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# Remodeling of motor cortex function in acute cerebral infarction patients following human urinary kallidinogenase

## A functional magnetic resonance imaging evaluation after 6 months<sup>★</sup>

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### Abstract

A total of 29 patients were treated within 48 hours after acute subcortical cerebral infarction with *Xuesaitong* or *Xuesaitong* plus human urinary kallidinogenase for 14 days. Neurological deficits, activity of daily living, and evaluations of distal upper limb motor functions at the 6-month follow-up showed that patients treated with *Xuesaitong* plus human urinary kallidinogenase recovered better than with *Xuesaitong* alone. In addition, functional MRI revealed that activation sites were primarily at the ipsilesional side of injury in all patients. Human urinary kallidinogenase induced hyperactivation of the ipsilesional primary sensorimotor cortex, premotor cortex, supplementary motor area, and contralesional posterior parietal cortex. Results showed that human urinary kallidinogenase improved symptoms of neurological deficiency by enhancing remodeling of long-term cortical motor function in patients with acute cerebral infarction.

**Key Words:** cerebral infarction; functional magnetic resonance imaging; human urinary kallidinogenase; motor function remodeling; neural regeneration

**Abbreviations:** HUK, human urinary kallidinogenase; fMRI, functional magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale

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## INTRODUCTION

Previous studies have shown that human urinary kallidinogenase (HUK) dilates arterioles in ischemic brain regions, improves brain microcirculation, reduces cerebral infarction volume, promotes vascular reconstruction of infarcted foci, improves neurological functions, increases cortical functional remodeling, and attenuates limb dysfunctions<sup>[1-4]</sup>.

Functional magnetic resonance imaging (fMRI) can reflect the evolution of cortical functional remodeling. Contralesional activation has been detected at 1–6 weeks after stroke, but ipsilesional activation has been detected at 3–6 months after stroke, which was accompanied by hand motor function recovery<sup>[5-10]</sup>. Ward *et al*<sup>[6-8]</sup> studied the correlation between degree of recovery and brain motor-related activation in chronic stroke patients, concluding that more bilateral primary and secondary motor centers are activated in patients that exhibit poor recovery, and activation patterns in patients with better recovery are similar to normal individuals.

fMRI has been used to estimate the influence of therapeutic treatments on functional remodeling<sup>[11]</sup>. Very little is known about the effects of HUK on long-term cortical motor functional remodeling. The present study utilized fMRI to evaluate remodeling of long-term cortical motor function in patients with acute cerebral infarction following HUK treatment.

## RESULTS

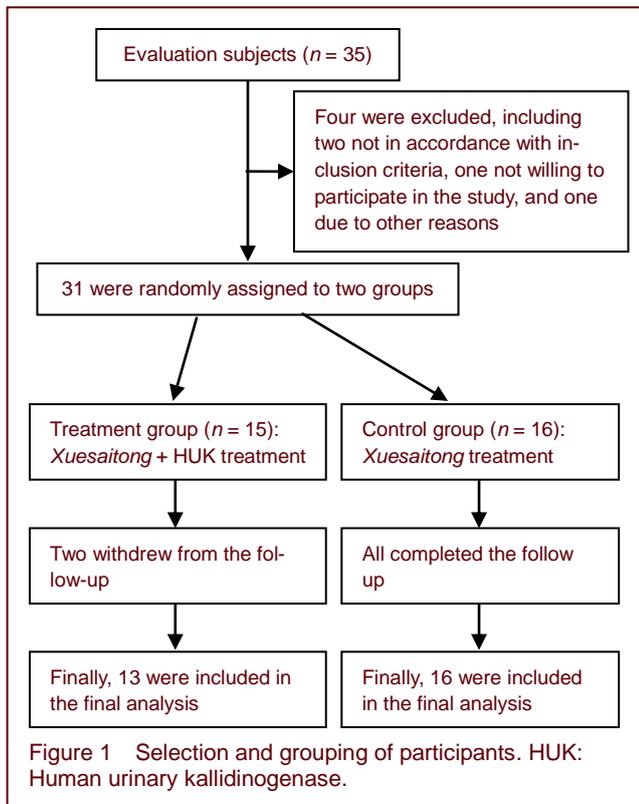
### Quantitative analysis of participants

A total of 29 patients with cerebral infarction were selected from the Department of Neurology, Guangzhou General Hospital of Guangzhou Military Command from November 2006 to July 2007. All 29 patients were included in the final analysis. Patient enrolling and grouping are shown in Figure 1.

