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Intensive training accelerates the recovery of motor functions following cerebral ischemia-reperfusion in MCAO rats

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Abstract. – OBJECTIVE: Cerebral ischemia-reperfusion is the major pathophysiological process in stroke and can cause severe and lasting sequel. However, an intensive exercise training can potentially effect a quick and efficient recovery. We used swimming training on rats with cerebral ischemia-reperfusion (CIR) and explore the underlying neuroprotective mechanism(s), including the effects of intensive training on the expression of semaphorin 3A (Sema3A) and its receptor Neuropilin-1 (NRP-1).

MATERIALS AND METHODS: The middle cerebral artery occlusion/reperfusion (MCAO/R) model was established by inserting a thread into the middle cerebral artery of Sprague-Dawley (SD) rats, and randomly dividing into the control group and training groups for different training intensities. The control group and the sham group received no training. All the rats in various groups were further randomly divided into three sub-groups for different postoperative time points (3, 7, and 14 days after operation). The apoptosis and the expression of Sema3A and NRP-1 were analyzed using immunohistochemistry (IHC), RT-PCR, and Western blotting methods respectively.

RESULTS: The intensive training resulted in significant neurological function improvements at all the time points after MCAO, compared to that in the control group ($p<0.05$), with training group 3 (highest training intensity) showing the most remarkable recovery. The Sema3A and NP-1 expressions were significantly lower than those of the control group at all the time points ($p<0.05$), with training group 3 having the lowest levels (best recovery).

CONCLUSIONS: Intensive training can reduce cerebral damage after ischemia and reperfusion in rats, inhibit the MCAO-induced Sema3A and NRP-1 expression, and accelerate the restoring process of motor nerve functions.

Key Words:
Cerebral ischemia and reperfusion, Intensive exercise training, Sema3A, NRP-1, Apoptosis.

Introduction

Stroke is a serious public health problem worldwide[1-8]. Although the incidence and mortality have been shown to decline in recent years, stroke can affect all ages and different populations[9-12]. Although its etiology remains unclear and there is no effective approach to preventing and treating this disorder[13-17], it is believed that cerebral ischemia-reperfusion is the major pathophysiological process in stroke. Numerous studies[18,19] have demonstrated that, after the central nervous system damage, the damaged axons will regenerate in a certain direction and to a certain extent and then establish new synaptic connections, thereby improving impaired nerve function and resulting in various degrees of recovery in the clinic.

Some reports[20,21] have shown that after a stroke, patients with early exercise training can promote recovery of motor function. There is ample evidence supporting the fact that exercise training can accelerate neurite outgrowth and synapse remodeling[22,23] and can promote the recovery of impaired motor functions of affected limbs[24,25]. However, the molecular mechanisms underlying the effects of exercise training on neurological recovery are not fully understood. The optimal training program, including the intensity of the exercise training and schedule, has not been established yet, to achieve the best results possible.

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There are many factors regulating nerve growth and regeneration[26-29]. Among many nerve growth inhibitory factors, Semaphorin 3A (Sema3A) is the first to be identified and is perhaps the best studied chemical repulsion factor[30,31]. It specifically mediates the cone collapse of axonal growth and inhibits neurite extension[32-34]. It has been demonstrated that Sema3A is associated with neuronal death and neuron regeneration[35-37], is involved in nerve repair and regeneration of axons, and thereby plays a major role in guiding the growth of inhibitory nerve[38-40]. As the receptor of Sema3A, Neuropilin-1 (NRP1) is a membrane-bound co-receptor to a tyrosine kinase receptor for both vascular endothelial growth factor (VEGF) and Sema3A family members and plays important roles in angiogenesis, axon guidance, cell survival, migration and invasion[41,42]. It has been shown that both Sema3A and NRP-1 participate in the repair and regeneration of damaged nerve axons and Sema3A expression is increased after transient cerebral ischemia[41], but it is not clear whether exercise training impacts on the expression and function of Sema3A and NRP-1.

Although the underlying mechanisms for neuroprotective and neurogenerative effects of intensive exercise training remain to be elucidated, it is believed that intensive exercise training can promote a quicker and more efficient recovery[43-46]. In the present work, we attempt to utilize middle cerebral artery occlusion (MCAO) to develop a middle cerebral artery occlusion/reperfusion (MCAO/R) model, and determine the effects of intensive swimming training on the recovery of motor functions after MCAO/R. To carry out a mechanistic investigation, we determine the effects of the exercise using different training intensity at various times after MCAO/R and training, and explore the changes in apoptosis and the expression of Sema3A and NRP-1. The dynamic analysis of these functional and biochemical parameters will facilitate the development of an optimal excise training program for stroke patients or other patients with injury resulting from cerebral ischemia in the clinic.

Materials and Methods

Animals and Preparation of the Cerebral Ischemia and Reperfusion Model

The experimental protocol was approved by the Animal Care and Management Committee of Qingdao University and was in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) revised in 1996. Male, Sprague-Dawley (SD) rats, weighing 220-250 g, were obtained from the Experimental Animal Center, Qingdao University (SCXK(LU)20140001), China, and housed in a temperature – (22-24ºC) and humidity – (60%-65%) controlled room under a constant 12 h light/12 h dark cycle and provided food and tap water ad libitum. The animal care and experiments were conducted according to the Guide for the Care and Use of Laboratory Animals of China.

The MCAO/R model was developed using the method of Longa et al[47]; the left middle cerebral artery occlusion (MCAO) was made for a 2 h period and then reperfusion was performed. The evaluation of the model was performed according to the Zausinger neurobehavioral scoring system[48]; and the MCAO/R rats with scores in the range of 1-4, after being awakened from anesthesia, were used in subsequent experiments. The sham-operated group underwent the same operation procedure without the MCAO.

Rehabilitation and Training Methods

The swimming training was adopted from a previous report[47]. The sham-operated group and the MCAO model group received no exercise training. For the analyses of neurological function, apoptosis, immunohistochemistry (IHC) and RT-PCR, the MCAO rats were randomly divided into the model group and three swimming training groups consisting of training group 1 (once a day, 5 min each), training group 2 (once a day, 10 min each), and training group 3 (twice a day, 10 min each). For the Western blotting analyses, the MCAO rats were randomly divided into the model group and two swimming training groups including general training group (once a day, 10 min each) and intensive training group (twice a day, 10 min each). The sham-operated groups were included in all the analyses. All the trainings started at 24 h after the reperfusion.

Neurological Function Assessment

According to the Zausinger scoring system[48], the neurological function for all rats was assessed by an investigator who was blinded to the group assignments of the animals and the experimental procedures. The Zausinger scoring criteria were

as follows: 0 score, no spontaneous walking; 1 score, moving freely and rotating contralateral to the lesion side; 2 score, grabbing the tail, rotating contralateral to the lesion side; 3 score, decreased ability to resist the pressure towards the contralateral side; 4 scores, no ability of straightening the paws of the contralateral side, and even the flexion of the whole body; and 5 score, no neurological deficits.

