

# Exercise Preconditioning Improves Behavioral Functions following Transient Cerebral Ischemia Induced by 4-Vessel Occlusion (4-VO) in Rats

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## Abstract

**Background:** There are evidence indicating that exercise decreases ischemia/reperfusion injury in rats. Since behavioral deficits are the main outcome in patients after stroke, our study was designed to investigate whether exercise preconditioning improves the acute behavioral functions and also brain inflammatory injury following cerebral ischemia.

**Methods:** Male rats weighing 250–300 g were randomly allocated into five experimental groups. Exercise was performed on a treadmill 30min/day for 3 weeks. Ischemia was induced by 4-vessel occlusion method. Recognition memory was assessed by novel object recognition task (NORT) and step-through passive avoidance task. Sensorimotor function and motor movements were evaluated by adhesive removal test and ledged beam-walking test, respectively. Brain inflammatory injury was evaluated by histological assessment.

**Results:** In NORT, the discrimination ratio was decreased after ischemia ( $P < 0.05$ ) and exercise preconditioning improved it in ischemic animals. In the passive avoidance test, a significant reduction in response latency was observed in the ischemic group. Exercise preconditioning significantly decreased the response latency in the ischemic rats ( $P < 0.001$ ). In the adhesive removal test, latency to touch and remove the sticky labels from forepaw was increased following induction of ischemia (all  $P < 0.001$ ) and exercise preconditioning decreased these indices compared to the ischemic group (all  $P < 0.001$ ). In the ledged beam-walking test, the slip ratio was increased following ischemia ( $P < 0.05$ ). In the ischemia group, marked neuronal injury in hippocampus was observed. These neuropathological changes were attenuated by exercise preconditioning ( $P < 0.001$ ).

**Conclusion:** Our results showed that exercise preconditioning improves behavioral functions and maintains more viable cells in the dorsal hippocampus of the ischemic brain.

**Key words:** Cerebral ischemia, exercise preconditioning, passive avoidance, recognition memory

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## Introduction

Transient cerebral ischemia is a prominent clinical syndrome, causing deficits in spatial learning and memory.<sup>1,2</sup> Unfortunately, as few treatment options are available for each sequela, greater attention must be devoted to stroke prevention.

Human and animal research substantiates that exercise improves neuroprotection and resistance to brain injuries induced by stroke.<sup>3–6</sup> A noticeable improvement in survival and a corresponding reduction in neuronal damage were observed in gerbils engaged in exercise preconditioning before a transient forebrain ischemia.<sup>7</sup> Physical exercise preconditioning also improves microvascular integrity and declines permeability of the blood brain

barrier after stroke.<sup>8</sup> Despite several studies addressing the effect of physical exercise on abnormal brain function, few studies have dealt with the effect of exercise preconditioning on the behavioral deficits induced by global ischemia. Since behavioral deficits are the main outcome in patients after stroke, we investigated the effects of exercise preconditioning on the acute behavioral deficits following global cerebral ischemia.

## Material and Methods

### Animals

Fifty male Wistar rats weighing 250–300 g were kept under standard laboratory conditions with free access to food and water. The room temperature was maintained at 37°C. Animals in each group were housed in the same animal care facility during a 12:12-hr light/dark cycle throughout the study. All experiments were approved by the ethical committee of Rafsanjan University of Medical Sciences. All efforts were made to minimize the pain and stress of the rats.

### Experimental groups

The rats were randomly allocated into the following 5 experimental groups (10 rats per each group). In the control group; there

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was no exercise and ischemia treatment. In the ischemia group, the animals underwent only ischemia by the 4-VO method. In the exercise group, the animals underwent 3 weeks of exercise, the details of which are explained in the following section. In exercise + ischemia group, the animals underwent 3 weeks of exercise before induction of ischemia. The sham operated group was treated similar to the previous group with the exception of electrocoagulation of the vertebral and occlusion of common carotid arteries.

#### Exercise protocol

The exercise was performed in two episodes. In the first week, the animals ran at a speed of 6–9 m/min for 20 min per day to adapt to the running exercise.<sup>9</sup> After that, the rats began formal training on a five-lane treadmill (IITC Life Science, Woodland Hills, USA) at a speed of 18 m/min for 30 min each day. The total running distance was 540 m per day. The formal training was performed over 5 consecutive days per week for 3 weeks.<sup>10</sup> The exercising and non-exercising animals were housed separately in standard cages. To monitor the potential stress induced by treadmill running, body weight was measured every 3 days.<sup>11</sup>