### Baseline data of participants

A total of 29 participants comprised 27 males and 2 females, aged 40–75 years, with subcortical cerebral infarction. The patients were assigned to treatment ( $n = 13$ ; 12 males and 1 female) and control ( $n = 16$ ; 15 males and 1 female) groups. Two-sample

*t*-test revealed no significant differences between the groups in terms of age, gender, or infarction volume ( $P > 0.05$ ; Table 1).



### Neurological functional evaluation

Prior to treatment, there were no significant differences between the groups in terms of Barthel index, National Institutes of Health Stroke Scale (NIHSS) scores, finger-tapping test, or grip strength ( $P > 0.05$ ). At 6

months post-treatment, the Barthel index and frequency of finger tapping significantly increased in both groups ( $P < 0.05$ ), NIHSS scores significantly decreased ( $P < 0.05$ ), and grip strength significantly increased ( $P < 0.05$ ). In particular, the treatment group significantly improved ( $P < 0.05$ ; Tables 2, 3).

Table 1 Baseline data of groups

Group	n	Age (year)	Infarction volume (cm <sup>2</sup> )
Treatment (lesioned left hemisphere)	7	58.50±9.97	3.21±0.92
Control (lesioned left hemisphere)	8	57.14±10.62	3.38±1.12
Treatment (lesioned right hemisphere)	6	54.43±10.23	3.34±0.57
Control (lesioned right hemisphere)	8	62.22±7.71	3.18±0.74

Results are expressed as mean ± SD. The two-sample *t*-test shows no significant differences between groups in terms of age or infarction volume ( $P > 0.05$ ).

### fMRI results of motor tasks using the hemiplegic hand

fMRI results showed that when the hemiplegic hand conducted motor tasks, both groups exhibited activation of the entire motor network, including the primary sensorimotor cortex, supplementary motor area, premotor cortex, cerebellum, insular, basal ganglia, posterior parietal cortex at the ipsilesional side, and the contralesional primary sensorimotor cortex. At 6 months post-treatment, activation was mainly observed at the ipsilesional side in both groups. In addition, hyperactivation of the ipsilesional primary sensorimotor cortex, premotor cortex, supplementary motor area, and posterior parietal cortex was greater in the treatment group than in the control group (Figures 2, 3, Tables 4, 5).

Table 2 Clinical evaluation of patient functional recovery

Group	n	NIHSS (score)		BI (score)	
		Prior to treatment	6 months post-treatment	Prior to treatment	6 months post-treatment
Treatment (lesioned left hemisphere)	7	9.33±2.16	2.16±0.75 <sup>ab</sup>	46.67±9.83	90.83±4.91 <sup>ab</sup>
Control (lesioned left hemisphere)	8	9.50±2.17	3.17±0.75 <sup>a</sup>	45.83±11.58	81.67±8.16 <sup>a</sup>
Treatment (lesioned right hemisphere)	6	9.50±2.51	2.00±0.89 <sup>ab</sup>	49.17±7.35	92.50±2.74 <sup>ab</sup>
Control (lesioned right hemisphere)	8	9.67±2.16	3.50±1.04 <sup>a</sup>	50.83±6.65	86.67±5.16 <sup>a</sup>

Results are expressed as mean ± SD. <sup>a</sup> $P < 0.05$ , vs. prior to treatment; <sup>b</sup> $P < 0.05$ , vs. control group (two-sample *t*-test). NIHSS scores ranged from 0 to 58; high scores indicate severe neurological deficit<sup>[12]</sup>. BI scores ranged from 0 to 100; high scores indicate good self-care ability<sup>[13]</sup>. NIHSS: National Institutes of Health Stroke Scale; BI: Barthel Index.