TUNEL and Immunohistochemistry Analyses

At 3, 7, and 14 days after reperfusion, five rats from each group were sacrificed and the brain tissues were processed for the analysis of apoptosis using the TUNEL assay kit and IHC assay kit. The TUNEL assay kit was provided by Roche Diagnostics (Roche Diagnostics, Indianapolis, IN, USA). The IHC analyses were performed by using anti-Sema3A antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-NRP-1 antibody (Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China). The cytoplasm stained brown was regarded as a positive cell. The negative control section was analyzed using phosphate buffered saline (PBS) instead of primary antibody.

Tunel assay was carried out as follows; Steps for TUNEL assay: The sections were deparaffinized into water and a beaker of 200 ml 0.01M, pH 6.0 citrate buffer, heated to 90-95°C, quickly placed into slices, using 680W (80% power), microwave irradiation for 1 min, 80 ml distilled water was added twice (20-25°C) for rapid cooling, the slides were placed in PBS (20-25°C). They have been washed with PBS 5 min × 3 times, 20% normal bovine serum was added at room temperature for 30 min and the TUNEL reaction mixture was added to the slice, incubated at 37°C for 90 min [negative photos, terminal deoxynucleotidyl transferase (TDT) was not added to the TUNEL mixture]. It was then washed with PBS for 5 min × 3 times, 3% H_2O_2 in methanol at room temperature for 10 min was used as block, it was then incubated at 37°C for 90 min, POD conversion agent was added and incubated at 37°C for 30 min, PBS washed 5 min × 3 times and DAB/H_2O_2 to make the colors stand out. Hematoxylin staining was carried out followed by conventional dehydration and it was then fixed in transparent neutral gum.

Immunohistochemistry (IHC) assay was undertaken as follows: dewaxing and hydration: Xylene I for 15 min, xylene for 15 min, 100% ethanol I for 3 min, 100% ethanol for 3 min, 95% Ethanol for 3 min, 85% ethanol for 3 min, 75% ethanol for 3 min and distilled water for 3 min. 3% Water was used and incubated at room temperature 10 min, to eliminate endogenous peroxidase activity. PBS was used to rinse the slices placed in PBS solution soak 3 min× 2 times. Heat antigen retrieval: citrate repair solution was used. The sections were immersed in 0.01 M citrate buffer, after an interval of 10 min, repeated once. After washing with PBS, and allowed to natural cool 3 min x3 times. Normal goat serum blocking solution was used, incubated at room temperature in a wet box for 20 min, in order to reduce non-specific staining. Then the excess liquid was poured out. Sema3A or anti-NP-1 diluted solution was added (1:150, 1:200) overnight at 4°C. Washing with PBS for 3 min × 3 times was carried out. Antibody working fluid was added and incubated at 37°C for 30 min. Washing with PBS carried out for 3 min × 3 times. Horseradish peroxidase labeled streptavidin working solution was added and incubated at 37°C for 30 min. Diaminobenzidine (DAB) staining was carried out for 5-20 min, when it could be observed that the slides turned yellow with the naked eye and the slides were observed under the microscope, if the staining was deemed satisfactory, the staining was terminated. Washing was done for 3 min × 3 times. Mild hematoxylin staining of nuclei was carried out for 3-10 min, observed under the microscope and timely terminated. Graded ethanol dehydration was carried out, with successive immersion in 80%, 95% ethanol I, II and 100% ethanol I, II each 3 min, xylene I, II each 3 min until transparent.

Determination of Sema3A and NRP-1 mRNA Expression Levels by RT-PCR

Under strict sterile conditions, the left cerebral cortex tissues were separated and stored at -80°C. Total RNA was extracted with Trizol reagent kit for purity and its concentration was measured to store at -80°C. After synthesis of first strand cDNA, the reverse transcription was performed at 42°C for 30 min, followed by inactivation of reverse transcriptase at 95°C for 5 min and cooling down at 4°C for 5 min. cDNA was kept at -20°C. The reverse transcribed cDNA was used as a template for PCR amplification. The sequences of the primers for Sema3A, NRP-1 and GAPDH are listed in Table I. The PCR products were analyzed by a 2% agarose gel and the image was recorded with the UVP gel imaging system ima-

ge (Upland, CA, USA) and analyzed with Image J image analysis system.

Determination of Sema3A and NRP-1 proteins by Western Blotting Analysis

The removed brains were kept at -80°C. The frozen rat brains were cut into 1mm thick slices; the infarct cortex and surrounding tissues were removed and homogenized. The brain homogenates were centrifuged at 15,294 g for 20 min to collect the supernatant. The total protein concentration of the supernatant was measured by BCA (bicinchoninic acid assay) method. After adjusting the protein concentration to 20 µg/10 µl, the samples were mixed with the loading buffer, boiled for 5 min, cooled down and then kept at -20°C until analysis.

The protein samples (10 µl) were fractionated by 8% SDS-polyacrylamide gel electrophoresis and transferred to a PVDF (polyvinylidene difluoride) membrane, which was then incubated with 5% nonfat dry milk for 1 h and then with primary antibodies (rabbit anti-mouse sema3A, rabbit anti-mouse NRP-1, dilution 1:500; rabbit anti-mouse β-actin, dilution 1:1000) overnight at 4°C. After washing, the membrane was incubated with the secondary antibody (horseradish peroxidase-labeled goat anti-rabbit IgG antibody at a dilution of 1:10000) at 37°C for 1 h. The chemiluminescence system was used to document the protein band density with a UVP gel imaging system and analyzed with ImageJ image analysis software. The Sema3A and NRP-1 protein levels were normalized to the internal control β-actin protein bands. The relative expression levels were normalized to that of the sham group.

Quantification of Cells

Olympus optical microscope was used to observe the ipsilateral cerebral cortex around the infarction area, five slices of non-consecutive areas were taken from each rat, for each slice, at a 400-fold magnification, 5 randomly selected non-overlapping field of view were chosen from the cortical ischemic peripheral zone. We, then, used Image Plus image analyzer to analyze the total cell count and the positive cells count and positive cells rate = (positive cells/total cells) × 100%.

Statistical Analysis

The experimental data are expressed as mean ± SD and tested for homogeneity of variance and normality. The differences among various experimental groups were analyzed using the t-test or one-way ANOVA. The SPSS17.0 statistical software (SPSS Inc., Chicago, IL, USA) has been used for all the statistical analyses. $p<0.05$ was considered statistically significant.

Results

Swimming Training Improves Neurological Functions after MCAO

The neurological (motor) functions were assessed using Zausinger scoring system. As illustrated in Figure 1, the sham-operated group had no neurological deficits. In the model group, the scores at all the time points were the lowest among all the groups. The scores of the training groups at all the time points were significantly higher than those of the model group at corresponding time points ($p<0.05$), with training group 3's score being the highest among all the MCAO/R rats. The order of the scores was as follows: Group 3 > Group 2 > Group 1 > model, which indicated that the effect of training was intensity-dependent.

