Low-Intensity Exercise Improves Neuroplasticity and Spatial Memory on Young Adult of Male Wistar Rats

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ABSTRACT

Kartinah NT, Faizah A, Ayu AD, Tahyatu B. Low-Intensity Exercise Improves Neuroplasticity and Spatial Memory on Young Adult of Male Wistar Rats. **JEPonline** 2018;21(5):19-28. The purpose of this study was to examine the effect of low-intensity exercise on neuroplasticity and memory performance in young Wistar adult rats. Neuroplasticity was determined by altering the expression of proteins such as synaptophysin, neuroligin-1, AMPAR, NMDAR, and PSD-95 in the rat hippocampus. Six-month-old rats were randomly divided into Sedentary (Sed) and Exercise (Ex) Groups. Exercise rats had better spatial memory compared to sedentary rats. This result was shown by a reduction in both the time travelled and the total error in the Water-E Maze task ($P<0.05$). Immunohistochemistry staining showed that exercise rats had higher optical density scores of both pre- and postsynaptic proteins compared to sedentary rats ($P<0.05$). In conclusion, the findings indicate that the low-intensity exercise improves spatial learning and memory, as shown by increased neuroplasticity and synaptic maturation in young Wistar adult rats.

Key Words: Low-Intensity, Neuroplasticity, Physical Exercise, Spatial Memory and Young Adult

INTRODUCTION

Physical exercise is an effective activity to achieve health and body fitness. Moreover, physical exercise can improve memory function (18) by increasing neuroplasticity in the hippocampus (15). Neuroplasticity occurs by forming new synaptic connections and by synaptic maturation (16). In recent studies, physical exercise was shown to have the potential to increase neuroplasticity by involving some proteins in pre- and postsynaptic membranes in old and young rats (5,13,15). However, there are still quite limited reports about the effects of physical exercise on neuroplasticity in young adult rats. This is an important concern because rats start showing a decrease in cognitive function at a young adult age (12).

The purpose of this study was to determine the effect of exercise on neuroplasticity and memory function in young adult rats. The intensity and duration of exercise need to be considered since both will influence the responses of neuroplasticity and memory function. Shimada et al. (22) showed that low-intensity physical exercise is better than high-intensity exercise in improving spatial memory function after ischemic brain injury. Thus, specifically, this study will investigate whether the effect of 6 wks of low-intensity exercise is enough to enhance the connection and maturation of synapses. One indicator of synapse plasticity is synaptophysin, which is a component of synaptic vesicles (20). This component can be used to assess the maturation of synapses, as well as neuroligin protein (7) and PSD-95 (6). Neuroligin and PSD-95 bonding increase the recruitment of AMPAR and NMDAR into postsynaptic membranes to form LTP for learning and memory functions (10).

METHODS

Animals
Twelve young adult (6-month-old) Wistar male rats with body weights of 150 to 250 gm were used in this study. The rats were housed with a 12-hr light/dark cycle. The room temperature was maintained at $25 \pm 1°C$. Food and water were supplied *ad libitum*. The study design and methods were approved by the ethic committee (Number 849/UN2.F1/ETIK/2016).

Exercise Protocol
All the rats experienced a 5-day acclimation period with an animal treadmill. The speed and duration were increased incrementally with a maximum of 10 m·min^{-1} for 10 min. After resting for 2 days, the rats were randomly divided into two groups: (a) Sedentary Group (Sed, n=6); and (b) Exercise Group (Ex, n=6). The exercised rats were trained on the treadmill at a speed of 20 m·min^{-1} for 30 min, 5 times·wk^{-1} for 6 wks. The rats in the Sed Group remained relatively inactive during the 6-wk period.

