Effect of complex aerobic physical exercise on PSD-95 in the hippocampus and on cognitive function in juvenile mice

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Abstract. Increased neuroplasticity induced by complex aerobic physical exercise is associated with improved cognitive function in adult mice. Increased cognitive function is assumed to be based on increased synapse formation. One of the regions of the brain that is important in cognitive function is the hippocampus, which plays a role in memory formation. Post synaptic density-95 (PSD-95) is an adhesion protein of the post-synaptic density scaffolding that is essential to synaptic stabilization. As we age, the PSD-95 molecule matures the synapses needed for the formation of the basic circuitry of the nervous system in the brain. However, during the growth period, synapse elimination is higher than its formation. This study aims to determine whether complex aerobic exercise can improve cognitive function and PSD-95 levels in the hippocampus of juvenile mice during their growth stage. The mice performed complex aerobic exercise starting at five weeks of age and continuing for seven weeks with a gradual increase of 8 m/min. At eight weeks it was increased to 10 m/min. The exercise was done for five days of each week. The subjects of the study were tested for cognition one week before being sacrificed (at 12 weeks). The PSD-95 in the hippocampus was measured with ELISA. The results showed that there was a significant difference in cognitive function, where p < 0.05, between the group that was given complex aerobic exercise and a control group that did not. However, the PSD-95 levels did not differ significantly between the two groups. The results of this study indicate that early complex aerobic exercise can improve cognitive ability in adulthood but does not increase the levels of PSD-95 in adults.

1. Introduction
PSD-95 is a protein molecule in the post-synapse that is important for maturation and for synaptic stabilization. The PSD-95 is known as a major scaffold molecule located at the post-synaptic density of the excitatory glutamatergic synapse [1], although the GABAergic synapse is not associated with PSD [2]. PSD-95 interacts with the NMDA (N-methyl-D-aspartate) receptors and a number of signal proteins that regulate the dendritic spine and the synaptic densities that play a role in the LTP (long term potentiation) [3] process in the learning and memory process. The PSD-95 molecule affects retention and moderates the various synaptic proteins that govern the structure and strength of the synapse [4]. The main role of PSD-95 is to accelerate the maturation of the synapses by increasing the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors. These receptors are found in many glutamatergic excitatory synapses [5]. AMPA receptors (AMPARs) have a relatively low affinity for glutamate [6] so, for effective activation, the AMPARs need to be located or positioned near or directly opposite the pre-synapse side of the glutamate release [5]. The retention of AMPARs on the post-synaptic membrane is triggered by the PSD-95 binding to stargazin which is a
transmembrane protein (TARPs) [7]. The localization and retention of AMPA in the correct position facilitates communication between synapses (pre-post synapses) to induce a long term potentiation process.

That PSD-95 plays a role in synaptic stabilization is also evident in the elimination of synapses [8]. As is known, the period of growth in the juvenile is a time of fairly high synapse elimination compared to the more stable adult period. This growth period is a time when a period of new spine formation is followed by the elimination of more spines [9]. The process of synapse formation and elimination is a competitive process for the selection of persistent synapses and for providing the basic circuits of the central nervous system (the brain) in adulthood. The process of synapse formation and elimination is known as the process of neuroplasticity, which is the process of brain adaptation to experience or environmental stimulus. Increased neuroplasticity is associated with improved cognitive function. One of the stimuli that improve neuroplasticity is aerobic physical exercise. Complex aerobic exercise in middle adult mice has been shown to improve neuroplasia, angiogenesis, and cognitive function as compared to simple aerobic exercise [10]. Increased cognition must be based on synapse formation. Research has shown that increased PSD-95 also improves cognitive function in the form of increased spatial memory in adult age animals [11]. However, during the growth stage, a greater elimination of synapses occurs relative to adulthood. This raises the question of whether the effect of aerobic physical exercise improves cognitive ability and PSD-95 levels in the hippocampus of mice during the growth stage. This study shows that complex aerobic exercise performed early does not increase PSD-95 levels but that it does have an effect in the form of better cognitive ability in adulthood.