Swimming Training Reduces MCAO-Induced Apoptosis

As shown in Table II, the apoptosis was significantly increased in the MCAO model rats,

Table I. Sequences and characteristics of primers for VEGF, NRP-1, and GAPDH.

Gene	Forward	Reverse	PCR product (bp)	Temperature (°C)
Sema3A	5′- AAAGTGGTTT CAGTCCCCAAG -3′	5′- AGTCAGTGGG TCTCCATTCCT -3′	318	61
NRP-1	5′- AGTGGCACAGG TGATGACTTC -3′	5′- GGCAGAATGTC TTGTGAGAGC -3′	151	54
GAPDH	5′-CACCCGCGAG TACAACCTTC-3′	5′- CCCATACCCA CCATCACACC-3′	207	58

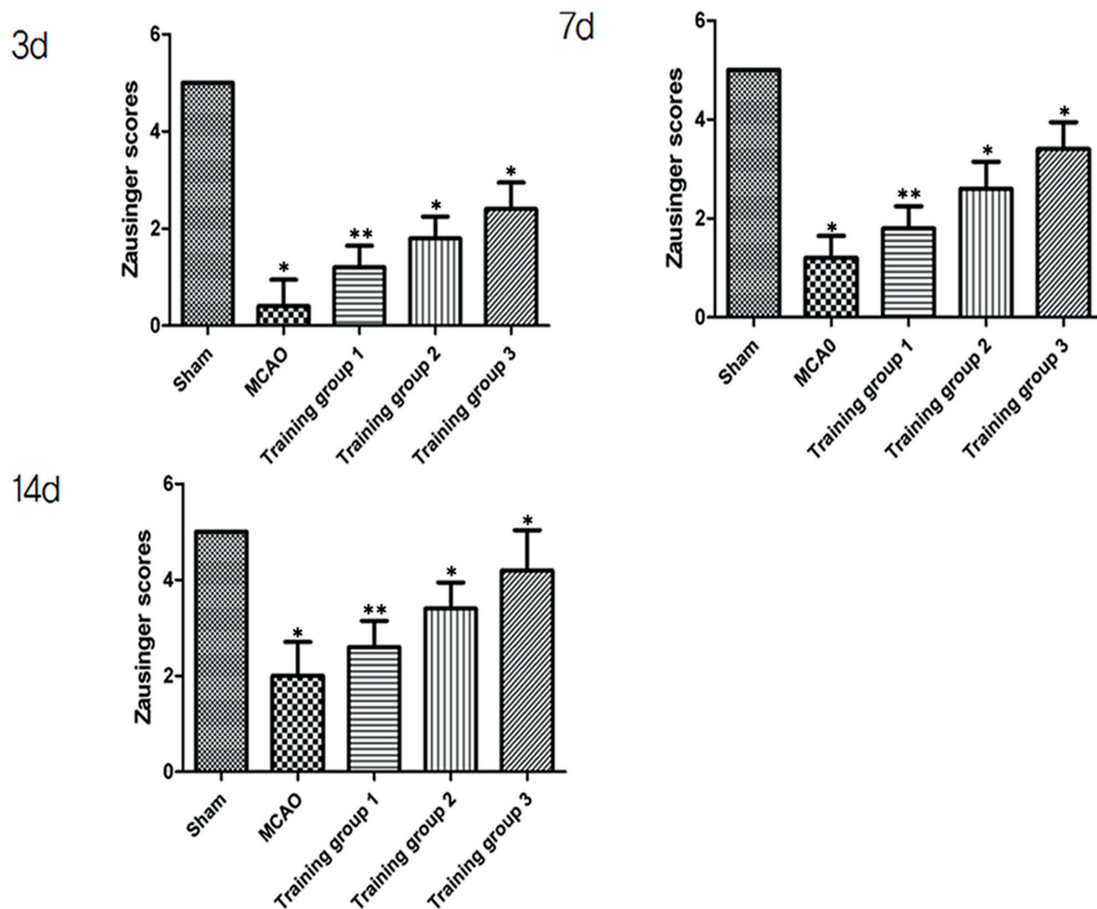

Figure 1. The scores of the training groups and the model group, compared with the sham-operated group. The same pattern was observed for the three time points (Days 3, 7 and 14). Statistical results are as follows: $p<0.05$, all the training and model groups vs. the sham-operated group; $p<0.01$, the training groups vs. the model group; $p<0.05$, the training groups 2 and 3 vs. the training group 1; and $p<0.05$, the training group 3 vs. the training group 2.

compared to the sham-operated group ($p<0.01$). The exercise training groups showed reduced apoptosis rates compared to the MCAO model group at all the time points ($p<0.01$), with the most effective results being seen in Group 3 (intensive training). The respective TUNEL staining images are shown in Figure 2.

Swimming Training Reduces the Expression Levels of Sema3A and NRP-1

Results of Immunohistochemical Assays for Protein Expressions

As shown in Table III, the percentages of Sema3A positive cells in the infarct area in the MCAO

Table II. Apoptosis (TUNEL staining) in brain tissues surrounding the infarct zone of rats in various groups (%, ±s).

Group	n	3d	7d	14d
Sham-operated	5	2.27 ± 0.79	2.21 ± 0.66	2.30 ± 0.83
Model	5	43.85 ± 2.27[a]	30.12 ± 1.88[a]	27.73 ± 1.80[a]
Training 1	5	37.22 ± 2.43[b]	28.45 ± 1.24[b]	25.61 ± 1.64[b]
Training 2	5	32.72 ± 1.75[b c]	27.13 ± 1.72[b c]	22.93 ± 1.28[b c]
Training 3	5	29.43 ± 1.38b[c d]	22.30 ± 1.21[b c d]	17.58 ± 1.70[b c d]

[a]$p<0.01$, compared with the sham-operated group; [b]$p<0.01$, compared with the model group; [c]$p<0.01$, compared with the training group 1; and [d]$p<0.01$, compared with the training group 2.

