Swimming Training Modifies Stress-induced Changes in the Hippocampal Morphology and Spatial Memory in Rats

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Abstract

This study assessed the effect of swimming training on morphology (Glial fibrillary acidic protein (GFAP), dark cells and thickness of CA1 and DG) of the hippocampus and spatial memory performance in young male rats exposed to chronic stress. Adult male wistar rats were randomly assigned to five groups: swimming training (ST); exposure to chronic mild stress (CS); exposure to chronic mild stress followed by swimming training (CS + ST); exposure to chronic mild stress followed by a recovery period (CS + recovery); control group with no exercise or stress intervention. Spatial memory was measured by Morris Water Maze (MWM). **Results:** The lowest GFAP and number of dark cells, the highest thickness of CA1, DG, and spatial memory performance were observed in the ST group. In the CS + ST group, swimming training reduced GFAP and the number of dark cells, increased thickness of CA1 and DG, and memory performance, compared to control and CS + Recovery. In the CS group, GFAP and the number of dark cells were highest and thickness of CA1 and DG were lowest among the study groups. In the CS + Recovery group, GFAP, and the number of dark cells were lower, compared to the CS group. **Conclusion:** swimming training could attenuate astrogliosis and the number of dark cells in both stressed and non-stressed rats and its effect on non-stressed rats was more prominent than in stressed rats. Among young male rats, swimming training could improve stress-induced spatial memory performance and hippocampal morphology.

Introduction

Stress understood as the disbalance between environmental-external and internal demands and expectations, compared to the personal and subjective resources to cope with such demands and expectations (Lazarus and Folkman 1984). Repeated and unpredictable stress is associated with unfavorable psychological and physical health issues (Panossian et al. 2021). Physiological responses are not uniform, but depend on the type (physical, emotional, cognitive), the duration (acute vs. chronic), and the individual coping strategies (Chen et al. 2020). When environmental, physical and psychological stressors exceed individual coping strategies at middle and long term, then physiological homeostasis is impaired. As a results, increased production or inadequate removal of free radicals may lead to irreversible cellular degradation (Rudland et al. 2020), which in turn can impair neural morphology and function .

In the case of chronic psychological stress, upregulation of cytokine secretion affects the activity of glucocorticoid receptors and inhibit the activity of cortisol anti-inflammatory pathways. In this condition, sympathetic system hyperactivity leads to an increase in inflammatory mediators such as interleukin-1, and alpha-induced tumor necrosis factor (Donzis and Tronson 2014; Li et al. 2018), in general, and the increase of interleukin-1 in the hippocampus (Donzis and Tronson 2014), tumor necrosis factor-alpha, corticosterone, and corticotropin (Gross and Pinhasov 2016; Lv et al. 2019), in specific. Importantly, higher cortisol and inflammatory concentrations appear to be associated with smaller hippocampal volume (Sudheimer et al. 2014). As such, stress may affect the hippocampus through the hyperactivity of hypothalamic-pituitary-adrenocortical (HPA) axis. The key product of the HPA axis is cortisol (Gold et al.

2015; Bernatova et al. 2018). Thus, altering HPA axis activity is associated with cortisol fluctuations (Jurueña et al. 2020), and high levels of glucocorticoid appear to be causally associated with atrophic changes in the hippocampus region (Segev et al. 2014; Shen et al. 2017).

The hippocampus is the main neuronal structure for spatial memory (Benatti et al. 2019; Wellman and Moench 2019). Long-term spatial memory is defined as stable changes of spatial information processing over time. Such information processing consists of encoding, consolidation, storage and retrieval of specific spatial information (Voss et al. 2019). Stress can be affective on memory through affecting endocrine system (Wolf et al. 2016) and various regions of the hippocampus (Schoenfeld et al. 2017).

While both antidepressants (Zou et al. 2020), and physical activity (Jang et al. 2018) appear to favorably impact on the glucocorticoids secretion and sensitivity of their receptors (Jang et al. 2018), here, we focus on the impact of physical activity. More specifically, long-term exercise has the potential to favorably impact on the stress response in humans (Koc 2018). The beneficial effects of physical activity on neurophysiological levels could be summarized as follows: Physical activity can induce structural and functional plasticity in the hippocampus (Sah et al. 2017), instigate neurogenesis (Vivar and van Praag 2017), increase the volume of the hippocampus (Feter et al. 2018; Tang et al. 2019), favor angiogenesis and neuronal plasticity (Sah et al. 2017), and improve memory performance in rats (Jahangiri et al. 2019; Amirazodi et al. 2020; Park et al. 2020; Gokdemir et al. 2020). Physical exercise appeared to favorably impact on adult hippocampal neurogenesis, such to possibly counterbalance cognitive decline. Further, in rats, physical exercise could enhance long term potentiation (LTP) in the dentate gyrus (DG), increase spatial and related forms of learning and memory (Jahangiri et al. 2019; Amirazodi et al. 2020; Park et al. 2020; Gokdemir et al. 2020) and improve memory deficits and learning (Fu et al. 2017). Exercise may increase the expression of Glial fibrillary acidic protein (GFAP) in astrocytes (Saur et al. 2014) and alter the morphology of astrocytes via the reduction of the ramification and the length of astrocytes (Loprinzi 2019; Belaya et al. 2020). Thus, as the possible mechanisms related to the effect of exercise on memory, they were investigated in the present study. Further, we note that various types of exercising may induce different effects on neural and memory adaptations (Lin et al. 2012).

Swimming is one of the exercises associated with the following observations: 1. Increased secretion of the endorphins from the brain (references); 2. Increase of the feeling of pleasure (Nonato et al. 2016); 3. Secretion of antioxidant enzymes (Nonato et al. 2016); 4. Neurogenesis in the hippocampus (Snyder et al. 2009); 5. Decreased corticosterone levels, and 6. Improved cognitive behaviors in stress induced rats (Sigwalt et al. 2011). However, the effect of swimming as a rehabilitative intervention, and on the morphology and function of the hippocampus in previously induced stress condition in human or animals is not clear yet. Given this background, the following research question was answered: Does swimming training at constant intensity affect both the morphology of the hippocampus and the long-term memory performance in young male rats exposed to chronic stress?

