「 Effects of voluntary and forced exercises on motor function recovery in intracerebral hemorrhage rats 」
―自発運動ならびに強制運動が脳出血モデルラットの機能回復に及ぼす影響―

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Abbreviation

BDNF: 脳神経栄養因子（brain-derived neurotrophic factor）

F-Ex.: 強制的トレッドミル走行運動（forced treadmill running）

ICH: 脳出血（intracerebral hemorrhage ）

MDS: 運動障害スコア（motor deficit score）

mEPSC: 興奮性シナプス後電流（miniature excitatory postsynaptic current）

NACc: 側坐核（nucleus accumbens）

Non-Ex.: 非運動（no exercise）

PBS: リン酸緩衝食塩水（phosphate buffered saline）

V-Ex.: 自発的回転ケージ走行運動（voluntary wheel cage running）

Introduction

Mortality rates after intracerebral hemorrhage (ICH) have decreased with technological progress, but there is a large burden related to secondary insults that reduces a patient's quality of life after survival [1]. Motor paralysis is a severe after effect of ICH that reduces a patient's bodily functions as well as mental health, causing depression and apathy [2,3]. A rehabilitative approach was reported to be beneficial for promoting stroke recovery in general. However, functional recovery following stroke depends on the types of exercises performed [4]. Of note, it was reported that psychological factors such as motivation combined with exercise affected the recovery of motor function [5]. Forced and voluntary exercises are frequently used as effective rehabilitation training for animals, and wheel cage running is a type of voluntary exercise without stress [6]. Werme et al. reported that wheel cage running increased the expression of ΔFosB protein, a transcription factor that determines repeated neural activation, in the nucleus accumbens (NACc) [7]. In contrast, treadmill running is a type of forced exercise with stress. Ke et al. demonstrated that corticosterone, a stress hormone, was increased in rats that performed forced exercise after stroke compared with rats performing voluntary exercise [8]. These findings were reported in separate studies, and how different exercises affect rehabilitation and functional recovery related to neural activation and synaptic plasticity is poorly understood. Furthermore, the effect of psychological factors such as motivation and stress on the rehabilitation of motor recovery is unknown.

This study assessed motor functional recovery with voluntary or forced rehabilitation after collagenase-induced striatal ICH in rats. In addition, we investigated the mechanisms involved in how different exercises affected motor functional recovery. Some of the results were already published in abstract form [9].

Methods

1. Subjects and experimental protocol

Thirty-seven male Sprague Dawley rats (230–280 g, 8 weeks of age, Crea Japan, Tokyo) were housed under a controlled temperature (25°C) during a 12-hour light/dark cycle with free access to food and water. The experimental procedures employed in this study followed the guidelines for animal research by the Physiological Society of Japan and Hirosaki University Graduate School of Medicine. All efforts were made to minimize the suffering and number of animals used. All experiments were performed following the design shown in Fig. 1.