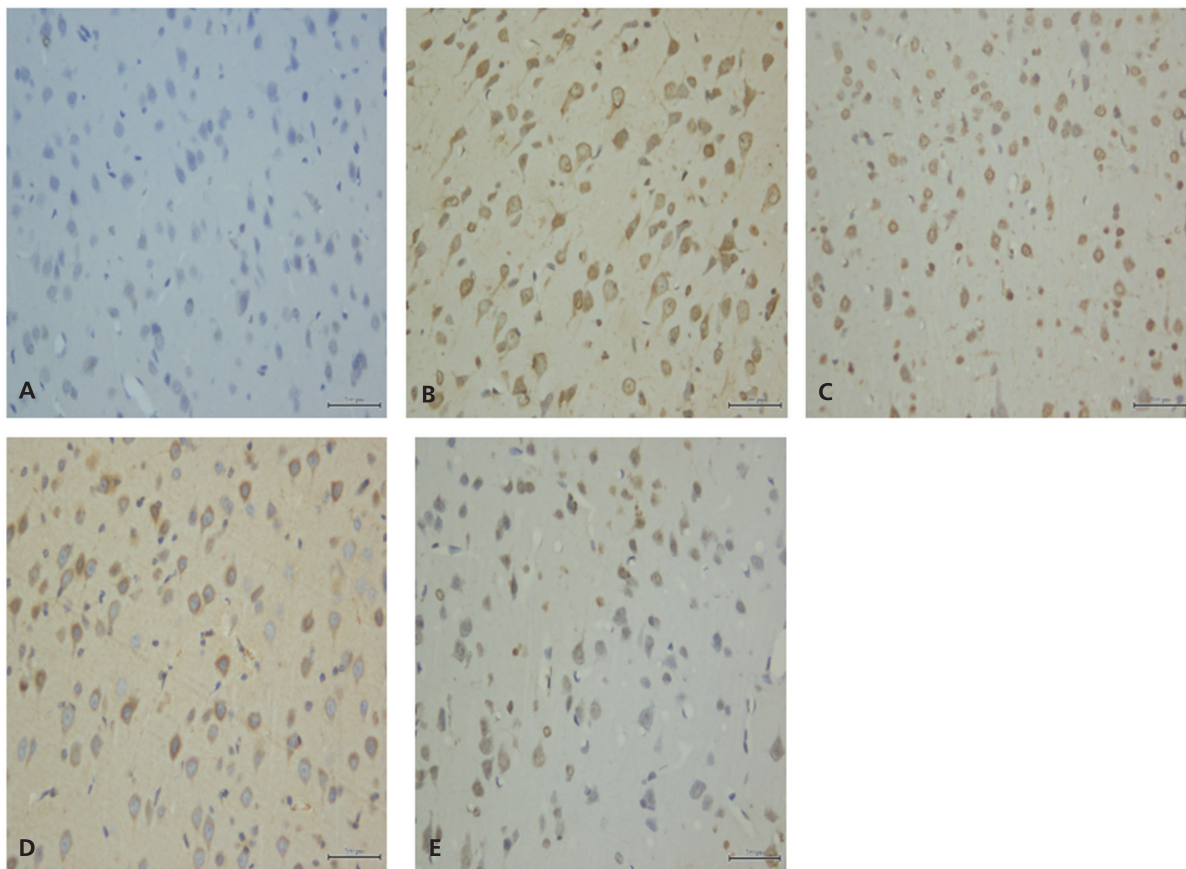

Figure 2. The detection of cell apoptosis in the peripheries of the cerebral infarct focus zone, SABC×400. Cytoplasmic dyed brown cells are counted as TUNEL positive. A, Sham-operated group; B, Model group on Day 7.

model group were significantly higher than those of the sham-operated group ($p<0.01$), with the figures being the highest on the third day and gradually deceasing. At all the time points, the percentages of Sema3A positive cells in the infarct area in all the training groups were significantly lower than those of the MCAO model groups ($p<0.01$), with the lowest figures (best effects) being seen in Group 3. The representative IHC images on Sema3A analyses are shown in Figure 3.

Similar IHC results were observed with NRP-1 staining. As shown in Table IV, the percentages of NRP-1 positive cells in the infarct area in the MCAO model group were significantly higher than those of the sham-operated group ($p<0.01$), with the figures being the highest on the third day and gradually deceasing over the time. At all the time points, the percentages of NRP-1 positive cells in the infarct area in all the training groups were significantly lower

Table III. The percentages of Sema3A positive cells in brain tissues surrounding the infarct zone of rats in various groups (%, $\bar{x}$±s).

Group	n	3d	7d	14d
Sham-operated	5	4.45±0.72	4.76±0.90	4.71±0.84
Model	5	36.60±1.55[a]	19.58±1.04[a]	11.01±0.92[a]
Training 1	5	33.73±0.89[b]	17.70±0.79[b]	10.30±0.89[b]
Training 2	5	26.51±1.14[b c]	12.18±1.17[b c]	8.78±0.81[b c]
Training 3	5	19.64±1.17[b c d]	9.73±3.83[b c d]	8.24±0.87[b c e]

[a]$p<0.01$, compared with the sham-operated group; [b]$p<0.01$, compared with the model group; [c]$p<0.01$, compared with the training group 1; and [d]$p<0.01$, [e]$p<0.05$, compared with the training group 2.