#### Induction of stroke

As described by Pulsinelli and Brierley,<sup>12</sup> transient global cerebral ischemia was induced by 4-VO. Rats were first anesthetized intraperitoneally with ketamine hydrochloride (80 mg/kg) and xylazine (4 mg/kg). Then, the skin was incised on the neck and the connective tissue and muscles were slowly pulled aside. The common carotid arteries were then freed from the surrounding tissues and the vertebral arteries were electrocauterized permanently. A small piece of silk thread was placed around each carotid artery to ease subsequent occlusion. After the surgical processes, incisions were closed, rats were allowed to recover for 24 hr, and food was withheld for 8 hr before inducing ischemia. On the following day, both common carotid arteries were occluded with aneurysm clips for 10 min to induce cerebral ischemia and then the clips were removed for reperfusion. Rectal temperature was maintained at 37°C with a temperature feedback heating pad during the procedure. Rats that had lost their righting reflex, had dilated pupils and were unresponsive to light were used in the experiments.<sup>2,13</sup> To verify the effectiveness of the occlusion method, relative regional cerebral blood flow of the middle cerebral artery (MCA) was monitored with a laser Doppler monitor (Moor instruments, UK) extradurally (1mm posterior to bregma, just lateral to the linea temporalis) in rats which had undergone transient 4-VO occlusion.<sup>14</sup>

#### Histological assessment

7 days after reperfusion, the rats were decapitated and their brains were quickly removed. The brain tissue was frozen on dry ice and cut into a series of 15- $\mu$ m-thick coronal sections with a cryostat (Cryostat, SLEE, Germany). Nine to eleven sections were collected from each sample in a systematic and uniform fashion. The sections were stained with Hematoxylin/Eosin (H & E) and studied under Olympus BH2 microscope. A single blinded investigator collected all data and stereologically analyzed all specimens. The optical fractionator technique was used to estimate the total cell population. Nucleoli were used as counting items. Only a clearly defined nucleus with pale and homogeneous nucleoplasm was counted.

#### Locomotor activity measurement

In order to measure the animals' motor activity, we put each animal in a Plexiglas box (35 cm  $\times$  35 cm  $\times$  35 cm with a black plastic base) and recorded its activity for 30 min with a camera positioned directly above the box. Subsequently the recorded film was analyzed using the Ethovision Software (Noldus, Wageningen, Netherlands).<sup>15,16</sup> For the time-course study, the locomotor activity of the ischemia-treated and sham operated animals was measured for 30 min on the 7<sup>th</sup> day after operation.

#### Novel object recognition task (NORT)

The object recognition task assesses recognition memory based on the natural tendency of animals to preferentially explore novel, rather than familiar, objects.<sup>17</sup> The experimental apparatus was a Plexiglas box (35 cm  $\times$  35 cm  $\times$  35 cm) with a black plastic floor, placed in a dimly illuminated room.<sup>15,16</sup> The objects to be discriminated were square and triangular iron blocks. The behavior of animals was recorded with a camera positioned directly above the box and subsequently analyzed using the Ethovision Software.

The object recognition task was completed in 3 phases with 24 hr intervals in between. During the habituation phase, the rats were allowed to freely explore the box in the absence of objects for 30min. On the training day (T1), each rat was placed in the box with two identical objects and allowed to explore for 5 min. The position and shape of the objects were changed after each animal was tested to prevent an order or side preference affecting the results. All rats were introduced into the box on the same spot, facing the same direction. On the test day (T2), each rat was returned to the box, which contained the familiar object from the training trial, with the position of the object consistent between both trials, and a novel object for 5 min. To eliminate olfactory trails, the box and objects were thoroughly cleaned with 70% ethanol between experiments.<sup>18,19</sup> To prevent use of visual cues, experiments were conducted in a room dimly illuminated by red light (< 1 Lux). In the presence of red light, objects appear invisible to albino rats.<sup>20</sup> The time spent exploring the individual or multiple objects was recorded. Exploration of an object was defined as pointing the nose to the object at a distance  $\leq$  2 cm. Climbing or sitting on an object was not considered as exploration. The object exploration time from the T2 phase is presented as preference index (exploration time of the novel object divided by total exploration time in T2 phase). Rats showing a total exploration time < 10s on either training or testing phase were excluded.<sup>21</sup>

#### Passive avoidance test

Cognitive function was assessed by use of a step-through passive avoidance task<sup>22</sup> on the 7<sup>th</sup> day after ischemia. The apparatus was an automated shuttle-box (Panlab, Spain), divided into an illuminated and a dark compartment of the same size and separated by a sliding door and a stainless rod grid serving as the base.

The adaptation phase was followed by a single trial in which the rats were put into the illuminated compartment and allowed to enter the dark compartment. After 1 hour of adaptation, the rat was placed in the illuminated compartment for the learning trial. The door to the illuminated compartment was closed automatically as soon as the rat stepped into the dark compartment. Then, a single inescapable scrambled foot shock (0.2 mA, 2s) was delivered through the grid base. We repeated the procedure until the latency to enter the dark box was more than 300s.<sup>23–25</sup>

The retention test was measured 24 hr after the learning trial.<sup>25</sup>

Each animal was put in the illuminated compartment and the door opened after 5s. Latency to enter the dark compartment was recorded to a maximum of 300s. No foot-shock was delivered when the retention test was being conducted. Animals that did not enter the dark chamber during the retention test were allotted a latency of 300s.<sup>22</sup>

#### Adhesive removal test

The sensorimotor function was evaluated by adhesive removal test.<sup>26</sup> Rats were tested 24 hr before surgery and at 1, 3, and 7 days after ischemia. In brief, a small adhesive label (1 cm × 1 cm) was attached to the radial surface of the left forepaw and the latency to touch and remove label from the left forepaw (in seconds) was recorded.

