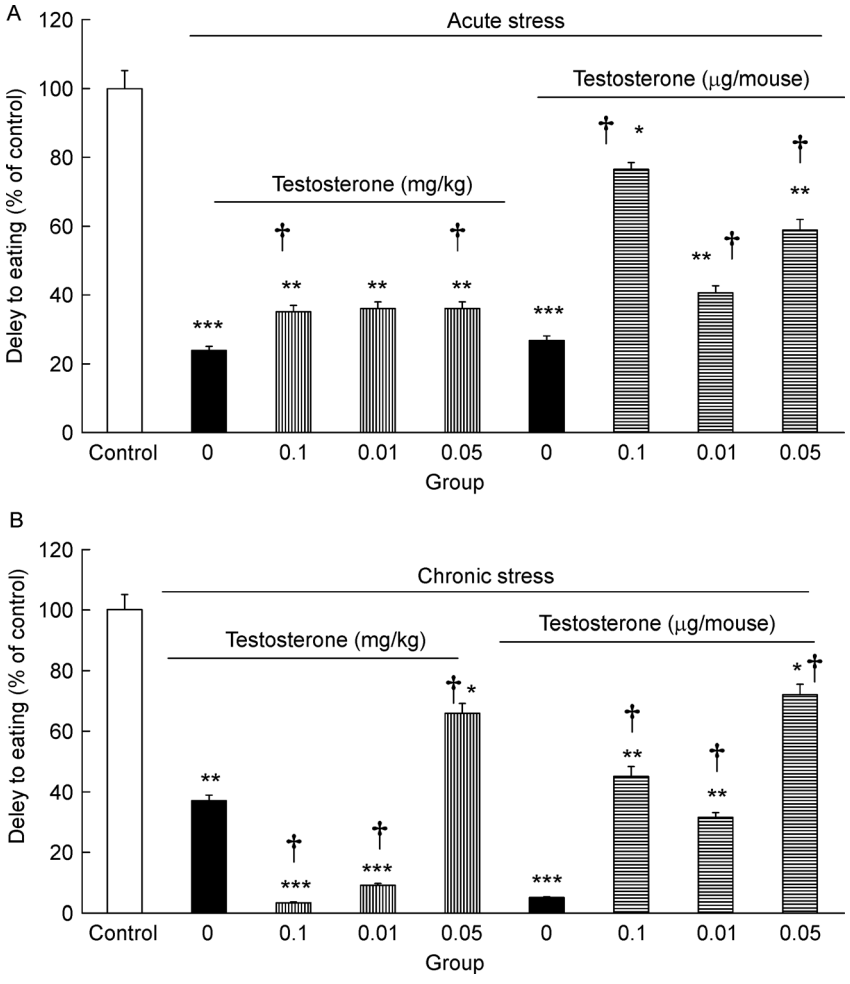

Figure 2 Effect of intraperitoneal and intraventricular injection of testosterone on delay to eating during the induction of acute (A) and chronic (B) stress. Mean±S.E.M. for 8 animals. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences compared to the control group. †$P < 0.05$ indicates a significant difference compared to the stress group.

testosterone at all doses (0.1, 0.05, 0.01 μg/mouse) significantly increased water intake (Fig. 4A). In the chronic stress experiments, the IP administration of testosterone had no effect on water intake compared to the stress group; however, the ICV administration of testosterone significantly reduced water intake compared to the stress group (Fig. 4B).

Effect of testosterone administration on weight changes after acute and chronic stress induction

The animals were weighed every day before the stress induction and their weight changes were measured over 4 days. Figure 5 shows the effect of different doses of testosterone on the animals' weight. Acute stress significantly increased the animals' weights compared to the control group (Fig. 5A), while chronic stress had no effect on bodyweight compared to the control group (Fig. 5B). On the other hand, in the acute stress experiments, the IP administration of testosterone at 0.1 and 0.01 mg/kg significantly reduced the animals' weights compared to the stress group (Fig. 5A). However, the ICV administration of testosterone had no effect on bodyweight compared to the stress group. As shown in Fig. 5, the IP injection of testosterone at 0.01 mg/kg significantly reduced the weights compared to the stress and control groups. However, the ICV injection of testosterone had no effect on animal weights.

Effect of IP and ICV injection of testosterone on brain/adrenal gland weight after acute and chronic stress

As shown in Figs. 6A and 6B, acute stress caused a significant decrease in brain and adrenal gland weights compared to the control group. However, chronic stress had no significant effect on brain and adrenal gland weights compared to the control group. In both acute and chronic stress, the IP and ICV injections of testosterone 0.01 mg/kg significantly increased the ratio of brain to adrenal gland weight compared to the stress and control groups.