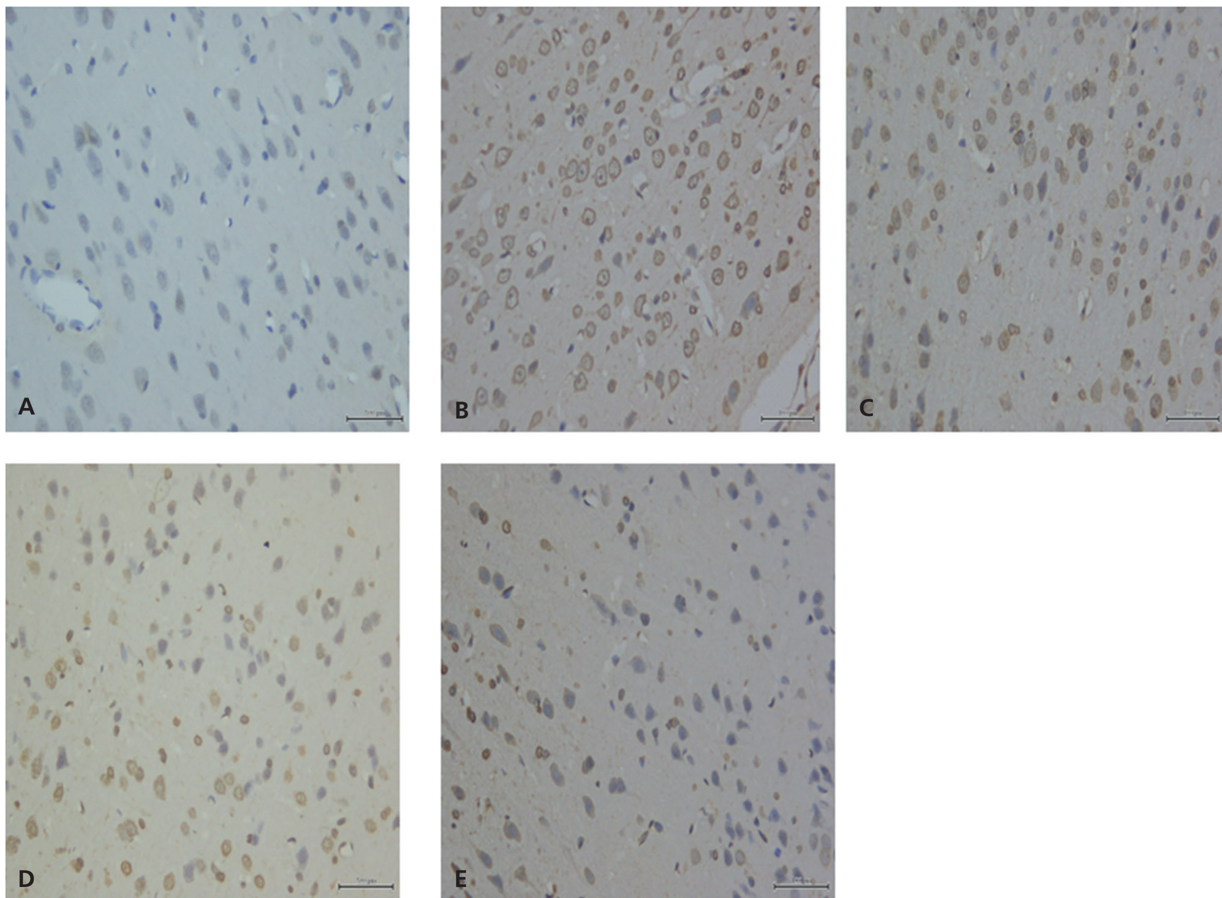

Figure 3. The detection of Sema 3A positive cells in the peripheries of the cerebral infarct focus zone, SABC×400. Cytoplasmic dyed brown cells are counted as Sema 3A positive. ***C***, Training group 1 on Day 7; ***D,*** Training group 3 on Day 7.

than those of the MCAO model groups (p<0.01), with the lowest figures (best effects) being seen in Group 3. The representative IHC images on NRP-1 analyses are shown in Figure 4.

Results of RT-PCR Assays for mRNA Expressions

As shown in Figure 5, the Sema3A mRNA levels were the highest in the MCAO model group at all the time points (p<0.01). The same figures in the training groups were significantly lower than those of the MCAO model groups (p<0.01), with the lowest levels being seen in the Group 3 (intensive training). Similar results were observed with the NRP-1 mRNA expression (Figure 6). The results indicated that exercise training resulted in the reduction of Sema3A and NRP-1 mRNA expression levels in an intensity-dependent manner.

Table IV. TThe percentages of NRP-1 positive cells in brain tissues surrounding the infarct zone of rats in various groups (%, $\bar{x}$±s).

Group	n	3d	7d	14d
Sham-operated	5	4.36±0.63	4.74±0.83	4.66±0.81
Model	5	53.31±1.44[a]	46.52±1.38[a]	34.50±1.70[a]
Training 1	5	47.76±1.09[b]	40.14±1.41[b]	31.74±1.43[b]
Training 2	5	40.45±1.31[b c]	34.12±1.42[b c]	25.90±1.26[b c]
Training 3	5	33.95±6.86[b c d]	27.95±1.29[b c d]	18.90±1.44[b c d]

[a]p<0.01, compared with the sham-operated group; [b]p<0.01, compared with the model group; [c]p<0.01, compared with the training group 1; and [d]p<0.01, compared with the training group 2.

Results of Western Blotting Assays for Protein Expressions

As shown in Figure 7, the Sema3A protein levels were significantly elevated in the MCAO model group rats compared to the sham-operated group (p<0.01). The swimming training group showed reduced Sema3A expression compared to MCAO model group (p<0.05), with the intensive training group having the lowest levels among all the MCAO rats. Similar results were observed with the NRP-1 protein expression (Figure 8). The results indicated that exercise training resulted in the reduction of Sema3A and NRP-1 protein expression levels in an intensity-dependent manner.

Discussion

There are many reports suggesting that after a stroke, patients with early exercise training may have a better recovery of motor function[20,21] and intensive exercise training may generate better and quicker improvement on neuro-regeneration and neurological function recovery[43-46]. Several clinical studies have demonstrated that rehabilitation may promote brain plasticity after cerebral infarction[49]; the improvement on the neuronal structure may be the neurological basis for functional recovery[50]. The present study was aimed at determining the correlation between exercise intensity and the response of the brain tissue, gene expression and the motor functions using a MCAO/R rat model. In the present study, we made four new findings. Firstly, exercise training improved the neurological functions in the MCAO/R rats in intensity- and time-dependent manner. Secondly, the apoptosis was significantly increased after MCAO/R in rats, which was reversed by exercise training in intensity- and time-dependent manner. Thirdly, the mRNA and protein expression levels of Sema3A and

