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Original Paper

Post-Weaning Two Weeks' Social Isolation Stress Causing Anxiety-Like Behavior, Locomotion, and Cognition Impairment in Rats

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Introduction: Social and psychological stressors are associated with cognitive impairment and anxiety. This study aimed to determine the effect of two weeks' socially-isolated post-weaning rats on their learning, memory, locomotion, and anxiety-related behavior.

Materials and Methods: Young male and female Wistar rats (21 days old), separated from different mothers (with no relationship), were divided into 4 groups: groups 1 and 2 comprised colonies of six male and six female rats in separate cages, lived for 2 weeks; groups 3 and 4 comprised stressed males and females housed individually in separated opaque cages (one rat lived alone in each cage for 2 weeks) in the same room. Then, we evaluated their passive avoidance and spatial memories, anxiety-like behaviors, and motor activity. The obtained data were analyzed by one-way ANOVA followed by Tukey's post hoc test. P values less than 0.05 were considered significant between all the test groups.

Results: Both avoidance and spatial memories were impaired in stressed (single lived) male and female rats significantly ($P<0.01$ and $P<0.001$, respectively) while they showed no motor dysfunction or change in swimming speed. Stressed male and female rats showed more severe anxiety-like behavior versus populated rats ($P<0.01$), while their locomotion behavior in open field tests was not different.

Conclusion: These results show that social stress during subjects' growth can negatively influence cognition, locomotion, and anxiety behaviors. It is suggested that infants and youths must keep away from psychological stressors during development to prevent behavioral impairment.

Keywords: Social stress, Memory, Anxiety, Locomotion, Rats

Introduction

Encountering different types of stresses is inevitable during human lives. Various brain regions contribute to the regulation of the stress response. Areas such as the amygdala, prefrontal cortex, and hippocampus are of particular importance (McEwen, 2001). Such areas are also compromised in neurodegenerative diseases, like Alzheimer, Parkinson, and Huntington diseases (Vonsattel & DiFiglia, 1998; Olanow & Tatton, 1999).

Stress is a condition or feeling experienced when a person perceives that demand exceeds the personal and social resources that the individual can use in the prevailing circumstance (Vonsattel & DiFiglia, 1998). Living organisms in stressful situations undergo some physiological, morphological, and biochemical modifications to survive. These modifications evolve to reduce the demands and maintain the homeostatic environment through a series of physiological and or behavioral responses (Keating, 2008; Sánchez-López et al., 2012). But inadequate, excessive, or prolonged activation of the stress

system can disturb normal physiological and behavioral functions (Takahashi et al., 2002).

In response to insults, stress and neurodegenerative diseases also share common pathological changes, such as Reactive Oxygen Species (ROS) production, inflammation (Sánchez-López et al., 2012), mitochondrial dysfunction (Keating, 2008), and apoptotic cell death pathways (Takahashi et al., 2002).

Stress induction during brain development predisposes the brain to various neuropathological conditions (Arborelius et al., 1999). Physical and psychological stresses during the growth period and brain development are associated with a broad range of diseases, such as mental disorders, anxiety, and depression (Jeong et al., 2006), as well as neurodegenerative diseases in the later phases of life (Swaab et al., 2005). According to a growing body of evidence, psychological stress is implicated as a potential contributing factor to the development of Alzheimer disease (McLaughlin et al., 2007; Palmer et al., 2007; Dong & Csernansky, 2009; Ray et al., 2011).

Environmental Enrichment (EE) housing paradigms (such as the existence of toys in the living environment or living and growing in social living conditions) have long been shown beneficial effects for brain function involving neural growth and activity, learning and memory capacity, and developing stress resiliency (Magarinos et al., 1997; Zanca et al., 2015). It has been suggested that EE produces an adaptive response to chronic stress allowing for increased Glucocorticoid Receptors (GR) levels, which lowers neuronal excitability reducing gluA2 and protein kinase M zeta trafficking. It was shown that EE adaptive response to stress as a potential underlying mechanism protects synaptic plasticity and memory function (Zanca et al., 2015). However, the effect of 2 weeks' social stress on the central nervous system and its impact on avoidance memory, locomotion, and anxiety deficits induced through mandatory solitary life has not been scientifically documented so far. Thus, this study aimed to investigate the effects of 2 weeks' social stress (individual living) on spatial and passive avoidance memory, locomotion, and anxiety-like behavior in young 21 days old rats.

