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Research report

Willed-movement training reduces motor deficits and induces a PICK1-dependent LTD in rats subjected to focal cerebral ischemia

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HIGHLIGHTS

- Willed-movement training can improve the motor performance of the ischemic rats.
- Willed-movement training can enhance LTD and PICK1 protein in the ischemia hemisphere.
- The enhanced LTD is activity-dependent through PICK1 mechanisms.

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ABSTRACT

Willed-movement (WM) training has been implicated in the promotion of motor function in human stroke survivors and focal ischemic rats. However, the molecular basis of changes in synaptic transmission following WM training remains unclear. In addition, studies examining the influence of rehabilitative training, such as skilled motor learning, on long-term depression (LTD) of synapses in the primary motor cortex have produced conflicting results. To identify the possible effects of willed movement on motor recovery, on expression of the protein interacting with C kinase 1 protein (PICK1), and on PICK1 related LTD, littermate rats were randomly divided into four groups: normal control, middle cerebral artery occlusion (MCAO), WM and environmental modification. Neurological and neurobehavioral assessments were performed for the rats with occlusion of the right middle cerebral artery. Double-labeling immunofluorescence staining was performed to detected expression of PICK1 and NeuN. Extracellular recordings were used to detect the basal extracellular field excitatory postsynaptic potentials and LTD with or without PICK1 inhibitor FSC231. The results showed that willed-movement training facilitated motor recovery after MCAO in rats, increased the PICK1 protein levels, and enhanced LTD in the ischemia hemisphere. The enhanced LTD for the rats after willed-movement training was attenuated by FSC231. Our results indicated that willed-movement training can enhance activity-dependent LTD through PICK1-dependent mechanisms in the ischemic hemisphere of rats.

1. Introduction

Motor skill training [1] or living in an enriched housing environment [2] can result in structural and functional plasticity of neurons in the motor cortex. Willed-movement (WM) training is defined as a voluntary motor training through which an individual pays attention to a goal and makes an effort to accomplish it [3]. WM therapy [4] using food motivation is similar to motor skill training [1] but involves more coordinated activities of the whole body and limbs. Similar to

Abbreviations: ACSF, artificial cerebrospinal fluid; EM, environmental modification; fEPSP, field excitatory postsynaptic potential; IP, ischemia penumbra; LFS, low-frequency stimulation; MCAO, middle cerebral artery occlusion; NC, normal control; PI, peri-infarcted; WM, Willed-movement.

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room- and ladder-enriched environments, WM therapy has been shown to significantly upregulate the mRNAs for α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptor subunits GluR1 and GluR4 in the ischemia penumbra (IP) region at a subacute stage [4]. Additionally, WM training has been clinically proven to be a promising approach to increase motor recovery after ischemic stroke [5]. These studies suggested that WM training might play a role in synaptic plasticity.

Protein interacting with C kinase 1 (PICK1) has been proven to be one of the key proteins in the regulation of synaptic plasticity [6–8]. PICK1 [9] is an AMPAR-binding [6,7] protein originally identified by its interaction with PKCα [9]. A role for PICK1 in synaptic plasticity has been established in an inhibitory avoidance task where PICK1 is essential for hippocampal-dependent learning in adult mice [6]. PICK1 also plays an essential role in regulating LTD in cerebellar [10] and hippocampal [11] synapses. Several studies have presented evidence that skilled motor learning over the course of 5 days increased the amount of LTD in the primary motor cortex contralateral to the trained [12,13] limb. Another study argued that stress-related effects produced by food deprivation and handling enhanced the induction of LTD in motor cortex pathways rather than the skilled motor learning [14]. In addition, no studies have tested PICK1-mediated LTD in focal ischemic animals following motor training. Therefore, in the present study we investigated the regulating role of PICK1 on LTD in rats after focal cerebral ischemia following WM training.

2. Materials and methods

2.1. Design

All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). These experiments were performed in accordance with the guidelines for the care and use of animals approved by the animal ethics committees of Central South University. A total of 114 adult male Sprague Dawley rats weighing 200 to 250 g were used in the study. All animals were kept on a 12 h light/dark cycle at 23 ± 2 °C room temperature. Rats with a neurological deficit score of 2 and 3, as evaluated at 2 h after recirculation, were used in this study. Littermate rats were randomly assigned to one of four groups: normal control rats that did not receive middle cerebral artery occlusion (MCAO) (NC, $n = 18$), rats that received only MCAO (MCAO, $n = 18$), rats that additionally experienced environmental modification (EM, $n = 18$), and rats that additionally received willed-movement therapy (WM, $n = 18$). The housing and feeding protocols of the rats in the EM and WM groups were similar to a previous study [4]. After training, EM and WM rats were housed in standard cages with 4 or 5 animals per cage, the same as the MCAO rats.