#### Ledged beam-walking test

A ledged beam-walking test was performed to evaluate motor function.<sup>27-29</sup> The beam-walking apparatus consists of a beam connected to a platform on one end. The beam is wide at the starting point and tapers gradually to a narrow end near the goal, which makes the task more sensitive. A black box opening toward the beam is installed on the platform. The rats were pre-trained for three days to traverse the beam and were tested 24 hr before surgery and at 1, 3, and 7 days after ischemia. The performance was videotaped and later analyzed by calculating the slip ratio of the impaired hind limb (number of slips/number of total steps).

#### Statistical analysis

The statistical analysis was performed using the SPSS software version 11.5. The result of Kolmogorov-Smirnov test confirmed the normality of the data in all groups (all  $P > 0.05$ ). All data are expressed as mean ± SEM, and  $P$ -values smaller than 0.05 were considered to indicate statistical significance. Differences between the groups were determined using one-way ANOVA and  $t$ -tests. All *post hoc* comparisons were made using Tukey's *post hoc* test. The activity level was compared between T1 and T2 phases of NORT using paired  $t$ -test.

## Results

#### Weight measurement

The body weight of exercising and non-exercising animals increased slowly with no significant differences during the 3-week exercise (Table 1).

#### Cerebral blood flow measurement

Cerebral blood flow values were expressed as the baseline 100%

(2 min before ischemia) and decreased to 14% ( $14.5 \pm 0.3$ ) during 10 min of ischemia.

#### Histological assessment

H&E staining was used to examine whether exercise preconditioning protects neuronal damage in ischemic rats. In the ischemia group (Figure 1A), a marked neuronal injury was observed at 7<sup>th</sup> day after ischemia, with significant cell dense and cell shrinkage. We also observed eosinophilia which is one of the inflammation elements after transient global ischemia (Figure 1B). The extent of neuronal damage was quantified by counting the number of nuclei in hippocampus sections (Figure 1C). A marked reduction in the number of viable cells in the mentioned regions was observed in the ischemia group ( $P < 0.001$ ). This reduction was significantly prevented by exercise preconditioning ( $P < 0.001$ ).

#### Locomotor activity

The locomotor activities were assessed by measuring the distance travelled during 30 min on the 7<sup>th</sup> day after operation using the Plexiglas box. We did not observe any significant difference in locomotion in any of the five groups ( $P = 0.3$ ) (Figure 2A).

#### Novel object recognition test

Tactile learning was assessed in the object recognition task one week after ischemia.

#### Object recognition task: Trial phase (T1)

The total time spent exploring both similar objects in T1 (Table 2) did not show any statistically significant difference among the 5 experimental groups ( $P = 0.76$ ). During T1, no reliable difference was found among the 5 experimental groups in terms of the frequency of visits to the sample objects ( $P = 0.78$ ) (Table 2).

#### Object recognition task: test phase (T2)

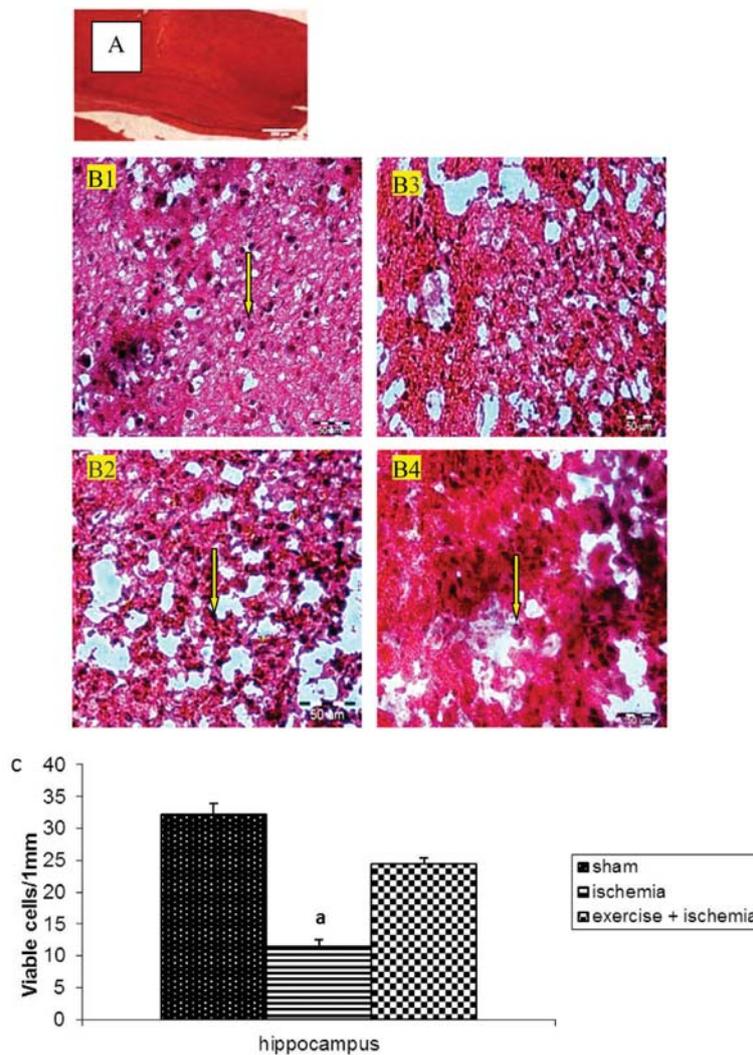
The total exploration time of both objects (familiar + novel) during test phase (T2) was not significantly different among the groups (see Table 2) (ANOVA,  $P = 0.41$ ). During T2, no reliable difference was found among the 5 experimental groups in terms of the frequency of visits for both familiar and novel objects (ANOVA,  $P = 0.05$ ) (Table 2).