Water-E Maze
The spatial memory test was performed using the Water-E Maze (WEM) that consisted of a main trench and three trench arms. The WEM was filled with water until the animal could not touch the bottom of the apparatus. The WEM had a ladder so that the rats could get out of the test device. The starting point was the M point in the middle trench, and the target (G) was a ladder that laid on one edge of the trench.
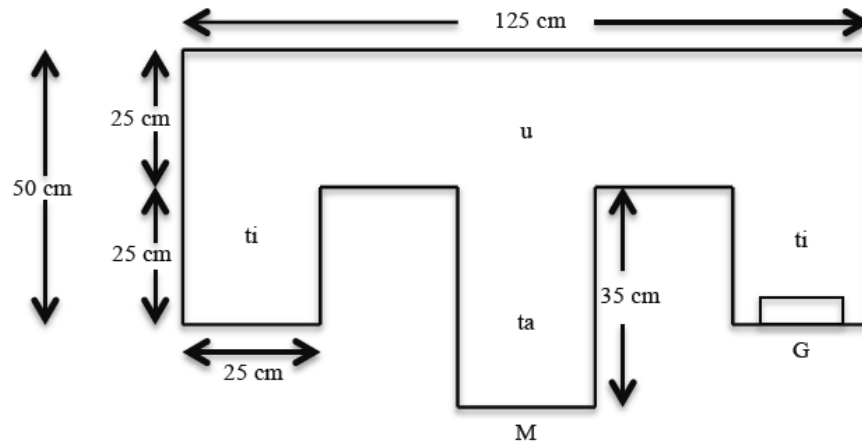
Figure 1. Water-E Maze (9)
u = Main Trench; **ta** = Middle Trench; **ti** = Edgetrench; **M** = Start; **G**= Goal

The acclimation for introducing the animals to the WEM included the placement of the rats in the WEM for no more than 3 min·d^{-1}. The test was performed for all the groups during every week of the exercise period (weeks 0, 2, 4, and 6). Each test consisted of 3 reps for each animal without intervals lasting for no more than 2 min for each repetition. The time travelled and total error to find the ladder were the parameters used to measure the rats' memory function (9).

Immunohistochemistry
Immunohistochemistry was used to identify the levels of pre- and postsynaptic protein expressions. All the rats were decapitated, and the brains were removed immediately. The brains were immersed in 4% paraformaldehyde until they were embedded in paraffin. Paraffin sections (4 μm) of the hippocampus were used for immunohistochemistry. The paraffin sections were washed for 5 min in 0.01 M phosphate-buffered saline (PBS, pH 7.4), and treated with 3% hydrogen peroxide in 0.01 M PBS including 10% methanol. They were washed three times for 5 min, each in 0.01 M PBS followed by 10 min of pre-incubation with buffer (Tris-EDTA pH 9.0), and then washed for 5 min in 0.01 M PBS.

The brain sections were then incubated with the following primary antibodies: anti-syp antibody (1:1200; Abcam), anti-neuroligin-1 antibody (1:750), anti-PSD 95 antibody (1:500; Abcam), anti-NMDAR antibody (1:250; Abcam), and anti-AMPAR antibody (1:1200; Abcam) for 60 to 75 min at room temperature. After a 5-min rinse in 0.01 M PBS, the sections were incubated with biotinylated secondary antibody for 15 min. Then, they were incubated with avidin-biotin peroxidase complex for 15 min at room temperature.

Immunoreactivity was visualized using enzyme substrate kits. Negative control sections were treated in the same way as described above, except that the antibody against anti-antibody was omitted. Then, the sections were counterstained with hematoxylin. For pre- and postsynaptic protein immunostaining, the sections were measured under a light microscope at a magnification of 400x. The protein immunostaining was graded semi quantitatively using the following scoring system: (a) 1, no staining (grade 1); (b) 2, weak (grade 2); (c) 3, moderate (grade 3); and (d) 4, intense (grade 4) in the hippocampal CA1 sector using a computer associated image analyzer software (ImageJ, IHC Profiler Plugin).

