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STAT3 signal that mediates the neural plasticity is involved in willed-movement training in focal ischemic rats*

Qing-ping TANG[1,2], Qin SHEN[3], Li-xiang WU[2], Xiang-ling FENG[4], Hui LIU[4], Bei WU[5],
Xiao-song HUANG[6], Gai-qing WANG[7], Zhong-hao LI[8], Zun-jing LIU[†‡8]

([1]Department of Rehabilitation, Brain Hospital of Hunan Province, Hunan University of Chinese Medicine, Changsha 410007, China)
([2]Department of Physiology, School of Basic Medical Sciences, Central South University, Changsha 410078, China)
([3]Department of Neurology, Xiangya Hospital, Central South University, Changsha 410008, China)
([4]Cancer Research Institute, Xiangya School of Medicine, Central South University, Changsha 410078, China)
([5]Department of Otolaryngology, Xiangya Hospital, Central South University, Changsha 410008, China)
([6]Department of Neurology, Brain Hospital of Hunan Province, Hunan University of Chinese Medicine, Changsha 410007, China)
([7]Department of Neurology, the Second Hospital, Shanxi Medical University, Taiyuan 030001, China)
([8]Department of Neurology, China-Japan Friendship Hospital, Beijing 100029, China)
[†]E-mail: liuzunjing@163.com

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Abstract: Willed-movement training has been demonstrated to be a promising approach to increase motor performance and neural plasticity in ischemic rats. However, little is known regarding the molecular signals that are involved in neural plasticity following willed-movement training. To investigate the potential signals related to neural plasticity following willed-movement training, littermate rats were randomly assigned into three groups: middle cerebral artery occlusion, environmental modification, and willed-movement training. The infarct volume was measured 18 d after occlusion of the right middle cerebral artery. Reverse transcription-polymerase chain reaction (PCR) and immunofluorescence staining were used to detect the changes in the signal transducer and activator of transcription 3 (STAT3) mRNA and protein, respectively. A chromatin immunoprecipitation was used to investigate whether STAT3 bound to plasticity-related genes, such as brain-derived neurotrophic factor (*BDNF*), synaptophysin, and protein interacting with C kinase 1 (*PICK1*). In this study, we demonstrated that STAT3 mRNA and protein were markedly increased following 15-d willed-movement training in the ischemic hemispheres of the treated rats. STAT3 bound to *BDNF*, *PICK1*, and synaptophysin promoters in the neocortical cells of rats. These data suggest that the increased STAT3 levels after willed-movement training might play critical roles in the neural plasticity by directly regulating plasticity-related genes.

Key words: Motor training, Signal transducer and activator of transcription 3 (STAT3), Brain-derived neurotrophic factor (BDNF), Protein interacting with C kinase 1 (PICK1), Neural plasticity
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1 Introduction

Motor training is known to be able to enhance the neuronal structural and functional synaptic plasticity in the motor cortex. Willed-movement (WM) training is a type of motor training, in which the individual makes an effort to accomplish motor tasks (Tang *et al.*, 2005; 2007). WM therapy for

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[‡] Corresponding author

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ORCID: Qing-ping TANG, http://orcid.org/0000-0002-8729-1490

rats can improve neurobehavioral performance, upregulate the messenger RNAs (mRNAs) for the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor subunits GluR1 and GluR4 (Tang *et al.*, 2007), and increase the levels of protein interacting with C kinase 1 (PICK1) (Tang *et al.*, 2013) and synaptophysin (data not shown) proteins in the ischemia hemispheres at their subacute stage. Additionally, WM training has been clinically demonstrated to be a promising approach to increase the motor recovery of stroke subjects with cognitive function deficits (Tang *et al.*, 2005). These studies suggested that WM training might play a role in synaptic plasticity. The activity-dependent synaptic changes are a function of the precise synaptic transmission (Bliss and Collingridge, 1993; Huang *et al.*, 2015). Neuronal transmission networks are involved in the molecular signaling that translates activities into long-lasting changes (Gao *et al.*, 2010).