A comparison of the discrimination ratio between the five groups revealed that after ischemia, this index decreased by  $34\% \pm 2\%$  (from  $60.2 \pm 2.8$  to  $39.8 \pm 1.6$ ) compared to the control group (ANOVA,  $P = 0.56$  followed by Tukey HSD  $P = 0.024$ ). Exercise improved this index in ischemic animals ( $57.3 \pm 6.6$ ); however, it was not significant compared to the control group ( $P = 0.9$ ; Figure 2B). These findings suggest that transient cerebral ischemia

**Table 1.** Body weight of animals during 3 weeks of exercise (g).

Days	Group				
	Control	Sham	Exercise	Ischemia	Exercise + ischemia
First day	263 ± 5.7	258.7 ± 5.4	269.5 ± 6.1	279.4 ± 4.6	265.3 ± 4.7
3 <sup>rd</sup> day	—	260.2 ± 5.6	273.3 ± 6.3	—	267.1 ± 4.8
6 <sup>th</sup> day	—	264.8 ± 5.9	275.5 ± 6.4	—	268.7 ± 5.1
9 <sup>th</sup> day	—	269.1 ± 6.1	279.6 ± 6.6	—	270 ± 5.6
12 <sup>th</sup> day	—	272 ± 6.3	282.6 ± 6.8	—	273.1 ± 5.7
15 <sup>th</sup> day	—	275.9 ± 6.4	284.3 ± 7.1	—	274.9 ± 7.2
18 <sup>th</sup> day	—	279 ± 6.6	287.5 ± 7.2	—	276.9 ± 6.1
21 <sup>st</sup> day	—	282.1 ± 6.9	291 ± 7.4	—	279.2 ± 6.2
Last day	269.6 ± 6.1	289.8 ± 7.3	299.8 ± 7.9	286.5 ± 5.5	287.4 ± 6.5

Data are expressed as mean ± SEM.



**Figure 1.** A) Hematoxylin and eosin staining in the hippocampal CA1 area, B1-4) coronal sections from the hippocampus in the sham, ischemic and exercise + ischemia groups, respectively. B4 shows eosinophilia in the hippocampus of the ischemic group. The pictures in each group are presented at the same magnification (50µm). The arrow head shows viable, pyknotic cell and eosinophilia in the sham and ischemia groups, respectively. C) Viable cells per square millimeter of the hippocampal CA1 area. *n* = 4 per group. <sup>a</sup> significant difference in number of viable cells between the ischemia and sham groups (*P* < 0.001). # significant difference in number of viable cells between the ischemia and exercise + ischemia groups (*P* < 0.001).

**Table 2.** Frequency and exploration times in T1 and T2.

		Control	Exercise	Ischemia	Sham	Exercise+ischemia
Trial phase (T1)	Total exploration time (s)	42.5 ± 3.5	44.8 ± 4.6	52.8 ± 9.7	47.6 ± 7.5	49.7 ± 8.04
	Frequency of visits to both objects	65.3 ± 7.7	56.2 ± 6.1	75.7 ± 12.9	82.3 ± 14.07	73.4 ± 10.8
	Travelled distance (m)	1342.3 ± 50.7	1375.6 ± 74.7	1297.1 ± 119.6	1435.8 ± 160	1116.8 ± 54
Test phase (T2)	Time to visit familiar object (s)	15.7 ± 1.9	17.8 ± 2.5	22.9 ± 3.2	19.2 ± 3.2	29.3 ± 8.4
	Time to visit novel object(s)	24.2 ± 2.7	27.01 ± 4.1	17.4 ± 2.9	27.1 ± 5.5	26.9 ± 5.6
	Total exploration time (s)	39.9 ± 3.8	44.8 ± 5.2	40.4 ± 5.4	46.3 ± 6.5	56.2 ± 9.8
	Frequency of visits to familiar object	22.8 ± 2.7	28.5 ± 3.4	48 ± 9.4 <sup>a</sup>	36.5 ± 8.08	31.7 ± 6.7
	Frequency of visits to novel object	42.5 ± 7.07	27.7 ± 4.1	27.7 ± 6.7	35.3 ± 10.01	41.7 ± 9.2
	Travelled distance (m)	1171.1 ± 53.1	1204.3 ± 70.9	1176.5 ± 140.9	1104.6 ± 43.2	1064.4 ± 84.1

Data are expressed as mean ± SEM. T2 was conducted 24 hr after T1. <sup>a</sup> significant difference in frequency of visits to familiar object between the ischemia and control groups (*P* = 0.05).

impaired the animals' ability to discriminate between novel and familiar objects while exercise preconditioning prevented this disability in rats.