Materials and Methods

Male and female Wistar rats from the time of weaning (60-80 g, 21 days old) separated from different mothers (with no relationship) were housed in standard opaque cages under controlled temperature (22±2□C), humidity (50-55%), and a 12 h light/dark cycle (light on 07:00–19:00), with food and water provided ad libitum. The rats acclimated to the facilities for 1 week and then were randomly assigned to the experimental groups with 2 weeks' populated or social stress living: group 1 (Pop. F) comprised female rats with social life (six rats as a colony in one opaque cage for 2 weeks; group 2 (Pop. M) comprised male rats with social life (six rats as a colony in one opaque cage for 2 weeks; group 3 or stressed females (Single F) comprised female rats with single life housed individually in separate opaque cages for 2 weeks; group 4 or stressed male (Single M) comprised male rats with single life housed individually in separate opaque cages for 2 weeks.

Stress Paradigm

For induction of isolation stress, the rats were kept in individual cages without any neighbors. Since rats are social creatures, isolation stress is a psychological stress condition for them (Grippo et al., 2007; Kalshetti et al., 2015). All cages of populated life were preserved in the same room while the cages of single life were kept under situation so that the rats could not visit each other through the walls of cages when living and growing solely for 2 weeks. This procedure causes single rats living in each cage alone received psychological stress. Experimentation was approved by the Ethics Committee of Islamic Azad University following the international guidelines for animal experiments (IR-IAU-Arak-95-10-658). All efforts were made to minimize animal suffering and to reduce the number of animals used. At the end of 2 weeks living in populated or single cages, the behavioral tests were done. The timeline and design of experimental protocols are shown in Figure 1.

Passive Avoidance Performance

To assess the memory retention of animals, we performed the Passive Avoidance Task (PAT). This method was used following Rabiei et al. (2014) procedure with a little modification (Rabiei et al., 2014). In this test, the animal learns that a specific place should be avoided since it is associated with an aversive event. The shuttle box as a passive avoidance apparatus consisted of two light (Plexiglas) and dark compartments (20×30×30 cm) separated by a guillotine door (Borj Sanat Azma Co., Tehran-Iran). The floor of both light and dark (i.e., conditioning chamber) compartments was made of stainless-steel bars (0.5 cm diameter) separated by a distance of 1 cm. A single electric shock (50 Hz, 1 mA, 3 s) was delivered to the floor of the dark compartment by an isolated stimulator.

Inhibitory Avoidance Training

The rats in all groups were allowed to become familiar with the laboratory environment for 5 minutes 1 h before the training.

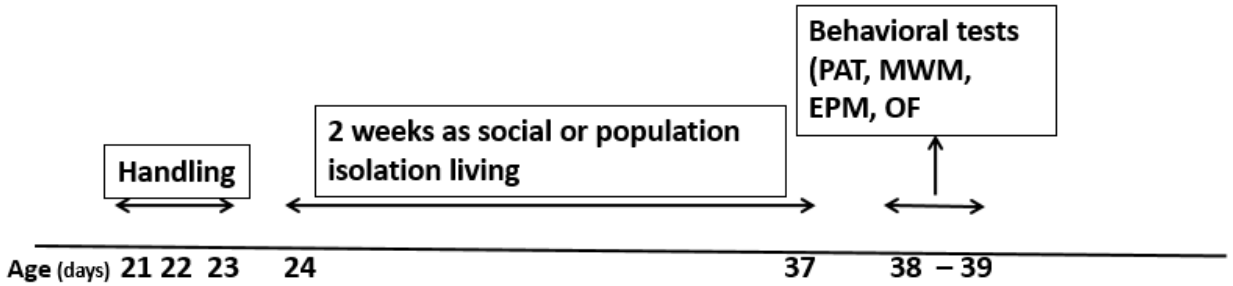

Figure 1: Timeline and design of experimental protocols

Each animal was placed in the light compartment for 20 s, after which the door was opened. The time that the animal waited before entering the dark (shock) compartment was recorded as the Initial Latency (IL). Then the animal was removed from the experiment while it waited for more than 300 s to enter the other side. Once the animal completely entered the dark compartment, the door was closed, and 1 mA foot shock was delivered for 3 s. Two minutes after the peace, the rats were removed from the dark compartment and put in their home cages.