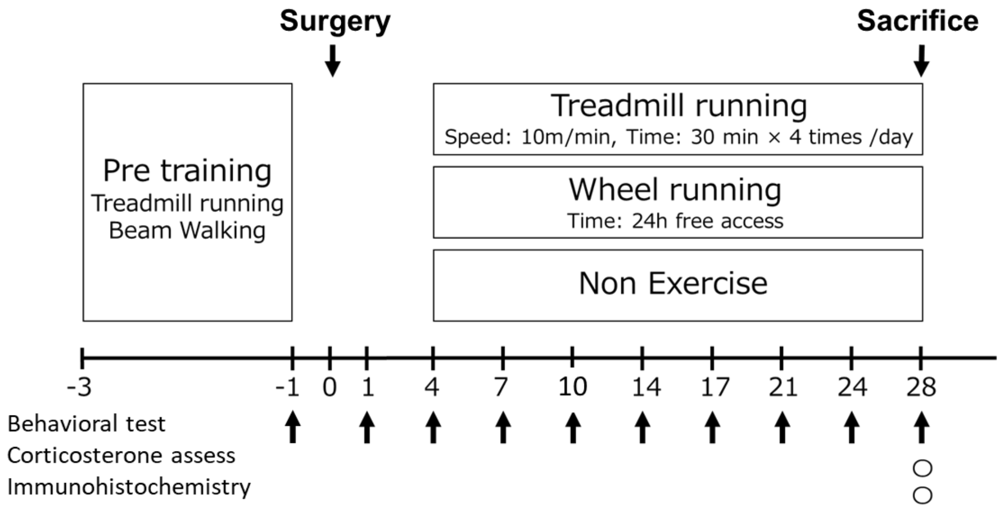

Fig. 1 Experimental design

2. Surgery

Hemorrhage was induced as previously described [9-11]. Thirty-seven animals were anesthetized with a combination of three anesthetics (medetomidine 0.15mg/kg, midazolam 2.0mg/kg, butorphanol 2.5mg/kg) and then placed on a stereotaxic frame. Collagenase type IV (Sigma-Aldrich, St. Louis, MO, USA) was diluted to 200 U/ml in sterile saline, and a total volume of 1.2 µl was injected into the right striatum thorough a small hole drilled 3.0 mm lateral to the midline, 0.2 mm anterior to the coronal, and 6.0 mm deep from surface of the brain.

3. Behavioral assessment

Motor deficit score

The motor deficit score (MDS) [10,11] included (1) observation of spontaneous ipsilateral circling, graded from a score of 0 (no circling) to 3 (continuous circling); (2) contralateral hindlimb retraction, which measured the ability of the animal to replace the hindlimb after it was displaced laterally by 2–3 cm, graded from a score of 0 (immediate replacement) to 3 (replacement after minutes or no replacement); (3) beam walking ability, graded from a score of 0 for a rat that readily traversed a 2.4 cm wide, 80 cm long beam to 3 for a rat unable to stay on the beam for 10 s; and (4) bilateral forepaw grasp, which measures the ability to hold onto a 2 mm diameter steel rod, graded from a score of 0 for a rat with normal forepaw grasping behavior to 3 for a rat unable to grasp with its forepaws. The scores from four subscales were added together to give the MDS (maximum possible score, 12). This test was performed at 1, 4, 7, 10, 14, 17, 21, 24 and 28 days after surgery.

Beam walking test

A narrow (a 1.0 cm wide, 120 cm long) and wide (a 2.4 cm wide, 120 cm long) Beam Walking test was carried out at 1, 4, 7, 10, 14, 17, 21, 24, and 28 days after surgery. The training was carried out before the surgery and lasted until they traversed the beam without more than two slips [10,11]. The performance was rated on a 7 point scale: (1) the rat was unable to place the affected hindlimb on the horizontal surface of the beam; (2) the rat placed the hindlimb on the beam and maintained balance but was unable to traverse the beam; (3) the rat traversed the beam dragging the affected hindlimb; (4) the rat traversed the beam and placed the affected hindlimb on the horizontal surface of the beam once; (5) the rat crossed the beam and placed the affected hindlimb on the horizontal surface of the beam to aid less than half of its steps; (6) the rat used the affected hindlimb to aid more than half of its steps; and (7) the rat traversed the beam with no more than two foot slips. This test was performed at 1, 4, 7, 10, 14, 17, 21, 24, and 28 days after surgery.

4. Rehabilitative training

Twenty-six rats were divided into three groups: Forced treadmill running group (F-Ex., n = 8), voluntary wheel cage running group (V-Ex., n = 8), and no exercise group (Non-Ex., n = 10). In the trained groups, rehabilitative training was carried out at 4–28 days after surgery.

Treadmill running

Animals in the F-Ex. group were run on a five-lane treadmill (Muromachi Kikai, Tokyo), at a speed of 10 m/min for 30 min under the electrical shocks. The training was performed 4 times/day with an interval of more than 60 min between sessions.

Wheel cage running

Animals in the V-Ex. group were housed in a cage with free access to a running wheel (Lafayette Instrument, USA). The mean number of revolutions were calculated and the running distances were recorded each day (mean 1224 ± 86 m).

5. Evaluation of the stress and motivation status after rehabilitation

Evaluation of the stress and motivation status after rehabilitation

Fifteen rats were used to evaluate the changes of stress and motivational status after rehabilitation: F-Ex., V-Ex., and Non-Ex. (each group, n = 5). The immunoreactivity of ΔFosB in the NACc was used as a marker of activated neuron on the reword system, and corticosterone concentrations were analyzed to assess the stress level with rehabilitation.