2. Materials and Methods

2.1. Animal Experiments

The subjects of this study were male *Mus musculus* mice, strain Swiss Webster, aged 4 weeks old with 4 samples in each group. One group of mice experienced the physical aerobic exercise treatment from 5–12 weeks (AK 5–12), and the other group was the control, tested at 12 weeks (K12). Mice were selected as the subjects for the experiment because mice are more active than rats. The health of the mice was maintained both before and during treatment so they did not become sick. The mice were fed standard food with water ad libitum. The cages were kept clean and were set to 12 hours of light and 12 hours of darkness. The ambient temperature was maintained at 23 ± 1 °C. Other matters in the experiment were adapted to the code of ethics of the commission for the handling and use of experimental animals. The study passed the ethical review of the Medical Research Ethics Committee of the Medical Faculty of UI with number: 866/UN2.F1/ETIK/2015.

2.2. Complex Aerobic Physical Exercise Procedures

Prior to the first treatment with complex aerobic exercise, the treadmill tool was used for 5 days, *v* = 5 m/min for 5 minutes per day. The AK group exercised physically by running in the treadmill for 30 minutes a day for 5 days a week. The exercise consisted of a warm up and cool down, each for 5 minutes, (*v* = 5 m/min) and a work out of 20 minutes, with speed: 8 m/min for the first 3 weeks, and 10 m/min for the next 4 weeks [7,10]. The control group was put on the treadmill for 10 minutes every day.

2.3. Cognition Test Procedure with Paired Associative Cognitive Test

This procedure is a modification of the PACT procedure by Irfannuddin [10], during the habituation stage (2 days) the mice were left in the starting room to enjoy a pellet until it ran out. After that, the mice were allowed to explore the area looking for a space containing a bonus pellet. All the doors were opened and every well contained a bonus pellet. This exercise was done twice a day for two days. During the training stage, the mice were left in the starting room with walls that had patterns on them while enjoying the pellet until it ran out. The door to the well that contained the prize pellet was then opened, and the door to the room that did not have a prize in the well was left closed, so the
mouse was guided to the correct well to find the prize. In the next experiment, the door to the room with the pellet in the well had a patterned code and again the door to this space was left open. The mice were expected to learn that if they choose a space that did not match the code in the initial space, they would not find pellets. In the test phase (immediately after the training session) the doors were opened in both directions. The mice were placed in the first room and the patterned walls were closed while they enjoyed the food pellet. After the pellet was finished, the space barriers were lifted and the mice were allowed to explore the entire space. It was expected that the mice might have had a relational memory it would leads them to find the right well just as they had done in the exercise. The performance of the mice was recorded on the basis of the time of the time they took to reach the well containing the prize and the number of times that they found the well containing the bonus pellet on their first attempt. The training and test phase was done with only one of the four potential images in the starting room. The test was conducted 5 times a day with each research subject.

2.4. Elisa Inspection Procedure
At the end of the treatment period, the study subjects were sacrificed through dislocation of the thoracic cervical bone. The hippocampus tissue was taken from the brain as soon as possible. The tissue was homogenized and the ELISA assay run for the PSD-95 or large Homolog 4 (DLG4) according to the SEG168Mu mouse manual from Cloud-Clone Corp.

3. Results and Discussion
3.1 Results
This study measured the cognition ability with the PACT tool. The results from the measurement of cognitive ability with the latent time parameter (sec) were as follows: the effect of the complex aerobic physical exercise from an early age (5 weeks) did not increase the cognitive ability compared to the control group at age 12 weeks (Figure 1) and did not increase the levels of PSD-95 (Figure 2).