Table 3 Evaluation of functional recovery in distal upper limbs

Group	n	Frequency of finger tapping (times/10 seconds)		Grip strength (kg)	
		Prior to treatment	6 months post-treatment	Prior to treatment	6 months post-treatment
Treatment (lesioned left hemisphere)	7	18.50±1.37	34.67±3.44 <sup>ab</sup>	16.27±3.19	39.48±7.90 <sup>ab</sup>
Control (lesioned left hemisphere)	8	19.85±3.18	29.83±3.76 <sup>a</sup>	18.38±4.62	28.77±4.43 <sup>a</sup>
Treatment (lesioned right hemisphere)	6	19.36±3.08	35.33±4.13 <sup>ab</sup>	13.38±2.37	25.78±3.31 <sup>ab</sup>
Control (lesioned right hemisphere)	8	19.92±3.35	29.50±4.42 <sup>a</sup>	12.97±2.69	19.87±5.01 <sup>a</sup>

Results are expressed as mean ± SD. <sup>a</sup> $P < 0.05$ , vs. prior to treatment; <sup>b</sup> $P < 0.05$ , vs. control group (two-sample *t*-test). Frequency of finger tapping is times of finger tapping in 10 seconds; high frequency represents high flexibility<sup>[14]</sup>. Grip strength examines strength; increased strength represents better recovery of motor function<sup>[14]</sup>.



Figure 2 Functional magnetic resonance imaging results of patients with lesioned left hemisphere in motor tasks by hemiplegic hand (red: activation region).

(A) Activation mainly at the ipsilesional side in the treatment group, involving primary motor sensory areas. In addition, bilateral supplementary motor area, premotor cortex, and cerebellum are also activated ( $P < 0.001$ ; uncorrected).

(B) Activation mainly at the ipsilesional side in the control group, involving primary motor sensory areas in accompany with activation in supplementary motor area, premotor cortex, posterior parietal cortex, and cerebellum ( $P < 0.001$ ; uncorrected).

(C) Hyperactivation of the ipsilesional primary sensorimotor cortex, bilateral premotor cortex, ipsilesional supplementary motor area, posterior parietal cortex, and cerebellum is induced in the treatment group compared with the control group ( $P < 0.001$ ; uncorrected).

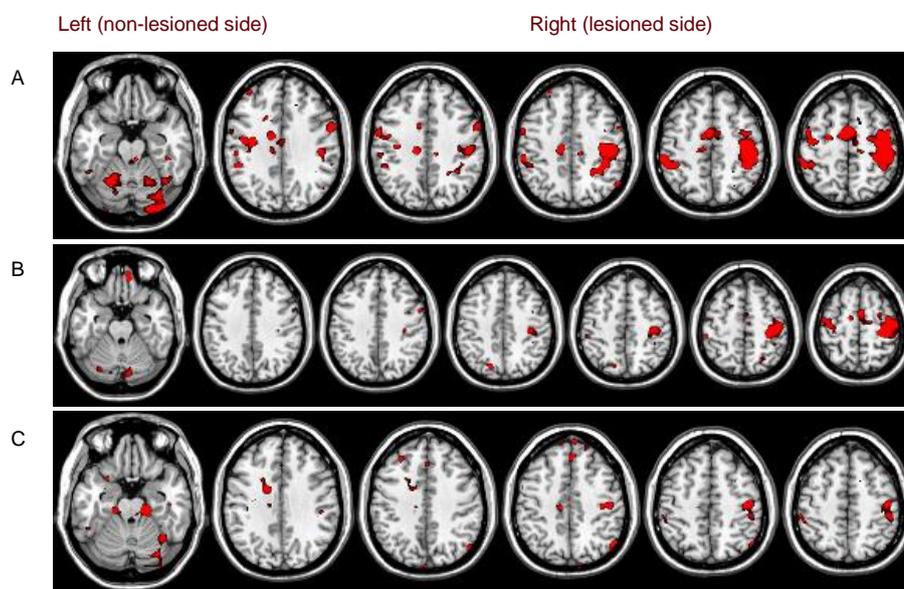


Figure 3 Functional magnetic resonance imaging results of patients with a lesioned right hemisphere performing motor tasks using hemiplegic hand (red: activation region)

(A) Activation at the ipsilesional side in the treatment group, involving extensive activation in ipsilesional primary motor sensory areas that accompanies activation in the bilateral supplementary motor areas, premotor cortex, posterior parietal cortex, and cerebellum ( $P < 0.001$ ; uncorrected).