Signal transducer and activator of transcription 3 (STAT3) has been identified as a transcription factor involved in the maintenance of early embryonic neocortical development (Yoshimatsu *et al.*, 2006; Oatley *et al.*, 2010) and neuronal survival (Yadav *et al.*, 2005). STAT3 signaling mediates axon elongation (Selvaraj *et al.*, 2012; Quarta *et al.*, 2014) and plays a pivotal role in early neural circuit formation (Bouret *et al.*, 2012). These studies suggest that STAT3 is involved in neural plasticity. However, studies have not tested the change of STAT3 expression levels following motor training, nor whether STAT3 is involved in regulating plasticity-related genes. In the present study, we investigated the possible role of STAT3 on neural plasticity in WM-trained rats which had suffered focal cerebral ischemia.

2 Materials and methods

2.1 Design

Ninety-eight adult male Sprague Dawley rats weighing 200 to 250 g were used in this study. All animals were housed at (23±2) °C room temperature with a 12-h light/dark cycle. A right middle cerebral artery occlusion (MCAO) was performed as in our previous study (Tang *et al.*, 2007). Only 36 survived rats with a neurological deficit score of 2 or 3 two hours after recirculation were recruited in this study.

Littermate rats were randomly assigned to three groups before ischemic surgery: rats that received only right MCAO, rats that additionally received environmental modification (EM), and rats that additionally received WM therapy. The housing, feeding, and training protocols of the rats in the EM and WM groups were the same as that in a previous study (Tang *et al.*, 2013). The rats were sacrificed 18 d after the surgery. The climbing frequency and neurological and neurobehavioral examinations were assessed according to our previous studies (Tang *et al.*, 2013).

2.2 Measurement of infarct volume

The infarct volume was measured using a 20 mg/ml 2,3,5-triphenyl tetrazolium chloride (TTC) staining protocol 18 d after MCAO. The rats were deeply anesthetized with sodium pentobarbital (60 mg/kg) and decapitated. The brain was then removed and coronally sliced into five 2-mm thick coronal sections. The slices were immersed in the TTC solution for 30 min and then fixed with 10% paraformaldehyde. The infarct volumes were calculated by measuring the infarct areas on each of the slices, multiplying these values by the slice thicknesses, and adding the infarct areas of all slices together. To eliminate the effect of brain edema, the corrected infarct volume was calculated using the following formula: intact contralateral hemisphere volume−(ipsilateral hemisphere volume−measured infarct volume) (Schabitz *et al.*, 1999).

2.3 Reverse transcription-polymerase chain reaction (RT-PCR)

The tissue samples of the ischemic penumbra (IP) were dissected according to the method descripted by Ashwal *et al.* (1998). Briefly, a longitudinal cut approximately 2 mm from the midline in the right hemisphere was made. Then a transverse diagonal cut was made to separate the penumbra from the core. The RNA extraction was performed as in our previous study (Tang *et al.*, 2007). Briefly, on Day 18 after surgery, rats were sacrificed and total RNA was extracted from the IP. Then the RNA was pelleted by centrifugation, washed and redissolved in 20 μl of diethylpyrocarbonate (DEPC)-treated water. The concentration and purity of RNA were confirmed and total RNA quality was assessed.

The RevertAid™ First Strand cDNA Synthesis Kit (Thermo, Cat. No. K1622) was utilized to synthesize complementary DNA (cDNA) according to the kit instructions. The 2-μg RNA was added to the cDNA synthesis reaction and one-twentieth volume of the final cDNA product (1 μl per reaction) was added to PCR reactions.