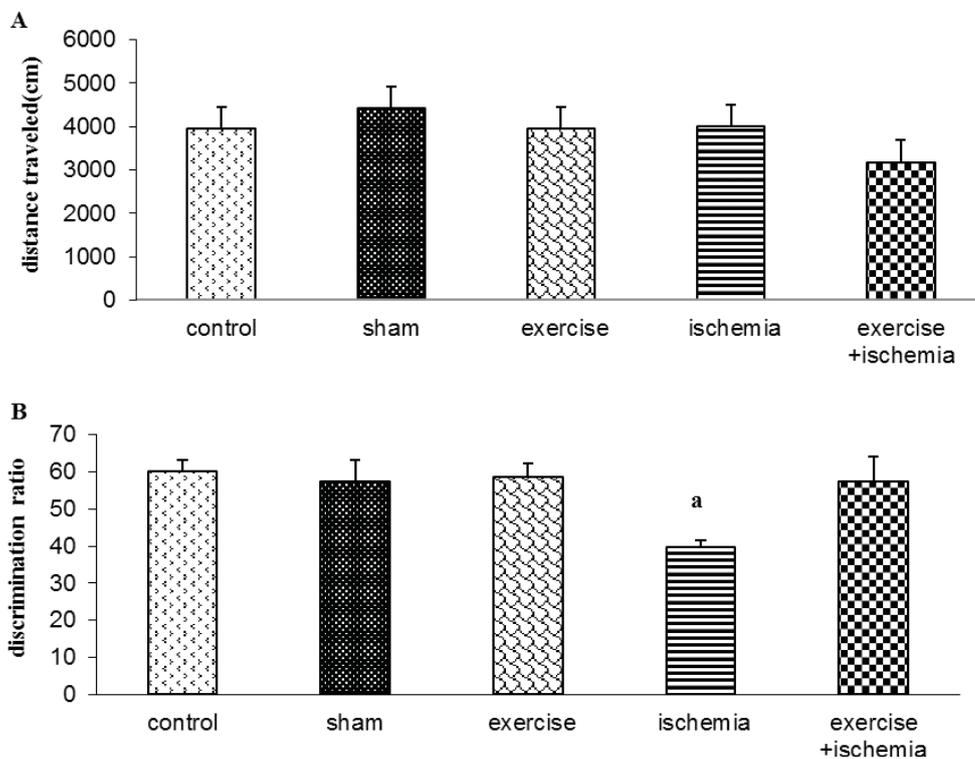
Activity level changes during NORT were studied by comparing the travelled distances between trial phase (T1) and test phase

(T2).<sup>19</sup> In any of the five groups, we did not observe any significant difference between T1 and T2. Furthermore, we investigated the differences between the control and other groups. In all experimental groups, the travelled distance was not significantly different (in T1, *P* = 0.26 and in T2, *P* = 0.82; Table 2).

**Table 3.** Touch and remove time on the adhesive removal test in 5 experimental groups before operation and at 1, 3, and 7 days after operation.

Groups		Before ischemia	Day 1	Day 3	Day 7
Control	Touch time (s)	7 ± 0.5	7.8 ± 0.8	7.9 ± 1.3	8.4 ± 0.6
	Remove time (s)	11.3 ± 0.7	10.6 ± 0.8	11 ± 0.5	12.5 ± 0.7
Sham	Touch time (s)	6.9 ± 0.8	10.2 ± 1.3	9.8 ± 1.4	11.6 ± 0.9
	Remove time (s)	8.5 ± 1	11.9 ± 3.1	12.1 ± 1.4	12.4 ± 2.8
Exercise	Touch time (s)	8.3 ± 0.7	7.4 ± 0.9	7.1 ± 0.6	8.3 ± 0.5
	Remove time (s)	13.1 ± 1.4	9.5 ± 1.05	9 ± 1.6	10.8 ± 2.7
Ischemia	Touch time (s)	5.1 ± 1.2	54.3 ± 2.7 <sup>a</sup>	50.4 ± 3.1 <sup>a</sup>	52.6 ± 1.9 <sup>a</sup>
	Remove time (s)	11.1 ± 1.6	82.7 ± 3.4 <sup>b</sup>	79.5 ± 2.8 <sup>b</sup>	81.7 ± 2.4 <sup>b</sup>
Exercise + ischemia	Touch time (s)	7.7 ± 1.5	21.6 ± 1.6 <sup>c</sup>	23.1 ± 1.5 <sup>c</sup>	26.8 ± 2.3 <sup>c</sup>
	Remove time (s)	10.2 ± 1.6	40.5 ± 1.9 <sup>d</sup>	45.5 ± 2.4 <sup>d</sup>	38.3 ± 1.1 <sup>d</sup>