Retention Test

Twenty-four hours later, each animal was placed in the light compartment, and the latency to enter the dark compartment was re-recorded as step-through latency (Suri et al., 2013). During these sessions, no foot shock was applied, and the test session ended when the animal entered the dark compartment (Lashgari et al., 2006).

Spatial Learning and Memory

The Morris Water Maze (MWM) was used for spatial learning and memory evaluation. It includes a black circular pool (1.4 m diameter) filled with water at 25±1°C and divided into four equivalent virtual quadrants. A hidden escape platform was placed in the middle of the target quadrant, and the testing room contained numerous cues. The animal's behavior was analyzed using a computerized tracking system (Maze Router Co. Tabriz, Iran). The MWM procedure lasted 2 days. On the first day, the animals were exposed to 8 training trials of 120 s; all animals passed the first four trials and then the next four to allow them to rest. In the first four trials, the animal was placed in different start points that were randomly changed in the second four trials. If the animals failed to find the platform within 120 s, the experimenter gently would guide the animal to the platform. All animals were left on the platform for 30 s and then removed from the pool for a 30 s rest period, after which the next trial commenced. On the second day, the animals were exposed to a 60-s probe trial without the platform. The analyzed variables in the training trials were swimming length, latency (time spent to find the platform), and average swimming speed. In the probe trial, the percentage of the time spent in the target quadrant during the 60 s of the probe trial was measured (Rizhova et al., 2007).

Anxiety-Like Behavior Test

To assess the anxiety-like behavior of rats, we employed the Elevated Plus Maze (EPM) apparatus. The EPM was a cross-shaped platform positioned 50 cm above the floor with two open arms (50×10×0.5 cm) and two closed arms (50×10×20 cm) on opposing sides of a central area (10×10 cm). This method was used following Yan Zeng et al. (2015) procedure with a little modification. At the start of the test, a rat was put on the central area facing one of the open arms and allowed to explore the maze freely for 5 min, during which its behavior was recorded by a video camera mounted 2 m above the maze. Following each session, the apparatus was wiped clean with 10% ethanol to remove any olfactory cues.

An experimenter blind to stressed groups handled the animals and analyzed the video recordings. The following parameters were considered; a) the number of open arms entries, 2) the amount of time spent in the open arms, 3) the number of closed arms entries, 4) the amount of time spent in the closed arms (Zeng et al., 2015).

Evaluation the locomotion

To assess the motor activity of rats, we used the Open Field (OF) apparatus. The rats were positioned in an acrylic box (50×50×20 cm) equipped with an activity detection device to measure the locomotion and exploratory activities. Total travel distance and rearing frequency were recorded, and high activities of these behaviors suggested increased movement and exploration. Rearing was counted by the number of unsupported rears (front paws off the floor) and supported rears (front paws on the wall).

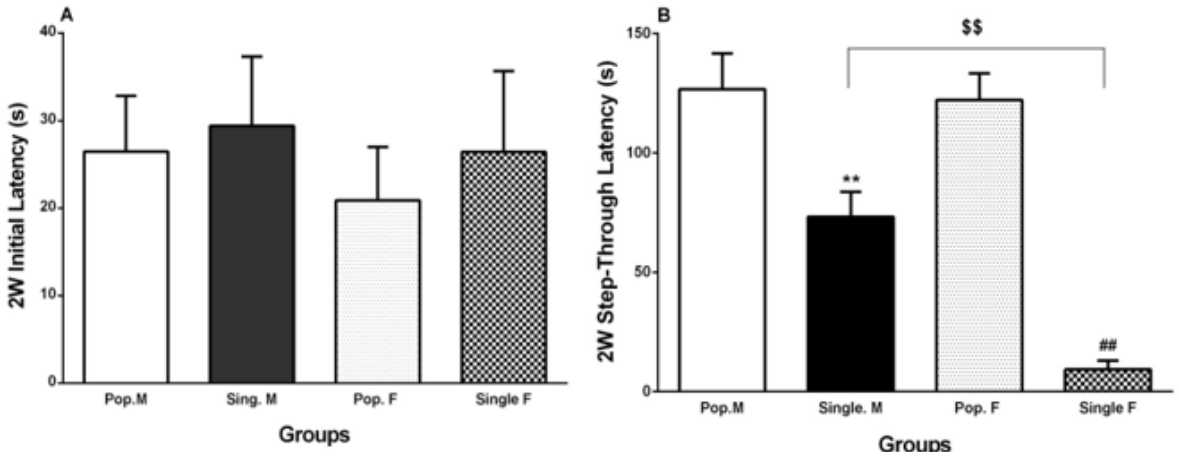

Figure 2: A) Initial Latency (IL) and B) Step-Through Latency (STL) in male and female rats groups lived for 2 weeks as populated (normal, 6 rats in each cage) or single rat in each cage (with social stress). Data were presented as mean±SEM and analyzed with one-way ANOVA followed by Tukey's post hoc test.