Method

Animals and procedures

In this experimental study, a total of 60 three-month-old male Wistar rats were used. After provision of animals from the related center, they were kept under normal light conditions (12h light/dark cycle) in laboratory cages at a temperature of $25 \pm 2^\circ \text{C}$, in order to comply with the test conditions. Water and food (consisting of a total of 16.6 kJ/g: carbohydrate 66.40%, fat 10.60% and protein 23%) were freely available. The number of animals in each group was determined according to the statistical test and the number of groups [18]; 6 rats were assigned to each group. After one week of maintenance in the laboratory, the rats were divided into the following five study conditions:

No exercise and no stress exposure (CON); Exposing to chronic stress followed by swimming training (CS + ST); Exposing to chronic stress (CS); Swimming training (ST); Exposing to chronic stress followed by a period of recovery time with neither training nor stress (CS + R).

The treatment for each group is summarized as follows:

CON: no treatment for 10 weeks; CS + ST: First: 3 weeks of stress; second, followed by 2 weeks of adaptation with water and swimming; third, followed by 4 weeks of swimming; ST: First: 2 weeks of adaptation with water and swimming, second, followed by 4 weeks of swimming; CS: 3 weeks of stress; CS + R: First, 3 weeks of stress; second, followed by 6 weeks of no further intervention.

For measuring memory, 24 hours after each intervention, Morris Water Maze (MWM) test was performed. Finally, six rats in each group received cardiac perfusion after anesthesia with ketamine xylazine. Then, their brains were removed and fixed in 10% formalin; after 24 hours, the tissues were sent to the laboratory for the measurement of astrogliosis, dark cells, thickness of CA1 and DG region of the hippocampus. To control the possible effect of age on the studied variables, the intervention schedule was adjusted so that all rats were euthanized at the same age and time frame. The summary of the study protocol is presented in Fig. 1.

Swimming training

Animals in the swimming groups performed 60 minutes of swimming per day, for 5 days a week, and for 4 consecutive weeks (Liu et al. 2013).

For controlling the effect of environment, sedentary (non-exercise) rats were placed in shallow water for 30 minutes a day in water at temperature of 31°C and were under controlled condition (Gobatto et al. 2001). To reduce stress without promoting physical training adaptations, all rats were adapted to water before initiating the main swimming program. This adaptation consisted of two parts. The first part focused on adaptation to water, which was performed for 4 days. On the first day, the animals were placed for 5 min into the swimming pool with shallow water in which they could stand. At the second day of adaptation, rats were placed 5 min in head-high water in which they could start swimming. At the third day, the water was deep enough, so that they had to swim for 5 min. At the fourth and last day of adaptation, the animals had to swim for 15 min. The water temperature was maintained at 31°C (Sigwalt

et al. 2011). The second part of the protocol focused on rat adaptation to the swimming exercise which performed for 6 days, and began with 15 min of swimming. The second, third, fourth, fifth and sixth days of adaptation consisted of 24, 33, 51 and 60 min of swimming exercise respectively(Liu et al. 2013).

CMS Protocol

Rats were exposed to mild chronic stress for 21 days, included 2 hours of paired caging, 18 hours of free access to food followed by 1.5 hours of restricted access to food (0.2 g pellet), 18 hours of water deprivation followed by 1.5 hours of empty bottle exposure, 21 hours of wet cage (300 ml of water added per 100 g of bedding), 36 hours of continuous exposure to light and 3 hours of 45° cage tilting. This program of stress was applied for four weeks (Segev et al. 2014) (Table 1).

Table 1
The chronic mild stress (CMS) protocol.

Day/time	0700	0800	1000	1100	1200	1300	1400	1600	1700	1800
Sunday								Fd, 18 h		
Monday	Oi, 36 h		Fr, 15 h							
Tuesday					Ph, 2 h				Wet, 21 h	
Wednesday				Till. 1.5 h						Wd, 18 h
Thursday			Eb. 1.5 h							
Friday						Wd, 21 h				
Saturday		Eb. 1.5 h								
Abbreviations: Eb, exposure to empty bottle; Fd, food deprivation; Fr, food restriction; Oi, overnight illumination; Ph, paired housing; Til, tilted cage; Wd, water deprivation; Wet, wet cage.										

Morris Water Maze (MWM) test

The MWM test, consisting of 3–6 consecutive days of oriented navigation trial and a 60s of probe trial, was performed to evaluate spatial memory as described previously by Morris(D’Hooge and De Deyn 2001) with some modifications. The water maze was a black circular pool (150cm in diameter, 50cm in height) filled with opaque water at 23 ± 1°C and surrounded by various visual cues. Moreover, the tank was divided into 4 equal quadrants (marked respectively as E, S, W and N) with two imaginary perpendicular lines. During the oriented navigation trial, each rat participated in 4 trials per day to learn locating the platform (9cm in diameter) that fixed in the quadrant E and submerged 1.5cm underneath

the surface of water. The animal was gently placed into the pool at the midpoint of the edge of quadrant, changed for each trial, and was allowed a maximum of 120s to locate the platform. Rats failing to find the platform within 120s were guided towards it and remained there for 15s, the same period as was allowed for the successful animals. 24hrs after the last navigation training trial, the platform was removed and each rat was subjected to the probe trial, during which the rats were placed into the tank at the midpoint of the edge of quadrant W and allowed to swim in the tank for 60s. The time spent in the target quadrant (quadrant E) and the time of crossing the location where the platform had been fixed were recorded as a measure of spatial memory. All measurements were recorded by an AHD camera 2-MP, Model No: cp-B20M3, Lens: 3.6 mm-3MP (HIKVISION, Hangzhou, China) (Fig. 2)

Tissue preparation

At the end of the experiments, after the intraperitoneal injection of ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), the animals were perfused transcardially with cold normal saline followed by 4% paraformaldehyde in 0.1 M PBS with pH of 7.4. Just after perfusion, the brain was dissected and immersed in 4% paraformaldehyde for 24 h at 4°C. Subsequently, tissue processing was performed and then, the tissues was embedded in paraffin. 8-µm-thick coronal sections was prepared. The sections were then mounted onto gelatin-subbed slides and used for immunohistochemistry and Nissle staining.