(B) Activation at the ipsilesional side in the control group, involving extensive activation in ipsilesional primary motor sensory areas that accompanies activation in the supplementary motor area, premotor cortex, and cerebellum ( $P < 0.001$ ; uncorrected).

(C) Hyperactivation of the ipsilesional primary sensorimotor cortex, ipsilesional premotor cortex, posterior parietal cortex, and cerebellum is greater in the treatment group than in the control group ( $P < 0.001$ ; uncorrected).

Table 4 Activation range and intensity of brain regions when the hemiplegic hand performed motor tasks (lesioned left hemisphere)

Location	Treatment group			Control group			Treatment/control group		
	Central coordinate (x, y, z)	Activation intensity (T)	Voxel	Central coordinate (x, y, z)	Activation intensity (T)	Voxel	Central coordinate (x, y, z)	Activation intensity (T)	Voxel
S1M1 <sup>a</sup>	-30, -33, 60	17.41	51	-36, -42, 63	14.34	32	-45, -21, 48	4.86	59
S1M1 <sup>b</sup>	39, -24, 57	3.11	14				54, -15, 51	4.72	18
SMA <sup>a</sup>	-3, 3, 48	10.45	38	-7, 3, 55	7.21	11	-3, -24, 45	4.64	24
PMC <sup>a</sup>	-45, 3, 54	9.58	17	-38, 8, 51	8.79	8	-21, 0, 60	4.42	10
Cerebellum <sup>b</sup>	6, -63, -15	10.98	22	15, -66, -12	12.8	10	6, -72, -21	3.79	22
PPC <sup>b</sup>	6, -42, 63	3.98	19	15, -48, 39	3.21	11	33, -51, 60	4.25	13
PPC <sup>a</sup>	-27, -48, 60	3.87	8	-30, -54, 48	3.07	4	-51, -33, 48	3.81	6
Insular lobe <sup>a</sup>	-39, -3, 6	3.74	8				-39, -6, 9	4.17	12

a: Ipsilesional; b: contralesional. Two-sample *t*-test was used. Treatment/control group statistical threshold:  $P < 0.01$  (uncorrected); activation range threshold: 10 voxel; threshold of treatment group and control group:  $P < 0.001$  (uncorrected); activation range threshold: 10 voxel. S1M1: Primary sensorimotor cortex; SMA: supplementary motor area; PMC: premotor cortex; PPC: posterior parietal cortex.

Table 5 Activation range and intensity of brain regions when the hemiplegic hand performed motor tasks (lesioned right hemisphere)

Location	Treatment group			Control group			Treatment/control group		
	Central coordinate (x, y, z)	Activation intensity (T)	Voxel	Central coordinate (x, y, z)	Activation intensity (T)	Voxel	Central coordinate (x, y, z)	Activation intensity (T)	Voxel
S1M1 <sup>a</sup>	39, -18, 69	17.01	63	30, -27, 60	15.31	43	45, -18, 48	5.11	38
SMA <sup>a</sup>	3, -21, 69	14.31	25	-4, -20, 66	10.58	19			
PMC <sup>a</sup>	36, -12, 63	14.83	28	41, -12, 52	10.74	25	30, -13, 61	4.25	29
PMC <sup>b</sup>	-39, -12, 60	11.79	10	-36, -15, 66	6.02	11	-39, -12, 60	3.51	6
Cerebellum <sup>a</sup>	22, -66, -26	10.72	41	17, -76, -26	7.34	16	30, -59, -28	3.30	17
PPC <sup>b</sup>	-51, -39, 51	10.45	18				-45, -42, 54	3.33	10
PPC <sup>a</sup>	42, -45, 60	13.98	7				51, -53, -45	3.09	3

a: Ipsilesional; b: contralesional. Two-sample *t*-test was used. Treatment/control group statistical threshold:  $P < 0.01$  (uncorrected); activation range threshold: 10 voxel; threshold of treatment group and control group:  $P < 0.001$  (uncorrected); activation range threshold: 10 voxel. S1M1: Primary sensorimotor cortex; SMA: supplementary motor area; PMC: premotor cortex; PPC: posterior parietal cortex.