****P<0.01 significant difference between single lived male group versus populated lived male group**

##P<0.01 significant difference between single lived female group versus populated lived female group

$$P<0.01 significant difference between single lived female versus male groups (n=6 in each group)

The total travel distance was automatically captured and analyzed using a digital video recording camera attached to a computer containing a video tracking software template (Ethovision 8.0; Noldus, Netherland). Animal behavior and activity were evaluated for 5 min (Wang et al., 2012).

Statistical Analysis

The obtained data were presented as mean±SEM. All data were checked for the homogeneity of variance and normality of distribution. Statistical analyses were conducted by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test in SPSS (version 21, SPSS Inc., Chicago, IL, USA). A P value less than 0.05 was considered to be statistically significant.

Results

Passive Avoidance Task: Figure 2 shows no significant differences between populated (normal) and 2 weeks' stressed male and female rats groups in the Initial Latencies (IL) (panel A), but there are significant differences (P<0.01) in Step-Through Latencies (STL) (panel B). On the other hand, the passive avoidance memory was more affected negatively in stressed females when compared with the stressed males (P<0.01).

Spatial Learning and Memory: As shown in Figure 3 (A-D panels), swimming length and escape latencies to find the platform increased in both stressed male and female rats significantly (P<0.01) when compared with populated (normal) males and females. According to the obtained data, the stressed females were more negatively affected compared with stressed male rats (panels A and B). The swimming speed was not changed in stressed male and female rats compared to the populated (normal) male and female rats (plan C). Time spent in the goal quadrant as a spatial memory parameter was impaired in both stressed male and female rats significantly (P<0.01) compared with populated (normal) male and female groups (panel D).

Anxiety-Like Behavior: Figure 4 shows the mean±SEM of entrance numbers of male and female rats in populated (normal) and single (social stress) living into open (panel A) and close (panel B) arms, time spent in open and close arms in both male and female rats with population or single living states (panels C and D) during Elevated Plus Maze (EPM) test. The entrance numbers and time spent in open arms decreased significantly in both stressed male and female rats (P<0.001 and P<0.01) compared to the populated male and female groups (panels A & B). Entrance numbers and time spent in closed arms increased significantly in both stressed male and female rats, (P<0.001 and P<0.01) compared to the populated male and female groups (panels C & D).

Locomotion: Figure 5 (A, B, and C) shows the mean±SEM of ambulation (line crossing), grooming, and rearing (exploration) frequencies of male and female rats in the populated (normal) and single (social stress) lived in the open field box. The locomotion activity did not significantly differ between male and female rats with populated or single lived (panel A).