For evaluating the Nissl substance in the cytoplasm of neurons, Cresyl Violet staining was employed. The sections of brain were hydrated with descending grades of alcohol and then, stained with Cresyl Violet. Finally, the tissues were dehydrated in alcohol of ascending concentrations.

Assessment of dark cells

Nissle stained sections was captured by a light microscope and the morphology of the cells was evaluated. The cells with shrunken and hyper basophilic feature with corkscrew-like dendrites was considered as dark cells. The percentage of dark cells per area was reported.

Immunohistochemistry

In order to assess the expression of glial fibrillary acidic protein (GFAP; a protein found in glial cells); the immunohistochemistry was done. Briefly, the brain sections were deparaffinized and incubated with 5% normal goat serum (NGS) and 1% bovine serum albumin (BSA) in PBS for 60 min. Then, rabbit anti-GFAP was applied at 4°C overnight. Subsequently, goat anti-rabbit IgG (FITC) was used for 60 min at RT and the nuclei was stained with DAPI. Immunoreactive cells was analyzed using a fluorescent microscope. The immunopositivity cells percentage per area was calculated by the $(\text{the number of positive cells} \times 100) / \text{the total number of nuclei}$ formula.

Statistical analyses

Shapiro–Wilk test was used to determine the distribution of normality. Leven’s test was performed to ensure the homogeneity of variances. Considering the normality and homogeneity of data, the one-way analysis of variance (ANOVA) test was used to compare the variables in the study groups. For paired

group comparisons, post-hoc analyses were performed with the least significant difference (LSD) test. The level of significance was set at $p < 0.05$. All statistical computations were performed with SPSS® 23.0 (IBM Corporation, Armonk, NY, USA) for Windows®.

Results

Body weight

Regarding the weight of the animals, before and after the treatment weights were compared and it was indicated that the weight did not change significantly in post compared to pretreatment condition in CON (230.33 ± 25.28 vs. 233.67 ± 24.44 gr, $p = 0.341$) and CS + R (284.36 ± 2.32 vs. 279.83 ± 2.31 gr, $p = 0.342$) groups. While, in the CS + ST group (229.50 ± 17.76 vs. 232.03 ± 26.67 , $p = 0.003$) and in CS group (229.50 ± 17.76 vs. 215.95 ± 12.42 , $p = 0.003$) and ST (256.58 ± 13.54 vs. 224.17 ± 7.46 gr, $p = 0.001$) the weight reduced significantly in post compared to pre training.

Gelial fibrillary acidic protein of CA1

GFAP of CA1 was significantly different between the five group conditions: ($F(4, 25) = 11.60$, $p < 0.001$). Post-hoc analyses showed that compared to the CON (9.43 ± 3.18) group, the GFAP of CA1 was significantly longer in the CS (15.83 ± 1.80 ; $p = 0.002$) group, and was significantly shorter in the ST (4.38 ± 0.52 ; $p < 0.01$) groups. Compared to the CS + ST (7.41 ± 4.02) condition, the GFAP of CA1 was significantly longer in the CS (15.83 ± 1.80 ; $p < 0.001$), and in the CS + Recovery (11.89 ± 4.38 ; $p = 0.02$) groups. Compared to the CS condition, the GFAP of CA1 was significantly shorter in the ST (4.38 ± 0.52 ; $p < 0.001$), and in the CS + Recovery (11.89 ± 4.38 ; $p = 0.03$) groups. Compared to the ST condition, the GFAP of CA1 was significantly longer in the CS + Recovery (11.89 ± 4.38 ; $p < 0.001$) group (Fig. 3).

To summarize, as regards the GFAP of CA1 as a measure of morphological change in hippocampus, it was highest in the CS condition, compared to the ST, CON, CS + Recovery and CS + ST conditions. By contrast, GFAP of CA1 was lowest in the ST condition, compared to the CS, CON, CS + Recovery and CS + ST conditions.

Gelial fibrillary acidic protein of DG

GFAP of DG was significantly different between the five group conditions: ($F(4, 25) = 14.98$, $p < 0.001$). Post-hoc analyses showed that compared to the CON (9.90 ± 3.38) group, the GFAP of DG was significantly longer in the CS (21.42 ± 6.43 ; $p < 0.001$) group.

Compared to the CS + ST (7.19 ± 3.83) group, the GFAP of DG was significantly longer in the CS (21.42 ± 6.43 ; $p < 0.001$), and in the CS + Recovery (13.34 ± 2.25 ; $p = 0.01$) groups. Compared to the CS condition, the GFAP of DG was significantly shorter in the ST (6.29 ± 1.54 ; $p < 0.001$), and in the CS + Recovery (13.34 ± 2.25 ; $p < 0.001$) groups. Compared to the ST condition, the GFAP of DG was significantly longer in the CS + Recovery (13.34 ± 2.25 ; $p = 0.004$) group (Fig. 3).

In summary, regarding the GFAP of DG as a measure of morphological change in hippocampus, it was highest in the CS condition, compared to the ST, CON, CS + Recovery and CS + ST conditions. By contrast, GFAP of DG was lowest in the ST condition, compared to the CS, CON, CS + Recovery and CS + ST conditions.