Oligonucleotide primers were designed on a computer (Primer 5 software) and synthesized by BGI (Shenzhen, China). The primers were designed for STAT3 cDNA as follows: sense, 5'-AAAGGACATC AGTGGCAAGA-3' and antisense, 5'-ACATCGGC AGGTCAATGGTA-3' with a length of 305 bp. As a control, the primers for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA were designed from the published sequences as follows: sense, 5'-ACCA CAGTCCATGCCATCAC-3' and antisense, 5'-TCC ACCACCCTGTTGCTGTA-3' with a length of 452 bp (Niture and Jaiswal, 2012). Specificity of the primers was determined by a BLAST (basic local alignment search tool) search. After preparation of the first-strand cDNA, the reaction solution was mixed with PCR reagents to make a 20-μl reaction solution containing 10 μl of 2× pre-Taq PCR master mix, 1 μl of each set of primer, 1 μl of template cDNA, and 8 μl of double-distilled water (ddH_2O). The reverse transcription reactions were carried on among the internal standard GAPDH and STAT3 in each cDNA production but in different separate tubes. PCR was performed in a T100 thermal cycler (Bio-Rad) by initial denaturation at 95 °C for 3 min, followed by 22 cycles of PCR amplification: denaturation at 95 °C for 30 s, annealing of primers STAT3 at 72 °C or GAPDH at 58 °C for 30 s, and extension at 72 °C for 1 min. PCR was completed for a final extension of 72 °C for 10 min. Negative controls for PCR were performed using templates derived from reverse transcription reactions lacking either reverse transcriptase or total RNA. Finally, PCR products were run on a 1.2% (12 mg/ml) agarose gel containing 0.2 μg/ml ethidium bromide, visualized under ultraviolet (UV) light (Bio-Rad), and analyzed with Quantity One 1-D analysis software (Bio-Rad).

2.4 Immunofluorescence staining

Immunofluorescence staining was performed on frozen sections of rat brain samples fixed by perfusion with 40 mg/ml fresh paraformaldehyde in 0.1 mol/L phosphate buffer solution (pH 7.4, 4 °C) (Tang *et al.*, 2013). The coronal sections (30 μm, −0.3 to 1.3 mm vs. bregma (Liu *et al.*, 2006)) of the brain were then cut on a Leica CM1900 cryostat (Leica Microsystems, Wetzlar, Germany). A free-floating immunofluorescence method similar to that previously described (Tang *et al.*, 2013) was utilized to detect STAT3. After the brain slices were incubated with a mouse anti-STAT3 antibody (Ab119352, 1:400; Abcam, Cambridge, MA, USA) for 48 h at 4 °C, the antibody was detected with an Alexa-594-labeled donkey anti-mouse antibody (A21203, 1:200; Invitrogen, Paisley, PA, USA) for 2 h at 37 °C. The stained slices were observed on an inverted fluorescence microscope (Eclipse T1, Nikon, Melville, NY, USA). Three bregma sections (1.2, 0.48, −0.24 mm) were selected and observed at 40×10 magnification for each rat. The regions with STAT3-positive cells expressed in the polymorphic layers of the cortical area and its adjacent corpus callosum, and the neural nuclei below the putamen in each section were scanned. The expression of STAT3 was evaluated by counting the number of STAT3-positive cells in each scanning field.

2.5 Cell culture

Mixed cultures of cortical neurons and glial cells were prepared from the cerebral cortices of embryonic Sprague Dawley rats at early embryonic stage (E18) (Doyle *et al.*, 2010) with minor modifications. Briefly, the dissected cortical tissue was dissociated with 1.25 mg/ml trypsin for 20 min at 37 °C. The cells were then plated in poly-D-lysine-coated 6-well clusters at a density of 7.5×10^5 cells per well and cultured in Neurobasal-A medium supplemented with 20 mg/ml B27 (Gibco-Life Technologies) and 0.5 mmol/L GlutaMAX™-I. Half the volume of the culture media was replenished 3 times per week and incubated at 37 °C with 5% CO_2.

2.6 Immunocytochemistry

Neurons at Day 7 were fixed in 40 mg/ml paraformaldehyde for 20 min, permeabilized for 10 min in 2 mg/ml Triton X-100, and blocked for 30 min in 5% normal donkey serum. The cells were then incubated with a mouse anti-NeuN (diluted 1:100; Millipore, Billerica, MA, USA) monoclonal antibody overnight at 4 °C. The cells were then treated with Alexa-594-labeled donkey anti-mouse antibody (diluted 1:200; Invitrogen) for 2 h at 37 °C. After removing the secondary antibody, the nuclei were stained with Hoechst

33342 (diluted 1:2000; Invitrogen), and the cells were visualized using a Nikon Eclipse TE2000-S inverted microscope (Nikon Instruments, Melville, NY, USA).