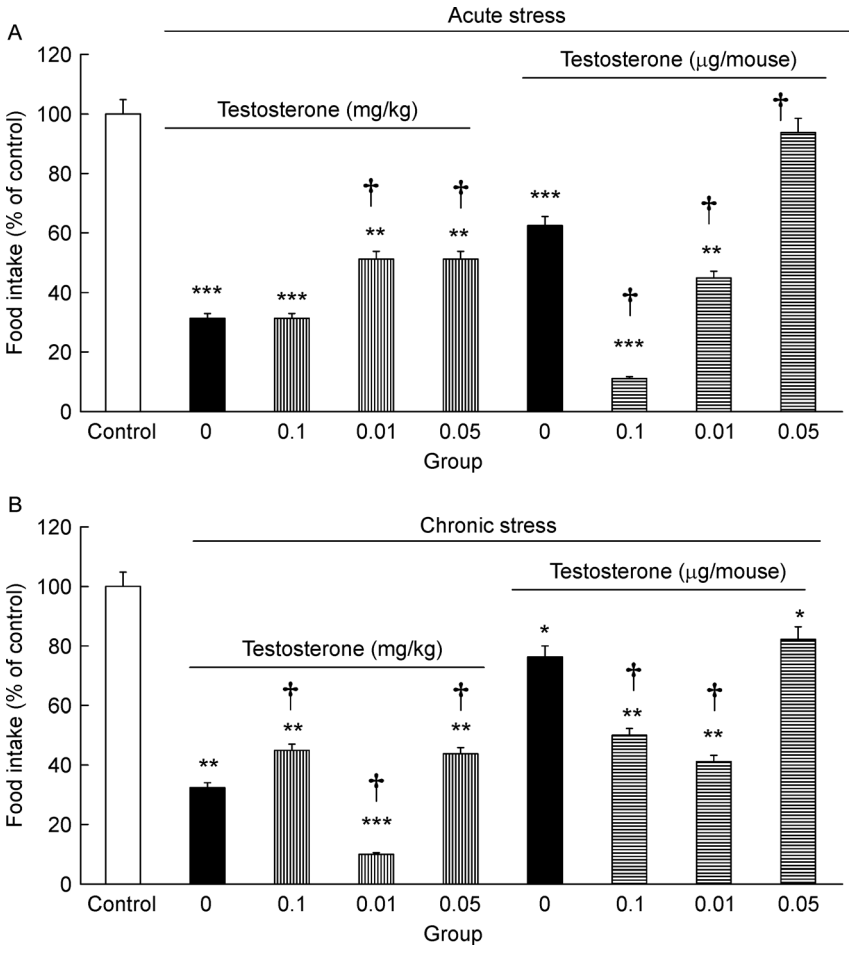

Figure 3 Effect of intraperitoneal and intraventricular injection of testosterone on food intake during the induction of acute (A) and chronic (B) stress. Mean±S.E.M. for 8 animals. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences compared to the control group. †$P < 0.05$ indicates a significant difference compared to the stress group.

Discussion

In this study, acute and chronic stress increases plasma cortisol levels in male mice. The IP injection of testosterone could not reduce the plasma cortisol concentrations. However, the ICV injection of testosterone reduced plasma cortisol concentrations. Stress can cause increased HPA axis activity, and an increased level of glucocorticoid hormones is one of the most important signs of stress (McEwen, 2007). In the present study, these mechanisms may increase plasma concentrations of cortisol in animals after stress induction. Several studies showed that androgens inhibit stress-stimulated ACTH and cortisol concentrations in animals (Handa et al., 1994; Papadopoulos and Wardlaw, 2000). Testosterone (through several mechanisms such as decreased CRH, increased glucocorticoid receptor concentrations, decreased arginine vasopressin [AVP], and suppression of cortisol secretion through its metabolite 3 beta androstanediol) reduced cortisol concentrations (Lund et al., 2004; Rubinow et al., 2005). It is possible that the ICV injection of testosterone reduces cortisol concentrations via these mechanisms.

Our results showed that chronic stress causes slight weight gain in animals, but this increase was not significant and probably due to stress-induced overeating. The IP injection of testosterone 0.01 mg/kg reduced the animals' weights in the stress experiments. However, the ICV administration of testosterone had no effect on the animals' weights in the chronic stress condition. Overeating caused by stress is common in human societies and may lead to metabolic disorders such as obesity and diabetes (Mikolajczyk et al., 2009). It is believed that the presence of high plasma and brain cortisol concentrations leads to high brain sensitivity that is reflected in increased nutritional intake and the tendency to the use special foods, increase sugar and fat cravings, and even increase fat stores. This is one mechanism by which stress increases fat and leads to metabolic dysfunction. Chronic stress, together with high glucocorticoid concentrations, usually reduces bodyweight gain in rats; in contrast, in stressed or depressed humans, chronic stress