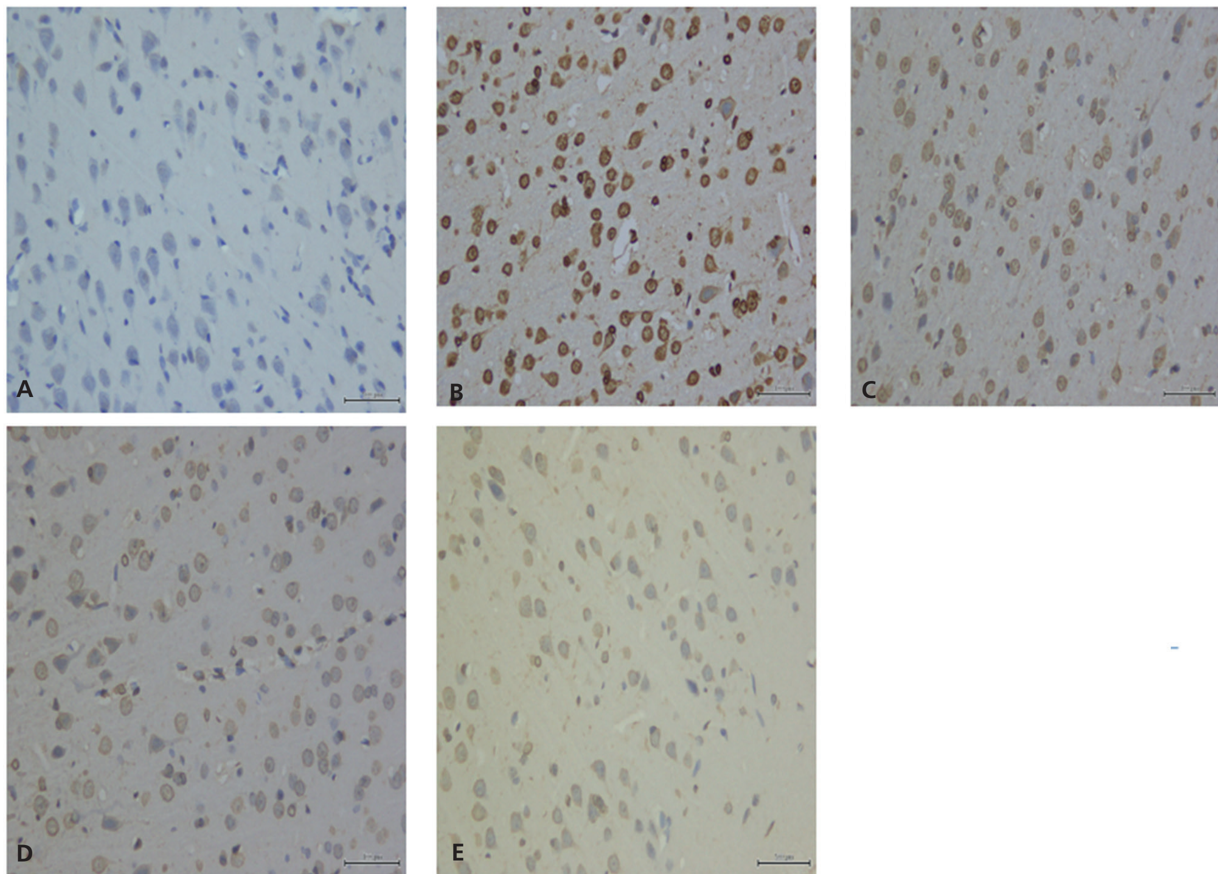

Figure 4. The detection of NRP-1 positive cells in the peripheries of the cerebral infarct focus zone, SABC×400. Cytoplasmic dyed brown cells are counted as NRP-1 positive. E Training group 1 on Day 7; F Training group 3 on Day 7.

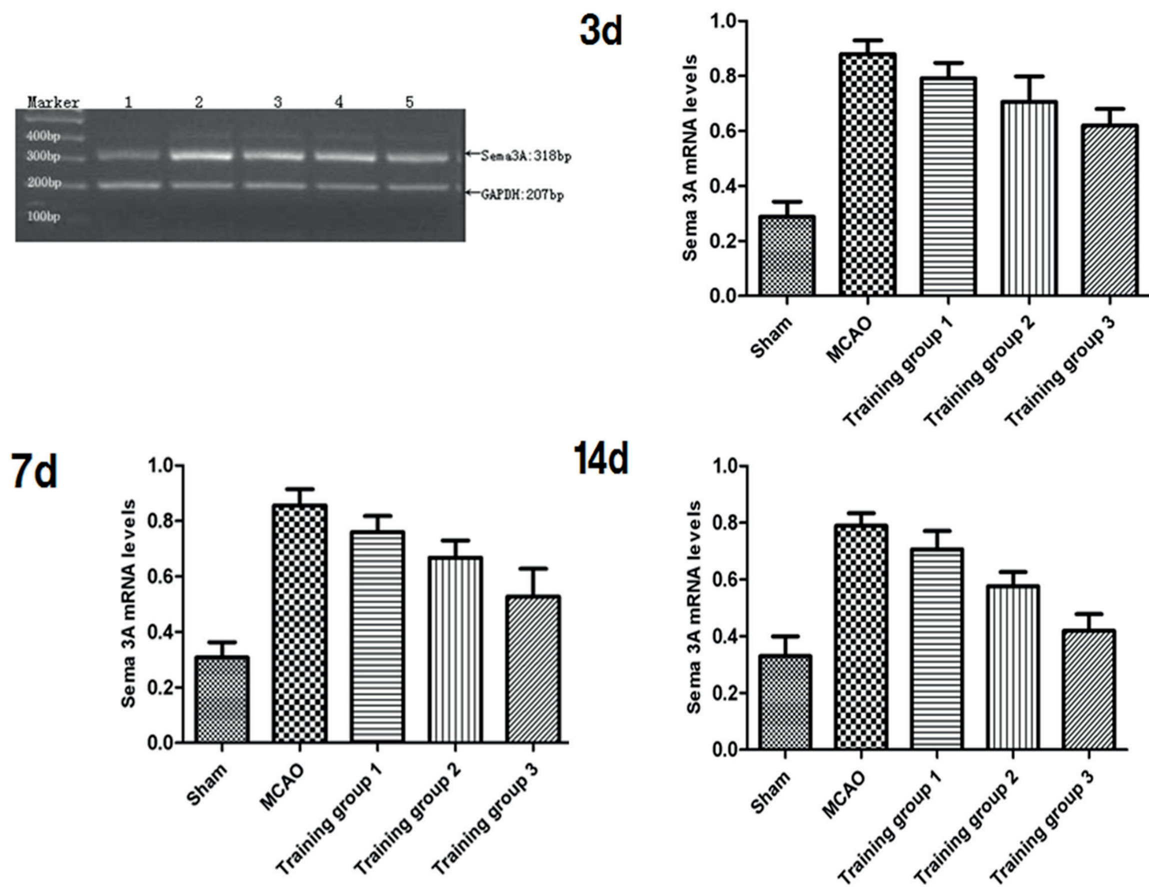

Figure 5. RT-PCR detection of Sema3A mRNA expression. Top panel: Representative PCR products of Sema3A and GAPDH on Day 14. Lanes: 1, Sham-operated group; 2, Model group; 3, Training group 1; 4, Training group 2; and 5, Training group 3. Bottom panels: Quantitative analysis of Sema3A-mRNA levels. Statistical results are as follows: On all the days, all the training groups compared with sham-operated and model groups, all $p<0.01$; On all the days, training groups 2 and 3 compared with training group 1, all $p<0.01$; On Days 3 and 7, Training group 3 compared with training group 2, $p<0.01$; On Day 14, Training group 3 compared with training group 2, $p<0.05$.

NRP-1 were significantly increased in MCAO/R rats, which were reversed by exercise training in an intensity and time-dependent manner. Finally, there were correlations between neurological scores, apoptosis, and the expression of Sema3A and NRP-1, which may provide a basis for future development of prognostic indexes and intervention approaches to stroke and other diseases involving MCAO/R.

In the present work, we found that the expression levels of mRNA and protein of both Sema3A and NRP-1 were significantly increased in MCAO/R rats, which were reversed by exercise training in intensity- and time-dependent manner. Sema3A plays a major role in inhibitory axon regeneration[30,31]. NRP-1 is involved in regulating axon and synapse formation[39,40,47,48,51,52]. Fujita et al[53] report that, after ischemic brain injury, the Sema3A and NRP-1 expressions in the ischemic cerebral cortex are significantly increased. Shirvan et al[54] have confirmed that, after ischemic stroke, the apoptosis of neurons is increased, and the increase in Sema3A is associated with neuronal cell apoptosis and necrosis, which could be blocked by the antibody against NRP-1. Jiang et al[55] have demonstrated that Sema3A can induce cell death of cortical neurons through NRP-1, while NRP-1 can also modulate the Sema3A-induced neuronal death through FER kinase. Other studies have confirmed that Sema3A and NRP-1 can induce cone collapse in axonal growth, thereby inhibiting axonal repair and regeneration[51,52,56]. In the present report, we have demonstrated that after intensive swimming training, the MCAO

