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Research report

Low-speed treadmill running exercise improves memory function after transient middle cerebral artery occlusion in rats

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HIGHLIGHTS

- The effects of exercise on memory function after stroke depend on exercise intensity.
- Low-intensity exercise could improve some types of memory impairment.
- High-intensity exercise improved memory based only on negative reinforcement.
- Both low- and high-intensity exercise reduced the infarct volume.
- Low-intensity exercise promoted hippocampal plasticity linked to memory function.

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ABSTRACT

Physical exercise may enhance the recovery of impaired memory function in stroke rats. However the appropriate conditions of exercise and the mechanisms underlying these beneficial effects are not yet known. Therefore, the purpose of this study was to investigate the effect exercise intensity on memory function after cerebral infarction in rats. The animals were subjected to middle cerebral artery occlusion (MCAO) for 90 min to induce stroke and were randomly assigned to four groups; Low-Ex, High-Ex, Non-Ex and Sham. On the fourth day after surgery, rats in the Low-Ex and High-Ex groups were forced to exercise using a treadmill for 30 min every day for four weeks. Memory functions were examined during the last 5 days of the experiment (27–32 days after MCAO) by three types of tests: an object recognition test, an object location test and a passive avoidance test. After the final memory test, the infarct volume, number of neurons and microtubule-associated protein 2 (MAP2) immunoreactivity in the hippocampus were analyzed by histochemistry. Memory functions in the Low-Ex group were improved in all tests. In the High-Ex group, only the passive avoidance test improved, but not the object recognition or object location tests. Both the Low-Ex and High-Ex groups had reduced infarct volumes. Although the number of neurons in the hippocampal dentate gyrus of the Low-Ex and High-Ex groups was increased, the number for the Low-Ex group increased more than that for the High-Ex group. Moreover hippocampal MAP2 immunoreactivity in the High-Ex group was reduced compared to that in the Low-Ex group. These data suggest that the effects of exercise on memory impairment after cerebral infarction depend on exercise intensity.

1. Introduction

Memory dysfunction is sometimes caused after insults such as stroke, and severely affects functional adaptation [1]. This dysfunction has been observed in animal models of focal brain ischemia [2–4]. In response, it has been reported that physical exercise could enhance the recovery of impaired memory function in stroke rats [5,6]. For example, one previous study reported that treadmill exercise after stroke improved short-term memory by suppressing ischemia-induced apoptotic neuronal cell death in the dentate gyrus [5]. Another study reported that voluntary exercise after

Abbreviations: MCAO, middle cerebral artery occlusion; MAP2, microtubule-associated protein 2; BDNF, brain-derived neurotrophic factor; Low-Ex, low-intensity treadmill exercise; High-Ex, high-intensity treadmill exercise; Non-Ex, non-exercised; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; TTC, 2, 3, 5-triphenyltetrazolium chloride; DG, dentate gyrus; ORT, object recognition test; OLT, object location test; PAT, passive avoidance test.

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stroke improved spatial memory by promoting newborn cell survival in the dentate gyrus [6]. However, these studies are not sufficient to indicate the appropriate exercise conditions or the mechanisms underlying these beneficial effects. If the appropriate conditions are established, our study can more clearly demonstrate the efficacy of exercise for memory impairment after cerebral infarction.

Physical exercise may also enhance memory in humans [7,8] and intact rats [9–11] because it has the beneficial effect of inducing brain plasticity, such as including neurogenesis, dendritic branching, synaptogenesis, long-term potentiation (LTP) and increased levels of trophic factors in hippocampus [12]. These changes in hippocampal plasticity depend on the level of exercise intensity. A previous study indicated that low-intensity exercise enhanced neurogenesis and increased the expression of trophic factors in the dentate gyrus of hippocampus more than high-intensity exercise [13]. On the other hand, another paper reported that over-training enhanced memory function, and the enhanced memory was associated with increased brain-derived neurotrophic factor (BDNF) [14].

Previous studies have investigated the influence of exercise intensity on functional memory enhancement and hippocampal plasticity in intact rats, but little is known about stroke rats. Therefore, the purpose of this study was to investigate the effects of different intensities of treadmill exercise on memory function and plastic changes in the hippocampus after cerebral infarction in rats. We conducted several behavioral tests to characterize the effect of different exercise intensity levels on memory function, as assessed through the histology of the hippocampus.

2. Methods

2.1. Animals and experimental design

Adult male Wistar rats (7 weeks of age, 200–220 g) were used. The animals were housed at 25 °C during a 12 h light/dark cycle, with food and water made available ad libitum throughout the experiments. All procedures were in accordance with the animal care guidelines of Nagoya University Graduate School of Medicine. All efforts were made to minimize suffering and the number of animals used.

The animals were randomly assigned to four experimental groups: (1) low-intensity treadmill exercise following MCAO (Low-Ex, $n = 11$), (2) high-intensity treadmill exercise following MCAO (High-Ex, $n = 8$), (3) without exercise following MCAO (Non-Ex, $n = 10$), and (4) without exercise following no lesion (Sham, $n = 7$).

2.2. Surgery

MCAO surgery was performed using the following method. The animals were anaesthetized with 4% chloral hydrate (10 ml/kg, i.p.). Throughout the surgical procedure, rectal temperature was monitored and maintained at 37 °C using a circulating heating pad. The rats were subjected to a 90-min left MCA occlusion using an intraluminal filament, as previously described [15]. Briefly, the left common carotid artery (CCA), the external carotid artery (ECA) and the internal carotid artery (ICA) were exposed. The distal ECA branch was completely coagulated. The CCA and ECA were then tied off with a white thread. A 4-0 nylon monofilament with a rounded tip was inserted into the left ICA and advanced 20 mm past the carotid bifurcation until a slight resistance was felt, blocking the MCA at its origin. The filament was left in place for 90 min and then withdrawn. For sham occlusion, the left CCA was exposed following general anesthesia similar to the procedure for stroke induction. The ICA was separated at the junction of the left ICA and the left ECA but was not occluded. All of the animals were placed in a warm environment until they had fully recovered from anesthesia.