![Figure 1](image-url) Results of measurement of cognitive ability with the Paired Associative Cognitive Test. A. Latent time to find the space containing food gift for group K12 and AK 5-12, T test results independent test days 1 and 5, respectively, p = 0.155 and *p = 0.017 (p < 0.05 ). B. Proportion that found the correct space on their first attempt in the K12 and AK 5-12 groups, T test result independent of 1st test day p = 0.058 and Mann Whitney test result on 5th day *p = 0.044 (p < 0.05).
Figure 2. Comparison of PSD-95 levels in groups K12, and AK 5-12. Although lower than the control, statistical analysis showed that there was no significant difference in PSD-95 levels between the two groups p = 0.249 (p > 0.05).

3.2 Discussion

The process of neuroplasticity is measured by a quantitative measurement of PSD-95 levels in the hippocampus. PSD-95 is a major scaffold molecule located at PSD synapse glutamatergic excitator1 as a regulator of various pre- and post-synapse molecules required for synaptic contact stabilization [4]. The results showed that there was no increase in PSD-95 level in the AK 5-12 group of compared to the control (K12). Early complex aerobic exercise had no significant effect on the PSD-95 levels. This can be explained by the synthetic elimination process that is very dominant at this stage of development [12]. Differences in PSD-95 levels that are stimulated by early complex aerobic exercises are consistent with another study done in a complex environment with rats [13]. In that study, rats were placed in a complex environment for 3 months beginning at the age of 21 days (post weaning) and at 4 months they showed a difference in synapse density. Adult rats showed a greater increase in synaptic density than juvenile rats, suggesting a decrease in synapse density in the young animals [13]. Infant mice that were given tactile stimuli with a small brush for 15 minutes, three times per day for 10 days showed a decrease in synaptic density and this was still evident in adulthood [14]. Despite the decrease in synapse density, the early tactile stimulation improved the motor skills and the spatial navigation during adulthood [15].

Although they were not significantly different, the PSD-95 levels were higher in the control group compared with the AK 5–12 treatment group. This data indirectly supports the results of research by Yang, Pan and Gan [9], which states that motor training and new sensory experiences can increase existing or spinalized elimination of spine or filopodia for more than two days in the motor cortex or barrel cortex. However, this spinal elimination is followed by spine formation. The formation of spine and spinal elimination is indicated to stabilize the spine that retains the memory throughout life [9]. It can be assumed that if the filopodia, which are the foundation of the spine, have been eliminated, the PSD-95 that plays a role in the maturation of the spine will no longer be needed and may be eliminated. In the developmental period, the majorities of new protrusions (86–90%) are temporary and do not produce stable spine dendritic forms [16]. In addition, high levels of PSD-95 are still found in the synapses for a short while before being eliminated [8]. The control group K12 tended to have higher PSD-95 levels than the treatment group, which may show that in the growth period, the synapses are normally formed and are sufficiently mature but that there is no memory gain from those particular synapses. However, the synapses of the mice in the treatment group were suspected to be strong in relation to memory formation as induced by the complex aerobic exercise. In fact, the activity of the nerve cells can affect the dynamics of the spine on a time scale of a minute to a second [8] and PSD is one factor that determines spinal volume [17]. Because the synapses undergo rapid changes in response to the environment, it is not known how dynamic synapse circuits retain permanent memory [9].
Complex aerobic exercise is thought to trigger the synapse remodeling process and long-term plasticity requires synaptic remodeling through protein synthesis and degradation [18]. The proper turnover of the synapse protein process is essential for maintaining cellular homeostasis and cellular protein quality [19]. This homeostatic control aims to control the balance of excitation and inhibition of synapses. This is because an increase in the ratio of synaptic excitation/inhibition may lead to increased excitability of the nerve cells associated with a susceptibility to seizure activity [20]. PSD-95 seems to influence the balance of the excitation and inhibition of synapses. Another study stated that an increase in the NMDA-PSD-95 receptor complex is found in many epileptic patients [21]. The turnover process is controlled by a system of proteolysis in the plasticity of the nerve cells. During the process of nerve cell development, local proteolysis is very important, including at the time of dendrite pruning [19]. In addition to the dendrites, the degradation of proteins by a system of proteolysis called ubiquitin-proteasome system (UPS) for the growth cones in the axons serves to guide the navigation of the axons [22]. The process of PSD-95 degradation is controlled by various factors. When the expression of a serine protease inhibitor, neuroserpin, is increased it lowers the PSD-95 expression [23]. Neuroserpin molecules are expressed at both the developmental and adult stages [24]. However, increased neuroserpin expression that decreases PSD-95 expression does not interfere with learning and memory [23]. However, there is no known effect of physical exercise on neuroserpin molecules.

