ISSN: 2538-5887; Pathobiology Research. 2021;24(2):41-57

Voluntary Exercise Ameliorates Cognitive Performance and Long-Term otentiation (LTP) Impairments in Maternally Separated Adolescent Male Rats

ARTICLE INFO

Article Type
Original Research

Authors

Sarieh Shahraki[1,]
Khadijeh Esmaeilpour[2],
Mohammad Shabani[3],
Gholamreza Sepehri[4],
Sara Joushi[5],
Vahid Sheibani [6]*

1.Department of Physiology, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran
2.Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
3.Department of Physiology & pharmacology, school of medicine, Zabol University of Medical Sciences, Zabol, Iran

***Corresponding authors:**
Corresponding author at: Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran. Email: v_sheibani@kmu.ac.ir

Article History
Received: 2021/08/16
Accepted: 2021/08/31

ABSTRACT

Maternal separation (MS) is a model to induce early life stress (CNS) and is related to increased levels of anxiety and cognitive deficiencies. Voluntary exercise has been shown to be associated with learning and memory improvement in behavioral tests and electrophysiological experiments. Since it plays a significant role in learning and memory and enhances synaptic plasticity, the authors hypothesized that voluntary exercise may affect MS-induced changes in synaptic plasticity and cognitive performance**.**
Rat Male rat pups underwent the MS protocol for 180 min/day from postnatal day (PND) 1 to 21. Voluntary exercise was performed in the exercise (Ex and MS + Ex groups) from PND 29 to 49. Anxiety-like behavior, learning and memory were measured in adolescent rats. In addition, capability of long-term potentiation (LTP) induction was examined in CA1 region of the hippocampus.
MS induced higher anxiety-like behavior as well as impaired recognition, spatial and passive avoidance learning and memory, but did not affect locomotor activity. Voluntary exercise improved MS-induced deficits and increased the learning and memory of MS rats. It also decreased anxiety-like behavior in the open field test. The results revealed that long-term potentiation (LTP) was induced in all groups, except for MS. However, voluntary exercise induced LTP and had maintenance in MS + Ex.

Keywords: Maternal separation; Voluntary exercise; Long-term potentiation (LTP); Learning and memory; anxiety-like behavior

Introduction

Previous studies suggest that early-life stress (ELS) has detrimental effects on cognitive capacities such as learning and memory in human (1) and animals in afterward life (2,3). Studies have demonstrated that the destructive effects of incessant stress on memory is more conspicuous when the stress is connected to earliest stages (4,5). Due to the fact that when stress is connected in basic periods of brain development (that is, in childhood and youth), it'll have long-term troublesome impacts on brain structure and the individual's behavior (6).Animal models provide opportunities to understand the mechanisms in which ELS would affect brain and cognition. Maternal separation (MS) is a common ELS model (7).

In bolster of this, Sousa et al. suggested that MS in earliest stages, from postnatal day (PND)-2 to PND-14, decreases spatial memory arrangement in male rats 26 days after the end of separation when the rats are 40-70 days old. This finding appears that MS stress has a long-term detrimental effect on spatial memory in male rats (5). One of the most components causing the long-term detrimental effects of ELS on spatial memory is the impacts of stress on the neuronal structure and function of the hippocampus, which is involved in memory processing (8). These impacts may be somewhat due to the pups' lack of healthy sustenance since the maternal care changes after separation from the dams (9,10).

MS is additionally associated with disturbances in the development of the hypothalamic-pituitary-adrenal (HPA) axis (11). Hippocampus, as one of the key brain regions in cognitive tasks (12), is as defenseless to the impacts of stress hormones. Long term exposure to high levels of glucocorticoids, just like the MS circumstance is associated with learning and memory deficits and hippocampal atrophy in adulthood (5). MS can modify hippocampal neuronal circuitry, diminish neurogenesis within the hippocampus and actuate modifications in long-term potentiation (LTP) (12). MS impairs LTP in CA1-CA3 neural connections and hippocampal-dependent memory in old rats (5). It also impairs LTP within the dentate gyrus region of adolescent male rats (13). In addition, MS diminishes LTP at the CA3 neural connection in adolescent mice (12). It has been shown that MS avoids LTP-reinforcement initiated by swim-stress in adolescent male rats (14).

LTP involvement in the learning and memory processes is confirmed and is considered to be among the biological basis of learning and memory (15). Hippocampus-dependent spatial learning and memory are closely related with hippocampal LTP, a disability which regularly leads to memory impairments (16). Gruss and his colleagues found that rats which were seprated for 24 hours on PND 9 showed impaired hippocampal LTP in adolescence period (14). Moreover, Herpfer and his team experienced an impaired excitatory postsynaptic potential LTP(EPSP LTP) by MS from PND 1 to 14 (17), whereas Baudin et al. (year) found a facilitated LTP in hippocampal-medial prefrontal cortex (PFC) pathway actuated by MS on PND 1–14 (18).

The exercise advantageous effects on numerous physiological systems, including the brain and nervous system are well-illustrated (19). Exercise could lead to advantageous alterations in synaptic plasticity (20) and could modify some types of synaptic plasticity such as LTP (19). It has been shown that exercise improves LTP in neurodegenerative diseases (21). Animal models might be utilized for either forced or voluntary exercise, each having particular highlights (22,23). The defensive impacts of distinctive sorts of exercise on different behavioral tests such as Morris water maze (MWM) (24,25), radial arm maze (26), Barnes maze (27), and passive avoidance have been proven (28).

Ang et al. in 2006 explained concisely0 that forced exercise might influence learning and memory by expanding the number of cholinergic neurons (29), and Mabandla et al. in 2010 explained that voluntary exercise diminished the neurotoxic impacts of 6-hydroxy dopamine in separated adult male rats (30). Moreover, Grace et al. (2009) suggested that MS had little impact on separated rats, whereas voluntary exercises enhanced both spatial and cognitive memory (31). Given the significance of adolescence in terms of improvement in neural-mediated pathways, and according to few results earliest stages of MS has annihilating impacts on cognitive pointers later in life (32), therefore, in the present study we

investigated the effects of voluntary exercise on learning and memory and synaptic plasticity of adolescent male rats that underwent MS during infancy. According to above evidence highlighting the importance of the MS effects and voluntary exercise on neural and behavior system, and based on the authors' knowledge. Thus, the aim of the present work was to study whether voluntary exercise during adolescence could reverse or compensate the MS effects on learning and memory processes and synaptic plasticity in adolescent male rats.

Materials and methods

Animal models

In our experiment, Wistar rats were utilized. They were maintained under a sequence of 12 h of light and 12 h of dark cycle and the temperature was set at 23 ± 2°C. They had ad libitum access to food and water. Experimental methods were performed according to the related rules to care of animal models under study and were approved by the Animal Ethics Committee of Kerman University of Medical Sciences (ethics code: IR. KMU.REC.1399.318). In order to apply the MS protocol, male and female rats were mated. Pregnant females were kept separately before parturition. PND 0 was allotted to litters born before 3:00 PM every day. Male MS litters were separated from dams for 180 min/day (08:30–11:30 AM), from PND 1 to PND 21. To start MS, dams were evacuated from the home cage, after which the pups were moved to a cage in an incubator, where the temperature was kept at 30 ± 0.5°C (52 ± 4% humidity) (33) to avoid hypothermia. The dam was kept in a separateroom during MS to restrain olfactory or ultrasound vocalization exchanges between the dams and their pups. After 3 h, first pups were returned to the home cage followed by the dam reunion.

Voluntary exercise

The rats within the wheel exercise gather were exclusively put in home cages associated to a running wheel (20 cm in distance across; 9 cm in width) daily (24 h) for 3 weeks. The number of transformations was recorded and wheel transformations were numbered independent of the course of the wheel. All the rats were accustomed to the exploratory environment for 30 min in 2 days before exercise convention onset in order to play down nonspecific stress reactions (34).