2.7 Chromatin immunoprecipitation

A chromatin immunoprecipitation (ChIP) assay was performed using the ChIP kit from Upstate (Billerica, MA, USA) according to the manufacturer's protocol. Briefly, neocortical cells on Day 7 in vitro were used in ChIP experiences, and cells were cross-linked with 10 mg/ml formaldehyde for 10 min at 37 °C. The cells were then homogenized in lysis buffer. The lysates were sonicated at 152 W for 6 min (cycles of 5 s "on" and 9 s "off", SCIENTZ-IID, Scientz, China) on wet ice. After pre-clearing the chromatin with protein G agarose, the sonicated chromatin (100 μl) was immunoprecipitated with 10 μg of STAT3 mouse antibody (Cell Signaling Technology Inc., Beverly, MA, USA), 1.0 μg of positive control (anti-RNA polymerase, Upstate), or 1.0 μg of negative control (normal mouse IgG, Upstate) in duplicate. Bound chromatin was eluted and was reverse cross-linked overnight at 65 °C. The ChIPed DNA was purified using spin columns and eluted with elution buffer. The purified DNA was used for quantitative PCR (qPCR).

2.8 PCR of immunoprecipitated DNA

Immunoprecipitated DNA was subjected to PCR analyses in a 20-μl reaction solution containing 2 μl DNA template, 1 μl of each set of primer, 7 μl H_2O, and 10 μl GoTaq® GreenMaster Mix (Promega, USA). The PCR reaction program consisted of an initial denaturation at 94 °C for 5 min, and 32 repeated cycles as follows: a heat denaturation at 94 °C for 30 s, annealing of STAT3-synaptophysin at 55 °C for 30 s, STAT3-brain-derived neurotrophic factor (BDNF) at 46 °C for 30 s, STAT3-PICK1 at 58 °C for 30 s, and GAPDH at 58 °C for 30 s, with extension at 72 °C for 30 s. PCR was completed for a final extension at 72 °C for 2 min. The PCR fragments were analyzed by electrophoresis on a 4% (40 mg/ml) agarose gel containing 0.2 mg/ml ethidium bromide and visualized under a UV light (Molecular Imager® ChemiDoc™ XRS+ System, Bio-Rad, USA). The control primers were designed for the rats' *GAPDH* gene in immunoprecipitated DNA, including a forward primer 5'-ACCACAGTCCATGCCATCAC-3' and a reverse primer 5'-TCCACCACCCTGTTGCTGTA-3' with a length of 452 bp (Niture and Jaiswal, 2012). The primers were designed according to the putative STAT3 binding sites at *BDNF*, *PICK1*, and synaptophysin promoters using the TFSEARCH server (http://cbrc3.cbrc.jp/research/db/TFSEARCH.html). The binding sites, the exact position of the primer design for each gene, and sequences of these primers are given in Table 1. The specificity of the primers was confirmed by a BLAST search.

2.9 Statistical analysis

All data were analyzed using the SPSS 19.0 statistical package. Data were presented as the mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used for the expression data, followed by the least significant difference (LSD) test for post-hoc comparisons. Statistically significant differences were established at $P<0.05$. The effect size was computed using partial eta squared (η^2) value.

3 Results

3.1 Infarct volumes

The mean infarct volumes were (68.62±10.07), (67.95±10.81), and (66.88±14.86) mm^3 for the MCAO, EM, and WM rats, respectively. Significant differences were not found among the three groups (Fig. 1; $df=2$, $F=0.03$, $\eta^2=0.00$, $P>0.05$).

Table 1 STAT3 binding sites and primers used for quantitative PCR for ChIP

Gene	GenBank No.	Binding site	Primer position	Primer sequence	Size (bp)
BDNF	NC_005102.3	TTCCAAGAA	5874–6067	Forward: 5'-GCCTGGCAACTCTAA-3' Reverse: 5'-TCCTAATGCGTTCTATG-3'	194
PICK1	NC_005106.3	TTCCCTGAA	2613–2909	Forward: 5'-CTGGCTGTGGGACGGACT-3' Reverse: 5'-AAGAGCACAGGGCTTCAGG-3'	287
Syn	NC_005120.3	TTCCTCTAA	1335–1509	Forward: 5'-AACTGAGCGGTCCTCCTAC-3' Reverse: 5'-TCGCACCCTGTCTGTCTT-3'	175

3.2 RT-PCR of STAT3 mRNA

At 18 d after perfusion, the STAT3 mRNAs of the three groups were significantly different from each other in the IP regions (Fig. 2; $df=2$, $F=4.58$, $\eta^2=0.34$, $P<0.05$). Further LSD indicated that STAT3 mRNA of WM rats in IP region (0.83±0.03) was upregulated at an average level of 13.70% compared with MCAO rats (0.73±0.09), $P<0.01$. No significant differences were found in STAT3 mRNA expressional levels between MCAO and EM rats (0.79±0.04), nor between EM and WM in IP regions (Fig. 2b).