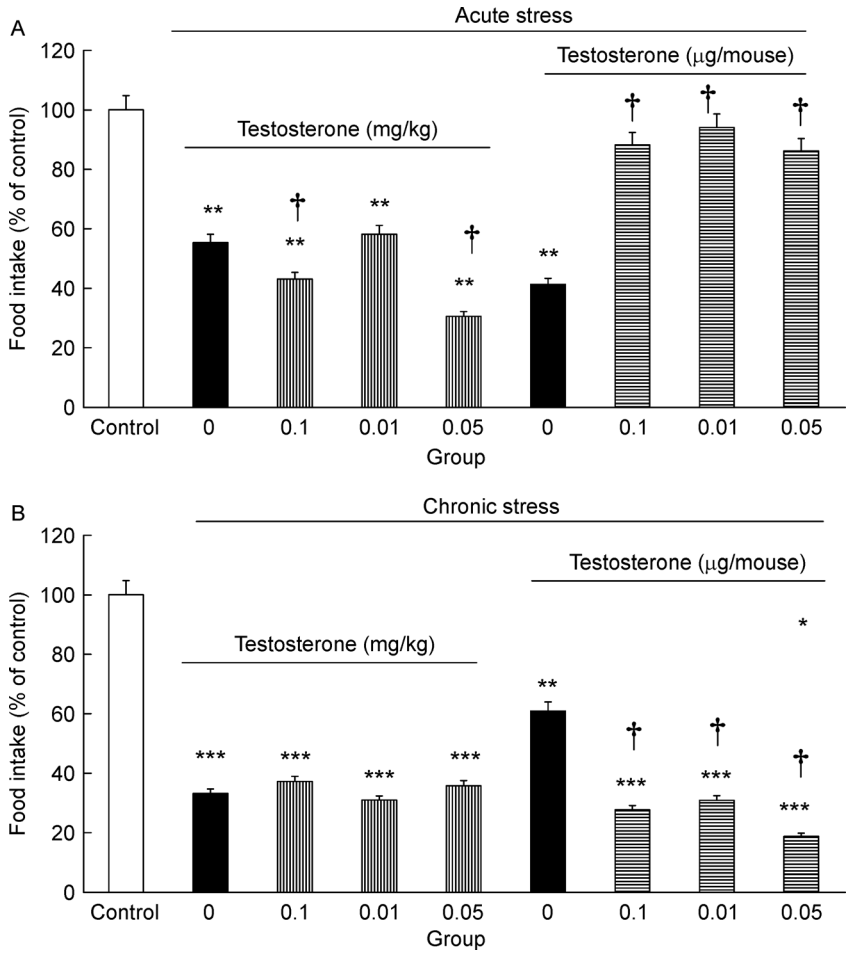

Figure 4 Effect of intraperitoneal and intraventricular injection of testosterone on water intake during the induction of acute (A) and chronic (B) stress. Mean±S.E.M. for 8 animals. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences compared to the control group. $\dagger P < 0.05$ indicates a significant difference compared to the stress group.

induces either elevated comfort food intake and bodyweight gain or reduced intake and bodyweight loss. Comfort food ingestion, which produces obesity, decreases corticotropin-releasing factor (CRF) mRNA in the rat hypothalamus (Dallman et al., 2003). Also, ghrelin is an important molecular mediator that leads to weight gain. Ghrelin is an appetizer peptide that is synthesized mainly in the stomach and observed in the blood of healthy individuals, but its circulating concentrations have been found to increase following stress (Chuang and Zigman, 2010). Ghrelin plays a role in food intake, weight gain, and obesity in rodents. Since chronic stress can lead to exposure to high cortisol levels in the brain and body and has direct and indirect effects on reward systems, the combination of these factors (high cortisol and more calories) can increase the amount of abdominal fat and leads to obesity (Adam and Epel, 2007).

Studies have shown that chronic stress reduces food intake in animals. Several studies have shown that stress can inhibit bodyweight gain and food intake in rodents (Krahn et al., 1990; Dallman et al., 1992). This reduction in food intake may be due to stress adaptation in animals. Acute stress can reduce food intake 1 day after stress in male mice. In the current study, the IP injection of testosterone had no significant ability to inhibit the effects of chronic stress on food intake, whereas the ICV injection of testosterone 0.05 μg/mouse increased the food intake. The HPA axis plays an important role in appetite regulation. The CRF is responsible for the anorexia in stress. The injection of CRF into the brain reportedly reduces appetite (Majzoub, 2006). However, there are sexual differences in this regard, and some studies have shown that food consumption, especially in women, increases during stress (Habhab et al., 2009). Testosterone receptors are involved in the control of food intake. For instance, androgen levels are involved in food intake and metabolism in women, which results in weight gain (Cotrufo et al., 2000; Hill, 2010). The arcuate nucleus plays a pivotal role in the control of food intake by opposing orexigenic and anorexigenic neuronal circuits. The anorexigenic neurons express cocaine- and amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC). The activation of POMC/CART neurons signals to the downstream neuronal pathways that suppress food intake. Hill (2010) showed that androgens could affect