Experimental groups

At the waning time on PND21, breast-feeding was halted for all the pups and they were housed in different groups with respect to their gender. Male pups (two litters per each dam) with n=7 sample size per condition were studied during adolescence (PND 50–60) They were divided into 4 groups including Control, Voluntary exercise (EX), MS, and MS + voluntary exercise (MS+EX). Control and voluntary exercise groups were kept intact until weaning; at that point, the voluntary group was allowed to experience voluntary exercise from PND 29 to 49. The MS + voluntary exercise group experienced MS from PND 1–21; at that point, this group was allowed to experience voluntary exercise from PND 29 to 49. Distinct sets of animals were utilized to perform behavior-related experiments and the passive avoidance test and also for electrophysiological study (Fig. 1).

Behavioral assessments

The open-field, novel object recognition (NOR), and Morris water maze (MWM) tests were performed individually on PND 50 for 3 successive days (open-field: PND 50, NOR: PND 51, and MWM test: PND 52). In the passive avoidance test, rats experienced electrical shock stimulation, so this test was performed in a group separated from those who underwent other behavior-related tests. The experiment was performed on PND 53, 54.

Open-field experiment

The open-field test was used to assay the exploratory, locomotor activity and anxiety-like behavior in rodents in this experiment. Cubic box was used in this study [90*90*30 cm] . Total movement distance and the velocity of movement were used to assess locomotor activity. The distance moved in the inner zone and also the number of grooming behavior was utilized to assess anxiety-like behavior of the rodent. These elements were recorded by a video camera on top of the field, also the animals' performance was

recorded by Noldus Ethovision system ver. 7.1. (35,36).

NOR test

The NOR task assesses the rodents' ability to recognize a novel object in the environment. The task procedure consisted of three phases: The habituation, familiarization, and test phases. In the habituation phase, each rat was free to explore the empty wooden arena. During the familiarization phase, a single rat was put in the arena containing two identical objects (A + A) for 5 min (37). the rat was returned to the arena with two objects after a retention interval of 45 min (37), for 3- min test phase, one similar to the sample and the other novel (A + B). The novel versus familiar object place was counterbalanced between trials. Normal rats spent more time exploring the novel object during the first few minutes of the test phase, and when this bias was observed, the animal was able to remember the sample object (38). Animals that did not explore any of the objects during the training and/or test phases were excluded from the analysis (39). NOR Performance was quantified using the discrimination ratio. The ratio was defined as the exploration time for each object divided by the total time spent for exploring both objects in the training and test phases (34,39–41). In the test phase, for example, the ratio was calculated using the following formula:

Discrimination ratio test phase: Time novel/Time total Discrimination ratio training phase: Time familiar/Time total

MWM test

This maze in this study was a black round pool (160 cm in diameter, 80 cm in height, 40 cm depth) filled with water and was kept at room temperature (22 ± 1 °C). A black square-shaped platform (10 cm in diameter) was placed in the northeast quarter of the pool and immersed into the water by 1.5 cm to be fine-drawn at the water level. The experiments were performed in a dimly lit room with spatial cues attached to the walls around the maze at different points. The behavior of the rats was recorded using the Noldus Ethovision system ver. 7.1. In order to evaluate learning, the animals were tested in three subsequent blocks in one day. Every block included four consecutive trials each of which lasting 60 s (within-experiment interim = 30 s). The rats were released randomly in the tank while facing the wall of each quarter. Once every rat recognized the platform, it was left there for 20 s and later transferred to its cage to rest for about 10 s before starting the subsequent trial. In some cases where a rat did not discover the platform within 60 s, was guided to the platform by the researcher. Time and distance to discover the hidden platform as well as the velocity of movements were recorded and analyzed afterward.

A single trial was done 2 h after the final trial to evaluate short-term spatial memory in the MWM. In this trial, the platform was expelled and the rat was permitted to swim for 60 s. The percentage of time and distance spent in the target quadrant (quadrant 4) was analyzed as an index of spatial memory retention (39). After the probe trial, the rats had to undergo visible platform test in order to assess their ability to escape to a visible platform while the platform was raised 2 cm above the water and was visible with an aluminum foil. This experiment was performed to evaluate any possibility of treatment interference with sensory and motor coordination or motivation of male rats (39).

Passive avoidance test

In the passive avoidance test, the rats underwent an electrical shock stress condition, so this test was carried out in a separated group from the other groupswho experienced other behavioral tasks.

A shuttle box apparatus with a dimension of 100 * 25 * 25 cm was used. The device consisted of two divisions, a bright and a dark compartment, which were separated with a guillotine door. The light part was illuminated by a 100-W lamp, 40 cm above the compartment and a transparent ceiling was used. The floors were made of 3-mm stainless steel rods with a distance of 1 cm. The dark part floor was connected to a stimulator for the production of electrical shock. The animal was put in the light compartment of the device and after 5 s, the door was opened and the animal entered the dark part. Afterward, the door was closed and no electric shock was applied. Then the rat was removed from the device and 30 min

later the procedure was repeated again. On the third entry into the dark section, an electric shock was applied to the animal (0.5 mA, 1.5 s duration). The rats should have learned to avoid entering the dark compartment and the time threshold was120s to make sure that animals have learned to avoid entering the dark box in training phase. After 24 h, the animal was tested for retention memory (42). It was placed in the light section of the apparatus and after 30 s, the door was opened. The duration after opening the door until the animal entering the dark section was recorded as step-through latency (STL) with a maximum of 300 s. If the animal did not enter the dark section during this period, STL was considered 300 s. The number of entrances into the dark sector was recorded as shocks, which indicates the contextual learning of the animals, time spent in dark compartment was also recorded. Long staying in dark part is a sign of memory impairment (42).

Electrophysiology

A separated group of animals (n = 6 for each group) was submitted for the electrophysiological experiment. An in vivo study of electrophysiological recording of field excitatory postsynaptic potentials (fEPSPs) from area CA1 of the hippocampus was performed according to Rajizadeh et (34) study. For electrophysiological recordings, Wistar male rats were anesthetized with urethane (1.2 g/kg) (Sigma–Aldrich) and placed in a stereotaxic device. Two holes were drilled in their skulls under sterile conditions, in order to place stimulating and recording electrodes according to the atlas of Paxinos and Watson (43)The rectal temperature was kept at 36.5 ± 0.5°C throughout the experiment (Harvard Apparatus). For field potential recording from area CA1, a concentric bipolar stimulating electrode (stainless steel, 0.125 mm in diameter) was placed in the ipsilateral Schaffer collateral pathway (AP = 3 mm; ML = 3.5 mm; DV = 2.8–3 mm), and a stainless steel recording electrode was lowered into the stratum radiatum of area CA1 of the right hippocampus (AP = 4.1; ML = 3 mm; DV = 2.5 mm). The stimulating electrode was connected to a stimulator and the recording electrode was connected to an amplifier. A maximum fEPSP slope was acquired by stimulating the Schaffer collateral pathway and recording in area CA1. After a 30-min of stabilization period, the input-output (I/O) curves were obtained by gradually increasing the stimulus intensities with constant current (input) and recording fEPSP (output). Extracellular field potentials were amplified and filtered (at1 Hz to 3 kHz band pass). A baseline was established by giving a test stimulus every 10 s for 20 min at a stimulus intensity 50% of which was required to elicit a maximum response. Paired-pulse facilitation (PPF) experiments were conducted in rats that were afterward used for LTP experiments. PPF was measured by delivering ten consecutive evoked responses of paired pulses at 20, 50, 70, 100, and 150 ms inter-pulse intervals (IPIs) to the Schaffer collateral pathway at a frequency of 0.1 Hz (10 s interval). The fEPSP slope ratio [second fEPSP slope/first fEPSP slope; fEPSP2/fEPSP1] was measured at different inter-stimulus intervals. E-LTP was then evoked by applying a train of high frequency stimulation. The pattern of this stimulation consisted of 10 trains with 10-pulse, a frequency of 400 Hz and a pulse width of 0.2 milliseconds and intervals between stimulations of 7 seconds.

The maintenance of LTP was measured for 2 h after high frequency stimulation (HFS) by giving a test stimulus every 10 s. The values of the slope of the fEPSP at each point in the graphs were averaged from 10 consecutive traces. Computer-based stimulation and recording was achieved using Neurotrace software ver. 9 and Electromodule 12 (Science Beam Institute, Tehran, Iran). For analyzing the responses, Potentalise software from the same institute was used (34).