3.3 Immunofluorescence labeling of STAT3

The immunofluorescence signals for antibodies to STAT3 were mainly localized in the cytoplasm and the nucleus at the polymorphic layers of cortical area and its adjacent corpus callosum, neural nuclei below the putamen. The STAT3-positive cells of the three groups were significantly different from each other in the ischemic hemisphere (Fig. 3; $df=2$, $F=107.87$, $\eta^2=0.86$, $P<0.001$). The WM rats showed a more than 7-fold increase and the EM rats showed a more than 2-fold increase in STAT3-positive cells compared with the MCAO rats (MCAO 10.42±2.39, EM 23.67±6.08, and WM 71.75±17.46 in 40×10 microscope, $P<0.01$).

3.4 Cortical neurons and glial cells in the culture

NeuN has been recognized as an excellent marker for neurons in the central and peripheral nervous systems (Mullen *et al.*, 1992). In our study, the Hoechst and NeuN staining indicated that most of the culture cells were neurons (86.7%) and a few cells were glial cells (13.3%), as shown in Fig. 4.

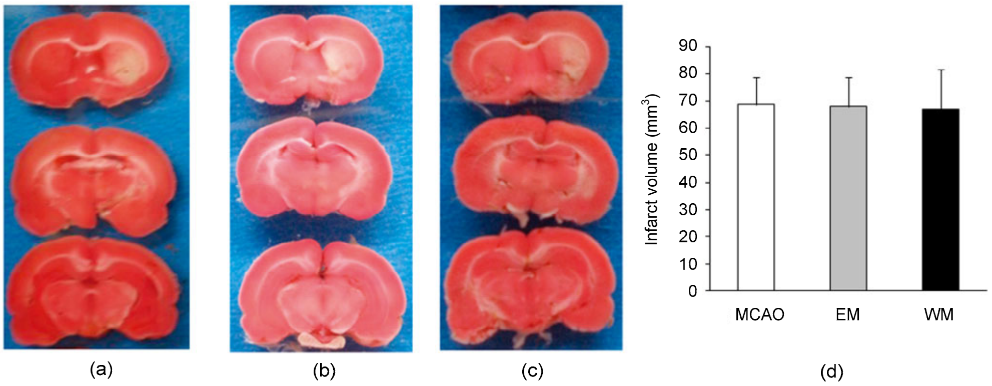


Fig. 1 TTC staining of the rats 18 d after surgery

Representative TTC staining of the MCAO (a), EM (b), and WM (c) rats, respectively. (d) Histogram demonstrates the infarct volumes of MCAO, EM, and WM rats 18 d after surgery. Data are expressed as mean±SD of 6 rats each group

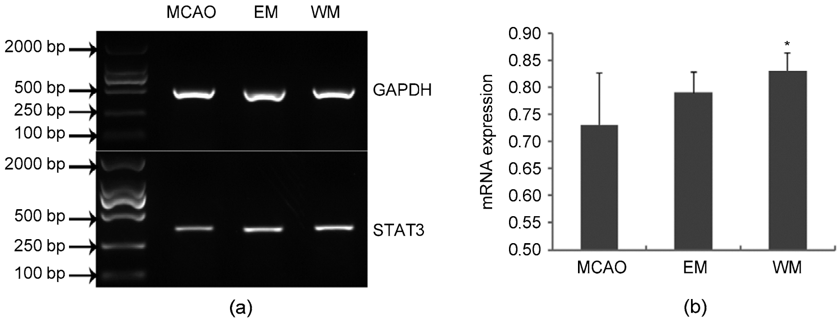
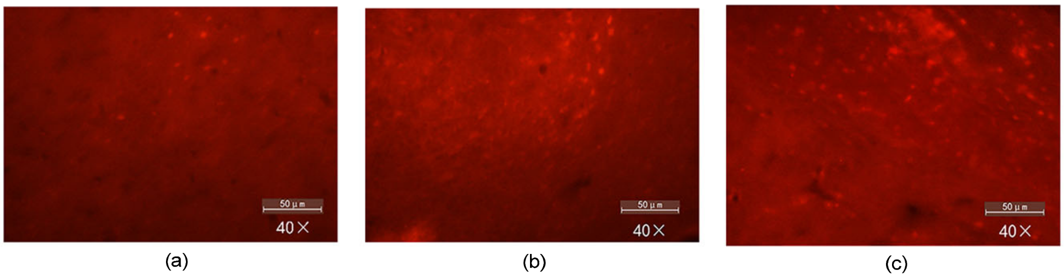