6. Immunohistochemistry

Rats were placed under deep anesthesia with sodium pentobarbital (45 mg/kg, i.p.), and then transcardially perfused with 0.9 % saline followed by 4% paraformaldehyde at 28 day after surgery. The brains were immediately removed from decapitated rats, post-fixed for 24 hours, and cryoprotected by incubation in 15% and then 30% sucrose diluted by 0.01 M phosphate buffered saline (PBS, pH 7.5) over 5 days at 4°C. Then, 40-μm thick coronal slices were taken from the nucleus accumbens (NACc) [12] and incubated with blocking buffer (5% goat serum in 0.01 M PBS) for 1 hour followed by a primary antibody (rabbit anti-FosB (1:250; Abcam, Boston, MA; ab184938)) with blocking buffer overnight at 4°C. After washing, the sections were incubated with anti-rabbit IgG secondary antibody (1:200; Vector Lab, Burlingame, CA) for 1 hour. The sections were incubated with avidin-biotin peroxidase complex reagents using a Vector ABC kit (Vector Labs) at room temperature. The horseradish peroxidase reaction was detected with diaminobenzidine and H_2O_2. After washing in 0.01 M PBS, the sections were counterstained with hematoxylin. The number of ΔFosB-positive cells were counted in the NACc using ImageJ (NIH, Bethesda, MD, USA). Twelve sections were selected randomly from 4 animals per group and the mean was calculated as the value of immunopositive cells within the observed area (10 × objective) per group.

7. Corticosterone Analysis

Corticosterone analysis was performed at 28 days after surgery. All rats were sacrificed under deep anesthesia within 1 hour of their final training. Blood samples were collected transcardially using a blood collection tube containing EDTA-2K after rehabilitation at the same timepoint throughout all experiments. The samples were centrifugated at 2000 ×*g* for 10 min, and then the plasma was separated out. The measurement of plasma corticosterone was performed using an Assay Max corticosterone ELISA kit (Assay Pro, St. Charles, MO, USA) in accordance with the manufacturer's protocol. The absorbance at a wavelength of 450 nm was measured using a Benchmark Microplate Reader (Bio-Rad, Hercules, CA, USA).

8. Quantitative Analysis and Statistical analysis

Statistical analysis was performed with SPSS for Windows (version 18.0, SPSS Inc., Tokyo, Japan). Behavioral assessments were analyzed by Tukey's test followed by ANOVA.

Data representing the amount of ΔFosB in NACc were expressed as the mean of immunopositive cells per mm^2 of section. The density of ΔFosB positive cells and the concentration of plasma corticosterone were analyzed by Tukey's test followed by ANOVA. Differences were considered statistically significant at $P < 0.05$.

Results

1. Exercise improves the recovery of motor function after stroke and voluntary training facilitates motor recovery.

We compared the effect of two exercises on motor recovery using Motor Deficit Score (MDS) and Beam Walking tests. MDS is a representative motor function assessment that consists of four components: spontaneous ipsilateral circling, contralateral hindlimb retraction, beam walking ability, and bilateral forepaw grasp. The MDS scores in the no exercise (Non-Ex.), forced treadmill running (F-Ex.), and voluntary wheel cage running (V-Ex.) groups are shown in Fig. 2A. The V-Ex. and F-Ex. groups had a lower MDS score compared with the Non-Ex. group. Furthermore, the V-Ex. group had a significantly lower score in the early phase compared with the F-Ex. group (Non-Ex. vs V-Ex. group: at days 7, 10, 14, 17, 21, 24, and 28. Non-Ex. vs F-Ex. group: at days 24 and 28. $P < 0.05$, Fig. 2A).

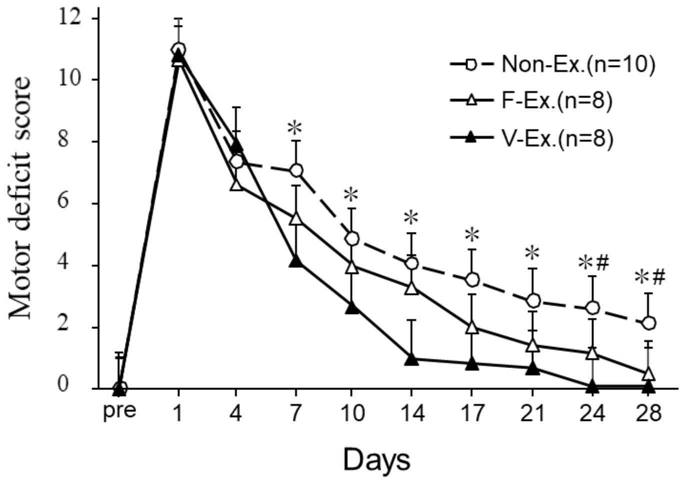

Fig. 2A The changing of motor deficit score after forced and voluntary rehabilitation.