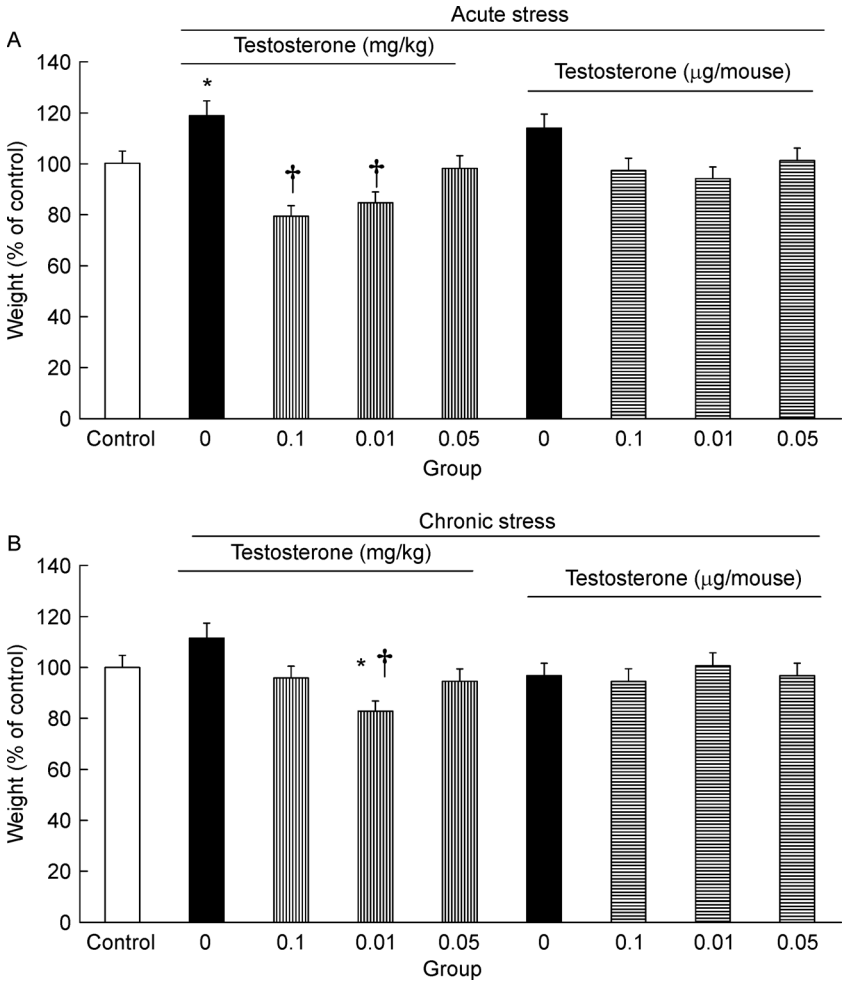

Figure 5 Effect of intraperitoneal and intraventricular injection of testosterone on weight change during acute (A) and chronic (B) stress. Mean±S.E.M. for 8 animals. $*P < 0.05$ indicates a significant difference compared to the control group. $\dagger P < 0.05$ indicates a significant difference compared to the stress group.

POMC gene expression and that androgen receptors regulate the transcription of target genes by interacting with DNA response elements.

In the present study, the effects of stress on the delay to eating were also investigated. Chronic stress increases the delay to eating in the stress group. As mentioned above, CRF has a significant effect on appetite suppression and is considered a strong neuropeptide appetite inhibitor. Also, glutamate drives the HPA axis stress responses through excitatory signaling via ionotropic glutamate receptor signaling. Moreover, glutamate innervation of the PVN undergoes neuroplastic alteration under chronic stress and may be involved in the sensitization of HPA axis responses (Evanson and Herman, 2015). Chronic stress may reduce the regulation of or changes in CRF receptor distribution in the hypothalamus and decrease the effect of CRF on the induction of anorexia during the chronic stress. The IP administration of testosterone at three doses reduced the delay to eating and the ICV injection of testosterone 0.1 or 0.05 μg/mouse increased the delay to eating time.

Our results show that acute stress can lead to reduced water intake in animals, a finding that is inconsistent with previous studies (Osanloo et al., 2015). The mechanisms involved in thirst are activated during stress and lead to the thirst sensation. The HPA axis plays an important role in adaptation to environmental stressors through the secretion of CRF and VP, which regulate ACTH release from the anterior pituitary and are involved in regulating the body's water intake. During stress stimulation, the simultaneous secretion of these two neurohormones from the PVN exacerbates the sensation of thirst and increases water intake (McEwen, 2012). In this study, the IP testosterone administration could not inhibit the effects of acute stress on reducing water intake, but the ICV injection of testosterone at three doses can indeed inhibit the effects of acute stress and result in increased water intake. We showed here that chronic stress also leads to a reduced water intake in male mice, while the ICV and IP injections of testosterone could not reduce water intake. Testosterone has a suppressive effect on stress-induced AVP biosynthesis in the median eminence (Viau and Meaney, 1996).