2.2. Training protocol

To accustom the rats to the training regime, rats in the EM and WM groups were given 3 days of preliminary training for 10, 20 and 30 min/day on the day 1, day 2 and day 3, respectively before surgery. The right MCAO was performed according to our previously study [4]. Rats in the EM and WM groups began to train 3 days after MCAO surgery for 30 min each day for 15 consecutive days. To minimize possible influence of the training on lesion size, which had been found in our preliminary study, we used a longer delay in the onset of training and shortened the duration of the manipulations compared to a previously study [4]. Following surgery and postoperative recovery, the WM rats could only obtain food by climbing a ladder or lateral cage wall to the top cover of the cage. To ensure that both EM and WM rats ate similar amounts of food and to ensure the attraction of the food for the WM rats, EM and WM rats were deprived of food for 12 h from 9 p.m. to 9 a.m. of the next day. Water was provided ad libitum for all rats. The NC and MCAO rats were not food deprived or handled. Video was recorded with a Sony video recorder (SONY-3CCD, Tokyo, Japan) from 9 a.m. to 9:30 a.m. for the WM and EM rats during the training time each day. The rats were killed on day 18 post-recirculation.

2.3. Neurological and neurobehavioral assessments

The frequency of climbing was assessed via video records [4]. Neurological examinations were performed 2 h after recirculation and then on days 1, 3, 7, 15, and 18 post-surgery until sacrifice using a modified neurological deficit scoring system according to Longa [15] and Berdenson [16]. Briefly, the neurological deficits were assessed on a seven-point scale: no neurological deficit = 0; forelimb flexion = 1; forelimb flexion with reduced resistance to lateral push = 2; circling toward the paretic side = 3; falling to the paretic side = 4; non-ambulatory and/or a depressed level of consciousness = 5; and death = 6. Investigators performing the outcome testing were kept blind to the group assignments.

2.4. Double-labeling immunofluorescence staining

Twenty four rats ($n = 6$ for each group) were deeply anesthetized with sodium pentobarbital (60 mg/kg) and were perfused through the ascending aorta with 50 ml of 0.01 M PBS (pH 7.4, room temperature) and then fixed by 300 ml of 4% paraformaldehyde in 0.1 M PB (pH 7.4, 4 °C). The brain was then dissected and postfixed in the same fixative solution for 24 h, and then placed in 20% and 30% sucrose solution at 4 °C until the brain sank to the bottom of the bottle. Coronal sections (30 μm, between −0.3 to 1.3 mm from bregma [17] of the brain were then cut using a Leica CM1900 cryostat (Leica Microsystems, Wetzlar, Germany). The slices were then placed in 0.01 M PBS (pH 7.4). Free-floating sections were rinsed with PBS (0.01 M, pH 7.4), permeabilized with 0.1% Triton X-100 in PBS, and blocked with 5% normal donkey serum. Then, the slice sections were incubated with a rabbit anti-PICK1 polyclonal antibody (Santa Cruz Biotechnology, diluted 1:50) combined with a mouse anti-NeuN (Millipore, Billerica, MA, diluted 1:100) monoclonal antibody for 48 h at 4 °C. The antibodies were detected with an Alexa-488-labeled donkey anti-rabbit antibody (Invitrogen; diluted 1:200) and Alexa-594-labeled donkey anti-mouse antibody (Invitrogen; diluted 1:200), respectively for 2 h at 37 °C. An inverted fluorescence microscope (Eclipse T1, Nikon, Melville, NY) and a confocal laser scanning microscope (LSM510, Zeiss; Jena, Germany) were used to observe the stained sections. For each rat, three bregma sections 1.2, 0.48, −0.24 were selected and counted at 20 × 10_magnification. The IC and IP fields, the adjacent fields close to the IP region and the nonischemic fields far from the IP region in the ischemic hemisphere in each section were scanned. Similarly, the corresponding fields for control rats in each section were also scanned. The expression of PICK1 was evaluated by counting the number of PICK1-positive cells in each scanning field. The number of NeuN-positive cells was counted within a 100 × 100 μm area at 5 random areas in the penumbra in each scanning field. Image-Pro Plus 6 software was used to automate the quantification of PICK1 and NeuN cells.

2.5. Extracellular Recordings

Forty eight rats ($n = 6$ for each subgroup with or without PICK1 inhibitor FSC231) were perfused transcardially with 50 ml artificial