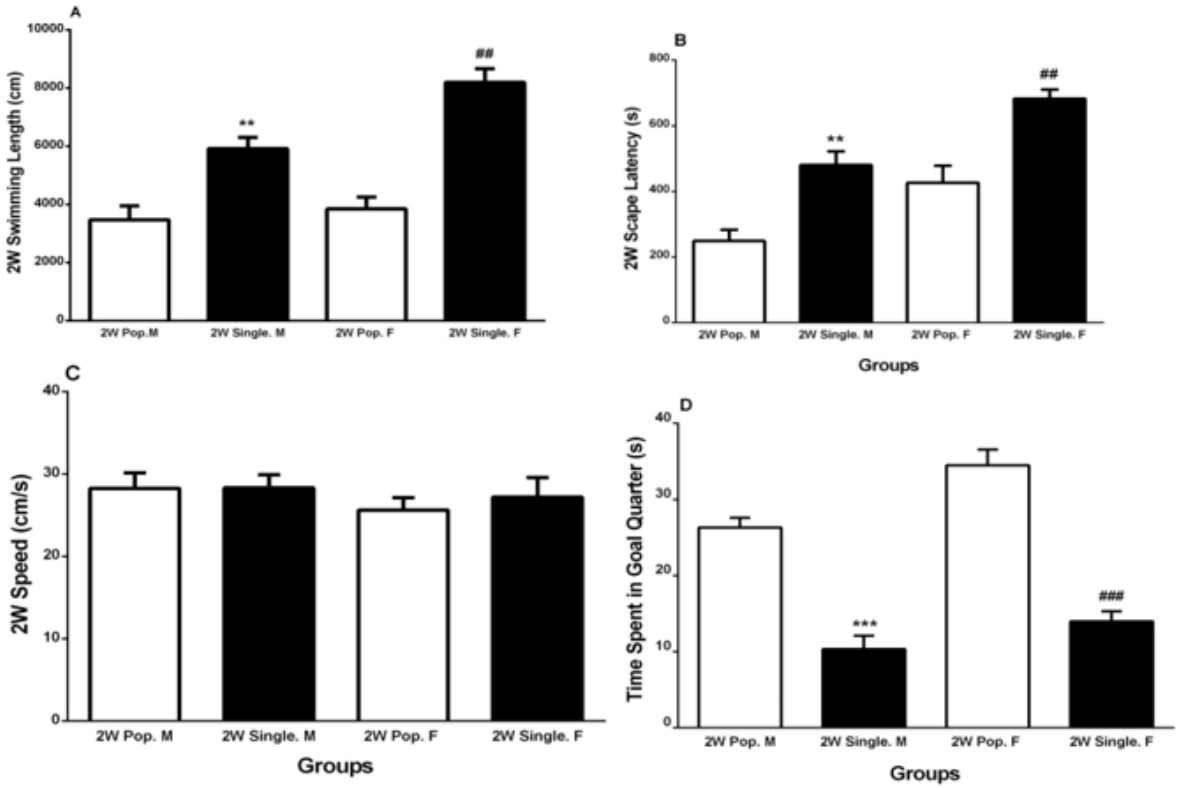

Figure 3: Panels A-D shows mean±SEM of swimming length, escape latency to find the platform, swimming speed, and probe trial in male and female groups lived for 2 weeks as populated (normal, 6 rats in each cage) or single rat in each cage (with social stress). Swimming distance and latency to the platform in panels A & B, swimming speed in panel C, and Time spent in goal quadrant during probe trial in panel D. Data were analyzed with one-way ANOVA followed by Tukey's post hoc test.

****P<0.01 and ***P<0.001 show a significant difference between single lived male group versus populated lived male group.**

##P<0.01 and ###P<0.001 show a significant difference between single lived female group versus populated lived female group (n=6 in each group).