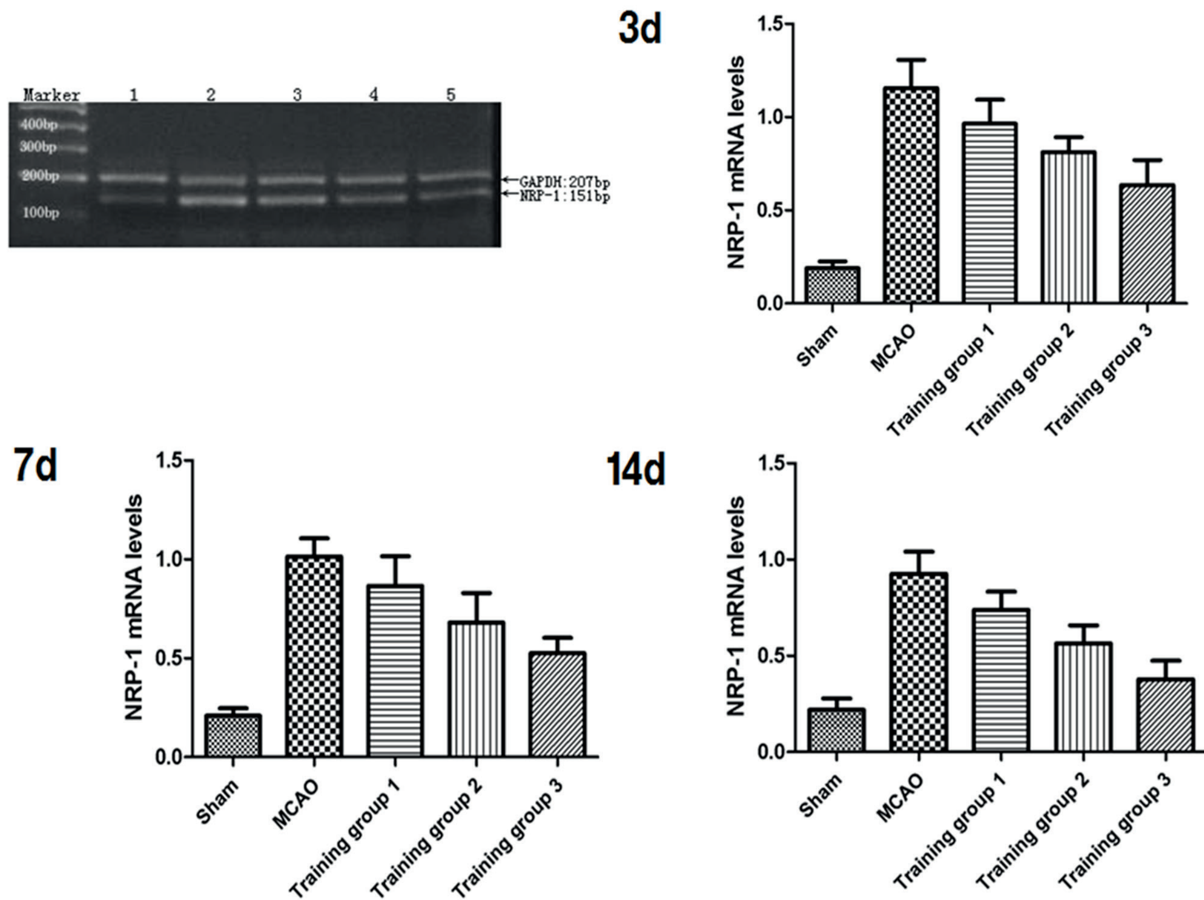

Figure 6. RT-PCR detection of NRP-1 mRNA expression. Top panel: Representative PCR products of NRP-1 and GAPDH on Day 14. Lanes: 1, Sham-operated group; 2, Model group; 3, Training group 1; 4, Training group 2; and 5, Training group 3. Bottom panels: Quantitative analysis of Sema3A-mRNA levels. Statistical results are as follows: On all the days, the model group compared with the sham-operated group, $p<0.05$; On all days, all the training groups compared with the model groups, all p<0.01; On all the days, training groups 2 and 3 compared with training group 1, all $p<0.05$; and on all days, Training group 3 compared with training group 2, $p<0.05$.

induced Sema3A and NP1 over-expression was significantly reduced, which indicated that the changes in Sema3A and NP1 expression might be responsible for reduced nerve injury and improved functional recovery observed in the animals undergoing intensive training.

It has been showed that early rehabilitation could improve patient's prognosis[20], but the intensity of the rehabilitation interventions has not been established. Lee et al[59] demonstrated that mild to moderate early exercise promotes recovery from cerebral ischemia in rats. Gertz et al[60] found that exercise training could increase nitric oxide synthase (NOS) in progenitor cells and endothelial cells in the bone marrow and spleen, accelerating the formation of new blood vessels, increasing vascular permeability and cerebral blood flow, and improving blood supply in the ischemic penumbra and surrounding areas, and thereby promoting the recovery of motor function. Quatermans et al[44] reported a systematic review demonstrating that, after stroke, early intervention with high-intensity training could be more effective in improving hemiplegic gait, walking ability and speed, and recovery of motor function. Sonoda et al[43] observed that, in stroke patients, the outcome of the weekly 7-day training program is significantly better than that of the weekly 5-day training program and the total treatment duration could be shortened, confirming that high-intensity exercise training could significantly improve the functional outcome of stroke patients. In the present study, we compared the outcomes of different swimming training