In addition to the neuroserpins, calpain proteinolysis molecules also regulate PSD-95 degradation both during development and during adulthood [25]. Calpain, which induces the destabilization of the spinal dendrites, can be caused by increased levels of the stress hormone, CRH (corticotropin releasing hormone) [26]. However, in the event of the activation of excess calpain molecules by the hormone CRH, the impact on the destabilization of the spinal dendrites can result in memory impairment [26]. Physical exercise increases the levels of the stress hormone, corticosterone [27,28] and adolescence is a period of high stress [27]. In this study, complex aerobic exercise performed during the growth stage was thought to induce physiological stress that played a role in the synapse remodeling that is important for learning and memory. In contrast, complex aerobic exercise improves cognitive function and can increase cognitive skill much better than in controls when performed at the growth stage. The results of cognitive tests performed do not give perfect results. The latent decrease in time and the correct proportional improvement on the test did not occur on every test day, especially in the control group, although there was also a pattern of ups and downs in the treatment group chart. This can be caused by many factors such as motivational factors, nutrition, physical exercise (in the treatment group) and social factors.

The complex aerobic exercise encouraged the locomotion abilities of the study subjects. Locomotion is related to the process of development of spatial cognition [29]. Therefore, many studies of aerobic exercise have resulted in improved spatial cognition. Complex aerobic exercise in adolescence is thought to stimulate the ability of executives. This is because the abilities of executives have begun to develop since they were children [30]. The long period of brain development is parallel to the long period of cognitive development [31]. Thus, brain stimulation from adolescence through complex aerobic exercise may facilitate the development of good executive functioning over time. Improved cognition is based on increased neuroplasticity, inevitably followed by increased synapse formation. This raises the question of how functional or stable the synapses in the control group are to form the memory associated with the relational (spatial) memory. In one study, there was no correlation between PSD-95 and global cognition score (r = 0.08, p = 0.68) and PSD-95 levels were found to be high in the study subjects diagnosed with dementia by the Mini–Mental State Examination (MMSE) [32]. In an overexpression study, PSD-95 inhibits LTP, possibly because of signal saturation receptor [33], and an increase of LTD may be the result of an increase in the number of AMPA receptors [34]. Other studies have shown that mice that do not express PSD-95 (knockout mice) with decreased AMPARs actually have a larger LTP increase compared to wild type mice [35].

Some of the new spines containing PSD were less stable for longer periods of time but were still more stable than those with no PSD [36]. This indicates that there are other factors that affect the stability of the spine [36]. However, there has been considerable research showing that aerobic
exercise can improve spatial memory capacity [10,11,37,38]. Quantitative measurements of PSD-95 may not describe adequately whether the spine with PSD-95 is a stable or functional spine able to support memory formation throughout life. This is because, in this study, the pre-synapse marker that supports PSD-95 is not measured as a scaffold molecule that stabilizes the synapse so that it is impossible to conclude how functional the spine or synapse is as formed in both the control group and the treatment group. In addition, neither measured neural cell signaling activity for long term potentiation, so it was not known whether PSD-95 levels in the treatment group had a better synapse transmission effect as compared to the control.

4. Conclusion
Complex aerobic exercise performed on mice from an early age can improve their cognitive function in adulthood, but this is not supported by elevated levels of PSD-95 in adulthood. This study strongly recommends further research on PSD-95 and other pre-synapse markers by means of immunohistochemistry and the measurement of neural cell signal activity in order to discover how it is that the effects of complex aerobic exercise are able to induce more functional synapses during the period of growth.

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