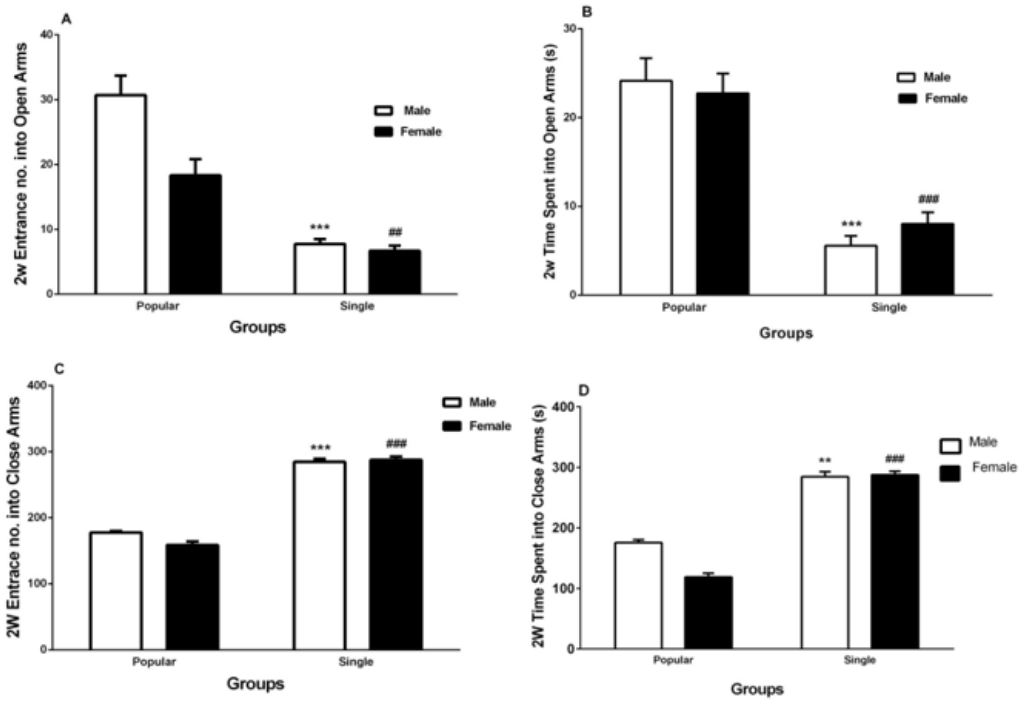

Figure 4: A) entrance numbers into Open Arms (OA); B) time spent in Open Arms (OA); C) entrance numbers into Close Arms (CA); D) time spent in Close Arms (CA) in anxiety-like behavior test on the Elevated-Plus Maze (EPM) in male and female rats groups lived for 2 weeks as populated (normal) with 6 rats in each cage (populated) or single rat in each cage (with social stress). Data were presented as mean±SEM and analyzed with one-way ANOVA followed by Tukey's post hoc test (n=6 in each group).

****P<0.01 and ***P<0.001 show a significant difference between single versus populated males.**

##P<0.01 and ###P<0.001 show a significant difference between single versus populated females.

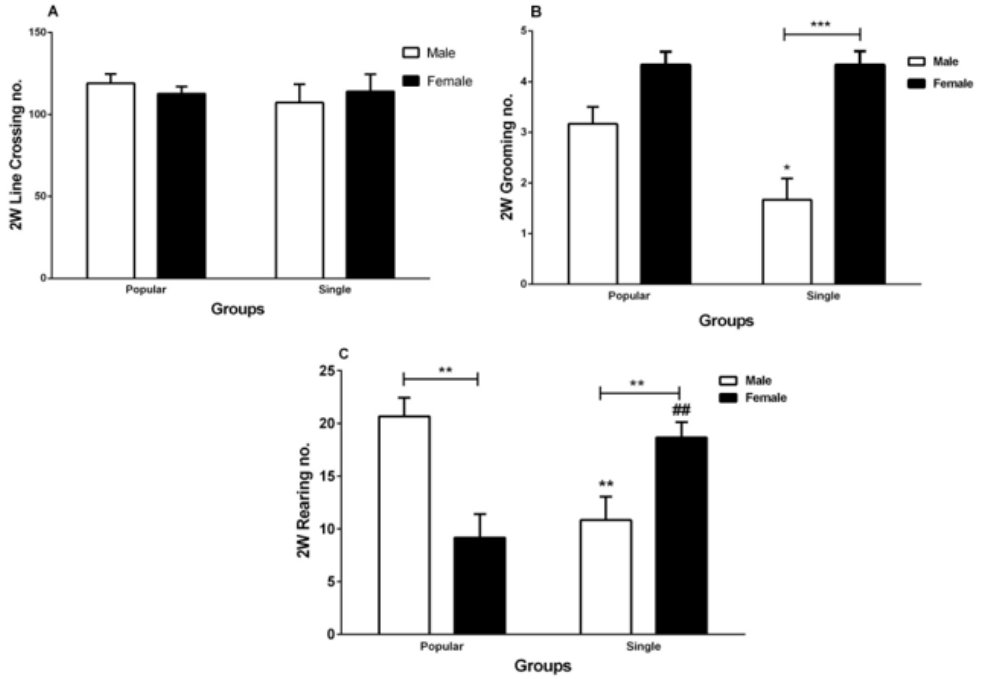

Figure 5: Ambulation frequency (line crossing number); A) as motor activities, grooming; B) and rearing frequencies; C) as an exploratory activity behavior during Open Field (OF) test in male and female rat groups lived for 2 weeks as populated (normal) with 6 rats in each cage (populated) or single rat in each cage (with social stress). Data were presented as mean±SEM and analyzed with one-way ANOVA followed by Tukey's post hoc test (n=6 in each group).

***P<0.05, **and ##P<0.01 show significant differences in grooming and rearing between male and female rats in popular and single lived groups, respectively.**

Grooming behavior increased in both populated and single lived females, but it was significant only in females with the single lived state (panel B, P<0.01). Rearing behavior was reduced in females than males in the populated group (P<0.01), but it increased significantly in female rats with 2 weeks of single living (social stress) versus male rats in the same group (P<0.01, panel C). Rearing was decreased significantly (P<0.01) in male rats with social stress (single living) compared to male rats with normal (populated) living. However, it was increased significantly (P<0.01) in female rats with social stress (single living) compare to female rats with normal (popular) living (panel C).