The protein expression has been measured by a binocular light microscope (Leica DM1000 LED) on Immunochemistry slide with enlarged 400 times. The images were photographed as five broad fields of view in various target areas of CA1 hippocampus. Each photo was composed of 1920x1080 pixels. Then, the protein expression area was calculated using the Image J program with a profile IHC technique

Statistical Analyses

The behavioral data and the immunostaining data were analyzed with SPSS 21.0 and were expressed as the mean ± standard deviation. The results were considered statistically significant at an alpha level of $P<0.05$. The results were represented graphically with GraphPad Prism. For the WEM task, the time travelled and the total errors were averaged within a group for each test. The time travelled was analyzed using the two-way repeated measures ANOVA test followed by the Bonferroni *post hoc* test, with group as the independent variable, and the total errors were analyzed using the Friedman test followed by Wilcoxon *post hoc* test with group as an independent variable. The pre- and postsynaptic protein data were analyzed using independent *t*-test for each CA1 hippocampus.

RESULTS

Spatial Memory Performance – WEM Task

The time travelled and the total error during the WEM task of the sedentary group and the exercise group are shown in Figures 2 and 3, respectively. All the rats in the Exercise Group showed a reduction in the time travelled between week 0 and week 6 ($P=0.000$; see Figure 2) and a reduction in the total errors to reach the ladder between week 0 and week 4 ($P=0.039$) and week 0 and week 6 ($P=0.042$; see Figure 3).
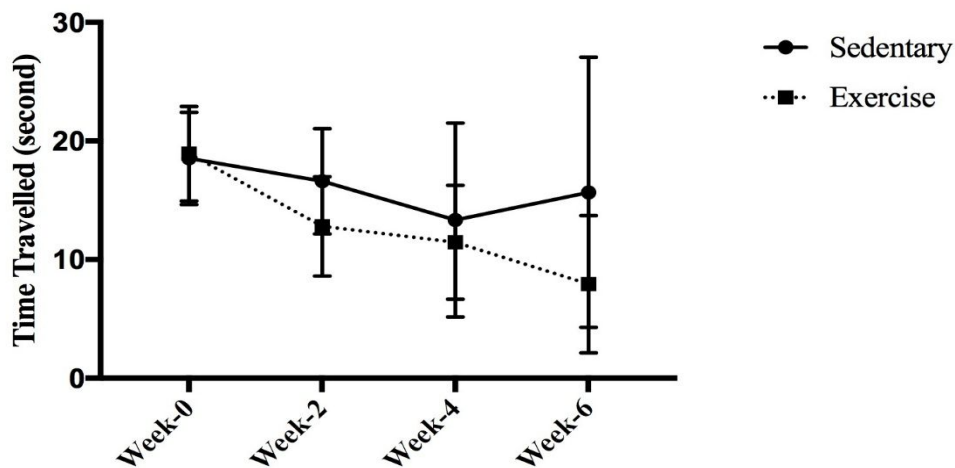

Figure 2. Effects of Exercise on the Average Time Travelled (sec). Each point represents the block mean ± SD of the WEM task. The Exercise Group (n=6) consistently resulted in a faster time travelled compared to the Sedentary Group (n=6), as confirmed by a two-way ANOVA ($P<0.05$).
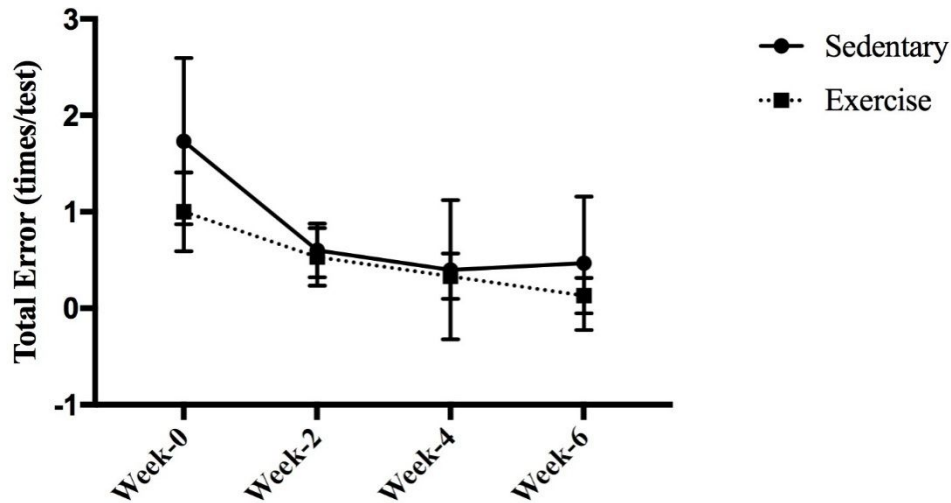
Figure 3. Effects of Exercise on the Total Errors during the WEM Task. Each point represents the block mean ± SD of the WEM task. For the total errors, lower numbers indicate better performance. The Exercise Group (n=6) exhibited better performance than the Sedentary Group (n=6) throughout the experiment, as confirmed by the Friedman test (P<0.05).