One day after surgery, the presence of hemiparesis was checked with a neurological deficits test developed by Longa et al. (Low-Ex, High-Ex or Non-Ex) [15]. Rats without clearly sustained hemiparesis (neurological deficit score of 0) were excluded ($n = 0$).

2.3. Treadmill exercise

The animals in the exercise groups (High-Ex and Low-Ex) were forced to run on a motorized treadmill for 30 min a day for 28 consecutive days starting on the fourth day after surgery. The low intensity exercise group ran at a speed of 2 m/min for the first 5 min, 5 m/min for the next 5 min, and 8 m/min for the remaining 20 min. The High-Ex group ran at a speed of 8 m/min for the first 5 min, 11 m/min for the next 5 min, and 22 m/min for the remaining 20 min [5,13]. There was not a progression of speed during these 4 weeks. Animals in the non-exercise groups (Non-Ex and Sham) were left on the treadmill for the same period of time without running.

2.4. Behavioral tests

Memory functions were examined during the last five days of the experiment (28–32 days after MCAO) by three tasks: an object recognition test (ORT), an object location test (OLT) and a passive avoidance test (PAT).

Behavioral paradigms were constructed so that each test had no impact on the other tests. The training trial for ORT was performed 28 days after MCAO, the PAT was 29 days after MCAO, and the OLT was 31 days after MCAO. All procedures were performed in a silent and dim room. The apparatus was cleaned with 70% alcohol before each animal was tested.

2.4.1. Object recognition test and object location test

The animals were assessed for recognition memory by ORT and spatial memory by OLT [16,17]. The experimental apparatus consisted of an open box (40 cm × 40 cm × 40 cm) made of wood with the inside painted grey. The floor was covered with woodchip bedding.

All animals were initially habituated to the apparatus and the test room once a day for 10 min for 2 consecutive days. During the habituation sessions, rats were allowed to freely explore the apparatus in the absence of objects. On the third day, two identical objects (A1 and A2) were placed in separate locations in the box and the rats were allowed to explore the objects for 3 min (training trial). These objects were placed close (approximately 10 cm) to two adjacent corners. Twenty-four hours after the training trial for on the ORT, the rat was placed back in the apparatus and exposed to the familiar object (A3) and a novel object (B) for 3 min (test trial). These objects were different shapes. For the OLT, the rats were returned to the box where one object remained in its original position (familiar location) while the other object was moved to a new position (novel location) during the test trial. For both tests, the time spent exploring each object and the total time spent exploring both objects was recorded. Exploration of an object was defined as pointing the nose to the object at a distance of 1 cm and/or touching it with the nose. Turning around, climbing or sitting on an object was not considered as exploration. The time spent exploring the novel object was expressed as the ratio of the total time spent exploring both objects. Rats with a total exploration time of 10 s on either training or testing were excluded. To avoid the presence of olfactory trails, the woodchips were stirred and the objects were thoroughly cleaned with 70% alcohol between rats. The objects were securely fixed to the floor of the box using tape, so that the animals could not move them around.

2.4.2. Passive avoidance test

The memory of animals was assessed based on negative reinforcement [4]. The experimental apparatus consisted of an illuminated compartment (40 cm × 25 cm × 25 cm) and a dark compartment (20 cm × 15 cm × 25 cm) with electro conductive grids on the floor. All animals were initially habituated to the apparatus and the test room. The rat was allowed to explore both the illuminated and the dark compartment for 5 min. On the training trial (24 h following habituation), the rat was placed in the illuminated compartment. Immediately after the rat entered the dark compartment, an electrical stimulation of 0.3 mA was applied for 5 s. In this way, the rat learned to associate pain with darkness. For 3 days after the training trial, the rat was placed back in the illuminated compartment, and the time to enter the dark compartment was measured (test trial). Animals who failed to enter the dark compartment within 300 s were assigned a maximum test latency score of 300 s.

2.4.3. Open field test

To test the influence of motor paralysis on the accuracy of memory evaluation, locomotor activity was measured using an open-field test [18] before memory assessment (27 days after MCAO).

The apparatus consisted of an open wooden box (80 cm × 80 cm × 40 cm). The area was divided into 25 squares painted on the floor. Each rat was gently placed in the center of the arena and was allowed to explore freely for 5 min. Its behavior was recorded by a video camera mounted above the open field. The number of line crossings was quantified as a measure of locomotor activity.

2.5. Infarct volume

After the final memory test (32 days after MCAO), rats were sacrificed under deep anesthesia followed by staining with 2, 3, 5-triphenyltetrazolium chloride (TTC) to determine the infarct volumes.

All animals were placed under deep anesthesia with 4% chloral hydrate (10 ml/kg, i.p.) and were perfused with 0.9% saline. The brains were carefully removed and then cut into 6 2-mm coronal sections from the frontal apex using a microtome. The flesh brain slices were then immersed in a 2% TTC (Sigma–Aldrich, Tokyo, Japan) solution at 37 °C for 10 min. The TTC-stained sections were scanned into a computer and quantified using Image J software (National Institutes of Health, USA). The ipsilateral non-infarcted areas and the areas contralateral to the occluded side were measured. The total infarct area was multiplied by the thickness of the sections to obtain infarct volume. To minimize the error introduced by edema and