

High-Intensity Interval Training on Neuroplasticity, Balance between Brain-Derived Neurotrophic Factor and Precursor Brain-Derived Neurotrophic Factor in Poststroke Depression Rats

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Background: High-intensity interval training (HIIT) improves functional and mental health in the patients with stroke. To investigate the potential mechanisms of HIIT on poststroke depression (PSD). *Methods:* Wistar rats were randomly divided into control, Sham, PSD, moderate intensity continuous training (MICT), and HIIT groups. After PSD model was successfully made, the maximum speed (S_{max}) and the blood lactate threshold corresponding speed (S_{LT}) were measured. Different intensity training protocols were performed on the MICT and HIIT groups, respectively. The behavioral tests (open field, forced swimming, and sucrose preference tests) were performed before and after training. Nissl staining was used to observe the changes of neuronal cell morphology in the left hippocampus. The expression of mature brain-derived neurotrophic factor (mBDNF), tropomyosin receptor kinase B (TrkB), precursor BDNF (proBDNF), pan-neurotrophin receptor 75 (p75NTR), NR2A, NR2B proteins, and BDNF, tissue plasminogen activator (tPA) mRNA in the hippocampus were detected by Western blotting, immunohistochemistry or RT-PCR after training. *Results:* After 28 days of training, higher center occupancy, immobility time, and level of proBDNF, p75NTR, and NR2B proteins, lower sucrose preference and level of mBDNF, TrkB, NR2A proteins, and BDNF, tPA mRNA were observed in the PSD group. Neuronal cells and Nissl body in the hippocampus were loosely arranged and lightly stained in the PSD group. The ethological findings, Nissl staining especially in the CA1 and dentate gyrus areas, expression of proteins and mRNA above in the MICT and HIIT rats were reversed. And the HIIT group changed more significantly compared with MICT. *Conclusions:* HIIT was superior to MICT in improving depression in the PSD rats might via increasing mBDNF/proBDNF ratio and further improving neural plasticity in the hippocampus.

Key Words: Stroke—depression—high intensity interval training—neuroplasticity
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Stroke has become the second cause of death after cardiac diseases¹ and poststroke depression (PSD) is the most common mental disorder, severely affecting the prognosis of patients with stroke. Accumulating evidence showed that the neural plasticity of hippocampus played a crucial role in the pathophysiology of depression. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophic family, is a vital regulator of activity-dependent neural plasticity and survival in the brain.² BDNF is originally synthesized as a glycosylation precursor protein (proBDNF) and then proteolytically cleaved to mature protein (mBDNF) through the tPA/plasminogen cascade.³ mBDNF activates its high-affinity receptor tropomyosin receptor kinase B (TrkB) to promote growth, survival, differentiation

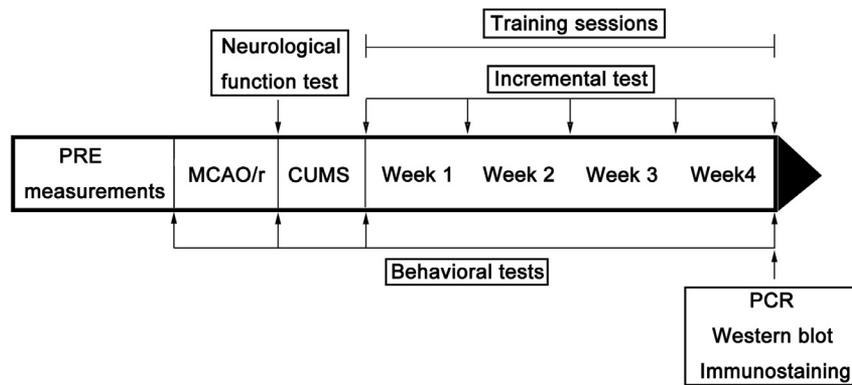


Figure 1. Experimental protocol illustrating the timeline for the behavioral tests, incremental test, brain sample, and training sessions.

of select neuronal types, and facilitated long-term potentiation (LTP) induction in the hippocampus. In contrast, proBDNF binds preferentially with the p75NTR, triggering proapoptotic, synaptic withdrawal and facilitating long-term depression (LTD) in the hippocampus.⁴ Both LTP and LTD require *N*-methyl-D-aspartate subtype glutamate receptor (NMDAR) activation. The activation of NR2A-containing NMDARs leads to LTP formation, and the activation of NR2B-containing NMDARs produces LTD.⁵ Our previous work hypothesized and found that the mechanisms controlling the cleavage of proBDNF and balance between mature BDNF and proBDNF in the hippocampus are likely to play important roles in the development of depression.

Stroke rehabilitation guidelines suggested that patients with stroke, capable of participating in physical activity, exercised in moderate intensity at 40%-70% of peak oxygen uptake (VO₂peak) or heart rate reserve, or 11-14 on the 6-20 scale of the Borg Rating of Perceived Exertion for 20-60 minutes, 3-7 days per week,⁶ which called MICT. However, preliminary evidence supported that HIIT, a new strategy that maximizes exercise intensity through short bursts of concentrated effort alternated with recovery periods of rest or light exercise, might be more significantly effective and safe than MICT in the clinical context for both functional and mental health in the patients with stroke.^{7,8}

There are no studies addressed the effects of higher intensity training on the regulation of BDNF isoforms compared with moderate intensity training. Thus, the primary aim of this pilot study was to investigate the relationship between PSD and mBDNF/proBDNF ratio in the hippocampus after MICT and HIIT. And the second was to evaluate if the 2 different intensity treadmill exercise regimens could induce different changes in neural plasticity and the relative levels of mature BDNF and proBDNF in the hippocampus.

Materials and Methods

Animals

Male Wistar rats (250 ± 20 g, between 2 and 3 months), purchased from Pengyue Experimental Animal Center (Jinan, China), were initially housed in standard plastic

cages in a temperature-controlled environment (22°C ± 2°C) with 50% ± 10% humidity under a 12/12 h light/dark cycle (lights on 7:00 A.M.) with free access to food and water except during experimental procedures. Animal certification number: SCXK (Lu) 20140007. All experiments were performed according to the National Institutes of Health guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and approved by the Institutional Animal Care and Use Committee of Qingdao University. The rats were allowed to adapt to the laboratory conditions for 7 days prior to use. We chose the male rats for the potential influences of progesterone in females on mood regulation⁹ and the differential regulation of BDNF in male and female rodents.¹⁰

Overall, 98 rats were used, and 55 of them were included. Each rat was randomly assigned to control group (n = 10), Sham group (n = 10), PSD group (n = 11), MICT group (n = 12), HIIT group (n = 12). The experimental process illustrating the timeline showed in the Figure 1.

