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The Effects of Voluntary, Involuntary, and Forced Exercises on Motor Recovery in a Stroke Rat Model

Zheng Ke, Shea-Ping Yip, Le Li, Xiao-Xiang Zheng, Wing-Kin Tam, Kai-Yu Tong, Se*nior Member, IEEE*

Abstract—**Stroke rehabilitation with different exercise paradigms has been investigated, but a comparison study on motor recovery after voluntary, involuntary, and forced exercises is limited. The current study used a rat brain ischemia model to investigate the effects of voluntary wheel running, involuntary muscle movement caused by functional electrical stimulation (FES), and forced treadmill exercise on motor recovery and brain BDNF changes. The results showed that voluntary exercise is the most effective intervention in upregulating the hippocampal BDNF level, and facilitating motor recovery after brain ischemia.**

I. INTRODUCTION

STROKE is a leading cause of long term morbidity and disability among aged people in many countries and consumes enormous economic and human resources every year. Different exercise rehabilitations have been substantiated effective in facilitation of recovery after stroke in animal models [1-3]. Among different exercise paradigms, voluntary wheel running, forced treadmill running, and involuntary muscle movement caused by electrical stimulation are the commonly adopted exercise models. Apart from their physical benefits, these exercises have been separately demonstrated to improve cognitive function and facilitate neural rehabilitation after brain damage [1-3]. It is important to know which rehabilitation intervention is more effective in facilitating motor recovery and up-regulating brain neurotrophic factor (BDNF) which is a leading factor in learning and memory, after brain ischemia.

Many studies on animals showed beneficial effects of treadmill exercise such as a smaller brain infarct volume or better neurological function either before or after stroke when compared with spontaneous recovery [2,4]. However, some studies suggested that such beneficial effects only existed in low-intensity treadmill (15m/min) running rats, and moderate-intensity treadmill (25m/min) would elevate serum corticosterone [5]. Wheel running is generally regarded as a type of voluntary exercise in animal models, and it does not activate systemic stress [6]. Although some study suggests that voluntary wheel running is not efficient in reducing brain infarct volume compared with forced treadmill running, Marin and his group concluded that there was no direct relationship between brain infarct volume and motor recovery [1]. Moreover, Arida's group found that voluntary exercise showed superior effects in terms of plastic changes in the dentate gyrus [7]. In accordance with this, Huang and his colleagues showed that upregulation of BDNF lasted seven and two days in wheel group and treadmill group respectively [8]. Involuntary exercise such as functional electrical stimulation (FES) by stimulating the paralyzed muscle by a specific stimulation pattern has also been involved in stroke rehabilitation program. In animal studies, rats receiving such electrical stimulation during brain ischemia showed decreased infarct volume and better behavioral outcomes [3].

To date, effectiveness of voluntary, involuntary and forced exercises has not been fully compared under an animal brain ischemia model. This study is to investigate the functions of these three rehabilitation interventions in the regulation of brain BDNF expression and their effects in facilitating motor recovery. The comparison results would help to provide more information for the clinical practice in the future.

II. METHODOLOGY

A. Surgical Preparation

All procedures performed were approved by the Animal Ethics Review Committee of the University. 150 young male Sprague-Dawley (SD) rats with body weight between 280-320g provided by the Central Animal Facility of the University were involved in this study. Briefly, all the rats were firstly trained to run on the treadmill (600m) and in the wheel (600m) as accommodation. Rats that did not run the minimum amount of distance of the treadmill and in the wheel were dropped out. Rats that passed the accommodation (n=117) were randomly divided into two groups: 27 rats in the involuntary exercise group (I-Ex, figure 1), and 90 rats in the other group. Electrode-implantation surgery was carried out on the I-Ex when rats were anesthetized by 10% chloral hydrate (0.4 mg/kg for induction and 0.02 mg subsequently). Incisions were made on the skull to expose the bregma, and on the left hindlimb to expose the tibialis anaterior (TA) and medial gastrocnemius (MG). Four teflon-coated stainless

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Z. Ke is with the Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China.