Fig. 2 Expression of STAT3 and GAPDH detected by RT-PCR

(a) Representative RT-PCR of mRNAs of STAT3 (305 bp) and GAPDH (452 bp) in the IP region of ischemic rats from MCAO, EM, and WM groups. The intensity of band for STAT3 in the WM rats is significantly higher than that in the MCAO rats. (b) Histogram demonstrates the intensity of STAT3 mRNAs in the IP region of ischemic rats for MCAO, EM, and WM rats. Data are expressed as mean±SD of 6 rats each group. $^*P<0.01$, vs. MCAO group



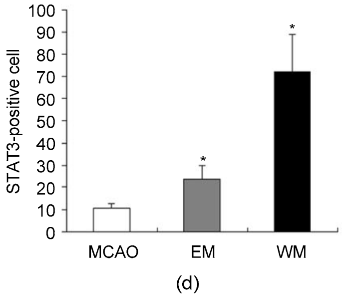


Fig. 3 Immunofluorescence signals of STAT3 at the polymorphic layers of the cortical area and its adjacent corpus callosum in the ischemic hemisphere for MCAO, EM, and WM rats
Representative immunofluorescence staining of STAT3 for MCAO (a), EM (b), and WM (c) rats, respectively. (d) Histogram demonstrates the number of STAT3-positive cells in the ischemic hemisphere for MCAO, EM, and WM rats. Data are expressed as mean±SD of 6 rats each group. $^*P<0.01$, vs. MCAO group

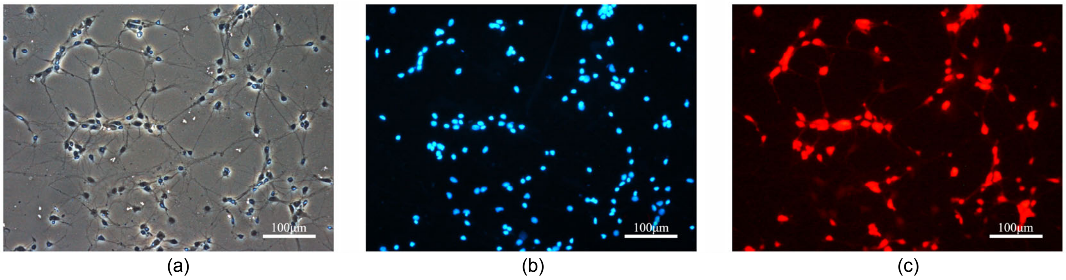


Fig. 4 Cortical neurons and glial cells on Day 7
(a) Cortical neurons and glial cells. (b) Hoechst staining nuclei (blue) of the cortical neurons and glial cells. (c) NeuN (red) cells with cytoplasm and nuclear staining in the neurons (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