Dark cells of CA1

Dark cells of CA1 was significantly different between the five groups ($F(4, 25) = 68.92, p < 0.001$). Post-hoc analyses showed that compared to the CON (20.55 ± 4.33) group, the dark cells of CA1 was significantly longer in the CS ($51.55 \pm 6.62; p < 0.001$), CS + Recovery ($30.07 \pm 6.69; p < 0.001$) groups, and was significantly shorter in the ST ($9.51 \pm 0.82; p < 0.0001$) group.

Compared to the CS + ST (20.24 ± 2.37) group, the dark cells of CA1 was significantly longer in the CS ($51.55 \pm 6.62; p < 0.001$), and in the CS + Recovery ($34.07 \pm 6.69; p = 0.02$) groups, and was significantly shorter in the ST ($9.51 \pm 0.82; p < 0.001$) group. Compared to the CS group, the dark cells of CA1 was significantly shorter in the ST ($9.51 \pm 0.82; p < 0.001$), and in the CS + Recovery ($34.07 \pm 6.69; p = 0.03$) groups. Compared to the ST group, the dark cells of CA1 was significantly longer in the CS + Recovery ($34.07 \pm 6.69; p < 0.001$) group (Fig. 4).

To summarize, considering the Dark cells of CA1 as a measure of morphological change in hippocampus, it was highest in the CS condition, compared to the ST, CON, CS + Recovery and CS + ST conditions. By contrast, dark cells of CA1 was lowest in the ST condition, compared to the CS, CON, CS + Recovery and CS + ST conditions.

Dark cells of DG

Dark cells of DG were significantly different between the five group conditions: ($F(4, 25) = 128.13, p < 0.001$). Post-hoc analyses showed that compared to the CON (19.15 ± 4.34) group, the dark cells of DG were significantly longer in the CS ($47.71 \pm 4.14; p < 0.001$), CS + Recovery ($27.4 \pm 1.56; p < 0.0001$) groups and was significantly shorter in the ST ($9.21 \pm 2.06; p < 0.001$) group.

Compared to the CS + ST (20.64 ± 2.64) condition, the dark cells of DG were significantly longer in the CS ($47.71 \pm 4.14; p < 0.001$), and in the CS + Recovery ($27.4 \pm 1.56; p = 0.02$) groups, and was significantly shorter in the ST ($9.21 \pm 2.06; p < 0.001$) group. Compared to the CS condition, the dark cells of DG were significantly shorter in the ST ($9.21 \pm 2.06; p < 0.001$), and in the CS + Recovery ($27.4 \pm 1.56; p = 0.03$) groups. Compared to the ST condition, the dark cells of DG were significantly longer in the CS + Recovery ($27.4 \pm 1.56; p < 0.001$) group (Fig. 4).

To summarize, regarding the dark cells of DG as a measure of morphological change in hippocampus, it was highest in the CS condition, compared to the ST, CON, CS + Recovery and CS + ST conditions. By contrast, dark cells of DG were lowest in the ST condition, compared to the CS, CON, CS + Recovery and CS + ST conditions.

Thickness of CA1

Thickness of CA1 was significantly different between the five groups ($F(4, 25) = 382.51, p < 0.001$). Post-hoc analyses showed that compared to the CON (70.14 ± 1.46) group, the thickness of CA1 was significantly shorter in the CS ($30.75 \pm 1.38; p < 0.001$), CS + Recovery ($53.20 \pm 3.09; p < 0.001$) groups, and was significantly longer in the ST ($92.19 \pm 4.82; p < 0.001$) group.

Compared to the CS + ST (72.35 ± 2.18) group, the thickness of CA1 was significantly shorter in the CS ($30.75 \pm 1.38; p < 0.001$), and in the CS + Recovery ($53.20 \pm 3.09; p < 0.001$) groups, and was significantly longer in the ST ($92.19 \pm 4.82; p < 0.001$) group. Compared to the CS group, the thickness of CA1 was significantly longer in the ST ($92.19 \pm 4.82; p < 0.001$), and in the CS + Recovery ($53.20 \pm 3.09; p < 0.001$) groups. Compared to the ST condition, the thickness of CA1 was significantly shorter in the CS + Recovery ($53.20 \pm 3.09; p < 0.001$) group (Fig. 5).

To summarize, regarding the thickness of CA1 as a measure of morphological change in hippocampus, it was highest in the ST condition, compared to the CS, CON, CS + Recovery and CS + ST conditions. By contrast, Thickness of CA1 was lowest in the CS condition, compared to the ST, CON, CS + Recovery and CS + ST conditions.

Thickness of DG

Thickness of DG was significantly different between the five groups ($F(4, 25) = 429.58, p < 0.001$). Post-hoc analyses showed that compared to the CON (68.78 ± 1.21) group, the thickness of DG was significantly shorter in the CS ($34.04 \pm 1.76; p < 0.001$), and CS + Recovery ($52.15 \pm 3.30; p < 0.0001$) groups, and was significantly longer in the ST ($93.78 \pm 3.87; p < 0.001$) group.

Compared to the CS + ST (71.69 ± 2.12) group, the thickness of DG was significantly shorter in the CS ($34.04 \pm 1.76; p < 0.001$), and in the CS + Recovery ($52.15 \pm 3.30; p < 0.001$) groups. And was significantly longer in the ST ($93.78 \pm 3.87; p < 0.001$) group. Compared to the CS group, the thickness of DG was significantly longer in the ST ($93.78 \pm 3.87; p < 0.001$), and in the CS + Recovery ($52.15 \pm 3.30; p < 0.001$) groups. Compared to the ST condition, the thickness of DG was significantly shorter in the CS + Recovery ($52.15 \pm 3.30; p < 0.001$) group (Fig. 5).

In summary, regarding the thickness of DG as a measure of morphological change in hippocampus, it was highest in the ST condition, compared to the CS, CON, CS + Recovery and CS + ST conditions. By contrast, Thickness of DG was lowest in the CS condition, compared to the ST, CON, CS + Recovery and CS + ST conditions.