Statistical analysis

The data were presented as mean ± standard error of mean (SEM). The normality of the parameters were examined with the Shapiro-Wilk normality test. For behavioral tests with normal data (open-field, NOR, and probe test in MWM), comparisons among the experimental groups were made by using a one-way analysis of variance (ANOVA) and for passive avoidance a Kruskal-Wallis test was used. A two-way repeated measures ANOVA test was used to compare the mean differences among groups in

MWM (groups and learning days as the factors). If there was statistical significance between the groups, Tukey's multiple comparison post-test was performed.

For electrophysiological study, a two-way repeated measures ANOVA test was conducted to determine the overall differences in the LTP time points (group and time as the factors). The results of single time points in groups were also analyzed with two-way ANOVA followed by Tukey's test for multiple comparisons, when required. All the values are presented as mean ± SEM and P-values less than 0.05 were regarded as significant.

Results

The effects of voluntary exercise on exploratory, locomotor activity and anxiety - like behavior of MS rats:

The open-field test was applied to assess anxiety-like behavior, locomotor and exploratory activity. A one-way ANOVA test results showed that there was no significant difference among groups in total distance and velocity in the open-field test (Fig. 2A and 2B). Rats of the MS group moved a shorter distance in the inner zone compared with the control group (Fig. 2C; $P<0.01$) and it was observed that voluntary exercise in the MS + EX group increased the inner zone distance movement compared with the MS group (Fig. 1C; $P<0.05$). The number of rearing in the MS group was less than the control group (Fig. 2D; $P<0.001$), and this index in the MS + EX group was more than the MS group (Fig. 2D; $P<0.05$). The number of grooming behavior increased significantly in the MS group compared with the control group (Fig. 2E; $P<0.001$) and sharply decreased in the MS + EX group compared with the MS group (Fig. 2E; $P<0.05$). (n = 7 in each group).

The effects of voluntary exercise on recognition memory of MS rats:

The NOR test was used to evaluate recognition memory. In the training phase with two identical objects, all groups spent almost a similar amount of time exploring the objects and no significant difference was seen among them (Fig. 3A).

In the test phase, NOR was assessed by replacing the objects of the first trial with a novel object and the result was reflected in a preference for exploring the novel object. The NOR memory was significantly disrupted in the MS rats. The MS rats spent less time investigating the novel object in comparison with the familiar object, and they were not significantly biased toward the novel object. In these animals, the discrimination ratio was significantly lower than the ratio the control group (Fig. 3B; $P<0.05$). Voluntary exercise ameliorated the MS-induced impairment of the NOR test (Fig. 3B; $P<0.05$) (one-way ANOVA followed by Tukey's test). (n = 7 in each group).

The effects of Voluntary exercise on spatial learning of MS rats:

MWM assessed spatial learning of the animals during the acquisition phase. The animals in all groups learned to find the hidden platform as reduction in their swimming distance as well as their escape latency across the blocks of training was observed; however, the present data showed that learning in the MS group was impaired (Fig. 4A and B, respectively).

Two-way ANOVA with repeated measures test indicated that the distance and escape latency of the MS group significantly increased in Block 2 (Fig. 4A, $P<0.05$ and Fig. 4B; $P<0.05$, respectively) and Block 3 (Fig. 4A; $P<0.01$ and Fig. 4B; $P<0.05$) compared with the control group. The ability of the MS rats to find the hidden platform was considerably improved through voluntary exercise in the MS + EX group. This was indicated by the marked decrease in their swimming distance in Block 3 (Fig.A; $P< 0.05$) and escape latency in Block 2 (Fig. 4B; $P<0.05$) and Block 3 (Fig. 4B; $P<0.05$ for the MS + EX) compared with the MS group in the MWM test. There was no significant difference in the swimming speed among all groups (Fig. 4C) (n = 7 in each group).

The effects of voluntary exercise on spatial short-term memory of MS Rats:

To assess spatial memory retention, the probe test was conducted and the mean percentage of distance and target quadrant time were analyzed. The probe test was done 2 h after the acquisition phase to examine short-term spatial memory retention. The obtained results included the time mean percentage (%)as well as distance and the

number of crossings in the target quadrant. The results of the probe test demonstrated that the MS rats moved significantly less distance, spent less time and had fewer crossings in the target quadrant compared with the control group (Fig. 4D; $P<0.01$, Fig. 4E; $P<0.05$, and Fig. 4F; $P<0.05$). However, this impairment was notably improved by voluntary exercise in the MS + EX group, since they moved more distance and had more crossings in the target quadrant than the MS rats (Fig. 4D; $P<0.05$ and Fig. 4F; $P<0.05$) (one-way ANOVA followed by Tukey's test). No significant difference in the latency was found in order to detect the visible platform among the experimental groups, so our manipulations did not induce any motor or sensory deficits on the experimental animals (Table 1).

The effects of voluntary exercise on passive avoidance learning and memory of MS rats:

The influence of MS and voluntary exercise on passive avoidance learning is shown in Fig. 5A. The Kruskal-Wallis test showed that the shock numbers significantly increased in the MS group compared with the control group (Fig. 5A; $P<0.001$). Voluntary exercise significantly decreased the shock number in the MS + EX group compared with the MS group (Fig. 5A; $P<0.05$). Moreover, Fig. 5B and 5C show the effect of MS and voluntary exercise on passive avoidance memory. MS significantly increased the time spent in the dark compartment (Fig. 5B; $P<0.01$) but significantly decreased STL (Fig. 5C; $P<0.05$) compared with the control group. However, voluntary exercise significantly decreased the time spent in the dark compartment (Fig. 5B; $P<0.05$) but significantly increased STL (Fig. 5C; $P<0.05$) compared with the MS group.($n = 7$ in each group).

CA1 basal synaptic transmission and PPF relationship:

To assay the effect of voluntary exercise and MS on basal synaptic performance, input-output (I-O) curves were plotted as changes in the slope of fEPSP against increasing stimulus intensities. Overall, a two-way ANOVA test showed that no significant difference was seen in the input/output relationship among all the rats. In addition, there was no significant change in the PPF ratios among all groups (Fig. 6A and 6B) ($n = 6$ in each group).

Early long-term potentiation (E-LTP):

LTP induction in the dorsal hippocampal CA1 area was confirmed by a significant increase in the fEPSP slope (more than 20%) and LTP maintenance was evaluated by recording the fEPSPs for 2 h following HFS.

HFS of the Schaffer-collaterals pathway evoked E-LTP as a marked increase in fEPSP slope which was examined for 2 h after HFS (maintenance phase of LTP). The results of our study showed that LTP was induced in all groups except MS group (Fig. 7 $P< 0.05$, MS vs Control). After applying HFS, the mean fEPSP slope in the MS group was significantly lower compared with the control group; However, voluntary exercise induced LTP and had maintenance in the MS + EX group, and it was significantly higher??? in comparison with the MS group (Fig. 7; $P<0.05$) ($n = 6$ in each group).

Discussion

The present study was conducted to determine the effects of voluntary exercise on cognitive performance and also LTP induction and maintenance in MS rats. According to our findings, MS could induce more anxiety-like behavior in the open-field test. In addition, MS rats showed learning and memory deficits in NOR, MWM and passive avoidance tests. Moreover, the hippocampal LTP magnitude in CA3-CA1 neural synapses activated by HFS was significantly lower in MS rats compared with the controls. Voluntary exercise led to LTP induction and maintenance in the MS + EX group, and it was significantly higher compared with the MS group.

Early life adversity could be a serious issue due to the passionate and cognitive effects found in subjects who encounter it around the globe (44). In an endeavor to investigate the natural premise of these alterations and attempt to diminish them within the future, several models of early stress have been created. One of the foremost broadly utilized models is MS (45). MS induces a wide extend of changes in behavior and one of the principle changes which has been considered is spatial learning. Alterations in spatial learning and memory have been shown in early stress

models; while, some researchers suggest that spatial memory isn't influenced, some recommend that prefrontal-related errands are modified after early stress (shifting and reversal learning, for instance), while classic reference memory in the MWM is protected. On the other hand, Other researchers propose that spatial memory is preserved and refer to social behavior as the leading influenced parameter. This discussion might be due to contrasts in the exact protocol used by each research group; alternatively, MS animals might be successful on memory tasks if they consume more metabolic energy than controls (46).

