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EFFECT OF AEROBIC EXERCISE ON SHORT-AND LONG-TERM MEMORY IN ADULT MALE WISTAR RATS

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ABSTRACT

Objective: Memory is an essential function of cognition in humans, but an age- and disease-related deterioration of this function is common. The currently known treatments have high failure rates, and thus, the slowing down of memory degeneration at an early age is the preferred preventive approach. Exercise, specifically aerobic exercise, has been proven to enhance memory via various pathways, such as neurogenesis, angiogenesis, and growth factor expression. The aim of this study is to assess the effect of aerobic exercise on short-term and long-term memory function in rats.

Methods: Twenty-four male Wistar rats aged 7 mo were randomly distributed into four groups: Control, short-term memory (C-S); Control, long-term memory (C-L); Aerobic, short-term memory (A-S); and Aerobic, long-term memory (A-L). The aerobic groups received exercise treatment for 30 min each five times per week, at a treadmill speed of 20 m/min. The treatment duration was 8 w. Short-term memory was assessed using the forced alteration Y-maze test, and long-term memory was assessed using the object location task.

Results: The findings showed that rats placed under the aerobic exercise regimen had significantly better long-term memory function at the end of 8 w ($p = 0.006$), while no significant difference was observed in short-term memory function between the aerobic exercise group and the control group.

Conclusion: The present study shows that aerobic exercise is beneficial in improving long-term memory function in rats.

Keywords: Aerobic exercise, Short-term memory, Long-term memory

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INTRODUCTION

Memory can be described as the ability to adapt to the ever-changing environment by modifying behavior based on the learning of new information and past experiences. According to its retention time, memory is categorized into short- and long-term memory [1]. Short-term memory has limited capacity and is responsible for temporary holding and processing of newly obtained information or information that was already stored, while long-term memory stores information for a longer period of time [2]. Some short-term memory is converted to long-term memory via a process called memory consolidation. This process is dependent on the hippocampus [3]. Even in adults, the hippocampus retains a high level of neuroplasticity, and it is, therefore, the best-studied structure with regard to neural plasticity [1].

Aging and various neurodegenerative diseases in humans are associated with progressive memory loss from a subtle to a severe degree that may even lead to dementia. The worldwide prevalence of dementia is expected to reach up to 131.5 million in 2050, with the majority of cases occurring in low-and middle-income countries. Currently, there are no treatment options, and clinical drug trials in the last decade have had a 99.6% failure rate. The high failure rate is because intervention/ treatment is usually administered later on in the disease course. Given this context, there has been a shift in the focus of treatment of neurodegenerative diseases to the delaying of dementia in younger people; that is, the focus is now on a preventive approach [4, 5].

Exercise, particularly chronic aerobic exercise, has been shown to have neuroprotective and neurorestorative effects on the brain [6]. It also leads to improvement in executive control and an increase in prefrontal and hippocampal volumes, specifically in the anterior hippocampus. This leads to better spatial memory function and induction of hippocampal plasticity [1, 6–8]. The mechanisms underlying these outcomes are numerous; for example, a decrease in inflammation, a decrease in oxidative stress, insulin/glucose dysregulation, and mitochondrial dysregulation. Exercise also increases the expression of neurotrophic factors, neurogenesis, and angiogenesis, and thus, enhances synaptic plasticity [1, 6]. In Alzheimer disease mice model, aerobic exercise was found to cause a decrease in the expression of amyloid-β and phosphorylated tau, improvement in spatial learning and exploration ability, increase in ATP production in the brain, increase in the number of synapses, and increase in the expression of GLUT1 and GLUT3 in the central nervous system (which enhances energy metabolism in the brain) [9]. In humans with early Alzheimer's disease, aerobic exercise improves memory performance and decreases hippocampal atrophy [5]. Aerobic exercise is a low-cost, low-risk, widely-available intervention, and when compared to pharmaceutical agents, it may even be more effective in improving memory function. Thus, exercise should be considered as a potential method to supplement current practices for the management and prevention of age-related memory impairment [5, 6].