Middle Cerebral Artery Occlusion/Reperfusion (MCAO/r) Surgery

The rats were deeply anesthetized (ketamine, 20 mg/100 g of body weight; xylazine, 2 mg/100 g, i.m.). Incisions were made at the neck midline to expose the left common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA). After ECA was ligated, a blunt nylon thread (Beijing Cinontech Co. Ltd., China) was inserted from the left ECA, through the bifurcation of CCA, and into the intracranial segment of ICA (19 ± 1 mm away from the carotid bifurcation) to block blood flow at the middle cerebral artery (MCA) for 90 minutes.¹¹ Then, the nylon thread was withdrawn to allow reperfusion. Finally, the skin was sutured. In the Sham group, CCA, ECA, and ICA were just exposed without being inserted by the thread to block the blood flow at MCA. The neurological deficit level of the rats was examined using the Longa's test 6 hours after MCAO/r surgery.¹¹ MCAO rats with scores between 1 and 3

participated in the experiment and were then given chronic unpredictable mild stress (CUMS).

CUMS

The rats in PSD, MICT, and HIIT groups were housed individually in a separate cage (cage size: 26 × 19 × 15 cm) and received random unpredictable stress for 28 days, including: 1 minute tail pinch (1 cm from the end of the tail), 2 hours physically restraint, 5 minutes cold swimming (4°C), 24 hours food deprivation, 24 hours water deprivation, overnight illumination, 45°cage tilt (24 hours), and damp bedding (24 hours). Rats received one of these stressors each day at different times and same stressor was not applied in 2 consecutive days. At the end of the CUMS protocol, OFT (day 29), FST (day 30), and SPT (day 31) were performed to assess mood-related behaviors. The rats in control and Sham groups were housed in a separate room without receiving any stress.

After MCAO/r surgery and CUMS, rats were excluded according to the following criteria: (1) extremely physical weakness (n = 7); (2) resisting running or unable to run on treadmill (n = 3); (3) no statistically significant differences before and after CUMS in more than one (≥ 2) behavioral tests (n = 14); (4) death (n = 19).

Exercise Protocols

To reduce stress and show the animals in which direction to run, the rats in MICT and HIIT groups were familiarized to exercise on the motor driven treadmill (Huaibei Zhenghua Biological Instrument Equipment Co. Ltd., China) by running at a speed of 9 m/min at 1% inclination for 15 min/day during three consecutive days before the experiments.

The protocol was performed after MCAO/r and CUMS with the following adaptations according to the previous published work¹²: the rat was placed on the treadmill and warmed up for 5 minutes at a rate of 9 m/min. Speed was then increased by 3 m/min per 3 minutes until the rat could not maintain the imposed speed despite mild stimulation with a wooden cane. And the end speed was considered as the rat's maximum speed (S_{max}). Blood was sampled (.2 μ L) at distal area of the tail vein at intervals of 20 seconds between each 3 minutes. Blood lactate concentration (mmol/L) was measured by a portable blood lactate device (Lactate Scout+, EKF diagnostics, Germany). The blood lactate concentration measured in the previous was regarded as lactate threshold (LT) when there was a significant inflection point or an increase of 1 mmol/L in the blood lactate concentration measured twice in succession, and the corresponding treadmill speed called S_{LT} .

HIIT: Sessions were composed of 4 × 4 minutes high intensity treadmill running ($S_{LT} + 60\% - 70\%$ ($S_{max} - S_{LT}$)) interrupted by 3 minutes of active recovery (S_{LT}) between

each intense series. The program was performed 5 times per week for 4 weeks.

MICT: Speed was fixed at 80%-90% S_{LT} to avoid lactate accumulation in the body. The program was performed every day for 4 weeks. In order to match the total amount energy consumption (W) between groups and only compare the influence of intensity, the daily exercise time of the MICT group was adjusted according to the energy consumption of the HIIT group (exercise + recovery) by the following formula:

$$W(\text{J/kg} \cdot \text{m}) = \text{mass}(\text{kg}) \times \text{speed}(\text{m}/\text{min}) \times \text{time}(\text{min}) \\ \times \text{treadmilltilt}(\text{°}) \times 9.8$$

Each program included a 5 minutes warm-up (50% S_{LT}) before the formal training and a 5 minutes cool-down (50% S_{LT}) after the training. To motivate the rats to run, their tails were stimulated using a soft bristle brush when necessary. The S_{max} and S_{LT} of the rats were retested every week to adjust the training intensity.

Behavioral Tests

The behavioral tests were carried out before the experiment, 24 hours after MCAO/r, next day after CUMS and 4 weeks after exercise training. Rats were placed in the test room to adapt for 30 minutes before the behavioral tests. All behavioral tests were operated between 8:00 A.M. and 12:00 A.M. After each test, rats were immediately returned to the original squirrel cage. The test sequence was as follows: (1) Open field test (OFT); (2) Forced swimming test (FST); (3) Sucrose preference test (SPT)

OFT

The experimental device consists of a plastic test box (45 × 45 × 50 cm) and an automatic video system. The rat was placed into the center of the open field to record its free movement for 10 minutes. The immobility time in the central area (centre occupancy), the frequency of erection (posture sustained with hind-paws on the floor) and the incidences of modification (including washing or mouthing of forelimbs, hind-paws, face, body, and genitals) were recorded and analyzed. The more immobility time spent in the central or less frequency of erection and incidences of modification are the indicators for depressive-like symptom.¹³ The box was thoroughly cleaned with 75% alcohol to remove any olfactory cues.

