CIMT促进脑缺血大鼠运动功能恢复与局部脑区葡萄糖代谢的相关性

李莹莹[1] 华 艳[2] 余克威[2] 鲍伟奇[3] 王瑜元[2] 胡 健[2] 胡世红[1] 白玉龙[2△]
([1]复旦大学附属上海市第五人民医院康复医学科 上海 200240; [2]复旦大学附属华山医院康复医学科,[3]PET中心 上海 200235)

【摘要】 **目的** 研究脑缺血再灌注(middle cerebral artery occlusion,MCAO)模型大鼠在强制性运动疗法(constraint-induced movement therapy,CIMT)干预前后局部脑区葡萄糖代谢水平与行为学评分的相关性,及MCAO模型大鼠自然恢复过程及CIMT干预后的运动恢复情况与脑区激活的相关性,并进一步探讨CIMT的作用机制。**方法** 将22只健康雄性SD大鼠随机分为实验组(6只)、对照组(6只)、假手术组(6只)和正常组(4只)。实验组和对照组通过线栓法建立大鼠MCAO模型,假手术组在手术中不阻断大脑中动脉,正常组不予特殊处理。术后对实验组和假手术组大鼠进行CIMT治疗。在术后第7天和第22天,利用错步实验(foot-fault test,FFT)和平衡木实验(beam balance and walking,BBW)进行前肢运动功能评估;采用微型正电子发射断层扫描仪(micro PET/CT)对大鼠大脑的葡萄糖代谢情况进行扫描;采用皮尔逊相关分析法分别将脑缺血组大鼠的行为学评分与各脑区葡萄糖代谢水平进行相关性分析。**结果** 术后第7天,实验组和对照组大鼠的BBW得分分别与左侧脑区的岛叶皮层、听觉皮层的葡萄糖代谢水平呈负相关,与右侧的脑区后腹部海马、上丘和下丘的葡萄糖代谢水平呈正相关,差异均有统计学意义;错步率分别与左侧脑区的躯体感觉皮层、岛叶皮层和眶额叶皮层的葡萄糖代谢水平呈负相关,与右侧的中脑葡萄糖代谢水平呈正相关,差异均有统计学意义。术后22天,实验组和对照组大鼠的BBW得分分别与左侧脑区的杏仁核、壳尾核、岛叶皮层和内嗅皮层的葡萄糖代谢水平呈正相关,且与右侧脑区的伏隔核和壳尾核的葡萄糖代谢水平呈负相关,差异均有统计学意义;错步率与右侧内嗅皮层呈负相关,差异具有统计学意义。**结论** CIMT促进大鼠运动功能恢复与双侧大脑半球的激活有关。CIMT促进脑缺血大鼠平衡功能的改善主要与右侧伏隔核的激活有关,其促进大鼠精细抓握功能的恢复可能与右侧内嗅皮层的激活有关。

【关键词】 强制性运动疗法(CIMT); 脑缺血再灌注; 微型正电子发射断层扫描仪(PET); 葡萄糖代谢; 运动功能; 皮尔逊相关性分析; 大鼠
【中图分类号】 R743 【文献标志码】 A **doi**:10.3969/j.issn.1672-8467.2022.06.010

Correlation between recovery of motor function and regional glucose metabolism induced by CIMT in rats with cerebral ischemia

LI Ying-ying[1], HUA Yan[2], YU Ke-wei[2], BAO Wei-qi[3], WANG Yu-yuan[2], HU Jian[2], HU Shi-hong[1], BAI Yu-long[2△]
([1]*Department of Rehabilitation Medicine, the Fifth People's Hospital, Fudan University, Shanghai* 200240, *China*; [2]*Department of Rehabilitation Medicine*, [3]*PET Center, Huashan Hospital, Fudan University, Shanghai* 200235, *China*)

【Abstract】 **Objective** To study the correlation between regional cerebral glucose metabolism and behavioral scores in middle cerebral artery occlusion (MCAO) model rats before and after the intervention of constraint induced movement therapy (CIMT), and the correlation between the natural recovery process