Some studies on MS rats have shown disorders in affective capacities, including more anxiety-like behavior (47,48), depressive-like behavior (49), and anhedonic behavior (48). Cognitive impedances such as learning impairments in MWM and NOR tests have also been reported (50). Moreover, some studies have shown the destructive effects of MS on social behavior (51). The results of Yang et al. revealed that MS does not affect the overall distance and motor activity which are in accordance with the results of our study, however the ratio of the border/center time was considerably reduced. These results are in contrast to other experiments of anxiety-like behaviors and not consistent with our results (52) Shin et al. found that MS mice (P2-P20, 4 h each day) displayed anxiety-like behavior in an elevated plus maze, which was further confirmed by the open-field test (P1-P14, 3 h each day) (12). Moreover, in a study by Aisa et al., the results showed a rise in anxiety-like behavior without affecting motor activity in maternally separated rats, which are consistent with our results (53). The reasons for these discrepancies require further clarification, although animal strains, MS protocol, and experimental environment might be implicated.

In MS conditions, all systems prepare themselves to respond more proficiently in stressful conditions. In case an animal is raised in early stressful conditions, the nervous, endocrine, and immune systems will be altered to manage with unpleasant situations in afterward life (54), these adjustments may be useless in non-stressful situations and appearingly lead to behavioral disabilities or neuropsychiatric-like disorders. One of these disorders is anxiety. MS actuates anxiety in adolescence and adulthood (55).

Another factor that could explain the anxious behavior is the increase in sympathomimetic neurotransmitters. MS animals show greater response in accumbens core dopamine levels when they face a stressor. Moreover, MS elevates striatal dopamine (56).

With respect to learning impairments, some researchers have found that MS does not impair object recognition in adults (57,58). While some other studies suggest that MS could impair object recognition (4,59).The biological substrate of this disability might incorporate the perirhinal cortex, the area with a well-established role in cognitive performance , particularly in those related to object recognition (60). Other investigations have reported the effect of MS on memory deficit in the novel object recognition test, which agrees with the results of the present study (52,61).

One explanation for recognition deficits could be the rise in acetylcholinesterase in the perirhinal cortex of MS subjects. When acetylcholinesterase inhibitors like galantamine are administered, memory alterations are reversed (62).

MWM is widely used for measuring spatial learning and memory (52). In this study, MS caused a deficit in spatial memory in maternally separated rats. In line with our study, Yang et al. in 2017 reported that MS (3 h/day, PND 3-21) caused impaired hippocampal synaptic activity, learning and memory and memory retrieval in adolescence (52). Studies by Aisa et al. indicated cognitive deficits and increased activity of the HPA axis. They reported that spatial memory in adult rats didn't change in the learning stage, but showed a significant difference with the control group rats in the recovery stage (53). The results of some other studies on spatial memory are in line with our findings (63,64).

Several previous studies have found that MS decreases spatial learning of adult rats, which is in accordance with our results (65). Also Joushi et al. suggested that MS could induce spatial memory impairments in adolescent male rats (39). Moreover, Hill et al. found that MS rats showed disruptions in spatial memory in Y-maze (66).

As stated in our present study MS P21 causes in avoidance memory impairment in maternally separated adolescent rats. On the other hand,

some authors have reported that MS P10 caused moderate impairment in P34 adolescent rats, while MS P21 has no effect on this process, which is contrary to our results. In addition, other findings indicated that MS in each period didn't affect the disruption of adult rats. The cause of this difference may be the age of the investigated animal or the daily period of MS (67).

In a study by Anna et al. MS increased the failure rate in maternally separated mice, which means that these mice obtained at least 10 times higher foot shocks so that they would not enter the dark part of the shuttle box (68). This finding confirms our results.

On the other hand, frontolimbic associations were impaired by stress (69). In the cortex-amygdala and cortex-thalamus pathways, MS diminishes LTP and raises long-term depression. In other words, MS diminishes synaptic alternations in adolescents. In MS animals, there's an overexposure to glucocorticoids, which can diminish synaptic plasticity (59). Nevertheless, some authors suggest that the size of LTP induced by high-frequency stimulation (HFS) was lower in maternally separated rats compared to control rats (5,70).

Brain development in mammals starts in intrauterine life and proceeds through adolescence (71). The hippocampus and PFC of rodents experienced significant development amid puberty. Learning and memory proceed to create all through this period (72).

Hippocampus plasticity is the basis of learning and memory processes and is significantly affected by early adverse events, such as stress (12). The obtained results demonstrated that MS eliminated the capability of LTP induction in the CA1 region of the hippocampus. Some researchers have found that the measured LTP actuated by HFS in MS rats was lower than control rats (5,13,39). Hippocampa l LTP has been appeared to diminish at the CA3-CA1 neural synapses as a result of MS (12). Another research revealed that both values of EPSP slope and population spike (PS) amplitude declined in maternally separated male rats (13).

In animal models, there are two distinctive exercise modalities: forced exercise and voluntary exercise. One of the key contrasts between these two exercise modalities is that voluntary exercise permits the animal to select the timing and measurement of the exercise, while forced exercise expels this aspect and normally depends on an aversive stimulus to condition the behavior. On the treadmill, this boost is electrical stun. Whereas this aversive boost is successful in conditioning running behavior, it could make an unpleasant situation for the animal. Voluntary running takes place when the animal makes a choice, more often in the dark cycle, but as a rule forced running takes place in the light cycle for researchers' comfort (73).

In spite of the fact that some evidences have proposed the significant development of cognitive performance, learning, and memory by physical exercise (74) offering considers appeared to have no impact in our study (75). Numerous examinations affirmed the positive impacts of different exercise models such as the treadmill or forced exercise (76)and voluntary exercise (77) on rodents' behavior. Some studies reported that treadmill exercise and voluntary exercise could reestablish cognitive impedances actuated by MS in MWM (73), novel object recognition (73), and passive avoidance (73) tasks in rats.

Physical exercise has been appeared to enhance learning as well as memory in both MWM (78) and passive avoidance tasks (79). Physical exercise induces functional and basic changes inbrain and its effects on the hippocampus is specific (19).The beneficial effects of exercise on physiological systems, including the central nervous system (CNS) and brain wellbeing, are well-demonstrated (19). Exercise has been revealed to upgrade hippocampus-dependent spatial memory in rodents in different models such as MWM, Y maze, and radial arm maze (80). It has been proposed that forced exercise and voluntary exercise actuate different effects in brain neuroplasticity and behavior (81). Treadmill exercise or wheel running improve hippocampus-dependent spatial learning and memory (82). It has been reported that voluntary exercise diminishes in both dopamine neuron degeneration and behavioral deficits (83).

Voluntary exercise has been shown to reduce anxiety levels and counteract the cognitive and impairments induced by stress and depression. Running has been appeared to improve

neurogenesis and enhance cognitive performance (84). One reason may be increased level of hippocampal BDNF observed in exercised rats (24). Some researchers illustrated that both voluntary and involuntary exercise influenced spatial learning and memory positively in adolescent rats, which exercise was also connected with expanded hippocampal insulin-like growth factor 1 (IGF-1) levels and higher numbers of hippocampal cells. These studies also reported an improvement in spatial learning and memory as a result of voluntary and involuntary exercise in both male and female rats (85,86).

In this study, we examined the combined effects of voluntary exercise and MS on behavioral function and synaptic plasticity in the CA1 area of the hippocampus. We found that voluntary exercise for 3 weeks rather ameliorated the MS-induced memory impairments, since spatial recognition and passive avoidance memories were improved. Moreover, voluntary exercise decreased anxiety-like behavior in maternally separated rats.

The number of studies regarding the combination of exercise and MS is scarce. Sosa et al. investigated the effects of an aerobic exercise (AE) shot on the consolidation and persistence of object recognition memory in maternally deprived (MD) rats. Their results showed that a single shot of exercise can modulate learning, promote the acquisition of memory in MD rats and leads to the persistence of this memory up to 21 days (87). Sadeghi et al. also found that voluntary physical exercise during adolescence protects against depressant effects of early-life stresses, at least partly through mitigating the innate immune responses in the hippocampus (88). The results of a step-down avoidance test demonstrated that short-term memory was decreased in the MS rats. However, treadmill exercise increased activity and ameliorated memory impairments (89). Nevertheless, Grace et al. reported that that MS had little effect on rats, whereas exercise enhanced both spatial and recognition memory. It seems that the reason for these differences is the MS duration (31).