S. P. Yip is with the Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China.

L. Li was with the Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China. He is now is with the Department of Rehabilitation Medicine, The First Affiliated Hospital, Sun Yat-sen University,Guangzhou, China.

X. X. Zheng is with the College of Biomedical Engineering and Instrument Science, Zhejiang University, Hangzhou, China

W. K. Tam is with the Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China.

K. Y. Tong is with the Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China. (corresponding author: phone: 852-27667662. email: k.y.tong@polyu.edu.hk)

steel wires (AW633, Cooner Wire, USA) were passed subcutaneously from the skull to the incisions on the limb. One end of each wire was fixed by stripping insulation off and looping them around the bellies and tendons of TA and MG, respectively, and the other ends were soldered to a 4-pin connecter and fixed on the skull with three screws, which were implanted on the surface of the skull, and fixed with dental cement [9].After suture, thresholds of stimulation voltages which allowed TA (1.5-6V) and MG (2-8V) contraction were tested and recorded (S8800 Stimulator, Astro-Med, USA.) when the rat were still under anesthesia. After 3 days rest, I-Ex rats (n=27) and other 90 rats were induced ischemic stroke with intraluminal suture middle cerebral artery occlusion/reperfusion (MCAo/r) model under the same anesthesia method mentioned above [10]. The rat's right MCA was blocked by a 4-0 monofilament nylon suture with its rounded tip for 90 minutes, and allowed reperfusion by withdrawing the suture. The damages produced in the brain include striatum and frontoparietal cortex. Except the I-Ex (n=14), the successful induced stroke rats were randomly divided into three groups: voluntary wheel exercise group (V-Ex, n=14), forced treadmill exercise group (F-Ex, n=15), and control group (Con, n=14). The three exercise group rats would receive a 7-day rehabilitation intervention (I1-I7), while Con rats were put in normal cage and allowed spontaneous recovery.

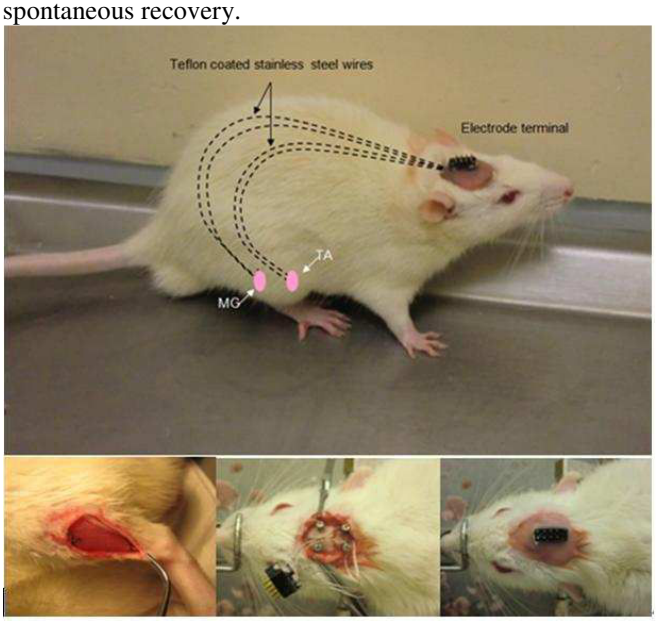

Fig. 1. I-Ex group rats. implantation of electrodes: four teflon-coated stainless steel wires are attached to the muscle bellies of TA and MG on the affected hindlimb, and the screws and headset for fixing the electrodes wire on the skull.