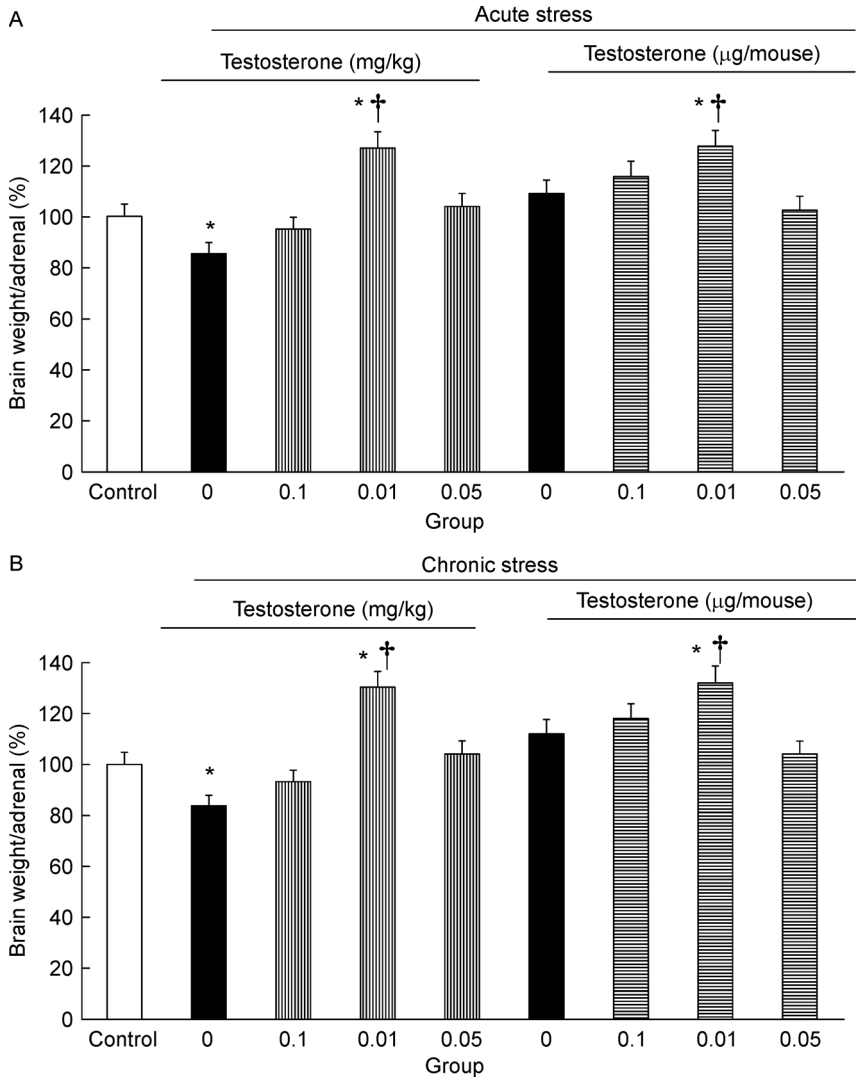

Figure 6 Effects of acute (A) and chronic (B) stress on changes in the ratio of brain volume/adrenal gland. Animals received different doses of intraventricular testosterone (0.1, 0.1, or 0.05 μg/mouse) 5 min and intraperitoneal testosterone (0.1, 0.01, or 0.05 mg/kg) 30 min before the stress induction. Mean±S.E.M. for 8 animals. $*P < 0.05$ indicates a significant difference compared to the control group. $\dagger P < 0.05$ indicates a significant difference compared to the stress group.

We also studied the effects of stress on brain/adrenal gland volume ratio. The hippocampus is a part of the brain involved in learning and memory (Meftahi et al., 2014; Eslamizade et al., 2015; Meftahi et al., 2015). Several studies have shown that the volume and weight of any part of the brain such as the hippocampus are reduced after chronic stress, demonstrating stress-induced dendrite atrophy and shrinkage, loss of dendrite spines in neuronal populations, and increased volume and weight of the outer part of the adrenal gland (Liston et al., 2006; Ulrich-Lai et al., 2006; Lee et al., 2009; Motahari et al., 2016).

Our results show that chronic stress can decrease the brain to adrenal gland volume ratio. On the other hand, the brain to adrenal gland weight ratio was also reduced in stresses animals. The IP and ICV administration of testosterone inhibited the damaging effects of stress; however, both increased brain/adrenal gland volume. These results showed that androgen receptors in the brain play an important role in inhibiting the damage caused by chronic stress.