Our results showed that MS affected negatively the induction and stability of LTP in adolescent male rats. Moreover, in the ex group, the LTP status was approximately the same as that of the control group. However, when exercise was applied with MS in the MS + Ex group situation didn't come back to the baseline, although LTP induction was improved compared to the MS group.

Plasticity is thought to be due to deleterious changes in intracellular signaling molecules (90) and receptors such as NMDA (N-methyl-d-aspartate) (91) and AMPA (_-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (92). Some studies have also affirmed the positive role of voluntary exercise on the brain (93). voluntary exercise appears to upgrade the plasticity of the brain in adult rats through several mechanisms(94).. To begin with, it can improve the neurogenesis within the dentate gyrus (DG) of the hippocampus (95). The capacity for both the induction and expression of LTP in the hippocampus is enhanced by voluntary exercise (94), and the changes in neurogenesis and LTP induction/expression are induced by concurrent increasing the expression of BDNF, NR2B, and GluR5 mRNA The beneficial effects of voluntary exercise (94)and forced exercise (96) on LTP induction and maintenance are also confirmed. Exercise challenges homeostasis; it fortifies the sympathetic nervous system to release adrenaline and noradrenaline (97)Noradrenergic projections emerging from the locus coeruleus adjust neuronal function by means of β-adrenergic receptors in brain that are involved in learning and memory, such as the hippocampus, prefrontal cortex (PFC), and amygdala (98). High-intensity exercise strengthens the HPA axis to emit corticotropin-releasing factor (CRF), vasopressin, and glucocorticoids (97). These hormones change a variety of physiological capacities to adjust homeostasis (99). Excitatory glutamate neural synapses play a rudimentary role in synaptic transmission, synaptic plasticity and behavioral adjustment (98). At glutamate neural synapses, the ionotropic AMPA receptors are involved in fast transmission, and changes in their trafficking have been proposed to be critical for synaptic plasticity (100). It is important to understand how stress can impact behavior since the stress-induced discharge of glucocorticoids regulates synaptic plasticity by modifying glutamate receptor function in limbic brain structures such as the hippocampus, amygdala, and PFC (101).

Glucocorticoids enhance the influx of calcium through glutamate NMDA receptors, facilitating both LTP and long-term depression within the hippocampus (101). High concentrations of corticosterone diminish LTP in hippocampal slices (100). Prenatal stress initiates depressive-like behavior and diminishes NMDA subunit (NR1 and NR2A) levels in some brain regions (102). AMPA receptors convert inner and outer part of the postsynaptic layer. Released norepinephrine during exercise fortifies the phosphorylation of GluR1 and transmits GluR1-containing AMPA receptors to the neural synapses which leads to the improvement of learning and memory processes (103). Intense stress causes fast inclusion of Ca2+-permeable AMPA receptors into the neural synapses to facilitate LTP in the hippocampus (104). Drugs anticipating glucocorticoid-induced surface dispersal of AMPA receptors from the neural synapse, could reestablish LTP in acutely stressed animals (100). These results affirm the complexity of the interaction between stress and glutamate receptor function. Prenatal stress also diminishes the beneficial effect of exercise on forelimb sensory-motor function (105). A similar interaction between stress and exercise was observed in the initiation of movement of the affected forelimb of rats subjected to the MS (30). Exercise has been shown to extend hippocampal dendritic spine density in adulthood, conceivably as a result of increased negative feedback regulation of the HPA axis, diminishing circulating corticosterone levels (106). Voluntary exercise (wheel running for 4 weeks) could also secure the hippocampus from the destructive effects of elevated corticosterone by enhancing hippocampal glucocorticoid receptor levels and diminishing the affectability of mineralocorticoid receptors (97). Some researchers foundout that ELS not only reduced the exercise-induced changes in neuron survival and behavior, but also changed brain neuroplasticity. It's been shown that voluntary exercise stimulated the mitogen-activated protein kinase/extracellular signal-regulated protein kinase 1/2 (MAPK/ERK1/2) signaling pathway in the rodent hippocampus and this stimulation was blocked in rats subjected to MS (107). Moreover, MS could alter protein levels in the ventral hippocampus and decreased proteins involved in structure, energy metabolism, and signaling in the PFC of adult rats. These changes were mostly reversed by exercise for 3 weeks during adolescence (108). Adolescence is distinguished as a basic period which stress could affect hippocampal plasticity. MS taken after by forced swim stress during adolescence extend hippocampal neurotrophin levels (109). Limited stress in adulthood potentiated the impacts of MS by expanding depressive-like behavior (110). It has been hypothesized that the identification of the critical period, intensity, and duration of stressors, as well as the brain areas and the molecular mechanisms affected by ELS, would lead to a better understanding of the stress effect on the risk of developing neuropsychiatric and neurodegenerative disorders later in life. On the other hand, not only the intensity but also the duration of exercise needs to be defined in order to realize the full structural and functional benefit of exercise for brain function. Patten et al. evaluated the effects of different periods of voluntary running (3, 7, 14, 28, and 56 days, respectively) on both structural (cell proliferation and maturation) and functional (*in vivo* LTP) changes in the dentate gyrus of adult male Sprague-Dawley hippocampus(74). They found that both short- and long-term periods increased cell proliferation in the dentate gyrus of the hippocampus. However, increase in neurogenesis required longer-term exercise protocols. Increase in immature neurons were not observed until animals had run for a minimum of 14 days. Similarly, short-term periods of wheel running did not facilitate LTP in the dentate gyrus of adult animals, and reliable increases in LTP were only observed after 56 days of exercise. These results provided a greater understanding of the exercise duration needed to enhance hippocampal dentate gyrus function. Furthermore, the results indicated that the produced new neurons in response to exercise didnt significantly contribute to synaptic plasticity until they mature (74).

In summary, voluntary exercise is an important pro-cognitive stimulus that improves cognitive deficits caused by MS. Although we cannot determine the exact modulatory mechanisms involved in acute exercise effects, it is clearly an

additional valuable measure to sustain the cognitive functions caused by ELS.

Conclusion

The present findings provided a detailed description of how MS can interfere with normal cognition. Voluntary exercise may be an appropriate therapeutic strategy for promoting cognitive function in cognitive disorders.

Funding statement

We would like to acknowledge the Kerman Neuroscience Research Center for financial support.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The protocols were carried out in strict compliance with the guidelines approved by the ethics committee of Kerman University of Medical Sciences (ethics code: IR.KMU.REC. 1399.318).

Conflict of interest

No conflict of interest was reported by the authors.

References

[1] Pechtel P, Pizzagalli DA. Effects of early life stress on cognitive and affective function: an integrated review of human literature. Psychopharmacology (Berl). 2011;214(1):55–70.

[2] Sandi C, Pinelo-Nava MT. Stress and memory: behavioral effects and neurobiological mechanisms. Neural Plast. 2007;2007.

[3] Judo C, Matsumoto M, Yamazaki D, Hiraide S, Yanagawa Y, Kimura S, et al. Early stress exposure impairs synaptic potentiation in the rat medial prefrontal cortex underlying contextual fear extinction. Neuroscience. 2010;169(4):1705–14.

[4] Solas M, Aisa B, Mugueta MC, Del Río J, Tordera RM, Ramírez MJ. Interactions between age, stress and insulin on cognition: implications for Alzheimer's disease. Neuropsychopharmacology. 2010;35(8):1664–73.

[5] Sousa VC, Vital J, Costenla AR, Batalha VL, Sebastião AM, Ribeiro JA, et al. Maternal separation impairs long term-potentiation in CA1-CA3 synapses and hippocampal-dependent memory in old rats. Neurobiol Aging. 2014;35(7):1680–5.

[6] Schwabe L, Joëls M, Roozendaal B, Wolf OT, Oitzl MS. Stress effects on memory: an update and integration. Neurosci Biobehav Rev. 2012;36(7):1740–9.