Score：average ± SEM, #:N-Ex. vs F-Ex., ＊:N-Ex. vs V-Ex.

One-way ANOVA followed by Tukey test

Next, we performed two Beam Walking tests (wide or narrow) to assess coordinated movement. In the wide Beam Walking test, the score was significantly higher in the F-Ex. and V-Ex. groups compared with the Non-Ex. group. The V-Ex. group had a greater score than the F-Ex. group in the early phase (Non-Ex. vs V-Ex. group: at days 14, 17, 21, 24, and 28. Non-Ex. vs F-Ex. group: at days 24 and 28. P < 0.05, Fig. 2Ba). The score of the narrow Beam Walking test, a more severe task, was also significantly higher in the F-Ex. and V-Ex. groups compared with the Non-Ex. group (Non-Ex. vs V-Ex. group: at days 10, 14, 17, 21, 24, and 28. Non-Ex. vs F-Ex. group: at days 24 and 28. P < 0.05. Fig. 2Bb). However, the V-Ex. group had a significantly greater score than the F-Ex. group at day 14 (F-Ex. vs V-Ex. group: at day 14. P < 0.05. Fig. 2Bb).

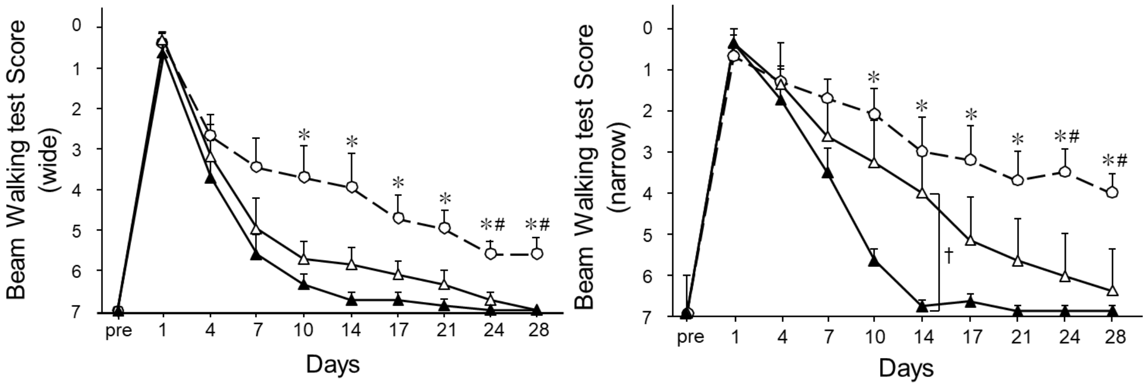

Fig. 2Ba Beam Walking test (wide) Fig. 2Bb Beam Walking test (narrow)

Score : average ± SEM, ＊: N-Ex. vs V-Ex., #: N-Ex. vs F-Ex., †: F-Ex. vs V-Ex. One-way ANOVA followed by Tukey test.

2. ΔFosB protein accumulates in the nucleus accumbens (NACc) of V-Ex. rats.

To examine the factors contributing to differences in recovery speed between the F-Ex. and V-Ex. groups, we investigated activation in the reward system. ΔFosB protein expression in the NACc, a representative brain area for motivation and reward systems, was analyzed by immunohistochemistry staining to determine whether voluntary and forced exercises influenced neural activation. Immunohistochemistry revealed the presence of ΔFosB positive cells in the NACc of control and running rats (Fig. 3Aa, Ab, Ac). At 28 day, there was limited expression of ΔFosB protein in the Non-Ex. and F-Ex. groups (Fig. 3Aa, 3Ab). Counts of immunopositive cells in the NACc revealed an increasing number expressed ΔFosB in the V-Ex. group. (n = 12, each group contained 4 rats, P < 0.05, Fig. 3Ac, 3B).