### fMRI results of motor tasks using non-hemiplegic hand

There were no significant differences in activated brain regions between the groups when performing motor tasks using the non-hemiplegic hand ( $P > 0.05$ ).

## DISCUSSION

HUK has been shown to improve blood supply in infarction sites and increase neuronal survival in the ischemic penumbra by dilating arterioles at the ischemic site<sup>[3, 15]</sup>, as well as provide conditions for remodeling around the infarction focus by promoting angiogenesis<sup>[4]</sup> and inhibiting apoptosis<sup>[3]</sup>. Clinical results have shown that HUK effectively and safely improves neurological deficits induced by acute cerebral infarction<sup>[16]</sup>. In the present study, HUK induced hyperactivation of the ipsilesional primary motor sensory cortex, indicating that HUK ameliorated more nerve fibers following ischemia and/or restored more innervations in the injury site compared with the control group. In addition, activation of the ipsilesional supplementary motor area, premotor cortex, and contralesional posterior parietal cortex was enhanced by HUK treatment, suggesting that HUK

regulated motor function remodeling in cerebral infarction patients.

Remodeling of brain motor functions has been associated with the injury site<sup>[17-20]</sup>. For instance, subcortical stroke patients exhibited extensive activation on fMRI following clinical recovery<sup>[21]</sup>, and some studies have shown that recovered patients exhibit activation patterns similar to normal individuals<sup>[5-10]</sup>. In the present study, however, both groups exhibited extensively activated motor networks when the hemiplegic hand performed motor tasks. The lesions were primarily located at subcortical sites (basal ganglia, corona radiata, or semioval center).

In addition, activation intensity and volume were significant in the supplementary motor area of the treatment group. The supplementary motor area and cingulate gyrus are linked to spinal cord motor neurons, and nerves dominating fingers and wrist muscles receive bilateral projection from the pyramidal tract<sup>[22-23]</sup>.

Following output interruption of the primary sensorimotor cortex, the ipsilateral cortex compensates for some of these functions<sup>[22, 24]</sup>. Bilateral supplementary motor areas connect through the internal capsule and provide information to the contralateral cortex for control of

voluntary movement<sup>[25]</sup>. Supplementary motor area remodeling has been detected during the early stages after cerebral infarction, although activation decreases at 4 months to 1 year after infarction<sup>[10]</sup>. The present study included patients, who had suffered from cerebral infarction within the past 6 months. The patients exhibited supplementary motor area activation during the chronic recovery stage. Further studies are needed, however, to determine the mechanisms of action. The posterior parietal cortex is responsible for perception imaging of positioning attention, spatial shifts, and motor skill learning processes<sup>[26]</sup>. A previous study showed that activation of the ipsilateral posterior parietal cortex Brodmann 40, as well as the second somatic sensory area SII, increases with time<sup>[10]</sup>. In the present study, the contralesional posterior parietal cortex was significantly activated in the treatment group, which suggested that HUK promoted functional remodeling after stroke. In conclusion, HUK promoted functional recovery, ameliorated limb dysfunctions, and improved remodeling of motor functions in patients with acute cerebral infarction.

## SUBJECTS AND METHODS

### Design

A randomized, controlled, prospective study.

### Time and setting

The study was performed at Guangzhou General Hospital of Guangzhou Military Command, China from November 2006 to January 2008.

### Subjects

#### **Diagnostic criteria of hemiplegic patients with cerebral infarction**

Blood supply disturbances in the brain are induced by various causes and often result in brain ischemia or ischemic necrosis, which corresponds with neurological deficits within one or several days. The injury is often confined to the blood supply region of a certain artery. In the present study, cerebral infarction was confirmed by MRI.