B. Rehabilitation Intervention

24 hours after the MCAo/r surgery, V-Ex rats were housed individually in a cage with a running wheel assembly and let free to run (manufactured by Kan Kee Sheet Metal Works, Hong Kong, figure 2). The circumference of the wheel was 1m, and the wheel was connected to a switch that counted each revolution [4]. F-Ex rats were forced to run on the motor-driven treadmill (KN-73, Natsume Ltd., China, figure 3) at a speed of 20m/min with a slope of 0°for a total of 30 minutes every day [11]. The 30-minute exercise was separated to three 10-minute sessions and with a 10-minute rest between each running sessions. The running duration on the treadmill was based on pilot experiment on rat wheel running and the experiment setup was intended to match the running distance between V-Ex and F-Ex. In this study, the distances were similar in V-Ex and F-Ex (average 612.24m and 600m, respectively) on the first day of intervention (I1). V-Ex rats gradually increased running distance in the intervention period from I2-I7. I-Ex rats were electrical stimulated with a biphasic stimulation with frequency 100Hz, pulse width 300μs and stimulation intensity based on muscle tests during anesthesia. The stimulation lasted a total of 30 minutes with a 10-minute rest between 10-minute stimulation sessions every day. The training and rest time in the stimulation protocol was planned to match with the F-Ex intervention protocol. The stimulation pattern (50ms TA stimulation, 150ms MG stimulation, and 300ms rest) imitated the gait pattern of rat running on the treadmill at the speed of 20m/min. The Con rats received no intervention and stayed in the normal cage.

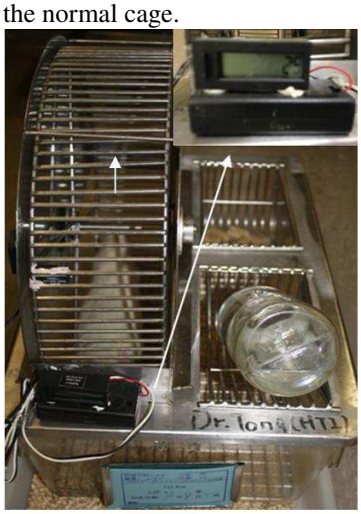

Fig. 2. the wheel training system: a switch counts and records the number of revolutions.

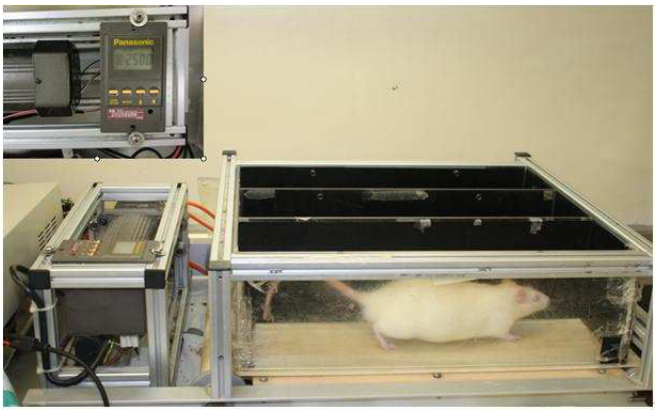

Fig. 3. The treadmill training system: rats on the treadmill runs with 0° slope at a speed of 20m/min.

C. Motor Recovery Test & BDNF Evaluation

The De Ryck's behavioral test was applied to evaluate the motor function recovery after stroke. The behavioral test was conducted daily during the 7-day intervention period (I1-I7) and repeated for three times after everyday intervention and the average score was recorded. The behavioral test was

based on the protocol of De Ryck et al., which is a 16-point scale including 8 tests with each scored from 0 to 2: 0 (maximum deficit), 1 (incomplete and/or delayed (>2 seconds) placing compared with the unaffected limb), and 2 (no deficit) [12]. Six out of 8 tests specifically evaluated forepaw's functions, including postural reflex, visual placing in the forward and sideways directions, tactile placing of the dorsal and lateral paw surfaces, and proprioceptive placing, while the other 2 examined the hindlimb's tactile placing of the lateral paw surfaces, and proprioceptive placing [3].

All rats were sacrificed by decapitation under anesthesia within 2 hours of their last intervention and assessment. After sacrifice, the rat's brain was obtained by removing the skull, and the cerebral cortex was carefully removed to extract the striatum and hippocampus. The striatum, the motor cortex of the affected area, and the hippocampus were obtained to assess the levels of BDNF by E_{max}® ELISA kit (Promega, Wisconsin, USA). All procedures were carried out according to the manufacturer's instructions.