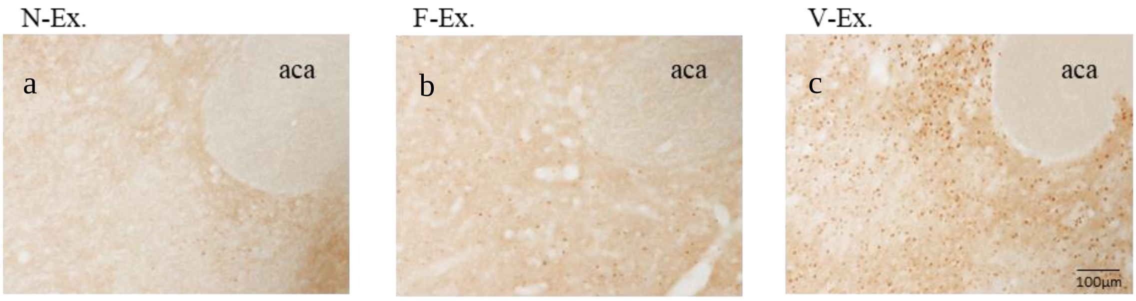

Fig. 3A Immunohistochemistry

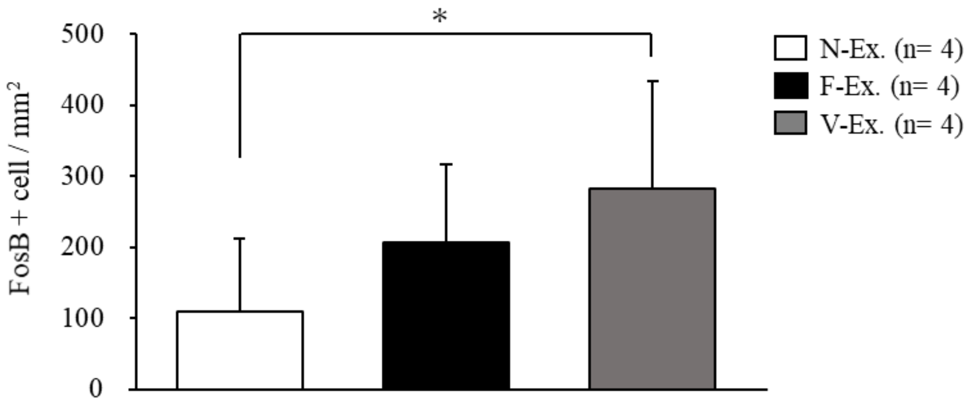

Fig. 3B Difference in motivation status between the forced and voluntary exercise.

Score：average ± SEM, ＊p < 0.05.

One-way ANOVA followed by Tukey test

To investigate whether running exercise had a negative effect on stress, we evaluated stress levels by measuring the concentration of plasma corticosterone. The concentration of plasma corticosterone in the F-Ex. group was significantly higher than in the Non-Ex. and V-Ex. groups (F-Ex. vs Non-Ex. group P < 0.01, F-Ex. vs V-Ex. group P<0.05, Fig. 3C). There were no differences between the N-Ex. and V-Ex. groups.

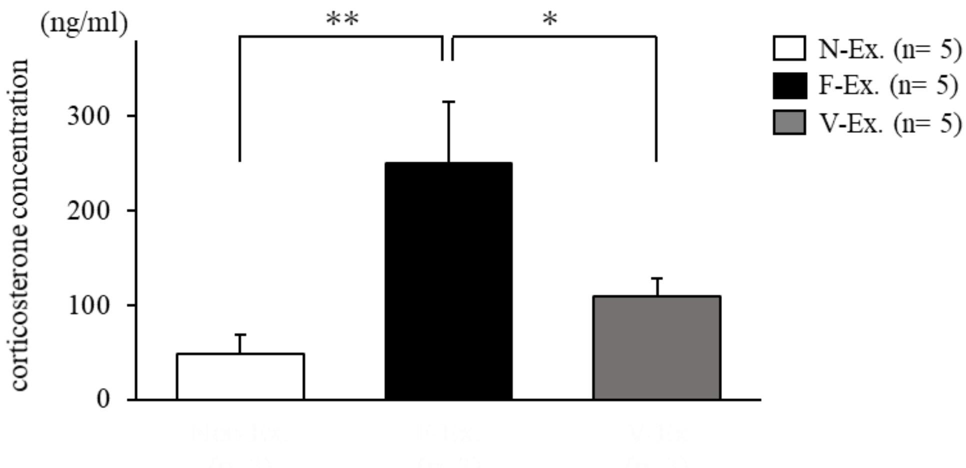

Fig. 3C Difference in stress status between the forced and voluntary exercise.