#### **Inclusion criteria**

Patients, aged 18–80 years, right handed, with first attack of cerebral infarction of the internal carotid artery within 48 hours, were included in the study. The patients exhibited neurological signs, with NIHSS scores  $\geq 4$ , but  $\leq 20$ <sup>[12]</sup>, and grade 2–4 muscle strength in distal upper limbs. Prior to onset, hand function was normal. The patients were free of continuous antipsychotic or anti-epileptic drugs prior to and after onset.

#### **Exclusion criteria**

Patients with intracranial hemorrhage, cerebral infarction involving the vertebral basilar system, multiple cerebral infarction, history of stroke or organic disease in central nervous system, conscious disturbances, or brief brain ischemia; NIHSS scores  $< 4$  or  $> 20$ <sup>[12]</sup>; Mini Mental State Examination scores  $< 24$ <sup>[27]</sup>; liver function failure, renal failure, heart function failure, or functional decompensation of other critical organs; cerebral embolism or suspected cerebral embolism;

complications due to atrioventricular block, atrial fibrillation, myocardial infarction, valvular disease, or infective endocarditis; heart rate  $< 50$  beats/min; alcoholism, drug addiction, drug dependence; psychosis, epilepsy; apraxia; contraindication for MRI; or hypersensitivity to various drugs. In addition, pregnant or lactating women were excluded, and patients participating in other clinical studies were excluded. In total, 29 patients were included in the study, which was performed in accordance with requirements of *Declaration of Helsinki*. Written informed consent was obtained from all participants.

### Methods

#### **Drug treatment**

All participants were intravenously injected with Xuesaitong (Wuhan Jianmin Medicinal Group Shiyang Kangdi Pharmaceutical, Wuhan, China) for basic treatment (400 mg Xuesaitong dissolved in 250 mL 5% glucose once daily for 14 consecutive days). The treatment group was also intravenously injected with 0.15 para nitroaniline unit<sup>[28]</sup> HUK, *i.e.* HUK required for hydrolyzing 1  $\mu$ mol Val-Leu-Arg-PNA in 1 minute at pH 8.0 and 37 °C, dissolved in 50 mL normal saline once daily for 14 consecutive days (angiotensin converting enzyme inhibitor was not allowed during treatment). Other drugs for cerebral infarction were not utilized, and the control group was subjected to basic treatment alone. Clinical neurological deficits, daily life activity, and motor functions in the distal upper limbs were evaluated prior to treatment and 6 months post-treatment. In addition, brain MRI was performed prior to treatment, and regions of interest area was measured using efilm software (Merge Healthcare Incorporation, Chicago, IL, USA). MRI was performed prior to and 6 months post-treatment.

#### **Neurological function evaluation**

Neurological deficits were evaluated using NIHSS<sup>[12]</sup>.

#### **Evaluation of activity of daily living**

Activity of daily life was evaluated using Barthel Index<sup>[13]</sup>.

#### **Evaluation of motor function of distal upper limbs**

Motor functions in distal upper limbs were evaluated using the finger-tapping test<sup>[14]</sup> and grip strength examinations<sup>[14]</sup>. In the finger-tapping test, the frequency of index finger tapping a computer mouse during a 10-second period was recorded<sup>[14]</sup>. Grip strength examination measured grip strength of the hand. During the testing, the arm was placed in any positions that did not contact the body. Results were recorded as kg weight. The orders of experiments were identical in each testing paradigm to prevent an order effect. To prevent a learning effect, the participants were allowed to practice three times prior to initial testing.

#### **MRI tasks**

A block design was utilized and included two sequences: the first sequence was performed using the left hand, and the second was performed using the right hand. Each sequence comprised 11 phases, and each phase lasted for 20 seconds, alternating between task and resting, for a total of 220 seconds (Figure 4).