D. Statistical Analysis

Results were expressed as means ± SD. SPSS (version 15.0) was used for statistical analyses in this study. One-way analysis of variance (ANOVA) with Bonferroni post-hoc test was used to compare the serum corticosterone level, and the BDNF levels in the hippocampus, striatum, and cortex among four groups. In the comparison based on the behavioral test, multivariate analysis of covariance (MANCOVA) incorporating all outcome measures recorded from I1-I7 was used to reduce the probability of type I error owing to multiple comparisons [13]. This is a technique for assessing group differences across multiple metric-dependent variables simultaneously, based on a set of categorical variables as independent variables. The within-subject factor was set as time and the between-subject factor was set as group. The I1 measurement of each respective outcome was entered as covariate. If the MANCOVA revealed a significant effect, post-hoc analysis using Bonferroni correction was used to indicate significant differences between particular groups. The level of statistical significance was set at 0.05.

III. RESULTS

A total of 57 ischemic stroke rats with motor deficits were assigned into four groups, and 45 of these rats finished the 7-day intervention. Twelve rats that died during the intervention period were not included in the BDNF analysis, and the intention-to-treat method was used to analyze their behavioral scores, which means that the scores of their unfinished days were the same as that of the last score that we recorded on the day before it died.

The motor function recovery outcome was presented as the behavioral score change and is shown in Figure 4. Significant differences among the four groups were found from day I3 and lasted until the end of the intervention period in the behavioral score (MANCOVA *P<0.0001*). At the end of the intervention, V-Ex had significant higher behavioral test score than I-Ex (*P=0.014*), F-Ex (*P<0.0001*), and Con (*P<0.0001)*. The brain BDNF levels are shown in Figure 5. Both V-Ex and I-Ex had higher hippocampal BDNF concentration than F-Ex and Con. Besides, I-Ex had significantly higher striatal and cortical BDNF concentrations than F-Ex and Con.

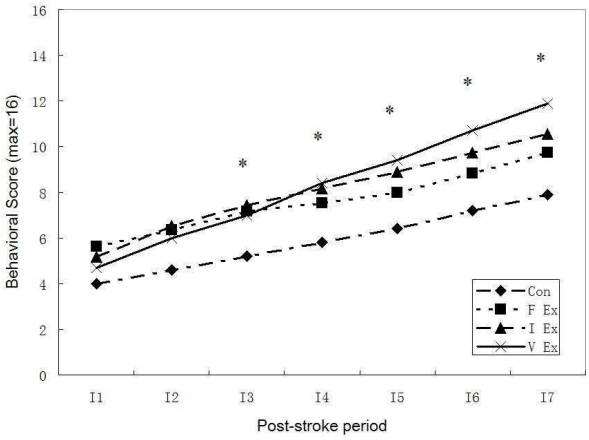

Fig. 4. The motor recovery was evaluated with daily behavioral score of the Con (♦), F-Ex (■), I-Ex (Δ), and V-Ex (x) during the 7-day intervention. *:a significant difference was revealed with MANCOVA from I3, and at the end of the training period, V-Ex has significant higher behavioral score than I-Ex, F-Ex and Con in the post-hoc.

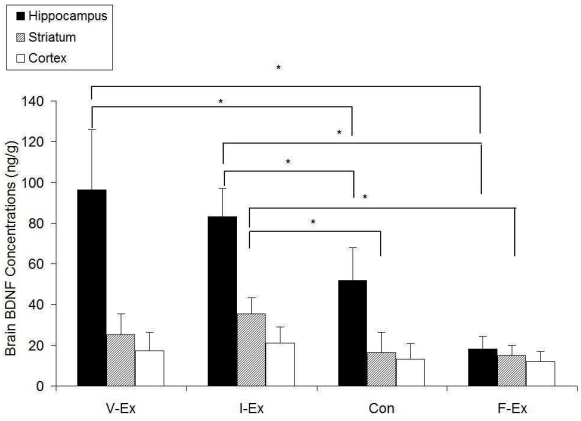

Fig. 5. The neurological status was evaluated with A: brain BDNF concentrations, and stress response was detected with B: serum corticosterone level. Values shown are mean ± SD *: a significant difference was revealed with one-way ANOVA.