Immunochemistry Staining

The results from the WEM task indicated that cognitive improvement occurred during the 6 wks of low-intensity exercise. To determine if altered expression of SYP, NLGN-1, PSD-95, AMPAR, and NMDAR were involved, we carried out immunohistochemistry. As shown in Figure 5, there was a significant inter-group difference in optical density scores of SYP (P=0.000), NLGN-1 (P=0.008), PSD-95 (P=0.001), AMPAR (P=0.005), and NMDAR (P=0.001). The Exercise Group had a higher optical density score than the Sedentary Group, both in pre- and postsynaptic proteins.
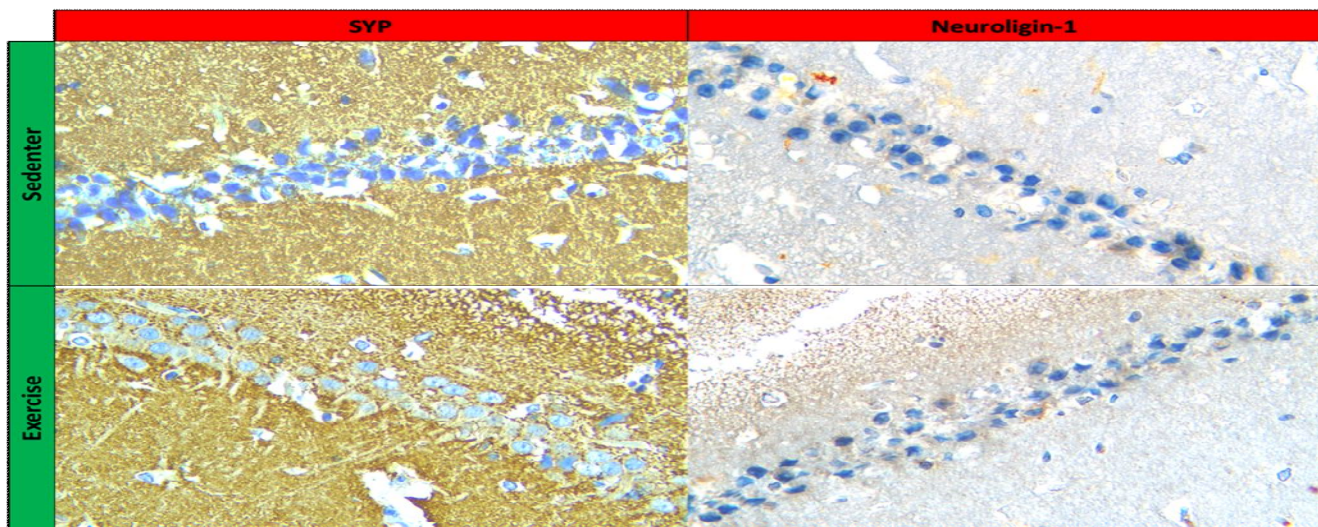