The general consensus is that exercise improves memory. The type, intensity, and duration of exercise determine whether the exercise regimen is beneficial for short-and long-term memory. Studies have shown that moderate exercise improves long-term spatial memory and working memory [10–12]. However, a meta-analysis revealed that regular exercise only had a small and even insignificant, effect on long-term memory [13]. Our previous studies have shown the benefits of an eight-week light-intensity long-duration aerobic exercise regimen in rodents, and thus demonstrated the favorable effects of regular aerobic exercise on short-term memory [14, 15]. However, the effect of the eight-week aerobic exercise regimen on long-term memory was not investigated. Therefore, the aim of the present study is to assess the effect of aerobic exercise on short-term and long-term memory function in rats.

MATERIALS AND METHODS

Animals

The study design and protocol for the treatment and care of animals were approved by the Health Research Ethics Committee of our university (approval no. KET-311/UN2. F1/ETIK/PPM.00.02/2019).

Twenty-four male Wistar rats aged 7 mo were acquired from the Health Research and Development Agency's animal laboratories (BALITBANGKES, Jakarta, Indonesia). The animals were housed in standard cages, were given free access to food and water, and were maintained under a 12/12-h light/dark cycle at a temperature of 23+1°C. Acclimatization was performed for one week before treatment to familiarize the animals with the treadmill, Y-maze, object location task (OLT) box, and other research conditions. The animals were then randomly distributed into four groups: control, short-term memory (C-S); control, long-term memory (C-L); aerobic, short-term memory (A-S); and aerobic, long-term memory (A-L). At the start of the experiment, the animals were tested for short- or long-term memory function, according to the group to which they were assigned. The aerobic exercise treatment lasted for 8 w. During aerobic exercise treatment, animals from the group were removed from their cages. At the end of the treatment, the animals from all the groups were again tested for short- or long-term memory function.

Aerobic exercise

A four-track rat treadmill was used for the aerobic exercise treatment. Exercise treatment was administered in the aerobic exercise groups for five days per week (Monday to Friday) for 8 w. The aerobic exercise regimen consisted of running on the treadmill at a speed of 20 m/min for a duration of 30 min. The treadmill speed was set at 8 m/min for both warming up and cooling down, with a duration of 5 min each, before and after the exercise regimen [14, 16].

Forced alternation Y-maze

Short-term memory function was assessed with the Y-Maze task. The Y-maze consists of a symmetrical three-arm maze in which there is an angle of 120° between any two arms. The maze was made of black wood veneer. Each arm was 40 cm in length, 8 cm in width, and 15 cm in height. To reduce anxiety, the intensity of the light in the room was set to 30±5 lux. Short-term memory function was evaluated using the forced alternation test. Rats were handled from 3 d prior to the test. The test was performed during the dark period and comprised of two phases: the sample trial (T1), which lasted for 5 min, and the second phase or the retrieval trial (T2), which also lasted for 5 min. There was an interval of 30 min between the two phases. During T1, the rat was positioned facing the wall at the end of the start arm and then left to explore two Y-maze arms. The third arm was blocked so as to prevent the entry of the rat into the third arm. After the 30-min interval, the rat was returned to the Y-maze and placed at the same starting position for T2. During T2, the block in the third arm was lifted and the rat could explore all three arms freely. If the rat climbed above the Y-maze wall, it would be immediately returned into the arm it was last in. The parameter assessed in this protocol was the time spent in the novel arm. An arm entry is defined as the entry of all four of the rat's extremities into the arm. Percentage of time spent in the novel arm (%) is defined as the amount of time spent in the novel arm divided by the amount of time spent in all arms during T2, multiplied by 100. Rats that did not enter all three arms in the first minute of T2 were excluded see the top part of **Error! Reference source not found.** [17].

Object location task

Long-term memory function was assessed using the OLT test with an open field box. The box was made of black wood veneer, and it was 100 cm in length, 100 cm in width, and 60 cm in height. Rats were handled from 3 d prior to the protocol. The test was performed during the dark period and consisted of three phases: habituation (H), training (T1), and trial (T2). In the H phase, the rat is placed in the box facing the wall in one corner (hereafter referred to as the "release corner") and is allowed to explore the box freely for 10 min. T1 was performed the next day: two 140-mL glass bottles with a height of 10 cm that was filled with colorful marbles were fixed to the floor at two adjacent non-release corners at a distance of 50 cm from each other. The rat was then placed facing the walls in the release corner, as performed during the H phase, and was allowed to explore the box and the objects freely for 10 min. After an interval of 24 h, T2 was performed using the same two objects, but one of the objects was moved to the other non-release corner so that the objects were fixed to the floor diagonally. The rat was then again placed facing the walls in the release corner, as performed during the H and T1 phases, and was allowed to explore the box and the objects freely for 10 min. The parameter assessed in this protocol was the discrimination index, which is defined as the amount of time spent with the object moved to a novel place divided by the total amount of time spent in exploring both objects multiplied by 100. Time spent with an object is recorded when the rat's nose is pointed toward the object at a maximum distance of 2 cm from it see the bottom part of **Error! Reference source not found.** [18, 19].