Score：average ± SEM, ## P < 0.01, # P < 0.05

One-way ANOVA followed by Tukey test

Discussion

In this study, we compared the effectiveness of forced and voluntary exercise on motor functional recovery in ICH rats. Next, we confirmed the difference of motor functional recovery by determining the status of motivation and stress through the rehabilitation period. Our results showed that both types of exercise performed on a treadmill or by wheel cage running significantly enhanced motor functional recovery compared with the Non-Ex. group after ICH (Fig. 2A, 2Ba, 2Bb). These results agree with previous reports where neurological and motor dysfunction were improved by wheel cage running or treadmill running [11,12]. Furthermore, in the narrow Beam Walking test (a difficult task), the effect on recovery was greater in the V-Ex. group compared with the F-Ex. group (Fig. 2Bb). Of note, it was reported that voluntary exercise improved motor coordination such as the locomotion in mice after spinal cord injury [13]. These differences might be involved in the recovery of the motor coordination by wheel cage running.

To investigate the differences in motor recovery between the V-Ex. and F-Ex. groups, the motivation and stress status of rats were measured after rehabilitation. The activation of the NACc area, a representative brain area for motivation, was assessed by the expression of ΔFosB protein in the NACc. It was reported that ΔFosB protein expression indicates neuronal activation in brain [14]. In the V-Ex. group, the density of ΔFosB protein in the NACc was significantly increased compared with the F-Ex. and Non-Ex. groups (Fig. 3B). The motivational aspects depend on reward processing in animal models and humans [15, 16]. It is well-known that rats and mice preferentially select wheel cage running [17,18]. In addition, the specific activation of the neural pathway from the ventral tegmental area (VTA) to the NACc increased the amount of voluntary wheel cage running [19]. These findings indicated that physical exercise is a rewarding behavior similar to drug abuse or feeding. Therefore, our results suggest that voluntary wheel cage running for functional recovery is associated with neural activation through the reward system.

Next, the status of stress was assessed by measuring the levels of plasma corticosterone, a stress hormone. Corticosterone is a key factor of many experiments assessing chronic stress [20], because it increases in response to stress, exercise, and food intake [21]. In this study, although rats ran the same distance on the wheel cage and treadmill during the rehabilitative period, the corticosterone level in the F-Ex. group was significantly higher than that in the V-Ex. group. Treadmill running was reported to increase corticosterone levels [22], which decreased the availability of brain-derived neurotrophic factor (BDNF) related to stress [23].

BDNF is an essential factor for the remodeling of neural networks and neurogenesis [24]. Physical exercise training by the voluntary wheel cage running significantly increased expression of BDNF in the brain in normal rats [25]. Therefore, our results suggest that voluntary exercise might promote functional recovery by increasing BDNF. However, forced running might elevate plasma corticosterone levels and diminish the beneficial effect of training on recovery from damaged corticospinal tracks. We consider that functional recovery after stroke was promoted by increased motivation through the reward center and diminished by stress via decreasing BDNF levels. These findings indicate that motor functional recovery depends on the intensity of exercise as well as psychological factors, such as stress and motivation, during rehabilitation.

This study showed that motivation and stress affected motor recovery, but the specific mechanisms involved remain unknown. We observed that the amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) in trained rats was greater than those in Non-Ex. rats using the whole-cell patch-clamp method at 14 days after ICH induction (data not shown). Thus, exercise after ICH might change the synaptic network related to spine size, density and/or neurogenesis. Further studies are needed to investigate neuronal plasticity in the brain during rehabilitation with psychological factors such as motivation and stress.

In summary, the present findings provide new and important information on the function of psychological factors during rehabilitation. We suggest that rehabilitation after stroke should involve training with physical intensity, and psychological effects such as motivation and stress should be monitored during exercise.