IV. DISCUSSION

The current study compared the effectiveness between voluntary, involuntary, and forced exercises under similar training intensity with the evaluation of the motor recovery, and their functions of regulating brain BDNF levels after brain ischemia in a rat model. Our results showed that all three types of exercise applied in this study had better motor recovery than the control group. The voluntary exercise induced higher hippocampal BDNF level, better improvement in motor recovery when compared with involuntary exercise, forced exercise, and control groups.

BDNF is a powerful differentiation factor distributed widely in the central nervous system, and its concentration is particularly high in the hippocampus [14]. Our results also showed that BDNF concentration was higher in the hippocampus than in the cortex and striatum for all four groups (figure 5). BDNF has been crucially implicated in

neuroplasticity and is present throughout our life to enable essential functions such as learning and memory [15]. Recent studies suggested that exercise-induced enhancement in learning and memory is dependent on an increased BDNF level in hippocampal BDNF level, and the behavioral recovery was also correlated with a cell proliferation in the rat hippocampus [16]. Moreover, BDNF had also been proved to consolidate short-term memories into long-term memories [13]. In adult rodents, BDNF also showed characteristics of neuroprotection which promoted survival of hippocampal, striatal, and septal neurons in culture and *in vivo* by protecting the brain against such insults as focal brain ischemia [17]. Evidence from other studies has revealed the effectiveness of spontaneous wheel running in the up-regulation of BDNF in the rat hippocampus [8]. Our results also showed that the hippocampal BDNF level in V-Ex rats was significantly higher than those of the Con and F-Ex. As first reported by Vanderwolf in 1969 [18], voluntary exercise activates a persistent firing pattern (known as theta-rhythm) in the rat hippocampus, and this firing pattern is dependent on cholinergic and GABAergic neurons. Such theta bursts lead to the secretion of BDNF in the hippocampus [19]. These evidences might be related to our results that V-Ex had the highest hippocampal BDNF level, and the highest last-day behavioral test score. Our results also indicated that the motor recovery seems to be more related to the hippocampal BDNF level than to the striatal or cortical BDNF levels, as V-Ex had high hippocampal but low striatal and cortical BDNF levels.

In this study, the results showed that I-Ex rats had the highest striatal and cortical BDNF levels. This might be attributed to the fact that electrical stimulation of the affected muscle groups brings about a series of physiological effects, including an increase in metabolism and cerebral blood flow (CBF) [20]. Electrical stimulation of peripheral nerves induces contraction of innervated skeletal muscles via motor nerve fibers, which consequently activates the CNS via sensory nerve fibers. With the stimulation of CNS, an increase of CBF is expected, and such linkage between neuron activities and CBF is also called activation-flow coupling. Burnett et al. demonstrated that, with the electrical stimulation of the rat's forepaw, the activation-flow coupling response was preserved over a broad range of baseline flow values during the MCAo/r [3]. As a result, a reasonable explanation is that electrical stimulation increases CBF, which consequently activates neurons and up-regulates chemicals such as trophic factors in these neurons.

The results of the present study demonstrated that voluntary exercise was the most effective intervention in facilitating motor recovery, followed by involuntary exercise through functional electrical stimulation. The results also showed that voluntary exercise could greatly up-regulate the hippocampal BDNF level, indicating that hippocampal BDNF level might relate to the motor recovery level. Forced treadmill exercise was shown to suppress BDNF circulation in the rat brain, which probably diminished the beneficial effect of training on persons after stroke. Future studies may explore the effects of these exercise interventions in the infarct volume and neurogenesis.

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