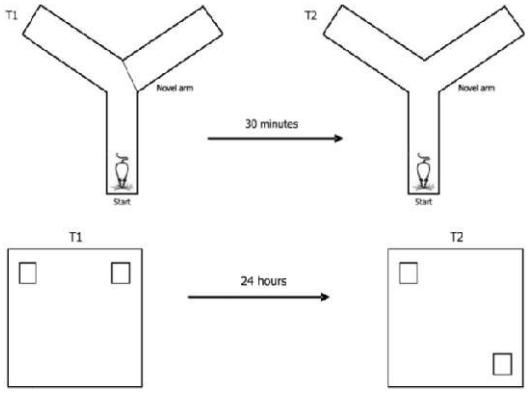

Fig. 1: Top: Forced-alternation test using the Y Maze; Bottom: Object location task (OLT). T1: Sample/training trial; T2: Retrieval trial

Statistical analyses

The normality of the data was assessed before they were statistically analyzed. One-way ANOVA was used to analyze body weight between the groups. An unpaired *t*-test was used to analyze the results of the memory test between the groups, and a paired *t*-test was used to analyze the results of the memory test between different time points.

RESULTS

Bodyweight

The rats' body weight was assessed at the beginning of the study (week 0) and at the end of treatment (week 8). No significant difference in body weight was observed between week 0 ($p = 0.661$) and week 8 ($p = 0.183$) see **Error! Reference source not found.**. The increase in body weight in the aerobic exercise groups was lower than that in the control groups at 8 w, but the difference was not significant.

Short-term memory

Our study showed that there were no significant differences in short-term memory function between the control and aerobic exercise groups at week 0 (p = 0.168) and week 8 (p = 0.715). There were also no significant differences in short-term memory function between week 0 and week 8 in both the control groups (p = 0.491) and the aerobic exercise groups (p = 0.478) see **Error! Reference source not found.**.

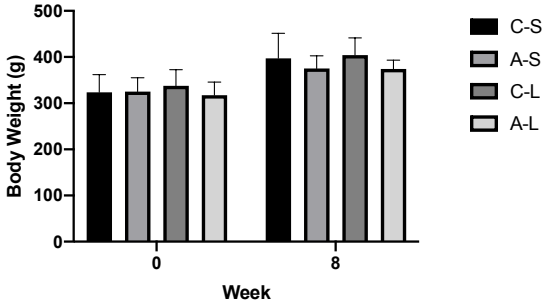

Fig. 2: Bodyweight of rats at the beginning (week 0) and at the end of the study (week 8), C-S = Control, Short-term memory; A-S = Aerobic exercise, Short-term memory; C-L = Control, Long-term memory; A-L = Aerobic exercise, Long-term memory, the mean values of parameters are presented, and the error bars represent standard deviation (determined by one-way ANOVA)

Long-term memory

No significant difference in long-term memory function was observed at week 0 between the control group and the aerobic exercise group (p = 0.961), but a significant difference in long-term memory function was observed at week 8 between the two groups (p = 0.006). No significant difference in long-term memory function between week 0 and week 8 was observed in both the control group (p = 0.478) and the aerobic exercise group (p = 0.208) see **Error! Reference source not found.**.

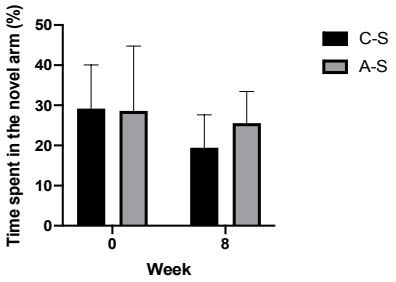

Fig. 3: Short-term memory function of rats at the beginning (week 0) and at the end of the study (week 8). C-S = Control, Short-term memory; A-S = Aerobic exercise, Short-term memory; C-L = Control, Long-term memory; A-L = Aerobic exercise, Long-term memory, the mean values of parameters are presented, and the error bars represent standard deviation. Data between groups were compared with an unpaired *t*-test, and data between time points were compared with a paired *t*-test