<sup>a</sup> significant difference in touch time between the ischemia and sham groups at 1, 3, and 7 days after operation ( $P < 0.001$ ). <sup>b</sup> significant difference in touch time between the exercise + ischemia and ischemia groups at 1, 3, and 7 days after operation ( $P = 0.001$ ). <sup>c</sup> significant difference in remove time between the ischemia and sham groups at 1, 3, and 7 days after operation ( $P < 0.001$ ). <sup>d</sup> significant difference in remove time between the exercise + ischemia and ischemia groups at 1, 3, and 7 days after operation ( $P < 0.001$ ). Data are expressed as mean ± SEM.



**Figure 2.** **A)** Locomotion in different experimental groups. The locomotor activity was measured by computing distance travelled in 30 min. **B)** Discrimination index in 5 experimental groups. Data are expressed as mean ± SEM. <sup>a</sup> significant difference in preference index between the ischemia and sham groups ( $P < 0.05$ ).

#### Passive avoidance test

In the passive avoidance test, a significant reduction in response latency ( $8.7 \pm 1.4$ s) was observed in the ischemia group, compared to  $239.0 \pm 9.0$ s for the sham-operated and  $278.8 \pm 8.5$ s for the control group ( $P < 0.001$ ; Figure 3A). The exercise preconditioning significantly ( $P < 0.001$ ) improved response latency ( $195.8 \pm 9.8$ s) in ischemia challenged rats (Figure 3B). Moreover, in the retention test, a significant increase was observed in the ischemia group in terms of the total time spent in the dark chamber ( $68.4 \pm 4.9$ s)

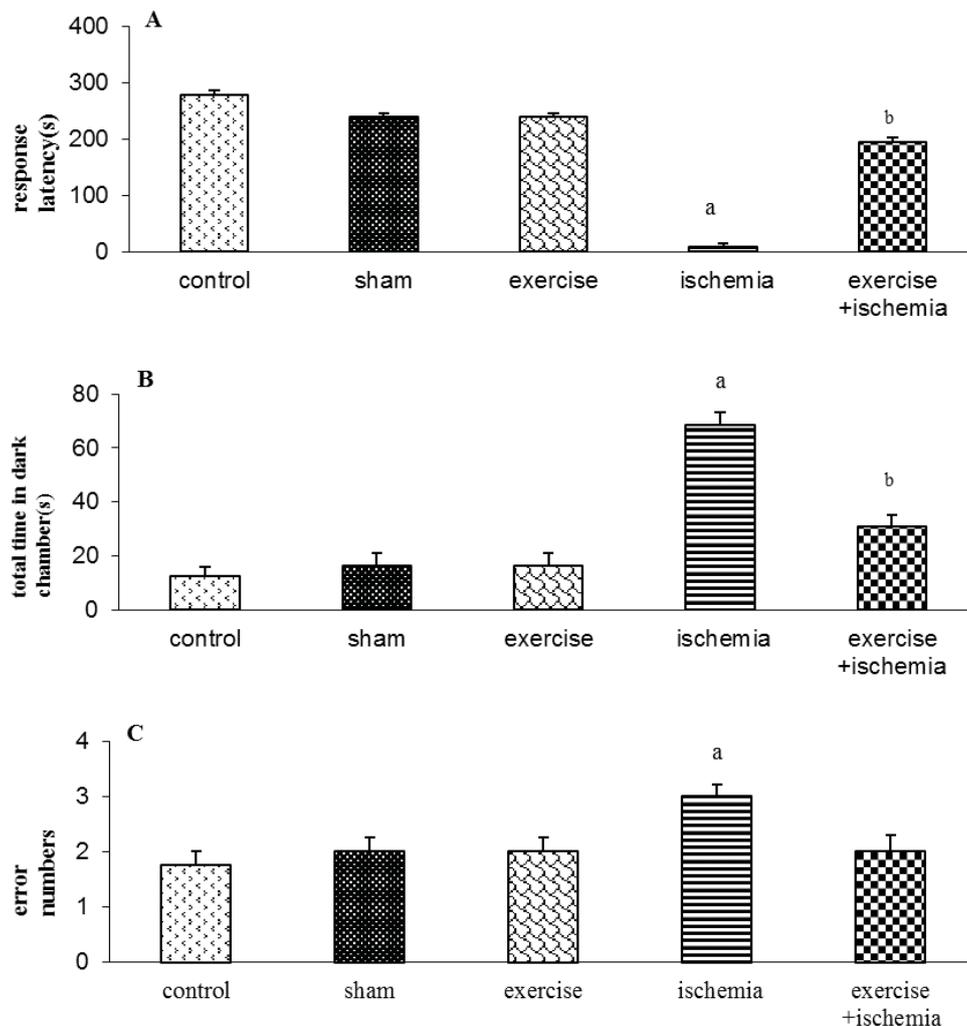
compared to the sham-operated ( $16.5 \pm 4.3$ s) and the control ( $12.5 \pm 3.2$ s) groups ( $P < 0.001$ ; Figure 3B). Exercise preconditioning reduced this index compared to the ischemia group ( $P < 0.001$ ).