Percentage of time spent in the target quarter (PSTQ) in Morris Water Maze

PSTQ was significantly different between the five group conditions: ($F(4, 25) = 9.63, p < 0.001$). Post-hoc analyses showed that compared to the CON (43.62 ± 3.91), the PSTQ was significantly shorter in the CS ($35.23 \pm 7.89; p = 0.032$), and was significantly longer in the ST ($53.19 \pm 4.46; p = 0.016$), and in the CS + ST ($55.63 \pm 9.16; p = 0.003$) groups.

Compared to the CS + ST condition, the PSTQ was significantly shorter in the CS (35.23 ± 7.89 ; $p < 0.001$), and in the CS + Recovery (47.97 ± 4.85 ; $p = 0.049$) groups. Compared to the CS condition, the PSTQ was significantly longer in the ST (53.19 ± 4.46 ; $p < 0.001$), and in the CS + Recovery (47.97 ± 4.85 ; $p = 0.016$) groups (Figs. 6 & 8).

To summarize, regarding the PSTQ as a measure of spatial memory, it was highest in the CS + ST condition, compared to the ST, CON, CS + Recovery and CS conditions. By contrast, PSTQ was lowest in the CS condition, compared to the ST, CON, CS + Recovery, CS + ST conditions.

The first time to reach the platform (FTRP) in Morris Water Maze

FTRP was significantly different between the five groups ($F(4, 25) = 3.81$, $p = 0.015$). Post-hoc analyses showed that compared to the CON (5.31 ± 2.7) group, the FTRP was significantly longer in the CS (18.25 ± 15.63 ; $p = 0.005$) group.

Compared to the CS + ST (4.96 ± 1.67) group, the FTRP was significantly longer in the CS (18.25 ± 15.63 ; $p = 0.004$) group. Compared to the CS condition, the FTRP was significantly shorter in the ST (5.28 ± 1.19 ; $p < 0.005$), and in the CS + Recovery (5.22 ± 3.06 ; $p = 0.006$) groups (Figs. 7 and 8).

To summarize, considering the FTRP as a measure of spatial memory, it was highest in the CS condition, compared to the CS + ST, CON, CS + Recovery and ST conditions. By contrast, FTRP was lowest in the CS + ST condition, compared to the ST, CON, CS + Recovery, and CS groups.

Discussion

The purpose of present study was to evaluate the effect of chronic stress and possible preventive effect of swimming training on some morphology of hippocampus indices including astrogliosis, dark cells, and thickness of hippocampus and spatial memory which was measured through GFAP, dark cells, thickness of CA1 and DG and Morris water maze test. In summary, the present study indicated the beneficial effects of swimming training on spatial memory and morphological change in rats under stress and rats not under stress, while the effect of swimming training was more prominent in non-stressed rats. Also, a part of previously published result of the study (Safari et al. 2020) indicated that swimming training decreased corticosterone in non-stressed rats while increased it in stressed rats. Recovery time without any treatment reduced astrogliosis, and dark cells.

Spatial memory

In the Morris water maze test, the decrease in time to reach the platform for the first time and also the increase in percentage of time spent in the quadrant where the platform was located as indication of increase in spatial memory.

The findings of the study revealed that CMS (in the CS group) decreased spatial memory. However, swimming training (in the ST group) and swimming training after a period of CMS (in the CS + ST group) could increase spatial memory, while these increases were smaller than those of the ST group and were similar to the control group in some indices.

The most important area for separating spatial memory patterns in the hippocampus is the DG area. As a result, its damage causes a disturbance in spatial memory(Voss et al. 2019; Ahn and Lee 2014). In DG of the hippocampus, new nerve cells are born in mammals, a process called adult neurogenesis(Sorrells et al. 2018; Boldrini et al. 2018). It has been suggested that this process plays a key role in pattern separation in spatial memory(Clelland et al. 2009). Also, suppression of adult neurogenesis genetic cause confusion in distinguishing between similar concepts and coding in the CA3 region(Niibori et al. 2012). Limited studies have been conducted focusing on the effect of physical activity and exercise on hippocampus and spatial memory patterns in humans. An interventional study with young adults found that 6 weeks of high-intensity exercise, which increased fitness, improved distinction(Déry et al. 2013). Studies using a cross-sectional design also suggest that more physical activity(Bernstein and McNally 2019) and higher fitness(Suwabe et al. 2017) are associated with better distinction in humans. Also, a physical activity session improved object recognition in young adults by altering the functional connection between the hippocampal region (DG and CA region) and the cortical regions involved in recall, which suggests that physical activity can affect functional changes of hippocampus without structural change in adults(Suwabe et al. 2017). However, findings of the present study indicated that increase of spatial memory occurred simultaneously by increase of the thickness and decrease in the number of dark cells and astrogliosis of different areas of the hippocampus.

Studies in rats have shown that voluntary wheel running and forced treadmill training increase neurogenesis in DG throughout life in both sexes(Vivar and van Praag 2017). Physical activity increases their dendritic arborization and cell body area in the hippocampus(Sah et al. 2017). This increase in morphology and number of nerve cells is associated with improved stimulus(Creer et al. 2010) and increased object recognition in mice(Bolz et al. 2015). Overall, these findings suggest that hippocampal neurogenesis can be facilitated by physical activity.

In rodents, physical activity improves hippocampal-dependent spatial memory on the Y maze test (Hatchard et al. 2014). On the other hand, exercise and physical activity can increase memory in the hippocampus by network wiring(Voss et al. 2019). In addition, exercise improves synaptic flexibility in adult-born neurons. In particular, voluntary exercise increases short-term synaptic flexibility from the lateral entorhinal cortex to adult-born neurons(Vivar et al. 2016). Exercise also increases innervation of the entorhinal cortex and areas important for spatial memory and the production of theta rhythms, including adult-born neurons, such as the caudomedial entorhinal cortex, middle septum, and upper internal mammalian nuclei(Vivar et al. 2016). In fact, evidence from animal models and in humans' primary results show that physical activity can cause the firing of nerve cells in the hippocampus is theta circuits(Rendeiro and Rhodes 2018). Exercise can also modify the expression of genes that are necessary for synaptic transmission in the lateral entorhinal cortex(Guerrieri and van Praag 2015). Exercise can

coordinate the activity of nerve groups to enhance memory processes and increase the integration of adult-born neurons into circuits in the hippocampus- entorhinal that would otherwise be damaged by aging(Leal and Yassa 2018). Briefly, swimming training could improve memory by some observed structural changes of hippocampus through possible mentioned mechanisms.