FST

Briefly, rat was placed into a transparent cylinder (37 cm diameter × 60 cm height, 40 cm water height, water 23°C ± 2°C) and its swimming was recorded for 6 minutes by a digital camera. The immobile behavior during the last 5 minutes was measured manually by an

experienced observer blind to the experiment design. Each rat was judged to be immobile when it ceased struggling and remained floating motionless in the water. Increasing immobility time shows symptoms of depression.¹³

SPT

The whole test took 3 days. On the first day, 2 bottles with 200mL 1% sucrose solution were available in each cage. Rats have free access to both bottles for 24 hours. On the second day, 1 of the bottles was replaced with fresh water. Rats were deprived of water and food for 22 hours on the third day. And then each rat was provided with one bottle of 100 mL 1% sucrose solution and one bottle of 100 mL fresh water. The 2 bottles were weighed before the experiment. After 1 hour, the positions of the bottles were reversed. And the 2 bottles were weighed again after 2 hours. The sucrose preference (%) which represents the anhedonia was then calculated as: sucrose consumption / (sucrose consumption + water consumption) × 100%. The decreased percentage of sucrose intake is the indicative of the depression-like behavior.¹³

Western Blot Assays

Rats in each group were sacrificed by decapitation after ketamine/xylazine injection (n = 5 each group). Their brains were quickly removed and the ischemic hippocampus were dissected and placed in Eppendorf tubes, frozen in liquid nitrogen, and stored at -80°C for further use. Brain tissue was homogenized with an electric homogenizer in RIPA lysis buffer (150 mM sodium chloride, 1.0% Triton X-100, .5% sodium deoxycholate, .1% SDS, 50mM Tris, pH 8.0). Centrifuge for 10 minutes at 12,000 rpm at 4°C in a microcentrifuge and aspirate the supernatant. Protein concentration was measured with the BCA protein assay kit (CW BIO, China). A total of 30µg of denatured proteins were separated in SDS-PAGE gel (4%-

20%, Willget Biotech, China) by electrophoresis under constant voltage (100 V in stacking gel for 15 minutes and 150 V in revolving gel for 45 minutes), and further transferred onto a PVDF membrane (Millipore). After blocking nonspecific binding sites with a 5% solution of nonfat milk for 1 hour at room temperature, the membranes were incubated overnight at 4°C with the appropriate primary antibodies diluted in blocking solution: anti-BDNF antibody (1:2000, Abcam), anti-proBDNF (1:500, Santa Cruz), anti-TrkB antibody (1:2000, Abcam), anti-p75NTR (1:1000, Santa Cruz), anti-NR2A (1:1000, Abcam), anti-NR2B (1:1000, Abcam), and anti-β-actin (1:1000, Abcam). After 3 times washes with TBS-T buffer (10 mM Tris, 150 mM NaCl, .05% Tween-20, pH 7.5), the blots were incubated for 2 hours at room temperature with secondary antibodies: a horseradish peroxidase-conjugated goat antimouse or rabbit IgG (1/5000, Bioss). The membranes were developed with ECL reagents (CW BIO, China) and imaged via UVP gel imaging system (UVP). Protein band densities were quantified by Image J software (NIH) and were expressed as a percentage of the control group.

RNA Extraction, Complementary DNA Synthesis, and Quantitative RT-PCR

Total RNA was extracted with TRizol kit (Invitrogen) and all DNA was removed from the samples. The absorption values of the diluent at 260 nm and 280 nm in the ultraviolet spectrophotometer were read to determine the concentration and purity of the RNA solution. Reverse transcriptase was used to transcribe qualified mRNA into cDNA in PrimeScript RT Reagent Kit (Takara, Otsu, Shiga, Japan). Reaction mix was incubated at 42°C for 15 minutes, at 85°C for 5 seconds. cDNA was diluted 20 fold in deionized water and stored at 20°C. PCR reaction was performed using quantitative RT-PCR (PE-ABI Prism 7300, Applied Biosystems, Foster City, CA) while Synthetic primer sequences were designed according to Primer Express version 3.0 software (Applied Biosystems), and the sequences were as follows (Table 1).

The target gene and housekeeping gene of each sample were respectively subjected to RT-PCR reaction. The response compound were constituted with diluted cDNA of each sample 2 µL, either forward or reverse primers 1 µL, SYBR Green Mix 12.5 µL, and ddH₂O water 8.5 µL. The amplification conditions were 95°C for 30 seconds followed by 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds. We got the corrective expression of each target gene (ratio of target gene to reference gene β-actin) (x ± s) through DNA gradient dilution standard curve, and calculated relative expression of experimental group compared to control group. Each sample was repeated twice and dissolution curves were analyzed for all samples in the end. Besides, Image J software was used for density analysis to quantify the data. Results were expressed as

Table 1. The primer sequences for RT-PCR

| Gene | Sequences |
|-------|--|
| BDNF | Forward: 5'-AACTCCAGT-CATCCTCTGTCTCC-3' Reverse: 5'-AGCCATCTCTAAGTCCA-CACCTC-3' |
| tPA | Forward: 5'-AGAGCCTGCAGGAACT-CAAG-3' Reverse: 5'-CTCCCATGTATCCCTGGTC-3' |
| GAPDH | Forward: 5'-AAGATTGTCAGCAATG-CATCC-3' Reverse: 5'-ACTGTGGTCATGAGCCCTTC-3' |

Abbreviations: BDNF, brain-derived neurotrophic factor; tPA, tissue plasminogen activator.

relative content of transcripts normalized by the reference gene β -actin, using the $2^{-\Delta\Delta Ct}$ method.