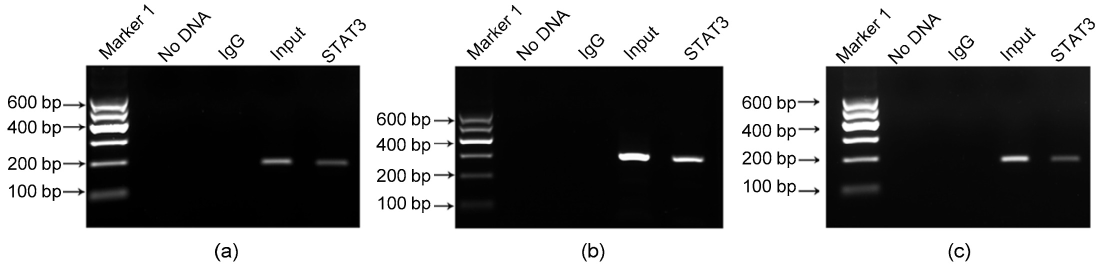
3.5 STAT3 binding to the *BDNF*, *PICK1*, and synaptophysin promoters

ChIP-PCR analyses were performed in neocortical cells to validate that STAT3 mediates the *BDNF*, *PICK1*, and synaptophysin genes. DNA was immunoprecipitated with anti-STAT3, normal mouse IgG, or anti-RNA polymerase using primers designed from the *BDNF*, *PICK1*, and synaptophysin promoter fragments. The *BDNF*, *PICK1*, and synaptophysin promoter sequences were amplified specifically from the STAT3 samples (Fig. 5), which indicated the binding of STAT3 to these plasticity-related genes in neocortical cells.

4 Discussion

This study indicated that the levels of STAT3 mRNA and protein markedly increased in the ischemic hemisphere following 15 d of WM training. Studies have demonstrated that STAT3 was elevated from approximately 0.5 h to several days after ischemia. The increased STAT3 levels were thought to be involved in the process of cerebral ischemia and reperfusion injury in the acute stage of ischemia (Suzuki *et al.*, 2001; Lei *et al.*, 2011; Shulga and Pastorino, 2012). However, the increase in STAT3 in the present study was found after 18 d of ischemic reperfusion. Additionally, although the infarct volumes and tissue loss were similar in all the rats in the three groups, a significant increase of STAT3 expressional level persisted in WM rats compared with MCAO or/and EM rats. Therefore, the enhancement of STAT3 protein was not involved in ischemia and reperfusion injury for WM rats.

Motor training can increase synaptic transmission and enhance neural plasticity (Jones *et al.*, 1999; Maldonado *et al.*, 2008; Tang *et al.*, 2013). Our



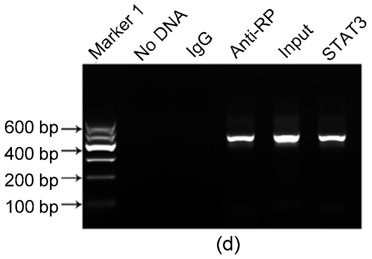


Fig. 5 Binding of STAT3 to the *BDNF*, *PICK1*, and synaptophysin promoters

The ChIP of synchronized neocortical cells was performed using an antibody to STAT3, a negative control antibody (IgG), or a positive control anti-RNA polymerase (anti-RP). A sample without DNA was tested as an additional control for DNA contamination, and an input sample was also detected. Immunoprecipitated DNA analyzed by PCR using primers that amplify the *BDNF* (a), *PICK1* (b), synaptophysin (c) promoters and *GAPDH* gene (d), respectively

previous data (Tang *et al.*, 2007; 2013) indicated that WM training for rats could improve neurobehavioral performance compared with EM and MCAO rats. STAT3 activation is associated with early embryonic and neocortical development (Yoshimatsu *et al.*, 2006; Oatley *et al.*, 2010), increasing cell survival (Yadav *et al.*, 2005; Dziennis and Alkayed, 2008), regulating neuronal differentiation (Chang *et al.*, 2014), and mediating axon elongation (Liu and Snider, 2001; Selvaraj *et al.*, 2012) and early neural circuit formation (Bouret *et al.*, 2012). Therefore, we speculated that the motor activity in the WM rats induced the increase of STAT3 protein that might be related to neural plasticity in the subacute stage of focal ischemia.