国家自然基金面上项目(81871841)
△Corresponding author E-mail: dr_baiyl@fudan.edu.cn
网络首发时间:2022-06-15 09:54:33 网络首发地址:https://kns.cnki.net/kcms/detail/31.1885.R.20220613.1755.012.html

and motor function recovery in MCAO model rats and the brain activation after CIMT intervention, and to further explore the mechanism of CIMT. **Methods** Twenty-two adult male Sprague-Dawley (SD) rats were randomly divided into an ischemic group treated with CIMT (CIMT, n=6), an ischemic group (Control, n=6), a sham-operated group (Sham, n=6), and a blank control group (Normal, n=4). The MCAO models of rats in the CIMT group and Control group were established by thread embolism method. The middle cerebral artery was not blocked during the operation for the Sham group, and the Normal group was not given any special treatment. After operation, rats in the CIMT group and Sham group were treated with CIMT. On the 7th day (d7) and the 22nd day (d22) after surgery, foot-fault test (FFT) and the beam balance and walking (BBW) test were used to evaluate the forelimb motor; micro positron emission tomography-computed tomography (micro PET/CT) imaging with fluorodeoxyglucose (^{18}F-FDG) was used to scan the glucose metabolism in different brain regions of rats; Pearson correlation analysis was used to analyze the correlation between behavioral scores and glucose metabolism level in the CIMT group and Control group. **Results** On d7, the BBW score in the CIMT group and Control group was negatively correlated with glucose metabolism in the left insular cortex and the auditory cortex, and positively correlated with glucose metabolism in the right posterior hippocampus, superior colliculus, and inferior colliculus, with statistically significant differences; the FFT score was negatively correlated with glucose metabolism in the left somatosensory cortex, insular cortex and orbitofrontal cortex, and positively correlated with glucose metabolism in the right midbrain, with statistically significant differences. On d22, the BBW score in the CIMT group and Control group was positively correlated with glucose metabolism in the amygdala, caudate putamen, insular cortex and entorhinal cortex, and negatively correlated with glucose metabolism in the nucleus accumbens (Acb) core shell and caudate putamen in the right brain region, with statistically significant differences; the FFT score was negatively correlated with the entorhinal cortex in the right hemisphere and the difference was statistically significant. **Conclusion** The recovery of motor function promoted by CIMT was associated with the activation of both cerebral hemispheres in rats. The improvement of balance function promoted by CIMT in rats with cerebral ischemia was mainly related to the activation of Acb core shell in the right hemisphere. The recovery of fine grasping function promoted by CIMT may be related to the activation of the right entorhinal cortex.

【Key words】 constraint-induced movement therapy (CIMT); cerebral ischemia-reperfusion; micro position emission tomography (PET); glucose metabolism; motor function; Pearson correlation analysis; rat

*** This work was supported by the General Program of National Natural Science Foundation of China (81871841).**

Ischemic stroke is recognized as one of the main causes of long-term disability and death worldwide[1-2]. It is triggered by thrombotic or embolic occlusion of cerebral arteries, and subsequently results in "learned non-use" of the affected limb without the appropriate treatment. Constraint-induced movement therapy (CIMT) has gained considerable popularity as a treatment approach to stroke rehabilitation. The idea of this approach is to use the affected extremity intensively and repeatedly, while restraining the unaffected extremity[3]. Recent studies have shown that CIMT can reduce motor deficits and enhance fine movement of the affected limb for hemiplegic stroke patients[4-5]. CIMT has also been shown to improve behavioral performance in ischemic rat models[6-9]. Although CIMT can improve neuroplasticity and functional recovery after cerebral ischemia, the process and pattern of rehabilitation are still unclear[10].

Micro ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) can clearly visualize glucose metabolism at different levels in different brain regions. Glucose metabolism in the brain can reflect the activity of brain functions to a certain extent, and thus provide direct evidence for the activation of brain functional areas after compulsory exercise.