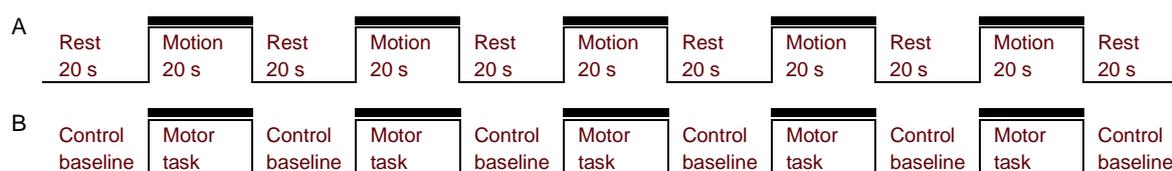


Figure 4 MRI block-design stimulation pattern (A) and data processing (B).

While performing the task, each subject clenched the fist at a rhythm of 1 Hz, followed by relaxing and maintaining motionless fingers while resting. The subject controlled time and motor frequency through visual information, which was compiled using DMDX software (DisplayMaster DirectX Department of Psychology Psycholinguistics Laboratory University of Arizona, Tucson, AZ, USA). The information was projected *via* a transparent screen to the computer projector, and the subject observed the information through a reflector in a coil placed at the head.

#### fMRI image acquisition

fMRI was performed prior to and 6 months after treatment using Siemens Sonata 1.5T superconducting magnetic resonance apparatus (Siemens, Munich, Germany), with a gradient field of 40 mT/m and a slew rate of 200 mT/m/ms. Standard head coil radio frequency was used, and the head motor was controlled. Initially, axial T1WI scanning of the head was performed with a repetition time of 683 ms, 11-ms echo time, 230-mm field of view, 192 × 144 matrix, 4-mm slice thickness, and 1-mm slice gap for 28 slices through the entire brain. Blood oxygen level dependent signals during motor and rest states were acquired using gradient echo in combination with echo planar imaging with parameters: 2 000-ms repetition time, 49-ms echo time, 210-mm field of view, 64 × 64 matrix, 4-mm slice thickness, and 1-mm slice gap for 28 slices through the entire brain. Area of regions of interest was measured using efilm software.

#### fMRI image processing and analysis

fMRI data were analyzed using Statistics parameter mapping 5 software of MATLAB platform (Wellcome Department of Cognitive Neurology, London, UK). Individual analysis: The first 10 images from each sequence were excluded to eliminate the influence of magnetic field strength changes. Preprocessing included head motion correction, space standardization, and space smoothing. Data from each patient was subjected to deconvolution and multiple linear regression analysis. A statistical value ( $F$ ) was obtained from each voxel, which was represented by color and overlaid onto three-dimensional structure image to generate a statistical parameter map. A threshold of  $P < 0.001$  (uncorrected) was established. The threshold of activation range was 10 voxels.

Intragroup analysis: A group activation map was obtained using a single sample  $t$ -test. The threshold was  $P < 0.001$  (uncorrected). The threshold of activation

range was 10 voxels.

Intergroup comparison: Activation images of treatment and control groups were compared using two-sample  $t$ -test and a hyperactivation image of HUK was obtained ( $[\text{Motion-rest}] \text{ treatment group} - [\text{Motion-rest}] \text{ control group} > 0$ ). Threshold was set to  $P < 0.001$  (uncorrected). The activation range threshold was 10 voxels. Activation volume (voxel) and activation intensity ( $T$  value) were quantitatively calculated.

#### Statistical analysis

Measurement data are expressed as mean  $\pm$  SD and analyzed using SPSS13.0 software (SPSS, Chicago, IL, USA). All data were subjected to Shapiro-wilk and Levene tests to verify normal distribution and variance. Enumeration data were represented by rate. Mean was compared using the two-sample  $t$ -test. A value of  $P < 0.05$  was considered statistically significant.

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**Author contributions:** Xuezhu Song provided, integrated, and analyzed data and wrote manuscript. Lixin Han provided data and contributed to statistical analysis. Yan Liu conceived and designed the study, revised the manuscript and was in charge of funds.

**Conflicts of interest:** None declared.

**Ethical approval:** This study received permission from the Ethics Committee of Guangzhou General Hospital of Guangzhou Military Command, China.

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