ACTH and corticosterone responses to acute stress in male rats are increased by gonadectomy and is reversed by 5α-dihydrotestosterone (the reduced nonaromatizable form of testosterone), a finding that is consistent with our results (Handa et al., 1994; Viau and Meaney, 1996). This shows that the suppressive effect of testosterone in males on stress HPA function is mediated by an androgen receptor–mediated effect under stress conditions. Acute exposure to stress elevates glutamate release in many parts of the brain such as limbic system, prefrontal cortex, hippocampus, and amygdala. If stress becomes chronic (more than a few hours or over long periods of time more than once a day), glucocorticoid levels increase severely and lead to stimulation of the glutamate

system, which damages different parts of the nervous system including the cerebral cortex and hippocampus (Venero and Borrell, 1999; Yuen et al., 2012). For this reason, reductions in brain size and weight after a period of chronic stress is predictable. Studies have shown that chronic stress increases adrenal gland weight by hyperplasia of the outer zona fasciculate and hypertrophy of the inner zona fasciculata and medulla. Stress-induced increases in adrenal medullary size and/or catecholamine content is frequently observed after other types of stress as well. Furthermore, following stress there is a generalized increase in medullary function, which suggests that medullary hypertrophy may be a general consequence of chronic stress (Miyamoto et al., 1999; Ulrich-Lai et al., 2006).

Wright et al. (1991) have shown that androgens can increase the volume, neuron number, and synapses of developing rat superior cervical ganglion. Hammond et al. (2001) also indicated that physiologic levels of testosterone could protect against serum deprivation–mediated neuronal apoptosis by interacting with androgen receptors. One of the proposed mechanisms is that androgens increase hippocampal neurogenesis by enhancing cell survival with no significant effects on cell proliferation in the dentate gyrus of male rodents (Spritzer and Galea, 2007). Thus, these findings are consistent with the present results demonstrating brain/adrenal volume decreases in stress.

Conclusions

The results of this study showed that the ICV and IP administration of testosterone in gonadectomized male mice can play an important role in the modulation of hormonal and metabolic effects caused by acute and chronic stress, including water and food intake, bodyweight changes, anorexia, and plasma cortisol level and the ratio of brain weight and volume to adrenal gland weight and volume during the induction of acute and chronic stress.

Acknowledgements

This work was supported by the Neuroscience Research Center of Baqiyatallah University of Medical Sciences, Tehran, Iran.

Compliance with ethics guidelines

Sara Salehi Shemiran, Gholam Hossein Meftahi, Hedayat Sahraei and Negin Ghobadi declare that they have no conflict of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

References

Adam T C, Epel E S (2007). Stress, eating and the reward system. Physiol Behav, 91(4): 449–458

Amouei N, Sahraei H, Alibeik H, Meftahi G H, Bahari Z, Mohammadi A (2016). Intrahippocampal and peripheral effects of nicotine injection on the metabolic and behavioral response to inescapable stress. Biosci Biotechnol Res Asia, 13(3): 1363–1371

Arun S, Burawat J, Sukhorum W, Sampannang A, Uabundit N, Iamsaard S (2016). Changes of testicular phosphorylated proteins in response to restraint stress in male rats. J Zhejiang Univ Sci B, 17(1): 21–29

Asalgoo S, Jahromi G P, Meftahi G H, Sahraei H (2015). Posttraumatic Stress Disorder (PTSD): Mechanisms and possible treatments. Neurophysiology, 47(6): 482–489

Baldwin D S (2001). Depression and sexual dysfunction. Br Med Bull, 57(1): 81–99

Chuang J C, Zigman J M (2010). Ghrelin's roles in stress, mood, and anxiety regulation. Int J Pept, 2010: 460549

Connan F, Lightman S L, Landau S, Wheeler M, Treasure J, Campbell I C (2007). An investigation of hypothalamic-pituitary-adrenal axis hyperactivity in anorexia nervosa: the role of CRH and AVP. J Psychiatr Res, 41(1-2): 131–143

Cotrufo P, Monteleone P, d'Istria M, Fuschino A, Serino I, Maj M (2000). Aggressive behavioral characteristics and endogenous hormones in women with Bulimia nervosa. Neuropsychobiology, 42(2): 58–61

Curtis AL, Bethea T, Valentino R J. Sexually dimorphic responses of the brain norepinephrine system to stress and corticotropin-releasing factor Neuropsychopharmacol. 2006;31(3):544–54

Dallman M F, Akana S F, Scribner K A, Bradbury M J, Walker C D, Strack A M, Cascio C S (1992). Stress, feedback and facilitation in the hypothalamo-pituitary-adrenal axis. J Neuroendocrinol, 4(5): 517–526

Dallman M F, Pecoraro N, Akana S F, La Fleur S E, Gomez F, Houshyar H, Bell M E, Bhatnagar S, Laugero K D, Manalo S (2003). Chronic stress and obesity: a new view of "comfort food". Proc Natl Acad Sci USA, 100(20): 11696–11701