Nissl Staining and Immunohistochemistry

The rats ($n=5$ each group) were deeply anesthetized and transcardially perfused with 400 mL of normal saline, .1 M phosphate buffered saline (PBS) and then fixed with 500 mL 4% paraformaldehyde solution in .1 M sodium phosphate buffer (PB), pH 7.4. The ischemic brains were removed and the brain tissue ($1.5 \times 1 \times .5$ cm) was taken 4 mm posterior to the optic chiasm, namely, the hippocampus plane. The tissue blocks were postfixed for 2 hours at 4°C in the same fixative and cryoprotected 24h at 4°C in 30% sucrose solution. After that, the tissue blocks were embedded in paraffin and sectioned to a desired thickness ($5\mu\text{m}$, Leica, Germany). For Nissl staining, sections were degreased through graded alcohol (70%, 95%, and 100% alcohol) for 3 minutes respectively and then hydrated through graded alcohol (95%, 70%, and 50% alcohol) for 3 minutes, respectively. Subsequently, the sections were stained in .1% toluidine blue solution for 20 minutes, quickly rinsed in distilled water and differentiated in 95% ethyl alcohol for 15 minutes. For immunohistochemistry, the serial coronal sections were then affixed onto the amino-propyl-tri-ethoxy-silane coated slides and incubated at 60°C for 2 hours after drying naturally. And then the slides were incubated with 3% H_2O_2 at room temperature for 10 minutes after deparaffinized and rehydrated. The sections were treated 10 minutes with sodium citrate buffer (10 mM Sodium Citrate, .05% Tween 20, pH 6.0) in the pressure cooker for antigen retrieval and blocked in PBS with .3% Triton X-100 and 5% normal goat serum for 10 minutes at room temperature and incubated with primary antibodies diluted in PBS with 5% normal goat serum and .1% Triton X-100 at 37°C for 2 hours using anti-BDNF antibody (1: 200, Abcam) and anti-proBDNF antibody (1:100, Santa Cruz). After rinsing 3 times in PBS with gentle agitation, slices were incubated with the corresponding horseradish peroxidase-conjugated secondary antibody (1: 300, Bioss) diluted in PBS at 37°C for 30 minutes. Thereafter, the sections were developed with diaminobenzidine for 5 minutes at room temperature and

counterstained with hematoxylin. After dehydrated, cleared and mounted, the slides were observed with Olympus Fluorview-500 confocal microscope (40 \times ; 1.0 NA) and quantitative analyzed by Image J software.

Statistical Analysis

All data were presented as mean \pm standard deviation (SD). Statistics analyses were performed on Statistical Program for the Social Sciences (Version 22.0, SPSS Inc, Chicago, IL). The data were analyzed statistically by 1-way analysis of variance for multiple comparisons followed by Tukey's post hoc test. Pearson's correlation coefficient was used to examine the relationship between mBDNF/proBDNF ratio and parameters of behavioral tests. Statistical differences were considered significant where $*P < .05$, $**P < .01$ compared with the control and Sham groups; $^+P < .05$, $^{++}P < .01$ compared with the PSD group; $^\#P < .05$, $^{##}P < .01$ compared with the MICT group.

Results

Running Speed and Blood Lactate Concentration

During 4 weeks, the speed during HIIT was higher than MICT (32%, 35%, 36%, 37%, respectively). In contrast, with the equal workload, the session duration of HIIT group was lower than MICT (-32%, -39%, -44%, -46%, respectively; Table 2).

The resting blood lactate concentration of the PSD group after 4 weeks of training ($3.7 \pm .5$ mmol/L) was higher ($F=11.67$, $P < .05$) than the control ($2.1 \pm .3$ mmol/L), Sham ($1.9 \pm .3$ mmol/L), MICT ($3.0 \pm .9$ mmol/L), and HIIT (2.6 ± 1.0 mmol/L) groups (Fig 2, A). Before training, S_{LT} and S_{max} of the PSD, MICT, HIIT groups were significantly lower than the control and sham groups ($P < .01$; Fig 2, B and C). After 4 weeks of treadmill training (D28), S_{LT} and S_{max} of PSD group did not change significantly (S_{LT} : $-1.8\% \pm 4.7\%$; S_{max} : $3.0\% \pm 5.1\%$, $P > .05$) compared with the one at the beginning of training (D1). However, S_{LT} ($23.7\% \pm 8.6\%$) and S_{max} ($24.0\% \pm 8.5\%$) changed significantly in the MICT (S_{LT} : $23.7\% \pm 8.6\%$; S_{max} : $24.0\% \pm 8.5\%$, $P < .01$) and HIIT groups (S_{LT} : $51.8\% \pm 14.3\%$; S_{max} : $34.1\% \pm 3.0\%$,

Table 2. Exercise protocols (speed, duration, and energy consumption) during HIIT and MICT

| | HIIT | | | | MICT | | | |
|------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Work speed (m/min) | 23.2 \pm 2.4 | 26.8 \pm 2.3 | 29.0 \pm 2.3 | 30.5 \pm 2.2 | 15.8 \pm 2.1 | 17.5 \pm 2.3 | 18.6 \pm 2.1 | 19.2 \pm 2.5 |
| Recovery speed (m/min) | 18.0 \pm 2.6 | 21.4 \pm 2.3 | 23.6 \pm 2.3 | 25.1 \pm 2.6 | | | | |
| Duration (min) | 28 | 28 | 28 | 28 | 37.0 \pm 4.0 | 38.9 \pm 4.9 | 40.2 \pm 4.1 | 40.8 \pm 4.4 |
| Workload (J) | 1726 \pm 173 | 2105 \pm 167 | 2383 \pm 170 | 2591 \pm 168 | 1726 \pm 173 | 2105 \pm 167 | 2383 \pm 170 | 2591 \pm 168 |

Abbreviations: HIIT, high-intensity interval training; MICT, moderate intensity continuous training.