The present study also found that chronic stress reduces spatial memory and the lowest spatial memory was related to the CS group. Chronic stress increases the expression of proinflammatory cytokines in the hippocampus such as TNF, IL-1B and IL6, which play an important role in memory and learning(Donzis and Tronson 2014; Li et al. 2018). Inflammatory cytokines can directly affect neural functions, LTP, the secretion of glutamate, glutamate receptor density and cell signaling pathways associated with learning and memory (Czerniawski et al. 2015). On the other hand, chronic exposure to stress disrupts various stages of hippocampal-dependent memory and also causes structural and functional changes in the hippocampus(Kleen et al. 2006). For example, chronic stress decreases neurogenesis in dentate gyrus and increases atrophy of apical dendrites in the CA3 region of the hippocampus(McEwen 2016). For this reason, it has been shown that chronic stress reduces spatial memory recovery by elevating platform (Torres-Berrio and Nava-Mesa 2019).

Another interesting finding of the present study indicated that swimming training following chronic stress (CS + ST) could increase partly spatial memory compared to the control group, but these increases were less than those of the ST group and were similar to the control group in some indices. Meanwhile, swimming training following CMS could increase spatial memory compared to CON, CS and CS + Recovery. Jahangiri et al (2019) indicated that moderate training improved memory impairments by attenuating the hippocampal cytokine levels and brain oxidative damage(Jahangiri et al. 2019). Numerous studies have reported that regular exercise can have an anti-inflammatory effect and modify the increase in levels of inflammatory cytokines (Chen et al. 2012; Segabinazi et al. 2020). The anti-inflammatory effects of regular exercise are caused by an increase in the anti-inflammatory cytokine IL-10 as well as a decrease in the levels of proinflammatory cytokines such as TNF- α , IL-1 β and IL-6. During exercise, skeletal muscle contraction releases IL-6, which increases the production of anti-inflammatory cytokines, including the interleukin-1 and IL-10 receptor antagonists, and inhibits the production of TNF- α (Gholamnezhad et al. 2014; Jahangiri et al. 2019). Also, Aerobic exercise has been reported to improve memory in humans and rats (Bae and Masaki 2019; Jahangiri et al. 2019; Amirazodi et al. 2020; Park et al. 2020; Gokdemir et al. 2020). Some cellular and molecular mechanisms have been suggested for this change due to aerobic exercise, including increased expression of markers of synaptic flexibility, including increased further expression of synaptic flexibility markers, such as synaptophysin (SYP) and postsynaptic 95-density protein-95 (PSD-95) and growth factors in the hippocampus(Serra et al. 2019). Swimming exercise ameliorates mood disorder and memory impairment by enhancing neurogenesis and serotonin expression and inhibiting apoptosis in social isolation(Park et al. 2020). Also, enhancing the hippocampal nerve density can be an important basis for improving learning and memory(Van Praag et al. 1999) which was approved by the present study findings.

Therefore, swimming could increase memory through several mechanisms such as the expression of synaptic flexibility markers such as synaptophysin (SYP), protein-95 postsynaptic density-95 (PSD-95), growth factors, neurogenesis, serotonin expression and inhibition of apoptosis.

Morphological change in hippocampus: GFAP, dark cells and thickness of hippocampus

The findings of the study revealed that CMS (in the CS group) could increase GFAP in CA1 and DG of hippocampus, dark cells in CA1 and DG of hippocampus and decrease thickness in CA1 and DG of hippocampus. However, swimming training (in the ST group) reduced GFAP in CA1 and DG of hippocampus, dark cells in CA1 and DG of hippocampus and increased thickness in CA1 and DG of hippocampus.

Swimming training after a period of three weeks of CMS (in the CS + ST group) could decrease GFAP in CA1 and DG of hippocampus, dark cells in CA1 and DG of hippocampus and increase thickness in CA1 and DG of hippocampus but these reductions were smaller than those of the ST group and were similar to the control group in some indices. A period of recovery time without any treatment after chronic stress (in the CS + Recovery group) could only reduce GFAP and Dark cells and increase thickness and special memory compared to the CS condition.

Gokdemir et al (2020) reported that exercise in multiple sessions may increase the number of cells in the hippocampus, prefrontal cortex and amygdala (Gokdemir et al. 2020). A study showed that people who performed an exercise program for about 6 weeks had a more stable brain structure than those who did not exercise (Erickson et al. 2014). But another study found that in the group that did no exercise, the hippocampal volume was reduced by about 2 percent, which was also associated with a decrease in BDNF (Wagner et al. 2015). It seems that the beneficial effects of exercise on the hippocampus are due to improved neurogenesis (Yang et al. 2014; Gokdemir et al. 2020), maturation of newborn nerve cells, increased dendritic complexity, increases astrocyte ramification and the length of their processes, a structural arrangement close to neural activity (Saur et al. 2014; Fahimi et al. 2017; Loprinzi 2019) and increased length of dendritic spines (Voss et al. 2013; Chowdhury et al. 2014).