Dalooei J R, Sahraei H, Meftahi G H, Khosravi M, Bahari Z, Hatef B, Mohammadi A, Nicaeili F, Eftekhari F, Ghamari F, Hadipour M, Kaka G (2016). Temporary amygdala inhibition reduces stress effects in female mice. J Adv Res, 7(5): 643–649

Derijk R H, van Leeuwen N, Klok M D, Zitman F G (2008). Corticosteroid receptor-gene variants: modulators of the stress-response and implications for mental health. Eur J Pharmacol, 585 (2-3): 492–501

Ehteram B Z, Sahraei H, Meftahi G H, Khosravi M (2017). Effect of intermittent feeding on gonadal function in male and female NMRI mice during chronic stress. Braz Arch Biol Technol, 60(0): e17160607

Erfani M, Sahraei H, Bahari Z, Meftahi G H, Hatef B, Mohammadi A, Hosseini S H (2017). Evaluation of the effect of time change in cognitive function in volunteers in Tehran. Glob J Health Sci, 9(2): 119–126

Eslamizade M J, Saffarzadeh F, Mousavi S M, Meftahi G H, Hosseinmardi N, Mehdizadeh M, Janahmadi M (2015). Alterations in CA1 pyramidal neuronal intrinsic excitability mediated by Ih channel currents in a rat model of amyloid beta pathology. Neuroscience, 305: 279–292

Evanson N K, Herman J P (2015). Role of paraventricular nucleus glutamate signaling in regulation of HPA axis stress responses. Interdiscip Inf Sci, 21(3): 253–260

Ghobadi N, Sahraei H, Meftahi G H, Bananej M, Salehi S (2016). Effect of estradiol replacement in ovariectomized NMRI micein response to acute and chronic stress. J Appl Pharm Sci, 6(11): 176–184

Habhab S, Sheldon J P, Loeb R C (2009). The relationship between stress, dietary restraint, and food preferences in women. Appetite, 52 (2): 437–444

Hammond J, Le Q, Goodyer C, Gelfand M, Trifiro M, LeBlanc A (2001). Testosterone mediated neuroprotection through the androgen receptor in human primary neurons. J Neurochem,77(5):1319–1326

Handa R J, Nunley K M, Lorens S A, Louie J P, McGivern R F, Bollnow M R (1994). Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. Physiol Behav, 55(1): 117–124

Hardy M P, Gao H B, Dong Q, Ge R, Wang Q, Chai W R, Feng X, Sottas C (2005). Stress hormone and male reproductive function. Cell Tissue Res, 322(1): 147–153

Hari Priya P, Reddy PS. Effect of restraint stress on lead induced male reproductive toxicity in rats. J Exp Zool A Ecol Genet Physiol 2012;317(7): 455–65

Hassantash M, Sahraei H, Bahari Z, Meftahi G H, Vesali R (2017). The role of dopamine D2 receptors in the amygdala in metabolic and behavioral responses to stress in male Swiss-Webster mice. Front Biol, 12(4): 298–310

Hill J W (2010). Gene expression and the control of food intake by hypothalamic POMC/CART neurons. Open Neuroendocrinol J, 3: 21–27

Kennedy SH, Dickens SE, Eisfeld BS, Bagby RM. Sexual dysfunction before ntidepressant therapy in major depression. 62TJ Affect Disord 1999;56:201–208

Krahn D D, Gosnell B A, Majchrzak M J (1990). The anorectic effects of CRH and restraint stress decrease with repeated exposures. Biol Psychiatry, 27(10): 1094–1102

Lee T, Jarome T, Li S J, Kim J J, Helmstetter F J (2009). Chronic stress selectively reduces hippocampal volume in rats: a longitudinal MRI study. Neuroreport, 25: 1554–1558

Liston C, Miller M M, Goldwater D S, Radley J J, Rocher A B, Hof P R, Morrison J H, McEwen B S (2006). Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. J Neurosci, 26(30): 7870–7874

Lund T D, Hinds L R, Handa R J (2006). The androgen 5alpha-dihydrotestosterone and its metabolite 5alpha-androstan-3beta, 17beta-diol inhibit the hypothalamo-pituitary-adrenal response to stress by acting through estrogen receptor beta-expressing neurons in the hypothalamus. J Neurosci, 26(5): 1448–1456

Lund T D, Munson D J, Haldy M E, Handa R J (2004). Androgen inhibits, while oestrogen enhances, restraint-induced activation of neuropeptide neurones in the paraventricular nucleus of the hypothalamus. J Neuroendocrinol, 16(3): 272–278

Majzoub J A (2006). Corticotropin-releasing hormone physiology. Eur J Endocrinol, 155(suppl_1): 71–76

McEwen B S (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev, 87(3): 873–904

McEwen B S (2012). Brain on stress: how the social environment gets under the skin. Proc Natl Acad Sci USA, 109(2 Suppl 2): 17180–17185

McEwen B S (2016). In pursuit of resilience: stress, epigenetics, and brain plasticity. Ann N Y Acad Sci, 1373(1): 56–64