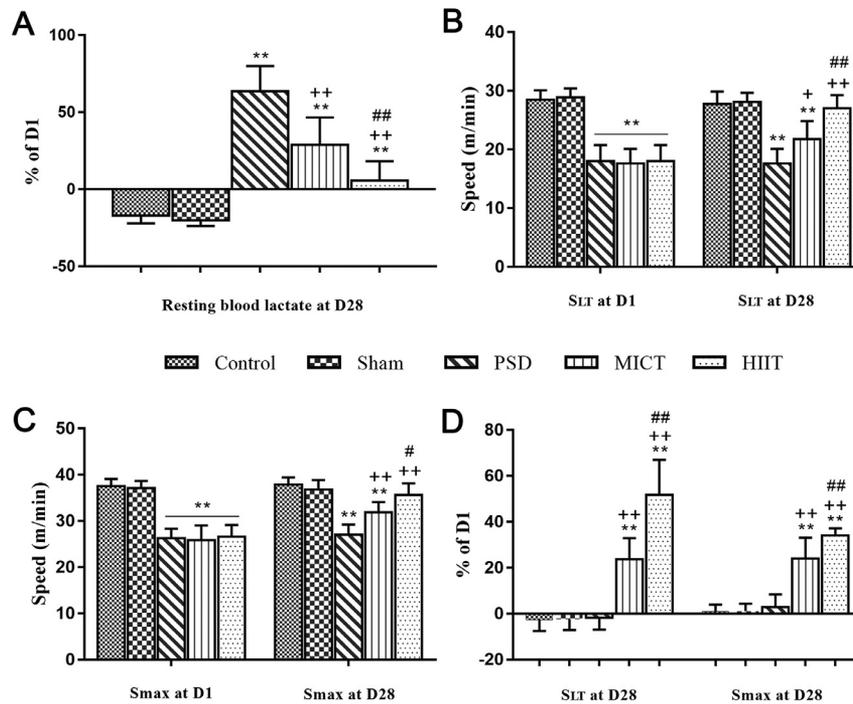


Figure 2. (A) Relative percentage (%) of resting blood lactate at D28 normalized to the D1. (B) S_{LT} (m/min) at D1 and D28. (C) S_{max} (m/min) at D1 and D28. (D) Relative percentage (%) of S_{LT} and S_{max} at D28 normalized to the D1.

S_{LT} indicates speed associated with the lactate threshold; and S_{max} , maximal speed.

$P < .01$). Moreover, the change rate of HIIT group was higher than the MICT group (S_{LT} : $q = 9.00$; S_{max} : $q = 5.33$, $P < .01$; Fig 2, D).

Behavioral Test

During the OFT, longer centre occupancy ($F = 39.67$, $P < .01$) and fewer active behaviors such as erection frequency ($F = 15.40$, $P < .01$), modification ($F = 13.47$, $P < .01$), and the excrement ($F = 4.75$, $P < .01$), were observed in the PSD group compared with the other groups at D28. And the data showed significant differences in the center occupancy ($q = 8.01$, $P < .01$), erection frequency ($q = 6.63$, $P < .01$), and modification ($q = 5.47$, $P < .01$) between the HIIT and MICT groups (Table 3).

Similarly, a significant increase in the immobility time ($F = 70.24$, $P < .01$) during the FST and a significant

decrease in the sucrose preference (%) ($F = 28.34$, $P < .01$) during the SPT at D28 were observed in the PSD. And there was a significant difference (FST: $q = 11$; SPT: $q = 7.64$, $P < .01$) between HIIT and MICT groups (Fig 3).

Western Blotting

After 4 weeks of training, the level of BDNF in the ischemic hippocampus was significantly up-regulated in the MICT ($q = 4.88$, $P < .05$) and HIIT groups ($q = 10.75$, $P < .01$) when normalized to the PSD group condition. And proBDNF expression was significantly down-regulated in the HIIT group ($q = 4.87$, $P < .05$), but no significant difference was observed between the MICT and PSD groups ($q = 2.36$, $P > .05$). There was a great significant difference between the HIIT and MICT groups in BDNF expression ($q = 5.88$, $P < .01$), instead of proBDNF expression ($q = 2.52$,

Table 3. Open field test in HIIT and MICT groups compared with control, Sham, and PSD groups

| | Centre occupancy (s) | Erection frequency | Modification (s) | Excrement |
|---------|----------------------|--------------------|------------------|-------------|
| Control | 74.0 ± 9.4 | 14.1 ± 2.0 | 55.3 ± 10.4 | 1.5 ± 1.2 |
| Sham | 70.5 ± 12.0 | 13.5 ± 2.5 | 58.4 ± 9.8 | 1.6 ± 1.3 |
| PSD | 120.2 ± 15.4** | 6.8 ± 3.2** | 28.7 ± 12.3** | 3.8 ± 1.8** |
| MICT | 90.0 ± 12.9*,++ | 10.3 ± 3.4 | 40.1 ± 13.5 | 3.1 ± 1.5 |
| HIIT | 60.2 ± 7.4++### | 16.8 ± 4.0+### | 60.5 ± 12.6++### | 1.6 ± 1.8 |

* $p < 0.05$, ** $p < 0.01$ compared with the control and Sham groups; + $p < 0.05$, ++ $p < 0.01$ compared with the PSD group; # $p < 0.05$, ### $p < 0.01$ compared with the MICT group.

Abbreviations: HIIT, high-intensity interval training; MICT, moderate intensity continuous training; PSD, poststroke depression.

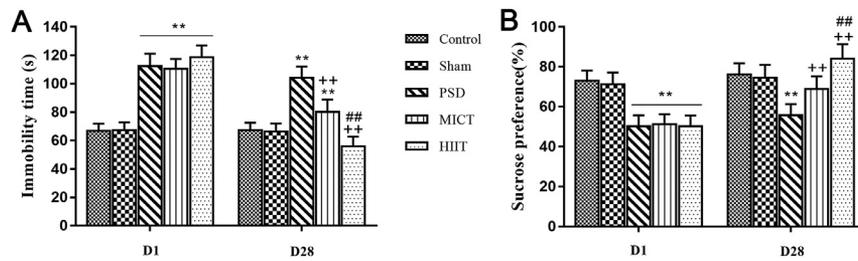


Figure 3. (A) The immobility time (s) during the FST. (B) The sucrose preference (%) during the SPT. Abbreviations: FST, forced swimming test; SPT, sucrose preference test.

$P > .05$). Similar to BDNF, the ratio of mBDNF/proBDNF was significantly decreased in the PSD group (PSD versus control: $q = 8.82$; PSD versus Sham: $q = 7.74$, $P < .01$) and significantly upregulated after MICT ($q = 5.07$, $P < .05$) and HIIT ($q = 13.44$, $P < .01$). In addition, the mBDNF/proBDNF ratio in the HIIT group was higher than that in the MICT group ($q = 8.37$, $P < .01$; Fig 4, A).