Discussion

In this study, two weeks' social stress significantly impaired passive avoidance and spatial memories in male and female rats with social stress. There were no changes in swimming speed and also an ambulation of both stressed males and females. The grooming and rearing in stressed males decreased significantly compared to populated males (P<0.05), while rearing was increased in stressed females versus populated females significantly (P<0.01).

Some investigators have evaluated locomotion, anxiety-related behavior (Gong et al., 2018), learning, and memory after 90 days socially-isolated post-weaning rats. They showed a significant difference in locomotion activity, while the anxiety scores and time spent in the goal quarter during the probe trial session of the MWM test were higher in the socially-isolated group compared to the control. They showed that the concentration of Brain-Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF) in the hippocampus decreased significantly after 90 days of social stress (Okudan & Belviranli, 2017; Cowell et al., 2019).

There is little evidence about subchronic social stress during childhood, adolescence and its effects in the later phases of life. The behavioral impairments may be due to a stress-related decrease in BDNF and NGF in the brain. These results are consistent with other research findings in this area (Vaynman et al., 2006; Daskalakis et al., 2015). Our finding showed that rats' offspring and adolescents exposed to 2 weeks of social and psychological stress would be affected by complications such as cognitive impairment and anxiety-like behaviors in developing offspring.

Aiming for effective stress management initially includes proper recognition of stress, the stressor, their manifestations, and effects on one's well-being. Poor stress management has been shown to contribute to the development and pathogenesis of several psychiatric disorders, including mood disorders, schizophrenia, depression, post-traumatic stress disorders, anxiety disorders, bipolar disorder, Alzheimer disease, Parkinson disease, and pathologic aging (Arborelius et al., 1999; Swaab et al., 2005; Jeong et al., 2006).

Stress, especially if subacute or chronic, can profoundly alter memory processing (McEwen & Magarinos, 2001; McGaugh, 2004; 2005; 2006). To our knowledge, the effects of stress on passive avoidance and spatial memories had not been studied. In some previous investigations, attempts have been made to determine the mechanisms of social stress effects during the growth period on behaviors. Some mechanisms to support our results may involve hippocampal neurogenesis (van Praag et al., 1999; During & Cao, 2006), brain tissue oxidative stress (Ogonovszky et al., 2005; Radak et al., 2006), and brain-derived neurotrophic factor levels (Berchtold et al., 2005; Huang et al., 2006).

In any case, the question that still arises is how the influence of stress on cognitive function can be ascribed to stress. Proper experiments are to answer this question. The physiological changes induced by stress are under normal circumstances self-limiting and adaptive, but when the stressful event overrides its threshold limits, it becomes irreversible, resulting in the pathogenesis of several disorders (Kim, 2020). The fact still remains that the basic trigger for these disorders is the exhaustion of energy supply and the collapse of energy metabolism following glucose deprivation in the circulation (Palmer et al., 2007). In the desire to augment the coping mechanism, the science of adaptation has emerged, which focuses on elucidating mechanisms that may help to counteract excessive and unnecessary responses to stress (Zeng et al., 2015). Adaptogens are substances that place an organism into a state of non-specific elevated resistance to the stressor by promoting homeostasis, which regulates the physiological processes in the body (Lakshmi & Sudhakar, 2010). They normalize body functions, strengthen systems and functions that are compromised by stress, and provide protective effects against a wide variety of stressors, such as physical, environmental, or emotional. In general, they increase an organism's ability to adapt and avoid damages incurred from stressful. They normalize body functions, strengthen systems and functions that are compromised by stress, and provide protective effects against a wide variety of stressors; such as physical, environmental, or emotional. In general, they increase the ability of an organism to adapt and avoid damages incurred from stressful exposure (Panossian *et al.*, 1999).

Conclusion

These findings suggest that social stress exposure to children and adolescences during growth period affects negatively at least some behaviors such as memory and anxiety. It is suggested that infants and youths must keep away from psychological stresses to prevent any behavioral impairment during development.

Acknowledgments

This article extracted from Mrs. Nehzat Sarkaki's psychology M.Sc. thesis from Islamic Azad University, Arak branch. This work was done in neurosciences Laboratory of Persian Gulf Physiology Research Center, Medical Basic Sciences Institute, Ahvaz, Iran.

Conflict of interest

The authors declared no conflict of interest.

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