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Author Contributions

CS and JY conducted all experiments. CS, MM, SK, KS, and KA contributed to the animal experiments. CS, KT, SS, and MC contributed to the biological and histological analyses. CS and JY analyzed the data. KT, MC, and JY supervised all aspects of this study. CS, KT, MC, and JY wrote the manuscript.

References

1) Bonita R. Epidemiology of stroke. Lancet, 339 :342–344, 1992.

2) Whyte EM, Mulsant BH, Vanderbilt J, Dodge HH, Ganguli M: Depression after stroke: a prospective epidemiological study. J Am Geriatr Soc, 52 :774-778, 2004.

3) Winstein CJ, Stein J, Arena R, Bates B, Cherney LR, Cramer SC, et al.: Guidelines for adult stroke rehabilitation and recovery. a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke, 47 : e98-e169, 2016.

4) Auriat AM, Wowk S, Colbourne F. Rehabilitation after intracerebral hemorrhage in rats improves recovery with enhanced dendritic complexity but no effect on cell proliferation. Behav Brain Res, 214 :42-47, 2010.

5) Gainotti G, Antonucci G, Marra C, Paolucci S. Relation between depression after stroke, antidepressant therapy, and functional recovery: J Neurol Neurosurg Psychiatry, 71:258-261, 2001.

6) Yanagita S, Amemiya S, Suzuki S, Kita I. Effects of spontaneous and forced running on activation of hypothalamic corticotropin-releasing hormone neurons in rats: Life Sci, 80 :356-363, 2007.

7) Werme M, Messer C, Olson L, Gilden L, Thoré P, Nestler EJ, et al.: Delta FosB

regulates wheel running J Neurosci, 22 : 8133-8138, 2002.

8) Ke Z, Yip SP, Li L, Zheng XX, Tong KY: The effects of voluntary, involuntary, and forced exercises on brain-derived neurotrophic factor and motor function recovery: A rat brain ischemia model. PLoS One, 6 :e 16643, 2011.

9) Sato C, Sumigawa K, Koeda S. Mikami M, Akahira K, Yamada J: Voluntary rehabilitation for promoting motor paralysis recovery in intracerebral hemorrhage model rats. Annual meeting of Society for Neuroscience, Washington D.C 20171103.

10) Tamakoshi K, Ishida A, Takamatsu Y, Hamakawa M, Nakashima H, Shimada H, et al.: Motor skills training promotes motor functional recovery and induces synaptogenesis in the motor cortex and striatum after intracerebral hemorrhage in rats. Behav Brain Res, 216 :34-43, 2014.

11) Takamatsu Y, Tamakoshi K, Waseda Y, Ishida K: Running exercise enhances motor functional recovery with inhibition of dendritic regression in the motor cortex after collagenase-induced intracerebral hemorrhage in rats. Behav Brain Res, 300 :56-64, 2016.

12) Hicks AU, Hewlett K, Windle V, Chernenko G, Ploughman M, Jolkkonen J, et al.: Enriched environment enhances transplanted subventricular zone stem cell migration and functional recovery after stroke. Neuroscience, 146 :31-40, 2007.

13) Engesser Cesar C, Ichiyama RM, Nefas AL, Hill MA, Edgerton VR, Cotman CW, et al.: Wheel running following spinal cord injury improves locomotor recovery and stimulates serotonergic fiber growth. Eur J Neurosci, 25 :1931-1939, 2007.

14) Chen J, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ: Chronic Fos-Related Antigens: Stable Variants of ΔFosB Induced in Brain by Chronic Treatments. J Neurosci, 17: 4933-4941, 1997.

15) Lopes DA, Souza TMO, de Andrade JS, Silva MFS, Antunes HKM, Sueur-Maluf LL, et al.: Environmental enrichment decreases avoidance responses in the elevated T-maze and delta FosB immunoreactivity in anxiety-related brain regions. Behav Brain Res, 344 :65-72, 2018.

16) Li Z, Zhang CY, Huang J, Wang Y, Yan C, Li K, et al.: Improving motivation through real-time fMRI-based self-regulation of the nucleus accumbens. Neuropsychology, 32 :764-776, 2018.

17) Herrera JJ, Sofiya F, Parsa RG, Tyler W, Peter JC, Nathan G, et al.: Neurochemical and behavioural indices of exercise reward are independent of exercise controllability. Eur J Neurosci, 43 :1190-1202, 2016.