McEwen B S, Milner T A (2007). Hippocampal formation: shedding light on the influence of sex and stress on the brain. Brain Res Brain Res Rev, 55(2): 343–355

McEwen B S, Nasca C, Gray J D (2016). Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex. Neuropsychopharmacology, 41(1): 3–23

Meftahi G, Ghotbedin Z, Eslamizade M J, Hosseinmardi N, Janahmadi M (2015). Suppressive effects of resveratrol treatment on the intrinsic evoked excitability of CA1 pyramidal neurons. Cell J, 17(3): 532–539

Meftahi G H, Janahmadi M, Eslamizade M J (2014). Effects of resveratrol on intrinsic neuronal properties of CA1 pyramidal neurons in rat hippocampal slices. Physiol Pharmacol, 18: 144–155

Mikolajczyk R T, El Ansari W, Maxwell A E (2009). Food consumption frequency and perceived stress and depressive symptoms among students in three European countries. Nutr J, 8(1): 31

Miyamoto H, Mitani F, Mukai K, Suematsu M, Ishimura Y (1999). Studies on cytogenesis in adult rat adrenal cortex: circadian and zonal variations and their modulation by adrenocorticotropic hormone. J Biochem, 126(6): 1175–1183

Mohammadian Z, Sahraei H, Meftahi G H, Ali-Beik H (2017). Effects of unilatral- and bilateral inhibition of rostral ventral tegmental area and central nucleus of amygdala on morphine-induced place conditioning in male Wistar rat. Clin Exp Pharmacol Physiol, 44(3): 403–412

Motahari A A, Sahraei H, Meftahi G H (2016). Role of nitric oxide on dopamine release and morphine-dependency. Basic Clin Neurosci, 7 (4): 283–290

Osanloo N, Sarahian N, Zardooz H, Sahraei H, Sahraei M, Sadeghi B (2015). Effects of memantine, an NMDA antagonist, on metabolic syndromes in female NMRI mice. Basic Clin Neurosci, 6(4): 239–252

Papadopoulos A D, Wardlaw S L (2000). Testosterone suppresses the response of the hypothalamic-pituitary-adrenal axis to interleukin-6. Neuroimmunomodulation, 8(1): 39–44

Paxinos G, Franklin K B JThe mouse brain in stereotaxic coordinates. 2nd Edition, San Diego Academic Press, 2001

Pourhashemi S F, Sahraei H, Meftahi G H, Hatef B, Gholipour B (2016). The effect of 20 minutes scuba diving on cognitive function of professional scuba divers. Asian J Sports Med, 7(3): e38633

Rai J, Pandey S N, Srivastava R K (2004). Testosterone hormone level in albino rats following restraint stress of long duration. J Anat Soc India, 53: 17–19

Rubinow D R, Roca C A, Schmidt P J, Danaceau M A, Putnam K, Cizza G, Chrousos G, Nieman L (2005). Testosterone suppression of CRH-stimulated cortisol in men. Neuropsychopharmacology, 30(10): 1906–1912

Sadeghi-Gharajehdaghi S, Sahraei H, Bahari Z, Meftahi G H, Jahromi G P, Ali-Beik H (2017). Effect of amygdaloid complex inhibition on nicotine-induced conditioned place preference in rats. J Appl Pharm Sci,7(03): 40–47

Salleh M R (2008). Life event, stress and illness. Malays J Med Sci, 15 (4): 9–18

Segerstrom S C, Miller G E (2004). Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull, 130(4): 601–630

Spritzer M D, Galea L A M (2007). Testosterone and dihydrotestoster-

one, but not estradiol, enhance survival of new hippocampal neurons in adult male rats. Dev Neurobiol, 67(10): 1321–1333

Ulrich-Lai Y M, Figueiredo H F, Ostrander M M, Choi D C, Engeland W C, Herman J P (2006). Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am J Physiol Endocrinol Metab, 291(5): E965–E973

Venero C, Borrell J (1999). Rapid glucocorticoid effects on excitatory amino acid levels in the hippocampus: a microdialysis study in freely moving rats. Eur J Neurosci, 11(7): 2465–2473

Viau V, Meaney M J (1996). The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. J Neurosci, 16(5): 1866–1876

Williamson M, Bingham B, Viau V (2005). Central organization of androgen-sensitive pathways to the hypothalamic-pituitary-adrenal axis: implications for individual differences in responses to homeostatic threat and predisposition to disease. Prog Neuropsychopharmacol Biol Psychiatry, 29(8): 1239–1248

Wright A F, Goedert M, Hastie N D (1991). Familial Alzheimer's disease. Beta amyloid resurrected. Nature, 349(6311): 653–654

Yuen E Y, Wei J, Liu W, Zhong P, Li X, Yan Z (2012). Repeated stress suppresses glutamate receptor expression and function in prefrontal cortex and impairs object recognition memory. Neuron, 73: 962–977