Our ChIP analysis confirmed that STAT3 bound to *BDNF*, synaptophysin, and *PICK1* in neocortical cells. BDNF (Nakai *et al.*, 2014), synaptophysin (Liang *et al.*, 2007; Gordon *et al.*, 2011; Kwon and Chapman, 2011), and PICK1 (Antoniou *et al.*, 2014; Xu *et al.*, 2014) have been proven to be the key proteins in the regulation of synaptic plasticity. Numerous studies indicated that motor exercise increased the expressions of BDNF (Gómez-Pinilla *et al.*, 2002; Ploughman *et al.*, 2009; MacLellan *et al.*, 2011; Wilhelm *et al.*, 2012), PICK1 (Volk *et al.*, 2010), and synaptophysin (Seo *et al.*, 2010), which promoted neuroplasticity and was beneficial for recovery after a stroke (MacLellan *et al.*, 2011). Our previous studies have proven that the expressional levels of PICK1 (Tang *et al.*, 2013) and synaptophysin proteins were elevated after WM training in the IP regions of ischemic rats. The STAT3-mediated activations of BDNF, synaptophysin, and PICK1 may therefore play a role in synaptic plasticity in WM training for focal ischemic rats. In WM training, STAT3 may affect synaptic vesicle efficiency via its synaptophysin-activating function (Liang *et al.*, 2007; Gordon *et al.*, 2011; Kwon and Chapman, 2011). Additionally, STAT3 may facilitate nerve regeneration, neuronal development, and synaptic transmission by a BDNF mechanism (Gómez-Pinilla *et al.*, 2002; Ng *et al.*, 2006; Ploughman *et al.*, 2009; MacLellan *et al.*, 2011; Waterhouse *et al.*, 2012; Wilhelm *et al.*, 2012). Furthermore, STAT3 may indirectly regulate receptors, transporters, and ion channel trafficking by activating PICK1 (Citri *et al.*, 2010; Hu *et al.*, 2010). Future studies could be designed to clarify which of these mechanisms are responsible for the neuroplasticity regulated by STAT3 after WM training.

Our findings agree with a study by Ng *et al.* (2006), in which the inhibition of STAT3 markedly attenuated BDNF-induced axon outgrowth in cultured hippocampal neurons. This finding suggested that BDNF signaling is regulated by the STAT3 pathway during axon elongation (Dominguez *et al.*, 2010). However, as far as we know, no studies have directly shown that STAT3 can bind to *BDNF*, synaptophysin, and *PICK1* promoters using ChIP assays.

In summary, our study discovered that STAT3 increases after WM training and is an important transcription factor for regulating plasticity-related genes, such as *BDNF*, synaptophysin, and *PICK1*.

These results indicate a novel gene regulatory mechanism mediated by STAT3 that is involved in neural plasticity. Furthermore, gene interference or knockout of STAT3 in WM rats is recommended to investigate whether these signaling pathways functionally regulate the neural plasticity following WM training.

Compliance with ethics guidelines

Qing-ping TANG, Qin SHEN, Li-xiang WU, Xiang-ling FENG, Hui LIU, Bei WU, Xiao-song HUANG, Gai-qing WANG, Zhong-hao LI, and Zun-jing LIU declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：调控神经可塑性的 STAT3 在意向性运动疗法干预后局灶性脑缺血大鼠中的表达

目　的：探讨意向性运动疗法干预后与神经可塑性相关的信号通路。

创新点：首次发现意向性运动疗法干预后信号传导与转录激活因子 3（STAT3）表达增高，以及 STAT3 直接调控神经可塑性相关基因。

方　法：将大脑中动脉梗死（MCAO）模型大鼠随机分为 MCAO 组、环境改变组和意向性运动疗法组。18 天后测量三组大鼠的脑梗死面积。应用逆转录聚合酶链反应（RT-PCR）和荧光免疫染色法分别检测 STAT3 的基因和蛋白的表达；应用染色质免疫共沉淀检测 STAT3 是否绑定脑源性神经营养因子（*BDNF*）、突触素以及蛋白激酶 Cα 相互作用蛋白 1（*PICK1*）。

结　论：研究结果显示：意向性运动疗法干预 15 天后，STAT3 的基因与蛋白均增高；STAT3 绑定皮层神经元 *BDNF*、*PICK1* 和突触素的启动子区；意向性运动干预后 STAT3 的升高可能与神经可塑性相关。

关键词：运动训练；信号传导与转录激活因子 3（STAT3）；脑源性神经营养因子（BDNF）；突触素；蛋白激酶 Cα 相互作用蛋白 1（PICK1）；神经可塑性