[7] Kosten TA, Kim JJ, Lee HJ. Early life manipulations alter learning and memory in rats. Neurosci Biobehav Rev. 2012;36(9):1985–2006.

[8] Lai M-C, Huang L-T. Effects of early life stress on neuroendocrine and neurobehavior: mechanisms and implications. Pediatr Neonatol. 2011;52(3):122–9.

[9] Figueiredo ÍL, Frota PB, da Cunha DG, da Silva Raposo R, Canuto KM, de Andrade GM, et al. Prolonged maternal separation induces undernutrition and systemic inflammation with disrupted hippocampal development in mice. Nutrition. 2016;32(9):1019–27.

[10] Aguggia JP, Suárez MM, Rivarola MA. Early maternal separation: neurobehavioral consequences in mother rats. Behav Brain Res. 2013;248:25–31.

[11] Doreste-Mendez R, Ríos-Ruiz EJ, Rivera-López LL, Gutierrez A, Torres-Reveron A. Effects of environmental enrichment in maternally separated rats: age and sex specific outcomes. Front Behav Neurosci. 2019;13:198.

[12] Shin SY, Han SH, Woo R-S, Jang SH, Min SS. Adolescent mice show anxiety-and aggressive-like behavior and the reduction of long-term potentiation in mossy fiber-CA3 synapses after neonatal maternal separation. Neuroscience. 2016;316:221–31.

[13] Cao X, Huang S, Cao J, Chen T, Zhu P, Zhu R, et al. The timing of maternal separation affects Morris water maze performance and long-term potentiation in male rats. Dev Psychobiol. 2014;56(5):1102–9.

[14] Gruss M, Braun K, Frey JU, Korz V. Maternal separation during a specific postnatal time window prevents reinforcement of hippocampal long-term potentiation in adolescent rats. Neuroscience. 2008;152(1):1–7.

[15] Cao X, Huang S, Ruan D. Enriched environment restores impaired hippocampal long-term potentiation and water maze performance induced by developmental lead exposure in rats. Dev Psychobiol J Int Soc Dev Psychobiol. 2008;50(3):307–13.

[16] Bliss TVP, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993;361(6407):31–9.

[17] Herpfer I, Hezel H, Reichardt W, Clark K, Geiger J, Gross CM, et al. Early life stress differentially modulates distinct forms of brain plasticity in young and adult mice. PLoS One. 2012;7(10):e46004.

[18] Baudin A, Blot K, Verney C, Estevez L, Santamaria J, Gressens P, et al. Maternal deprivation induces deficits in temporal memory and cognitive flexibility and exaggerates synaptic plasticity in the rat medial prefrontal cortex. Neurobiol Learn Mem. 2012;98(3):207–14.
[19] Cotman CW, Berchtold NC. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 2002;25(6):295–301.
[20] Griffin EW, Bechara RG, Birch AM, Kelly ÁM. Exercise enhances hippocampal-dependent learning in the rat: evidence for a BDNF-related mechanism. Hippocampus. 2009;19(10):973–80.
[21] Liu H, Zhao G, Cai K, Zhao H. Treadmill exercise prevents decline in spatial learning and memory in APP/PS1 transgenic mice through improvement of hippocampal long-term potentiation. Behav Brain Res. 2011;218(2):308–14.
[22] Landers MR, Kinney JW, Allen DN, van Breukelen F. A comparison of voluntary and forced exercise in protecting against behavioral asymmetry in a juvenile hemiparkinsonian rat model. Behav Brain Res. 2013;248:121–8.
[23] Leasure JL, Jones M. Forced and voluntary exercise differentially affect brain and behavior. Neuroscience. 2008;156(3):456–65.
[24] Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci. 2004;20(10):2580–90.
[25] Zheng S, Zhang F, Liu Q, Jian R, Yang M. Exercise training increases spatial memory via reducing contralateral hippocampal NMDAR subunits expression in intracerebral hemorrhage rats. Neuropsychiatr Dis Treat. 2019;15:1921.
[26] Ji E-S, Kim Y-M, Shin M-S, Kim C-J, Lee K-S, Kim K, et al. Treadmill exercise enhances spatial learning ability through suppressing hippocampal apoptosis in Huntington's disease rats. J Exerc Rehabil. 2015;11(3):133.
[27] Jacotte-Simancas A, Costa-Miserachs D, Torras-Garcia M, Coll-Andreu M, Portell-Cortés I. Effect of voluntary physical exercise and post-training epinephrine on acquisition of a spatial task in the barnes maze. Behav Brain Res. 2013;247:178–81.
[28] Zhang L, Feng Y, Ji W, Liu J, Liu K. Effect of voluntary wheel running on striatal dopamine level and neurocognitive behaviors after molar loss in rats. Behav Neurol. 2017;2017.
[29] Ang E-T, Dawe GS, Wong PTH, Moochhala S, Ng Y-K. Alterations in spatial learning and memory after forced exercise. Brain Res. 2006;1113(1):186–93.
[30] Mabandla MV, Russell VA. Voluntary exercise reduces the neurotoxic effects of 6-hydroxydopamine in maternally separated rats. Behav Brain Res. 2010;211(1):16–22.
[31] Grace L, Hescham S, Kellaway LA, Bugarith K, Russell VA. Effect of exercise on learning and memory in a rat model of developmental stress. Metab Brain Dis. 2009;24(4):643.
[32] Li M, Xue X, Shao S, Shao F, Wang W. Cognitive, emotional and neurochemical effects of repeated maternal separation in adolescent rats. brain Res. 2013;1518:82–90.
[33] Mustard JF. Experience-based brain development: scientific underpinnings of the importance of early child development in a global world. Early child Dev from Meas to action Washingt DC, World Bank. 2007;43–86.
[34] Rajizadeh MA, Esmaeilpour K, Haghparast E, Ebrahimi MN, Sheibani V. Voluntary exercise modulates learning & memory and synaptic plasticity impairments in sleep deprived female rats. Brain Res. 2020;1729:146598.
[35] Rajizadeh MA, Sheibani V, Bejeshk MA, Mohtashami Borzadaran F, Saghari H, Esmaeilpour K. The effects of high intensity exercise on learning and memory impairments followed by combination of sleep deprivation and demyelination induced by etidium bromide. Int J Neurosci. 2019;129(12):1166–78.
[36] Taheri P, Mohammadi F, Nazeri M, Zarei MR, Chamani G, Esfahlani MA, et al. Nitric oxide role in anxiety-like behavior, memory and cognitive impairments in animal model of chronic migraine. Heliyon [Internet]. 2020;6(12):e05654. Available from: https://doi.org/10.1016/j.heliyon.2020.e05654
[37] Esmaeilpour K, Sheibani V, Shabani M, Mirnajafi-Zadeh J. Effect of low frequency electrical stimulation on seizure-induced short- and long-term impairments in learning and memory in rats. Physiol Behav. 2017;168:112–21.
[38] Sivakumaran MH, Mackenzie AK, Callan IR, Ainge JA, O'Connor AR. The discrimination ratio derived from novel object recognition tasks as a measure of recognition memory sensitivity, not bias. Sci Rep. 2018;8(1):1–12.
[39] Joushi S, Esmaeilpour K, Masoumi-Ardakani Y, Esmaeili-Mahani S, Sheibani V. Intranasal oxytocin administration facilitates the induction of long-term potentiation and promotes cognitive performance of maternally separated

rats. Psychoneuroendocrinology. 2021;123:105044.

[40] Rehman NU, Esmaeilpour K, Joushi S, Abbas M, Al-Rashida M, Rauf K, et al. Effect of 4-Fluoro-N-(4-sulfamoylbenzyl) Benzene Sulfonamide on cognitive deficits and hippocampal plasticity during nicotine withdrawal in rats. Biomed Pharmacother. 2020;131:110783.

[41] Rajizadeh MA, Esmaeilpour K, Motamedy S, Borzadaranb FM, Sheibani V. Cognitive Impairments of Sleep-Deprived Ovariectomized (OVX) Female Rats by Voluntary Exercise. Basic Clin Neurosci. 2020;11(5):573.