Moreover, the relative expression of TrkB (HIIT versus PSD: $q = 13.61$, $P < .01$; MICT versus PSD: $q = 9.14$, $P < .01$) and NR2A (HIIT versus PSD: $q = 11.29$, $P < .01$; MICT versus PSD: $q = 6.01$, $P < .01$) protein within the ischemic hippocampus in the HIIT and MICT groups were significantly higher than that in the PSD group. Besides, the TrkB

($q = 4.47$, $P < .05$) and NR2A ($q = 5.28$, $P < .05$) expression in the HIIT group were higher than that in the MICT group. The relative expression of p75 ($q = 7.71$, $P < .01$) and NR2B ($q = 8.67$, $P < .05$) protein significantly decreased in the HIIT group compared with PSD group, while there was no significant reduction in the MICT group (p75: $q = 4.02$, $P > .05$; NR2B: $q = 3.65$, $P > .05$; Fig 4, B and C).

Correlation Between mBDNF/proBDNF Ratio and Parameters of Behavioral Tests

There was a negative correlation between mBDNF/proBDNF ratio and center occupancy during the OFT

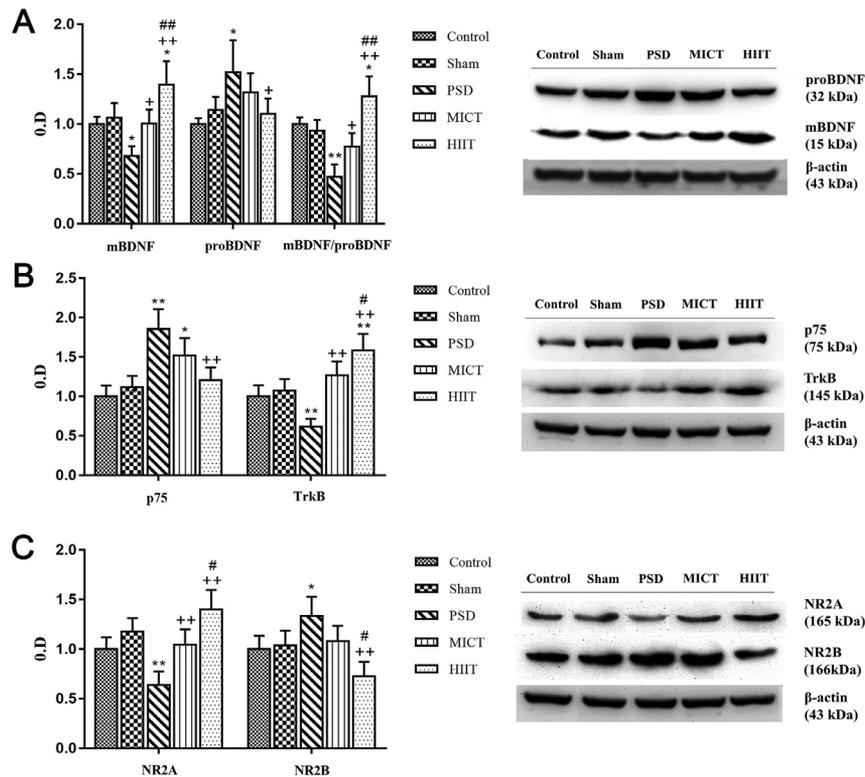


Figure 4. (A) BDNF and proBDNF expression normalized to control group condition and the mBDNF/proBDNF ratio (left). Representative immunoblot BDNF and proBDNF protein (and β -actin) (right). (B) TrkB and p75 expression normalized to control group condition (left). Representative immunoblot TrkB and p75 protein (and β -actin) (right). (C) NR2A and NR2B expression normalized to control group condition (left). Representative immunoblot NR2A and NR2B protein (and β -actin) (right).

Abbreviations: BDNF, brain-derived neurotrophic factor; mBDNF, mature brain-derived neurotrophic factor; proBDNF, precursor brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B.

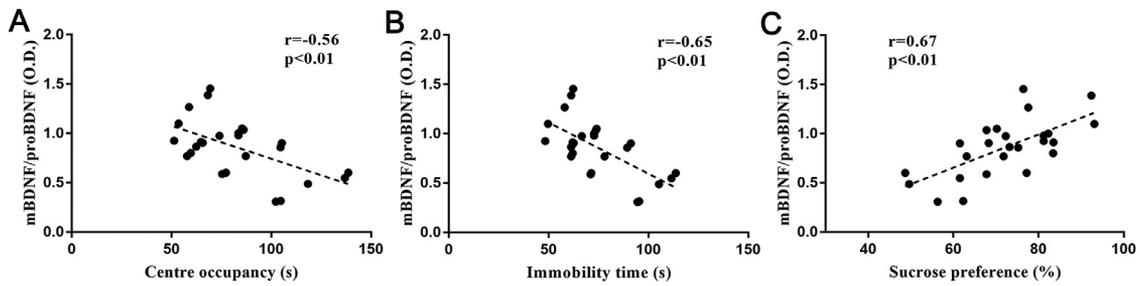


Figure 5. (A) Correlation between mBDNF/proBDNF ratio and center occupancy (s) during the OFT. (B) Correlation between mBDNF/proBDNF ratio and immobility time (s) during the FST. (C) Correlation between mBDNF/proBDNF ratio and sucrose preference (%) during the SPT.

Abbreviations: OFT, open field test; FST, forced swimming test; mBDNF, mature brain-derived neurotrophic factor; proBDNF, precursor brain-derived neurotrophic factor; SPT, sucrose preference test.

($r = -.56$, $P < .01$, Fig 5, A), as well as the immobility time during the FST ($r = -.65$, $P < .01$, Fig 5, B). A positive correlation was found between mBDNF/proBDNF ratio and sucrose preference during the SPT ($r = .67$; $P < .01$, Fig 5, C).

RT-PCR

Analysis of quantitative RT-PCR data showed that BDNF mRNA and tPA mRNA levels in the HIIT group were significantly higher than that in the other groups (BDNF mRNA: $F = 50.72$; tPA mRNA: $F = 28.76$, $P < .01$, Fig 6). And there were significant differences in BDNF mRNA and tPA mRNA levels between the MICT and PSD groups (BDNF mRNA: $q = 6.68$; tPA mRNA: $q = 6.43$, $P < .05$). Similarly, there was significant difference in BDNF mRNA expression between the MICT group and control, Sham groups (MICT versus control: $q = 4.63$, $P < .05$; MICT versus Sham: $q = 6.33$, $P < .01$), but no significant difference in tPA mRNA expression between the MICT group and control, Sham groups (MICT versus control: $q = 1.04$; MICT versus Sham: $q = 1.96$, $P > .05$, Fig 6).