Similarly, in the retention test, the number of errors in rats going to the dark chamber was significantly higher in the ischemia group ( $3 \pm 0.21$ ) compared to the sham-operated ( $1.75 \pm 0.25$ s) group (ANOVA,  $P < 0.016$  all  $P < 0.01$ ; Figure 3C). Exercise preconditioning improved this index compared to the ischemia group, although the difference was not significant (Figure 3C).

**Table 4.** Slip ratio percentage for hind limbs in 5 experimental groups before operation and at 1, 3, and 7 days after operation.

Groups	Before ischemia	Day 1	Day 3	Day 7
Control	10.7 ± 4.5	10.1 ± 2.5	9.3 ± 2.1	9.8 ± 2.1
Sham	6.9 ± 0.8	7.9 ± 0.6	8.3 ± 0.4	11.6 ± 4.3
Exercise	10.05 ± 5.5	8.9 ± 2.8	9.9 ± 3.3	8.7 ± 2.4
Ischemia	7.9 ± 6.08	25.9 ± 5.07 <sup>a</sup>	29.4 ± 8.7 <sup>a</sup>	34.9 ± 9.4 <sup>a</sup>
Exercise + ischemia	11.3 ± 5.3	23.6 ± 2.07	22.3 ± 4.6	21.2 ± 6.4

<sup>a</sup> significant difference in slip ratio % between the ischemia and sham groups at 1, 3, and 7 days after operation ( $P < 0.05$ ). Data are expressed as mean ± SEM.



**Figure 3. A)** Response latency in 5 experimental groups. Data are expressed as mean ± SEM. <sup>a</sup> significant difference in response latency between the ischemia and sham groups ( $P = 0.001$ ). <sup>b</sup> significant difference in response latency between the exercise + ischemia and ischemia groups ( $P = 0.001$ ). **B)** Total time in dark chamber in 5 experimental groups. Data are expressed as mean ± SEM. <sup>a</sup> significant difference in total time in dark chamber between the ischemia and sham groups ( $P = 0.001$ ). <sup>b</sup> significant difference in total time in dark chamber between the exercise + ischemia and ischemia groups ( $P = 0.001$ ). **C)** Error numbers in 5 experimental groups. Data are expressed as mean ± SEM. <sup>a</sup> significant difference in error numbers between the ischemia and sham groups ( $P = 0.01$ ).

#### Adhesive removal test

Table 3 demonstrates the effect of exercise preconditioning on sensory impairment in the adhesive label test. The latency to touch (ANOVA,  $P < 0.001$  followed by Tukey HSD  $P < 0.001$ ) and then remove (ANOVA,  $P < 0.001$  followed by Tukey HSD  $P < 0.001$ ) the sticky labels from forepaw was increased following induction of ischemia on the first, third and seventh days. Exercise preconditioning decreased the latency to touch ( $P < 0.001$ ) and then remove ( $P < 0.001$ ) the sticky labels from forepaw compared

to the ischemic group at the abovementioned time points. In the exercise group, exercise alone did not affect the latency to touch or remove the sticky labels from forepaw.

#### Ledged beam-walking

The motor function was tested using the ledged beam-walking test. Ischemic rats showed a significant increase in slip ratio compared to the control and sham operated animals at 1, 3, and 7 days after ischemia (ANOVA,  $P < 0.001$  followed by Tukey HSD  $P <$

0.02). Exercise preconditioning improved this index in ischemic animals, although the difference was not statistically significant ( $P = 0.27$ ; Table 4) at 1, 3, and 7 days after ischemia.

## Discussion

In this study, we investigated the effects of exercise preconditioning on behavioral deficits and cognitive disabilities induced by transient global ischemia in rats. Our result demonstrated that exercise preconditioning improved these deficits in ischemic rats.

Previous studies mainly concentrated on molecular and histological aspects of transient cerebral ischemia rather than the behavioral and cognitive aspects. As the behavioral tasks are suitable tools for investigating the consequences of cerebral ischemia, in this study we focused on cognitive and sensorimotor impairments. We observed behavioral and cognitive deficits following global cerebral ischemia. Passive avoidance learning and cognitive abilities (assessed by NORT) were impaired in the animals. In addition, the sensorimotor function (assessed by the adhesive removal test) was damaged and ischemic rats spent more time to touch and remove sticky labels from their forepaws. Motor coordination was evaluated by the ledged beam-walking test. This index was also impaired following ischemia.