[42] Mohammadi F, Ahmadi-Zeidabadi M, Nazeri M, Ghasemi A, Shabani M. Nitric oxide modulates cognitive, nociceptive and motor functions in a rat model of empathy. Int J Neurosci. 2020;1–10.

[43] Herman JP, Watson SJ. The rat brain in stereotaxic coordinates (2nd edn): by George Paxinos and Charles Watson, Academic Press, 1986.£ 40.00/$80.00 (264 pages) ISBN 012 547 6213. Elsevier Current Trends; 1987.

[44] Luby JL, Belden A, Harms MP, Tillman R, Barch DM. Preschool is a sensitive period for the influence of maternal support on the trajectory of hippocampal development. Proc Natl Acad Sci. 2016;113(20):5742–7.

[45] Nylander I, Roman E. Is the rodent maternal separation model a valid and effective model for studies on the early-life impact on ethanol consumption? Psychopharmacology (Berl). 2013;229(4):555–69.

[46] Banqueri M, Méndez M, Arias JL. Spatial memory-related brain activity in normally reared and different maternal separation models in rats. Physiol Behav. 2017;181:80–5.

[47] Huot RL, Thrivikraman K, Meaney MJ, Plotsky PM. Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. Psychopharmacology (Berl). 2001;158(4):366–73.

[48] Salm AK, Pavelko M, Krouse EM, Webster W, Kraszpulski M, Birkle DL. Lateral amygdaloid nucleus expansion in adult rats is associated with exposure to prenatal stress. Dev brain Res. 2004;148(2):159–67.

[49] Meaney MJ, Plotsky PM. Long-term behavioral and neuroendocrine adaptations to adverse early experience. Biol basis mind body Interact. 2000;122:81.

[50] Garcia VA, Hirotsu C, Matos G, Alvarenga T, Pires GN, Kapczinski F, et al. Modafinil ameliorates cognitive deficits induced by maternal separation and sleep deprivation. Behav Brain Res. 2013;253:274–9.

[51] Kambali MY, Anshu K, Kutty BM, Muddashetty RS, Laxmi TR. Effect of early maternal separation stress on attention, spatial learning and social interaction behaviour. Exp brain Res. 2019;237(8):1993–2010.

[52] Yang S, Li J, Han L, Zhu G. Early maternal separation promotes apoptosis in dentate gyrus and alters neurological behaviors in adolescent rats. Int J Clin Exp Pathol. 2017;10(11):10812.

[53] Aisa B, Elizalde N, Tordera R, Lasheras B, Del Río J, Ramírez MJ. Effects of neonatal stress on markers of synaptic plasticity in the hippocampus: implications for spatial memory. Hippocampus. 2009;19(12):1222–31.

[54] Korosi A, Naninck EFG, Oomen CA, Schouten M, Krugers H, Fitzsimons C, et al. Early-life stress mediated modulation of adult neurogenesis and behavior. Behav Brain Res. 2012;227(2):400–9.

[55] Sachs BD, Rodriguiz RM, Siesser WB, Kenan A, Royer EL, Jacobsen JPR, et al. The effects of brain serotonin deficiency on behavioural disinhibition and anxiety-like behaviour following mild early life stress. Int J Neuropsychopharmacol. 2013;16(9):2081–94.

[56] Ognibene E, Adriani W, Caprioli A, Ghirardi O, Ali SF, Aloe L, et al. The effect of early maternal separation on brain derived neurotrophic factor and monoamine levels in adult heterozygous reeler mice. Prog Neuro-Psychopharmacology Biol Psychiatry. 2008;32(5):1269–76.

[57] Klug M, van den Buuse M. Chronic cannabinoid treatment during young adulthood induces sex-specific behavioural deficits in maternally separated rats. Behav Brain Res. 2012;233(2):305–13.

[58] Pinheiro RMC, de Lima MNM, Portal BCD, Busato SB, Falavigna L, Ferreira RDP, et al. Long-lasting recognition memory impairment and alterations in brain levels of cytokines and BDNF induced by maternal deprivation: effects of valproic acid and topiramate. J Neural Transm. 2015;122(5):709–19.

[59] Danielewicz J, Hess G. Early life stress alters synaptic modification range in the rat lateral amygdala. Behav Brain Res. 2014;265:32–7.

[60] Hannesson DK, Howland JG, Pollock M, Mohapel P, Wallace AE, Corcoran ME. Anterior perirhinal cortex kindling produces

long-lasting effects on anxiety and object recognition memory. Eur J Neurosci. 2005;21(4):1081–90.

[61] Hulshof HJ, Novati A, Sgoifo A, Luiten PGM, den Boer JA, Meerlo P. Maternal separation decreases adult hippocampal cell proliferation and impairs cognitive performance but has little effect on stress sensitivity and anxiety in adult Wistar rats. Behav Brain Res. 2011;216(2):552–60.

[62] Benetti F, Mello PB, Bonini JS, Monteiro S, Cammarota M, Izquierdo I. Early postnatal maternal deprivation in rats induces memory deficits in adult life that can be reversed by donepezil and galantamine. Int J Dev Neurosci. 2009;27(1):59–64.

[63] Delavari F, Sheibani V, Esmaeilpour K, Esmaeili-Mahani S, Nakhaee N. Effects of maternal separation on nicotine-induced conditioned place preference and later spatial learning and memory function in adolescent male rats. Addict Heal. 2016;8(4):261.

[64] Wang Q, Li M, Du W, Shao F, Wang W. The different effects of maternal separation on spatial learning and reversal learning in rats. Behav Brain Res. 2015;280:16–23.

[65] Hui J, Zhang Z, Liu S, Xi G, Zhang X, Teng G-J, et al. Hippocampal neurochemistry is involved in the behavioural effects of neonatal maternal separation and their reversal by post-weaning environmental enrichment: a magnetic resonance study. Behav Brain Res. 2011;217(1):122–7.

[66] Hill RA, Klug M, Kiss Von Soly S, Binder MD, Hannan AJ, van Den Buuse M. Sex-specific disruptions in spatial memory and anhedonia in a “two hit” rat model correspond with alterations in hippocampal brain-derived neurotrophic factor expression and signaling. Hippocampus. 2014;24(10):1197–211.

[67] Banqueri M, Méndez M, Arias JL. Behavioral effects in adolescence and early adulthood in two length models of maternal separation in male rats. Behav Brain Res. 2017;324:77–86.

[68] Tchenio A, Lecca S, Valentinova K, Mameli M. Limiting habenular hyperactivity ameliorates maternal separation-driven depressive-like symptoms. Nat Commun. 2017;8(1):1–8.

[69] Soztutar E, Colak E, Ulupinar E. Gender-and anxiety level-dependent effects of perinatal stress exposure on medial prefrontal cortex. Exp Neurol. 2016;275:274–84.

[70] heydari A, Esmaeilpour K, Sheibani V. Maternal separation impairs long term-potentiation in CA3-CA1 synapses in adolescent female rats. Behav Brain Res [Internet]. 2019;376:112239. Available from: https://doi.org/10.1016/j.bbr.2019.112239

[71] Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev. 2000;24(4):417–63.

[72] Uysal N, Dayi A, ÖZBAL S, Cetin F, Aksu I, Yalaz G, et al. Development of spatial memory during adolescent period in rats. J Neurol Sci Turk. 2010;27(4):407–13.

[73] Rajizadeh MA, Esmaeilpour K, Masoumi-Ardakani Y, Bejeshk MA, Shabani M, Nakhaee N, et al. Voluntary exercise impact on cognitive impairments in sleep-deprived intact female rats. Physiol Behav. 2018;188:58–66.

[74] Patten AR, Sickmann H, Hryciw BN, Kucharsky T, Parton R, Kernick A, et al. Long-term exercise is needed to enhance synaptic plasticity in the hippocampus. Learn Mem. 2013;20(11):642–7.

[75] Titterness AK, Wiebe E, Kwasnica A, Keyes G, Christie BR. Voluntary exercise does not enhance long-term potentiation in the adolescent female dentate gyrus. Neuroscience. 2011;183:25–31.

[76] Rajizadeh MA, Afarinesh MR, Zarif M, Mirasadi A, Esmaeilpour K. Does caffeine therapy improve cognitive impairments in valproic acid rat model of autism? Toxin Rev. 2019;1–11.