The main goal for exercise in the CNS is the hippocampal region, especially the DG region (Roediger et al. 2021). Dendritic spine density (Zhao et al. 2006) and the rate of neurogenesis increase in this area as a result of exercise (Roediger et al. 2021). Although the exact mechanism of these morphological effects needs to be clarified, it seems that the most important mechanism in this process is to increase the synthesis and availability of neurotrophins, especially BDNF (Nijs et al. 2015). Because of this, astrocytes can play an important role in the production and maintenance of functional synapses (Filous and Silver 2016). In fact, the breakdown of astrocytes in the brain of mice can lead to a decrease in the thickness of DG and glutamate transporters, thus hampering the ability of these cells to remove excess glutamate released in the synaptic environment (Cui et al. 2001). Research has shown that both chronic exercise and increased BDNF expression can lead to increased levels of truncated and non-truncated TrkB receptors (Kim et al. 2015).

Chronic stress simultaneously leads to shortening of branches and a decrease in the number of astrocytes in rodents, while exercise can lead to a significant increase in astrocyte projections (number of bifurcation points and area covered These projections)(Tynan et al. 2013). In addition to the increase in astrocytic projections due to exercise activity, it seems that astrocytic projections are more directed to the DG region of the hippocampus, the region of the hippocampus that has the highest rate of BDNF synthesis and release after exercise activity(Fahimi et al. 2017). However, considering previous studies, significant increase in the total number of astrocytes was observed following prolonged exercise. In addition, it was found that the morphological changes of astrocytes are associated with improved BDNF - TrkB signaling in these cells(Fahimi et al. 2017). However, as has been pointed out by some researchers, important modulators such as animal species, age, and brain structure should be considered in interpreting such results(Viola and Loss 2014).

Another interesting finding of the present study indicated that swimming training following chronic stress (CS + ST) could reduce GFAP and dark cells and increase thickness in CA1 and DG of hippocampus compared to the control group. Preclinical studies have demonstrated that chronic stress causes dendritic atrophy and decreased spine density in pyramidal neurons of the hippocampus and the medial prefrontal cortex (mPFC)(McEwen 2016; Tornese et al. 2019). A study showed that 4 weeks of training increased the volume of the CA and DG in the CMS/Training group compared with those in the CMS/Standard group(Chen et al. 2017). Also, Liang et al (2017) reported that the group that exercised after receiving chronic stress had higher dendritic density than the group that did not exercise after chronic stress(Liang et al. 2019). However, the exact cellular mechanism of these changes has not been clarified yet. But degenerative changes in capillaries seem to be one of the primary causes of stress-induced neurological diseases. Poor capillary function and structure in such conditions can affect oxygen and blood support and lead to decreased metabolism and chronic anemia(Farkas and Luiten 2001). In addition, regular long-term exercise can have a greater effect on physiological activity and brain flexibility than acute exercise(Hopkins et al. 2012).

In the present study, nerve density was examined in six areas of the hippocampus. The results of this study showed that increased neurogenesis and decreased apoptosis occurred in DG, CA1 and CA3 regions in the exercise group compared to the control group. The results were consistent with the results of adolescent(Uysal et al. 2015) and adult rats(Van Praag et al. 1999; Kim et al. 2014; Kim et al. 2015) in previous studies. As a result, it seems that exercise after a period of chronic stress can reduce the destructive changes caused by stress in two ways:1) Increase of blood flow and metabolism in different areas of the hippocampus; 2) Increase of neuronal density in different areas of the hippocampus by increasing neurogenesis and reducing apoptosis

Chronic stress increases astrogliosis(Murrough et al. 2017; Lener et al. 2017), while exercise increases the size of astrocytes(Loprinzi 2019) and can also reduce astrogliosis(Loprinzi 2019) and reactive astrocytes(Belaya et al. 2020) by expressing GFAP. The mechanism of action of GFAP as well as the effective factors in its expression can be as follow.

The expression of glutamine synthetase (GS), which is an essential enzyme for the conversion of glutamate to glutamine by astrocytes, is altered in various neurological conditions such as brain hypoxia and ischemia, epilepsy and chronic neurodegenerative processes. Changes in GS expression in different areas of the brain are not positively correlated with GFAP expression (Rose et al. 2013). Also, the glutamate system is associated with depression caused by chronic stress (Murrough et al. 2017; Lener et al. 2017). Thus, while GS can be a positive indicator of astrocyte function, it is not a good indicator of astrogliosis. GS plays a vital role in the normal growth and function of the brain, and GS deficiency has been reported as a cause of death in both humans (Häberle et al. 2006) and mice (He et al. 2007). The role of reduced expression and function of GS in the pathogenesis of some CNS diseases has also been suggested. Immunohistochemical analysis of spinal cord or retinal and brain tissue reveals upregulation of GFAP and other symptoms of reactive astrocytes and astrogliosis in a wide range of neuropathologies such as stroke, cerebral hemorrhage, acute, sub-acute or chronic infections of the central nervous system, epilepsy, Alzheimer's disease etc... Cytokines, such as transforming interleukin (IL)-6, growth factor (TGF), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), and oncostatin M, trigger astrocyte activation in a rodent brain (Sriram et al. 2004; Hostenbach et al. 2014). In general, the effects of exercise on astrocytes, which include morphological changes, is associated with an increase or suppression in the level of GFAP in animals (Saur et al. 2014; Loprinzi 2019; Belaya et al. 2020; Lundquist et al. 2019).

This difference in GFAP levels can be attributed to astrocyte heterogeneity as well as differences in the type of exercise intervention. Most studies have used voluntary wheel running; In addition, factors such as daily access period, exercise duration, and age of start of intervention vary significantly between studies (Oakley et al. 2006). In response to CNS damage, astrocytes can be transferred to a reactive state, and this astrocyte reaction is associated with altered expression of many genes, increased GFAP expression, and functional and morphological changes (De Strooper and Karran 2016; Dossi et al. 2018). Therefore, it can be concluded that damage to the hippocampus due to chronic stress increases astrogliosis and reactive astrocytes, and exercise can prevent this process and reduce astrogliosis and reactive astrocytes and our results have proven this process. Other possible mechanism of exercise induced changes in morphology of the hippocampus may be related to corticosterone. As, one of our previous research findings revealed that CMS (in the CS group) could increase corticosterone levels in rats. However, swimming training (in the ST group) could decrease the corticosterone levels compared to the CS + ST and CS conditions. Swimming training after a period of three weeks of CMS (in the CS + ST group) could increase corticosterone compared to CON, ST and CS + Recovery. A period of recovery time without any treatment after chronic stress (in the CS + Recovery group) could reduce corticosterone compared to the CS and CS + ST groups (Safari et al. 2020).