Figure 4. Qualitative Changes of Presynaptic Proteins in the CA Hippocampus in the Sedentary Group and After 6 wks of Exercise in the Exercise Group.
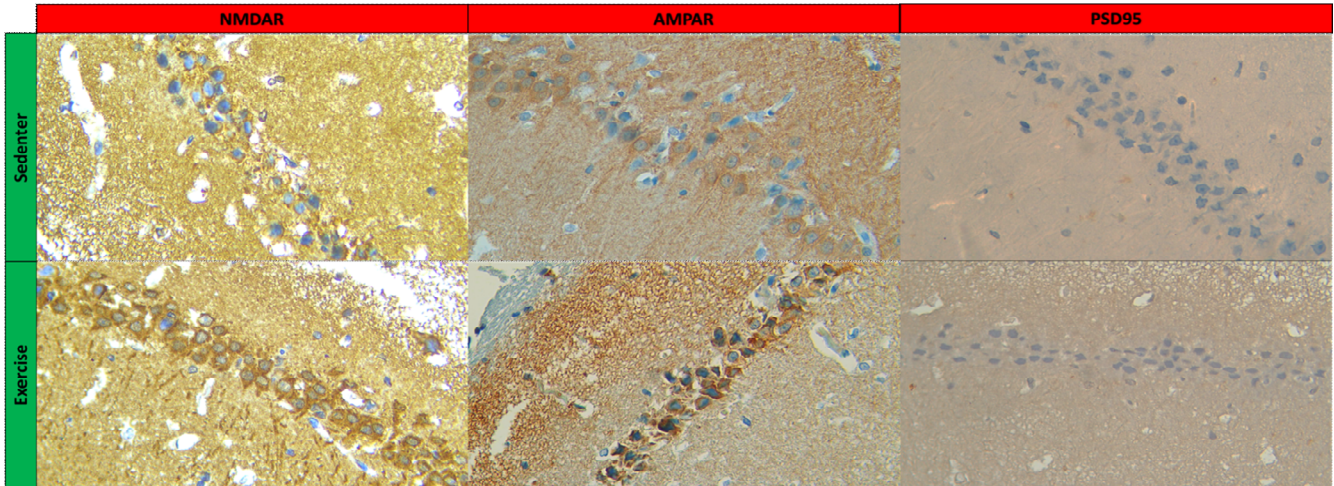
Figure 5. Qualitative Changes of Postsynaptic Proteins in the CA Hippocampus in the Sedentary Group and After 6 wks of Exercise in the Exercise Group.
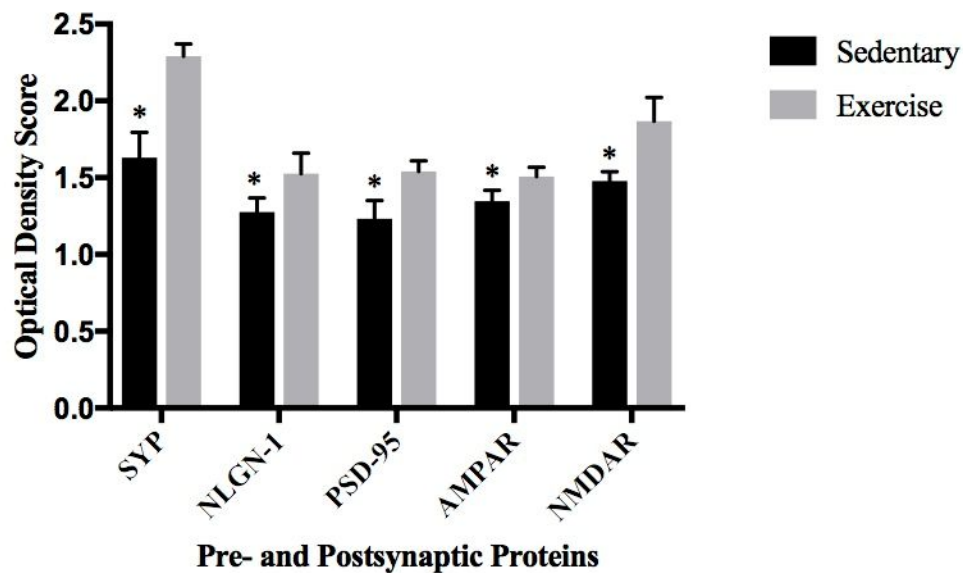