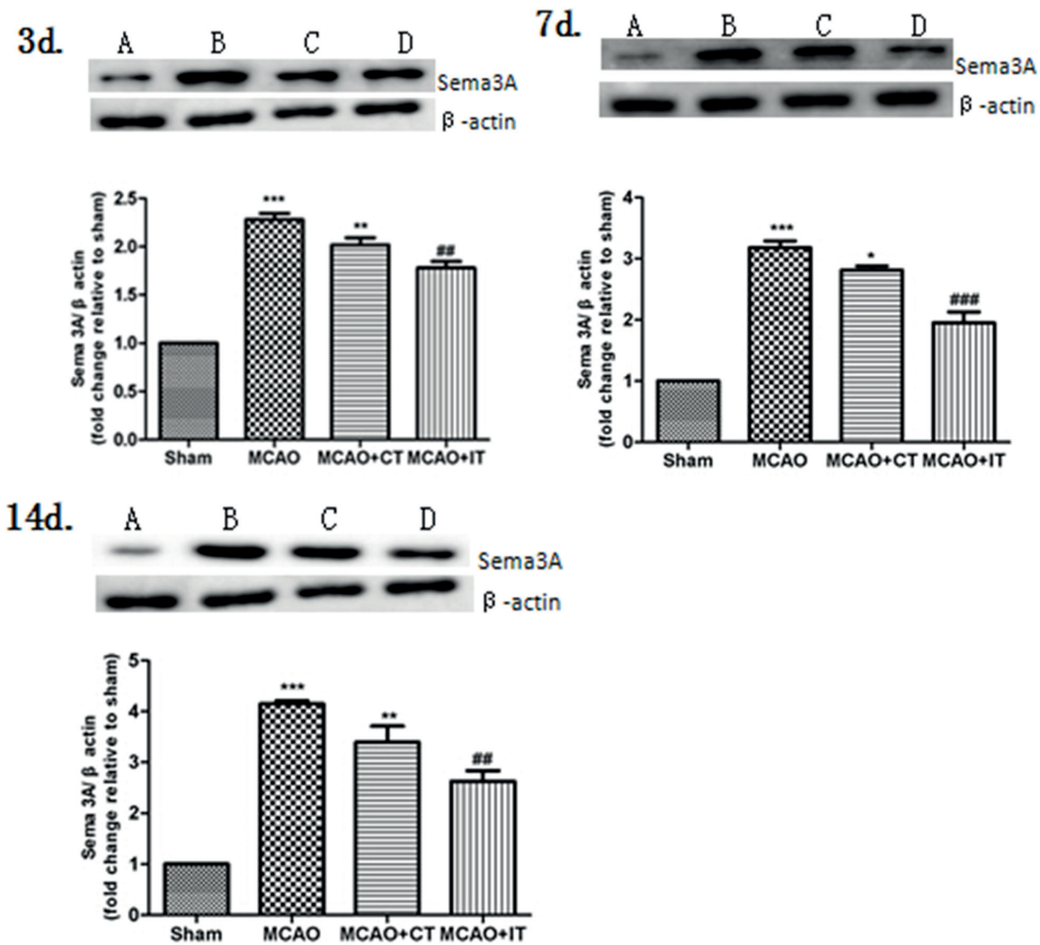

Figure 7. Western blotting detection of Sema3A and beta-actin protein expression. ***A,*** Sham-operated group; ***B,*** Model group; ***C,*** Regular training group (once a day, 10 min each); ***D,*** Intensive training group (twice a day, 10 min each). Statistical results: All days, $p<0.01$ B vs. A; Day 3, $p<0.01$, C vs. B, and $p<0.01$, D vs. C; Day 7, $p<0.05$ C vs. B; $p<0.01$, D vs. C; and Day 14, $p<0.01$, C vs. B; $p<0.01$, D vs. C.

intensities at different times in the MCAO/R rats. Although all the training methods could improve the motor function, reduce apoptosis and inhibit the mRNA and protein expression of Sema3A and NRP-1, the best results were seen in the intensive training group (Group 3), which indicated that high-intensity exercise training would accelerate the recovery after stroke.

Conclusions

Based on our findings from neurological function test and the IHC, apoptosis, and gene expression analysis, we concluded that intensive training could rapidly promote synapse formation and regeneration of new neural pathways, thereby accelerating functional recovery after ischemia. Our results also provided a molecular basis for the clinical treatment of early rehabilitation and potential targeted therapy in stroke patients.

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Author's contribution

QW, PW, PM, CH carried out the TUNEL and Immunohistochemistry analyses, participated in the RT-PCR and drafted the manuscript. PW and SY prepared the animal and the Cerebral Ischemia and Reperfusion Model. PM and CH participated in the data analysis.

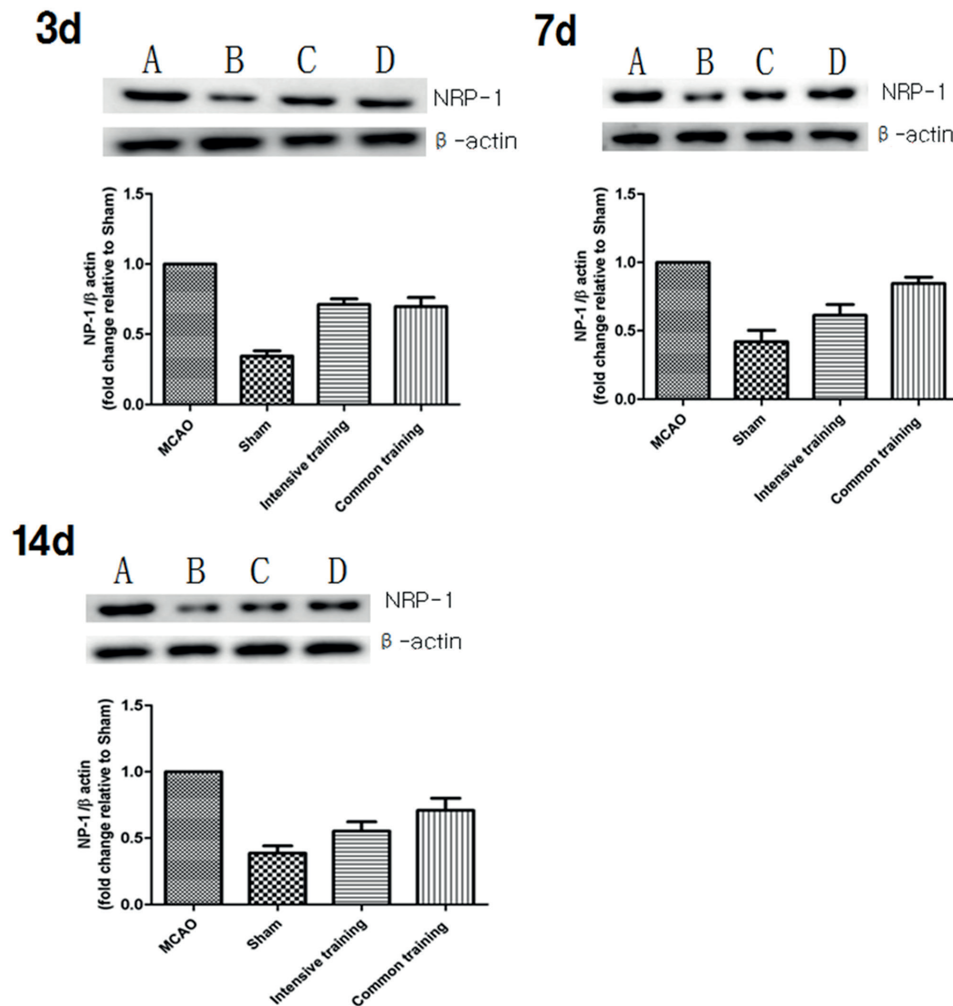

Figure 8. Western blotting detection of NRP-1 and beta-actin protein expression. ***A,*** Sham-operated group; ***B,*** Model group; ***C,*** Regular training group (once a day, 10 min each); ***D,*** Intensive training group (twice a day, 10 min each). Statistical results: All days, $p<0.05$, B vs. A; Day 3, $p<0.05$, D vs. A, and $p<0.05$, D vs. C; Day 7, $p<0.05$ D vs. A; $p<0.05$, D vs. C; and Day 14, $p<0.05$, D vs. A; $p<0.05$, D vs. C.

SY, PW and PM participated in the design of the study and performed the statistical analysis. SY and PW conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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