A limitation of the present study was not measuring many possible physiological factors such as brain-derived neurotrophic factors (along with its receptor) in the hippocampus which are recommended to be measured in the future studies.

One of the novelty and strength of the present study was that the effect of swimming on morphology of the hippocampus and spatial memory were evaluated simultaneously in stressed and non-stressed

conditions as well as comparing the effect of recovery time without any intervention. Another novelty of this study was selecting the mode of exercise as swimming and indicating the positive effect of swimming on morphology of hippocampus and spatial memory which was partly similar to land-based exercises in previous studies and swimming can be suggested to be used in future studies in this field.

Conclusions

According to MWM and hippocampus morphology findings, the present study shows the beneficial effects of swimming training on spatial memory and morphological change in rats under stress and rats not under stress, while the effect of swimming training was more prominent in non-stressed rats. Swimming training decreased astrogliosis, and dark cells in rats under stress and rats not under stress. Recovery time without any treatment compared to ST was not sufficient to reverse the effect of CMS anxiety behaviors, while reduced astrogliosis, and dark cells.

Declarations

Author Contributions Conceptualization, M.A.S., M.K.J., R.R., H.A., Z.Z., and S.B.; methodology, M.A.S., M.K.J., H.A. and R.R.; software, M.A.S., H.A., and M.K.J.; validation, M.A.S. and Z.Z.; formal analysis, M.A.S.; investigation, M.A.S.; resources, Z.Z., and M.A.S.; data curation, M.A.S., and M.K.J.; writing—M.A.S., M.K.J. and S.B.; writing—review and editing, M.A.S., M.K.J., R.R., H.A., Z.Z. and S.B.; visualization, M.A.S., and M.K.J.; supervision, M.K.J.; and S.B., Consultation: R.R., and H.A.; project administration, M.A.S.; funding acquisition, M.A.S., and S.B. All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest Statement There was no conflict of interest by the authors to declare.

Ethical approval

Statement of ethics The Ethical Committee of the University of Shiraz approved the study (registered number; 2689897; February 15, 2021), which was performed in accordance with the Recommendations of the Laboratory Animal Protection and Helsinki Trea. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Graduate committee board of Shiraz University registered by the number of 2689897 in Irandoc.

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Figures

Figure 1

Schematic view of experimental procedure swimming training (ST); exposure to chronic mild stress (CS); exposure to chronic mild stress followed by swimming training (CS + ST); exposure to chronic mild stress followed by a recovery period (CS + R).

Figure 2

Morris Water Maze test performance.

Figure 3

Glial fibrillary acidic protein(GFAP) of CA1 and DG (present). Data are expressed as the mean $\pm$ S.E.M. * $p < 0.001$, significantly different from the control group. ** $p < 0.001$, significantly different from the CS + ST group. *** $p < 0.001$, significantly different from the CS group. # $p < 0.001$, significantly different from the ST group. ## $p < 0.001$, significantly different from the CS + Recovery group. CS + ST: chronic mild stress followed by swimming training, CS: chronic mild stress, ST: swimming training, CS + Recovery: chronic mild stress followed by recovery time, CON: control.

Figure 4

Dark cell of CA1 and DG (percent). Data are expressed as the mean $\pm$ S.E.M. * $p < 0.001$, significantly different from the control group. ** $p < 0.001$, significantly different from the CS + ST group. *** $p < 0.001$, significantly different from the CS group. # $p < 0.001$, significantly different from the ST group. ## $p < 0.001$, significantly different from the CS + Recovery group. CS + ST: chronic mild stress followed by

swimming training, CS: chronic mild stress, ST: swimming training, CS + Recovery: chronic mild stress followed by recovery time, CON: control.

Figure 5

Thickness of CA1 and DG (present). Data are expressed as the mean ± S.E.M. * p < 0.001, significantly different from the control group. ** p < 0.001, significantly different from the CS + ST group. *** p < 0.001, significantly different from the CS group. # p < 0.001, significantly different from the ST group. ## p < 0.001, significantly different from the CS + Recovery group. CS + ST: chronic mild stress followed by

swimming training, CS: chronic mild stress, ST: swimming training, CS + Recovery: chronic mild stress followed by recovery time, CON: control.

Figure 6

Percentage of time spent in the target quarter (PSTQ) (percent). Data are expressed as the mean $\pm$ S.E.M. * $p < 0.001$ significantly different from the control group. ** $p < 0.001$, significantly different from the CS + ST group. *** $p < 0.001$, significantly different from the CS group. # $p < 0.001$, significantly different from the ST group. ## $p < 0.001$, significantly different from the CS + Recovery group. CS + ST: chronic mild stress followed by swimming training, CS: chronic mild stress, ST: swimming training, CS + Recovery: chronic mild stress followed by recovery time, CON: control.

Figure 7

The first time to reach the platform(FTRP) (second). Data are expressed as the mean $\pm$ S.E.M. * $p < 0.001$ significantly different from the control group. ** $p < 0.001$, significantly different from the CS + ST group. *** $p < 0.001$, significantly different from the CS group. # $p < 0.001$, significantly different from the ST group. ## $p < 0.001$, significantly different from the CS + Recovery group. CS + ST: chronic mild stress followed by swimming training, CS: chronic mild stress, ST: swimming training, CS + Recovery: chronic mild stress followed by recovery time, CON: control.

Figure 8

Schematic images of Morris Water Maze testing. CON: Control; CS + ST: chronic mild stress followed by swimming training; CS: chronic mild stress; ST: swimming training; CS + Recovery: chronic mild stress followed by recovery time, CON: control.