Figure 6. Effects of Exercise on Pre- and Postsynaptic Protein Immunostaining, as Measured by the Mean of the Optical Density Score in the CA1 Hippocampus. The Exercise Group (n=6) consistently showed a higher optical density score compared to the Sedentary Group (n=6), as determined by independent *t*-test (P<0.05).

DISCUSSION

In this study, we report that low-intensity exercise for 30 min is effective in improving spatial memory in rats. These findings are in line with a study conducted by Hosseinzadeh et al. (11), which showed that physical exercise with an intensity of 12 to 18 m·min^{-1} improved

spatial memory. The exercise duration in this study started with 10 min and gradually increased to 58 min for rats aged 3-month-old. However, studies on 6-month-old rats using high-intensity and long-duration exercise showed a decline in memory function. Thus, the administration of exercise intensity and duration is of concern, since both will impact the development of spatial memory. On the other hand, the findings in the present study indicate that low-intensity physical exercise (20 m·min^{-1}) for a duration of 30 min improves spatial memory function in young adult rats. This improvement is shown to be significant, starting at 6 wks.

The improvement of spatial memory function is associated with some structural changes, such as dendritic complexity and density in the DG, CA1, and CA3 hippocampal areas, in addition to an increase in neuroplasticity (1,14,23,24) that is associated with multiple proteins. One protein that plays a role in synaptic plasticity changes is synaptophysin (SYP), a component of glutamate vesicles in the presynaptic area. In the present study, we found that SYP had the highest density score compared with the other proteins. Increases in SYP may indicate morphological changes in synapses, such as the dilation of the active zone area and the spinal dendritic volume as well as the formation of new synapses (20). This finding suggests that light physical exercise with a duration of 30 min in young adult rats changes synaptic plasticity, which is in line with research by Fang et al. (8). They reported an increase in the expression of SYP protein in young adult rats after low-intensity aerobic exercise.

In addition, physical exercise can increase the maturation of synapses. PSD-95 accelerates the maturation of synapses by adding AMPAR to the postsynaptic membrane and by stabilizing the synaptic contact (4). Therefore, PSD-95 is often used as a marker protein for postsynaptic membranes (6). The results of the present study indicate that physical exercise can increase the expression of PSD-95 protein. These results are in agreement with a study by Fang et al. (8) and Shih et al. (23) that used light intensity for 30 min. Also, the Satriani et al. (21) showed that complex physical exercise increased PSD-95 levels in mice (8 to 15 wks old).

Synaptic maturation is also regulated by neuroligin-1, an adhesion protein molecule that plays an important role in regulating synaptic plasticity (2,7). The association between neuroligin-1 and PSD-95 increases the recruitment of AMPA and NMDA receptors (10). In the present study, we found that the increase in neuroligin-1 and PSD 95 was followed by an increase in the protein receptors AMPA and NMDA in postsynaptic membranes. Physical exercise increases NMDAR (15,17,19) and AMPAR. Both AMPAR and NMDAR then bind to glutamate, which is involved in LTP for learning processes and memory function (3).

CONCLUSIONS

Low-intensity exercise duration of 30 min for 6 wks enhanced spatial memory function in young male Wistar rats. This improvement occurred because of changes in neuroplasticity, as shown by increased SYP protein. In addition, there was also an increase in synaptic maturation, as shown by the increase in PSD 95 and neuroligin-1 that improved the recruitment of AMPAR and NMDAR, which play a role in LTP and memory function.

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