[77] Segal-Gavish H, Barzilay R, Rimoni O, Offen D. Voluntary exercise improves cognitive deficits in female dominant-negative DISC1 transgenic mouse model of neuropsychiatric disorders. World J Biol Psychiatry. 2019;20(3):243–52.

[78] Aguiar Jr AS, Castro AA, Moreira EL, Glaser V, Santos ARS, Tasca CI, et al. Short bouts of mild-intensity physical exercise improve spatial learning and memory in aging rats: involvement of hippocampal plasticity via AKT, CREB and BDNF signaling. Mech Ageing Dev. 2011;132(11–12):560–7.

[79] Saadati H, Babri S, Ahmadiasl N, Mashhadi M. Effects of exercise on memory consolidation and retrieval of passive avoidance learning in young male rats. Asian J Sports Med. 2010;1(3):137.

[80] Fordyce DE, Farrar RP. Enhancement of spatial learning in F344 rats by physical activity and related learning-associated alterations in hippocampal and cortical cholinergic functioning. Behav Brain Res. 1991;46(2):123–33.

[81] Howells FM, Russell VA, Mabandla M V,

Kellaway LA. Stress reduces the neuroprotective effect of exercise in a rat model for Parkinson's disease. Behav Brain Res. 2005;165(2):210–20.

[82] Liu Y, Chen H, Wu C, Kuo Y, Yu L, Huang A, et al. Differential effects of treadmill running and wheel running on spatial or aversive learning and memory: roles of amygdalar brain-derived neurotrophic factor and synaptotagmin I. J Physiol. 2009;587(13):3221–31.

[83] O'dell SJ, Gross NB, Fricks AN, Casiano BD, Nguyen TB, Marshall JF. Running wheel exercise enhances recovery from nigrostriatal dopamine injury without inducing neuroprotection. Neuroscience. 2007;144(3):1141–51.

[84] Duman CH, Schlesinger L, Russell DS, Duman RS. Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. Brain Res. 2008;1199:148–58.

[85] Cetinkaya C, Sisman AR, Kiray M, Camsari UM, Gencoglu C, Baykara B, et al. Positive effects of aerobic exercise on learning and memory functioning, which correlate with hippocampal IGF-1 increase in adolescent rats. Neurosci Lett. 2013;549:177–81.

[86] Uysal N, Kiray M, Sisman AR, Camsari UM, Gencoglu C, Baykara B, et al. Effects of voluntary and involuntary exercise on cognitive functions, and VEGF and BDNF levels in adolescent rats. Biotech Histochem. 2015;90(1):55–68.

[87] Sosa PM, Neves B-HS, Carrazoni GS, Gomes GM, Del Rosso G, Ramborger BP, et al. Maternal Deprivation Induces Memory Deficits That Are Reduced by One Aerobic Exercise Shot Performed after the Learning Session. Neural Plast. 2019;2019.

[88] Sadeghi M, Peeri M, Hosseini M-J. Adolescent voluntary exercise attenuated hippocampal innate immunity responses and depressive-like behaviors following maternal separation stress in male rats. Physiol Behav. 2016;163:177–83.

[89] Park S-S, Kim T-W, Park H-S, Seo T-B, Kim Y-P. Effects of treadmill exercise on activity, short-term memory, vascular dysfunction in maternal separation rats. J Exerc Rehabil. 2020;16(2):118.

[90] Zagaar M, Alhaider I, Dao A, Levine A, Alkarawi A, Alzubaidy M, et al. The beneficial effects of regular exercise on cognition in REM sleep deprivation: behavioral, electrophysiological and molecular evidence. Neurobiol Dis. 2012;45(3):1153–62.

[91] Ravassard P, Pachoud B, Comte J-C, Mejia-Perez C, Scoté-Blachon C, Gay N, et al. Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. Sleep. 2009;32(2):227–40.

[92] Hagewoud R, Havekes R, Novati A, Keijser JN, Van der Zee EA, Meerlo P. Sleep deprivation impairs spatial working memory and reduces hippocampal AMPA receptor phosphorylation. J Sleep Res. 2010;19(2):280–8.

[93] Miller RM, Marriott D, Trotter J, Hammond T, Lyman D, Call T, et al. Running exercise mitigates the negative consequences of chronic stress on dorsal hippocampal long-term potentiation in male mice. Neurobiol Learn Mem. 2018;149:28–38.

[94] Farmer J, Zhao X V, Van Praag H, Wodtke K, Gage FH, Christie BR. Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague–Dawley rats in vivo. Neuroscience. 2004;124(1):71–9.

[95] Niwa A, Nishibori M, Hamasaki S, Kobori T, Liu K, Wake H, et al. Voluntary exercise induces neurogenesis in the hypothalamus and ependymal lining of the third ventricle. Brain Struct Funct. 2016;221(3):1653–66.

[96] Babri S, Reisi P, Alaei H, Sharifi MR, Mohades G. Effect of forced treadmill exercise on long-term potentiation (LTP) in the dentate gyrus of hippocampus in male rats. Physiol Pharmacol. 2008;12(1):39–45.

[97] Droste SK, Chandramohan Y, Hill LE, Linthorst ACE, Reul JMHM. Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. Neuroendocrinology. 2007;86(1):26–37.

[98] Timmermans W, Xiong H, Hoogenraad CC, Krugers HJ. Stress and excitatory synapses: from health to disease. Neuroscience. 2013;248:626–36.

[99] De Kloet ER, Joëls M, Holsboer F. Stress and the brain: from adaptation to disease. Nat Rev Neurosci. 2005;6(6):463–75.

[100] Zhang H, Etherington LA, Hafner AS, Belelli D, Coussen F, Delagrange P, et al. Regulation of AMPA receptor surface trafficking and synaptic plasticity by a cognitive enhancer and antidepressant molecule. Mol Psychiatry. 2013;18(4):471–84.

[101] Tse YC, Bagot RC, Hutter JA, Wong AS, Wong TP. Modulation of synaptic plasticity by stress

hormone associates with plastic alteration of synaptic NMDA receptor in the adult hippocampus. PLoS One. 2011;6(11):e27215.
[102] Sun H, Guan L, Zhu Z, Li H. Reduced levels of NR1 and NR2A with depression-like behavior in different brain regions in prenatally stressed juvenile offspring. PLoS One. 2013;8(11):e81775.
[103] Hu H, Real E, Takamiya K, Kang M-G, Ledoux J, Huganir RL, et al. Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. Cell. 2007;131(1):160–73.
[104] Whitehead G, Jo J, Hogg EL, Piers T, Kim D-H, Seaton G, et al. Acute stress causes rapid synaptic insertion of Ca2+-permeable AMPA receptors to facilitate long-term potentiation in the hippocampus. Brain. 2013;136(12):3753–65.
[105] Mabandla M V, Kellaway LA, Daniels WMU, Russell VA. Effect of exercise on dopamine neuron survival in prenatally stressed rats. Metab Brain Dis. 2009;24(4):525–39.
[106] Wosiski-Kuhn M, Stranahan AM. Transient increases in dendritic spine density contribute to dentate gyrus long-term potentiation. Synapse. 2012;66(7):661–4.
[107] Makena N, Bugarith K, Russell VA. Maternal separation enhances object location memory and prevents exercise-induced MAPK/ERK signalling in adult Sprague–Dawley rats. Metab Brain Dis. 2012;27(3):377–85.
[108] Dimatelis JJ, Stein DJ, Russell VA. Chronic exposure to light reverses the effect of maternal separation on proteins in the prefrontal cortex. J Mol Neurosci. 2013;51(3):835–43.
[109] Faure J, Uys JDK, Marais L, Stein DJ, Daniels WMU. Early maternal separation alters the response to traumatization: resulting in increased levels of hippocampal neurotrophic factors. Metab Brain Dis. 2007;22(2):183–95.
[110] 110. Marais L, Van Rensburg SJ, Van Zyl JM, Stein DJ, Daniels WMU. Maternal separation of rat pups increases the risk of developing depressive-like behavior after subsequent chronic stress by altering corticosterone and neurotrophin levels in the hippocampus. Neurosci Res. 2008;61(1):106–12.