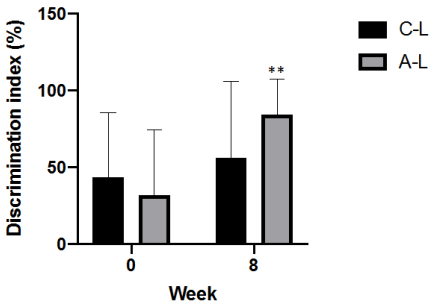

Fig. 4: Long-term memory function of rats at the beginning (week 0) and at the end of the study (week 8). C-S = Control, Short-term memory; A-S = Aerobic exercise, Short-term memory; C-L = Control, Long-term memory; A-L = Aerobic exercise, Long-term memory, **p<0.01 vs. C-L week 8, The mean values of parameters are presented, and the error bars represent standard deviation. Data between groups were compared with an unpaired *t*-test, and data between time points were compared with a paired *t*-test

DISCUSSION

The present study investigates the effect of aerobic exercise on long-term memory to further explore the benefits of long-term regular aerobic exercise on memory function. The exercise regimen used in this study was adopted from a previous study that has compared various exercise regimen and concluded that this particular regimen (a five-day, 30-min running at 20 m/min treadmill speed) showed the best improvement in short-term memory function [20]. The animals used in this study were 7-month-old Wistar male rats, which are considered to be good models for humans in their 20s (the age at which decline in memory function starts to occur) [21].

The findings of this study did not show any improvement in short-term memory function, and in fact, a decrease in short-term memory function was observed in both groups. This was not in line with our previous findings [14, 15]. However, the aerobic group showed a smaller reduction in short-term memory function than the control group, even though the difference was not statistically significant. This might mean that aerobic exercise delays memory impairment via its effect on neurodegenerative processes, which are known first to affect short-term or working memory [22]. Furthermore, other treatments, when combined with aerobic exercise, may have additive beneficial effects and further improve memory function. The other potentially useful treatments include diet modification (low-fat diet) [4, 23], antioxidant supplementation (selenium, astaxanthin) [24, 25], cholesterol-lowering treatment (simvastatin) [26], and enriched environment (such as bigger cage with running wheels and toys) [14-16]. These approaches would lower the levels of toxic radicals, enhance mitochondrial function and synaptic activity, and improve cognitive function [23]. Therefore, the use of such combined treatment regimens should be investigated further.

The process of memory consolidation is dependent on the hippocampus [3]. The hippocampus plays a key role in declarative memory, which involves relational representations. OLT is considered as a simple and effective test for assessing hippocampal-dependent spatial memory. The test relies on a rodent's inherent preference for novel objects without any other external reinforcement. It is believed that this approach can help avoid the complications associated with different emotional responses [18]. Regular aerobic exercise has been shown to improve long-term memory in rodents [11]. This was corroborated in the present study. Aerobic exercise improves memory function via numerous mechanisms, and one of these is an increase in neuroplasticity, particularly in the hippocampus. This effect may be achieved via an

increase in the expression of neurotrophins such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), insulin-like growth factor 1 (IGF-1), fibroblast growth factor 2 (FGF-2), and vascular endothelial growth factor (VEGF). These growth factors improve neuroplasticity by means of neurogenesis, neuronal proliferation, dendritic branching, and angiogenesis [1, 11]. Physical exercise appears to have the most pronounced effect on BDNF. BDNF protects neurons and plays a vital role in neuronal survival, axonal and dendritic expression, neurite growth and remodeling, neural differentiation, and neural plasticity by improving synaptogenesis and synaptic transmission efficacy. BDNF also improves energy metabolism in the brain by reducing food intake, increasing glucose oxidation, lowering blood glucose levels, and increasing insulin sensitivity [8]. Studies have shown that hippocampal BDNF levels are higher in rats exposed to running, which corresponds to spatial memory function [1, 27]. This was also proven by our previous study [15]. Further studies to investigate the downstream mechanism of BDNF on memory function should be conducted.

CONCLUSION

The present findings indicate that rats undergoing the aerobic exercise regimen showed better long-term spatial memory function. This implies that aerobic exercise may be beneficial in delaying memory impairment associated with neurodegenerative processes.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

All authors have none to declare.

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