Nissl Staining

Nissl staining showed that the neuronal cells in the 3 subfields of hippocampus (area CA1, area CA3, and the DG) in the PSD group were loosely arranged or missing, and Nissl bodies were lightly stained or even dissolved compared with that in other groups. We also found that the neuronal cells in the hippocampus of the HIIT group, especially in the CA1 and DG regions, were arranged more neatly and densely, and Nissl bodies were deeper

died than the control, Sham and MICT groups. While there was no significant visual difference in the CA3 region of the MICT and HIIT groups compared with the PSD group (Fig 7, A).

Immunohistochemistry

The immunohistochemistry for BDNF protein revealed a significant reduction in the PSD group compared with the other groups (CA1: $F = 14.45$; CA3: $F = 55.93$; DG: $F = 84.93$, $P < .01$). In contrast, HIIT promoted the BDNF levels in the 3 subfields of hippocampus. For qualitative staining, the expression of BDNF in the HIIT group was obviously higher than that in the PSD (CA1: $q = 10.72$; CA3: $q = 19.87$; DG: $q = 24.67$, $P < .01$) and MICT groups (CA1: $q = 5.45$; CA3: $q = 11.45$; DG: $q = 12.17$, $P < .01$), respectively (Fig 7, B).

Contrary to BDNF, the expression of the proBDNF significantly elevated in the CA3 ($F = 36.11$, $P < .01$) and DG ($F = 18.34$, $P < .01$) regions of the PSD group. After HIIT or MICT, the proBDNF levels decreased in the CA3 and DG regions, while there was no significant difference between the HIIT and MICT groups (CA3: $q = 1.84$; DG: $q = 3.02$, $P > .05$). Interestingly, no differences were observed in the CA1 area among all the 5 groups. Besides, the proBDNF level in the CA1 area was lower than that in the CA3 and DG areas (Fig 7, C).

In lined with the results of western blotting, the ratio of mBDNF/proBDNF in the 3 subfields of hippocampus was significantly decreased in the PSD group (CA1: $F = 12.15$; CA3: $F = 27.85$; DG: $F = 40.51$, $P < .01$) and

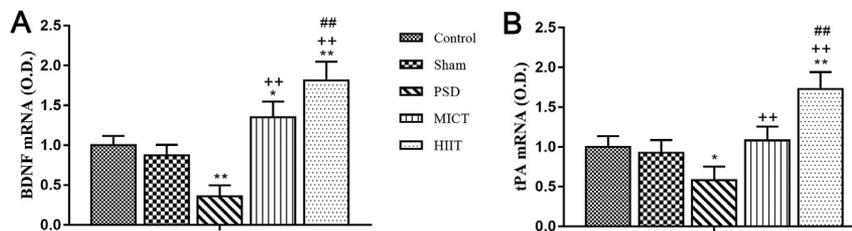


Figure 6. (A) BDNF mRNA expression normalized to control group condition. (B) tPA mRNA expression normalized to control group condition.

Abbreviations: BDNF, brain-derived neurotrophic factor; tPA, tissue plasminogen activator.

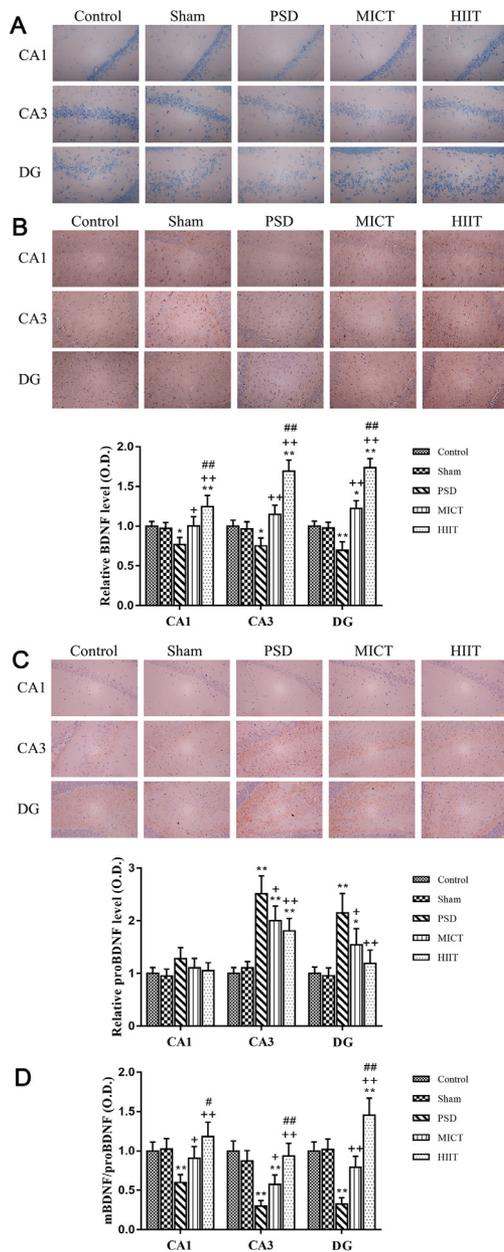


Figure 7. (A) Nissl staining in the 3 subfields of hippocampus. Scale bars = 200 μm . (B) Illustration of BDNF immunostaining in the 3 subfields of hippocampus. Scale bars = 200 μm (top). BDNF expression in the 3 subfields of hippocampus normalized to control group condition (bottom). (C) Illustration of proBDNF immunostaining in the 3 subfields of hippocampus. Scale bars = 200 μm (top). proBDNF expression in the 3 subfields of hippocampus normalized to control group condition (bottom). (D) mBDNF/proBDNF ratio in the 3 subfields of hippocampus normalized to control group condition.