18) Belke TW, Wagner JP: The reinforcing property and the rewarding aftereffect of wheel running in rats: a combination of two paradigms. Behav Processes, 68 :165-172, 2005.

19) Linde B, Azar O, Mieneke CML, Inge GWD, Ellen CW, Geoffrey VDP, et al.: Chemogenetic activation of dopamine neurons in the ventral tegmental area, but not substantia nigra, induces hyperactivity in rats. Eur Neuropsychopharmacol, 26 :1784-1793, 2016.

20) Brown DA, Johnson MS, Armstrong CJ, Lynch JM, Caruso NM, Ehlers LB, et al. Short-term treadmill running in the rat: what kind of stressor is it? J Appl Physiol, 103:1979-1985, 2007.

21) Love AC, Lovern MB, DuRant SE: Captivity influences immune responses, stress endocrinology, and organ size in house sparrows (Passer domesticus). Gen Comp Endocrinol, 252 :18-26, 2017.

22) Chen C, Nakagawa S, An Y, Ito K, Kitaichi Y, Kusumi I: The exercise-glucocorticoid paradox: How exercise is beneficial to cognition, mood, and the brain while increasing glucocorticoid levels. Front Neuroendocrinol, 44 :83-102, 2017.

23) Leasure JL, Jones M: Forced and voluntary exercise differentially affect brain and behavior. Neuroscience, 156 :456-465, 2008.

24) Gorski JA, Zeiler SR, Tamowski S, Jones KR: Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. J Neuroscience, 30 :6856-6865, 2003.

25) Shawne AN, Fernando GP, James C, Carl WC: Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Research, 726 :49-56, 1996.

Abstract

自発運動ならびに強制運動が脳出血モデルラットの機能回復に及ぼす影響

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脳卒中後の運動麻痺は、最適な治療法開発が喫緊の課題である。訓練に伴うやる気やストレスが機能回復に与える影響に注目が集まっており、心理的要素を加味した訓練効果の検証が必要である。そこで、本研究では半身に運動麻痺を呈する脳出血モデルラットを作出し、自発運動ならびに強制運動の実施による運動機能回復の違いを、運動に伴う心理的側面の影響から検証した。

雄SDラット（8 - 9週齢）を用いて、collagenase typeⅣを右線条体に注入し左半身に運動麻痺を呈する脳出血モデルラットを作製した（n= 41）。運動介入には強制運動としてトレッドミル（10 m /min）内での走行を30分/回、1日4セット行った（F-Ex.群、n= 13）を、自発運動としてケージに常設した回転かご内を自由に走行させた（V-Ex.群、n= 13, 走行距離：1223 ± 86 m）。コントロール群として運動介入を行わない非介入群を設けた（Non-Ex.群、n= 15）。運動機能評価にはMotor Deficit score（以下MDS）、Beam Walking Test （1.0cm、2.4cm幅）を

用い、評価時点は術前日および術後1-28日に行った。その結果、全ての運動評価項目において、非介入群に比べて強制ならびに自発運動群の成績は有意に向上した。加えて、四肢協調性の評価であるBeam Walking Testにおいては、1.0cm幅の難易度の高い課題における成績が強制運動群よりも自発運動群で有意に高く、自発運動群の回復効果が有意に高かった。麻痺改善効果の違いをストレスやモチベーションといった心理的要素から検証するために、モチベーション評価では、報酬系の責任領域である側坐核（以下NACc）中のdelta-FosB（以下ΔFosB）蛋白発現量を免疫染色法にて確認した。ΔFosBは神経活動の指標として広く用いられており、この蛋白量の増加は脳領域の賦活を表わす。ストレス状態の評価には、ストレスホルモンである血漿中コルチコステロン濃度を計測した。その結果、非介入群と強制運動群に比べて自発運動群でのΔFosB蛋白陽性細胞数が有意に多く、自動運動に伴うNACc賦活が確認できた。一方で血漿中コルチコステロンは、非介入群や自発運動群よりも強制運動群における濃度が有意に高く、強制運動に高いストレスを伴うことが明らかとなった。

以上の結果より、脳卒中後の機能回復にはトレッドミルによる強制運動よりも回転ケージによる自発運動の方が有効であり、この回復効果の違いには自発運動に伴うモチベーションや強制運動に伴うストレスといった心理的要因が影響している可能性が示唆された。