Ischemic injuries are often fatal, and patients who survive often suffer from sequelae such as impaired learning and memory as well as behavioral deficits caused by ischemic cell injuries in vulnerable regions of the brain.<sup>30</sup> Katsuta et al. (2003) and Wang et al. (2008) reported that global cerebral ischemia leads to extensive neuronal damage in hippocampal CA1 pyramidal neurons and other cortical neurons.<sup>1,2</sup> These damages cause behavioral deficits in learning and spatial memory.

Effective neuroprotection against neurodegenerative insults is one of the most clinically important goals in neuroscience research. Preconditioning is a concept that is recently discovered to play an important role in protecting the brain against neurodegenerative insults.

Interestingly, recent studies have indicated that exercise entails protective effects when used as preconditioning stimuli.<sup>4,11,31</sup> Similarly in the present study, we found that exercise preconditioning improves behavioral deficits following global ischemia.

Our results demonstrate the advantages of exercise preconditioning on behavioral, cognitive and sensorimotor deficits. We found that 3 weeks of exercise improves object recognition memory (assessed by NORT) in ischemic rats and therefore ameliorates cognitive impairment. A previous study reported that exercise preconditioning improves discrimination index in diabetic rats.<sup>32</sup> Our results indicate that the discrimination index in exercising animals is not significantly different compared to the non-exercising control group. On the other hand, Hopkins et al. (2010)<sup>33</sup> showed that exercising animals have a better discrimination index compared to non-exercising animals. However, they reported that the cognitive improvements resulting from the exercise regimen did not persist after 2 weeks of inactivity. Our results further demonstrate that the discrimination ratio did not increase even 1 week after inactivity. There are reports about the involvement of perirhinal cortex in novel object recognition task. It is possible that exercise affects different brain regions, such as the perirhinal cortex, and may thus improve functional injuries in perirhinal cortex following global cerebral ischemia.<sup>33</sup>

Our results indicated no difference in the locomotor behavior

among the 5 experimental groups when measured in the familiar phase of NORT; therefore, the obtained results could not be ascribed to exercise-induced changes or ischemia-induced changes in locomotor activity. Previous reports have demonstrated that the locomotor activity did not change in ischemic animals when measured 5 ± 7 days after ischemia.<sup>34,35</sup>

In the present study, we employed passive avoidance learning test to assess other aspects of cognitive and memory deficits following ischemia. Our results indicated beneficial effects of exercise preconditioning on the passive avoidance learning deficits. Response latency is the index of learning in this test, which diminished prominently in the ischemic rats in our study. This reduction reflects impaired memory following transient cerebral ischemia. Interestingly, exercise preconditioning improved response latency in ischemic rats and increased the latency for entering the dark chamber. In addition, exercise preconditioning improved the other indices (total time in dark chamber and error numbers) assessed by this test in the ischemic rats.

Ischemic injuries in the hippocampus lead to extensive impairment in tasks related to spatial memory. In a study by Kim et al. (2005), the authors observed that exercise improves short term and spatial memory by increasing neurogenesis and reducing apoptosis in the hippocampus.<sup>36</sup> Ding et al. (2005) found that exercise reduces inflammation injuries following stroke by reducing inflammatory mediators as well as decreasing the accumulation of leukocytes.<sup>11</sup> Another study has indicated that pre-ischemic exercise decreases matrix metalloproteinase-9 expression and improves blood-brain barrier dysfunction in stroke.<sup>4</sup> In this study, histological results also demonstrated that exercise preconditioning decreased the infiltration of inflammatory cells and maintained more viable cells in the ischemic hippocampus, cortex and sub-hippocampal structures.

We employed the ledged beam-walking test to evaluate motor integration and found that ischemia leads to motor impairment as the slip ratio increased following cerebral ischemia. Exercise preconditioning improved this impairment in ischemic rats to some extent, although it was not significant. Previous studies reported similar results about the effect of ischemia on the ledged beam-walking test.<sup>26,27,37</sup>

The adhesive removal test is a sensitive method which can be easily conducted without the need for expensive facilities and reflects sensorimotor impairment quite well. We performed this test at 1, 3, and 7 days after induction of ischemia because sensory dysfunction follows ischemia by 24–48 hours.<sup>38</sup> We observed sensorimotor impairment in ischemic rats as they spent more time to touch and remove sticky labels compared to the control rats. We also observed that exercise preconditioning ameliorated this impairment in the ischemic rats. Previous studies reported similar results about the effect of ischemia on adhesive removal test.<sup>26,39</sup>

Increased delay on the adhesive removal test following ischemia is associated with injuries of the anteromedial cortex, the caudal forelimb region of the somatic sensorimotor cortex and the rostral forelimb.<sup>40</sup> The cortical component is mainly reflected in the time difference from the animal's first touch with the sticky labels using its teeth to its removal.<sup>41</sup> Therefore, it is possible that exercise can improve functional lesions in these parts.

In summary, the elucidation of protecting effects of the exercise preconditioning on behavioral and cognitive deficits induced by cerebral ischemia may encourage people to participate in exercise programs.

## Conclusion

Our results showed that exercise preconditioning improved behavioral deficits and maintained more viable nerve cells in the hippocampus of ischemic rats.

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