Abbreviations: BDNF, brain-derived neurotrophic factor; mBDNF, mature brain-derived neurotrophic factor; proBDNF, precursor brain-derived neurotrophic factor.

significantly up-regulated after MICT (CA1: $q = 4.96$; CA3: $q = 4.96$, $P < .05$; DG: $q = 7.28$, $P < .01$) and HIIT (CA1: $q = 9.42$; CA3: $q = 11.46$; DG: $q = 17.53$, $P < .01$). And the mBDNF/proBDNF ratio in the HIIT group was higher than that in the CA1, CA3, and DG regions of the MICT

group (CA1: $q = 4.46$, $P < .05$; CA3: $q = 6.50$; DG: $q = 10.25$, $P < .01$; Fig 7, D).

Discussion

The present study is the first research to compare the effects of HIIT and MICT on the relative levels of mature BDNF and its precursor proBDNF in control versus PSD brain. Here, we demonstrated that HIIT was superior to MICT with regard to improving depression-like behaviors, neural plasticity, and increasing mBDNF/proBDNF ratio in the ischemic hippocampus in PSD rats. Given that HIIT might be more preferable and effective for post-stroke depression rehabilitation as a novel form of exercise training.

We chose the CUMS-induced depression model because of its widely application for evaluating the efficacy of antidepressant candidates as revealed by open-field, forced swimming, and sucrose preference tests.¹⁴ Our results demonstrated that HIIT could effectively produce antidepressant effects in rodents. Unlike the moderate intensity training recommended by some stroke rehabilitation guidelines,¹⁵ we quantified high-intensity training (>23 m/min) and low to moderate intensity aerobic training (<20 m/min) by measuring blood lactate thresholds (LT) that most patients can achieve during exercise in PSD rats. The intensity below LT located in the ranges of the moderate intensity (e.g., 40%-70% of $\text{VO}_{2\text{peak}}$). With the equal energy consumption, the total exercise duration in the HIIT group was obviously shorter than that in the MICT group. Moreover, clinical study showed that the HIIT could also elicit higher enjoyment than MICT,¹⁶ indicating that the HIIT is time efficient and might not be considered as a major barrier for patients. Furthermore, the lactate level decreased and S_{LT} increased after HIIT, indicating that HIIT can effectively improve the body's aerobic fitness.

Our current results demonstrated that different intensity or type of exercise had differential effects on the precursor and mature forms of BDNF. With the low levels of mature BDNF and BDNF mRNA, proBDNF in the PSD group still maintained a high level. Reasonable explanation might be that CUMS could block the proteolytic cleavage of proBDNF into mature BDNF, which was in line with the low level of tPA mRNA in the PSD group. The level of mBDNF/proBDNF significantly increased after 4 weeks HIIT compared with MICT. Besides, the mBDNF/proBDNF ratio was closely related to various parameters of behavioral tests. Thus, why do not we speculate that HIIT significantly improve the depression-like behavior in the PSD rats compared with MICT via enhancing the relative level of mBDNF and proBDNF in the hippocampus.

Accumulating previous evidences have proven that the tPA, widely expressing in the central nervous system, cleaves plasminogen to generate plasmin and in turn

converts the precursor proBDNF to mBDNF.¹⁷ So we boldly conjecture that tPA plays an important role in stress reaction and depression. The present study showed that the expression of tPA mRNA in the PSD group was significantly lower than that in the control and Sham groups. Accompanied by the improvement of depression behavior during the various behavioral tests, the level of tPA mRNA increased after HIIT and MICT. And the growth in the HIIT group was more obvious than that in the MICT group. Consistent with our results, blocking tPA expression can lead to depression-like behavior, while tPA over-expression is protective.¹⁸ In addition, BDNF has also been reported to stimulate tPA expression in neurons.¹⁹ Thus, there may be mutual stimulation between BDNF and the molecule involved in its post-translational processing.

The BDNF protein is encoded by a gene that is also called BDNF, found in humans on chromosome 11,²⁰ which is released from neurons in the adult central nervous system and widely distributed in hippocampus, cortex, and basal forebrain.²¹ Knockdown of BDNF expression, specifically in either CA1 or DG region of hippocampus, prevented antidepressant-like effect of desipramine and citalopram.²² ProBDNF knock-in mice depressed synaptic transmission, impaired LTP, and enhanced LTD in the CA1 region of the hippocampus.²³ In tune with our results, neuronal cells were loosely arranged or missing in the PSD group. Moreover, the low expression of BDNF and high level of proBDNF were observed in various subfields in the PSD group. In contrast, our study showed that HIIT induced significant increase of BDNF and decrease of proBDNF in the hippocampus, especially in the CA3 and DG regions, compared with MICT group. Further, the neuronal cells specifically in both CA1 and DG regions of hippocampus in the HIIT group were arranged more neatly and densely than that in the MICT group. The mBDNF/proBDNF ratio in the CA3 area of the HIIT group was slightly lower than that in the control group and nearly equal to the Sham group for the high level of proBDNF. Consistent with that, the result of Nissl staining showed that the neuronal cells in the CA3 region of hippocampus in the HIIT group were loosely arranged and lightly stained than that in the other groups. Moreover, the level of NR2A in the HIIT group was higher and the level of NR2B was lower than that in the MICT group. These findings implied that HIIT might improve the neural plasticity in the hippocampus through regulating the relative level of mBDNF and proBDNF. Supporting our point of view, the expression of tPA mRNA in the HIIT group was higher than that in the MICT group, for the tPA has also been demonstrated to cleave NMDA receptor, enhance glutamate transmission, and reduce the apoptotic cell death in cerebral ischemia models.^{24,25} It was noteworthy that there was no significant visual difference in CA3 observed in Nissl staining in the MICT and HIIT groups compared to the PSD group

despite of increasing expression of mBDNF. Further studies are needed to explore the role of area CA3 in neural plasticity.

In conclusion, our study first demonstrated the advantageous effect of both HIIT and MICT on depression, mBDNF/proBDNF ratio and neural plasticity in the hippocampus in PSD rats. It also suggested that HIIT is more preferable and effective as a novel form of exercise training compared with MICT. Based on the multiple effects of BDNF and important actions of tPA in the brain, it seems crucial to maintain the level of mBDNF and proBDNF within a homeostatic range, might being a potential therapeutic target for the treatment of mood disorders.

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