We previously reported the effect of CIMT on the behavior of rats with middle cerebral artery occlusion (MCAO) and the effect of cerebral glucose metabolism[11]. However, whether there is a correlation between the recovery of motor function and the activation of brain functional areas remains to be determined. This study is a continuation of our previous work. Its purpose is to further explore the correlation between motor function recovery and regional glucose metabolism induced by CIMT in rats with cerebral ischemia, so as to further understand the action mechanism of CIMT and lay a foundation for subsequent molecular mechanism research.

Materials and Methods

Animals Twenty-two male specific pathogen-free (SPF) Sprague-Dawley (SD) rats with body weights of 230−280 g were used in the experiment. They were provided by Sino-British Sippr/BK Lab Animal Ltd. Using a random number table, these rats were divided into an ischemic group treated with CIMT (CIMT, n=6), an ischemic group (Control, n=6), a sham-operated group (Sham, n=6), and a normal group (Normal, n=4). All rats were kept in a room temperature environment with a 12-hour light/dark cycle. They were given plenty of food and water. In the experiment, all operations were carried out during the day. The study protocol was revised and approved by the Animal Experimental Committee of Fudan University (Approval No. 201802173S). The experiment was conducted according to the Guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals[12].

Establishment of cerebral ischemia models Ischemia models were established by occluding the left middle cerebral artery in the CIMT and Control groups, as previously described[13]. The middle cerebral artery was not blocked in the Sham group, and no special treatment was given to the Normal group. The operation was performed by the same surgeon using the same specification surgical nylon monofilament. The insertion depth of the monofilament was 18−20 mm. The Sham group received the same treatments as the Control group, but the middle cerebral artery was not blocked. Before, during, and after the MCAO procedure, Laser-Doppler flowmetry (moorVMS-LDF Vascular Monitoring System; Moor Instruments Ltd., UK) was used to continuously monitor the regional cerebral blood flow (rCBF). The blood flow in the middle cerebral artery decreased by 70%−80% after the insertion of the monofilament and the ischemic period lasted for 1.5 h. The same level of blood flow was maintained throughout the ischemic period and was slowly returned to the base value after the monofilament was removed. If the thrombe was met with the above monitoring standard, the MCAO model was deemed successful[14]. The results showed that there were no significant differences between the Control and CIMT groups. The specific operation steps and results were described in our previous study[11].

Constraint of movement in the unaffected limb Starting from day 8 (d8) after cerebral ischemia, the ipsilateral (left) forelimbs and upper torsos of rats in the CIMT and Sham groups were subjected to constrained movement for 14 days using a plaster cast with a smooth cotton inner lining. The rats in the Control group received no CIMT training and the rats in the Normal group received no treatment. All animals could move freely in the cage. CIMT training consisted of fine grip training and daily activities. The foot-fault test (FFT) was used to evaluate grip training for the affected limb. Daily training sessions consisted of 16 to 20 grips repeated 5 times with an interval of 1 to 2 minutes. In the rest of the time, the rats engaged in daily activities, grooming, eating, etc. inside the cage. After 2 weeks of CIMT training, the cast was removed and a behavioral assessment and micro-PET scan were performed. For detailed methods please refer to our previous study [11].

Behavioral assessment In the present study, FFT was used to assess the fine motor function in the affected limb. A horizontal ladder with 34 rods separated from each other by 2 cm-intervals was used as previously described[15]. Under normal circumstances, rats could walk the whole distance by taking 9 to 12 steps in a row. Falling off or slipping through the rod once for the forelimb on the affected side was considered as one faulty step. The fall ratio for the affected limb was calculated from the ratio of faulty steps to the total number of steps. FFT was mainly used to measure the fine grasping ability of rat forelimbs. The gross motor function and balance ability was evaluated with the BBW. The apparatus for BBW was a beam with a width of 2.5 cm and a length of 150 cm, elevated 55 cm above the ground. The animals were rated using a 5-point scale. Higher scores indicated more severe neurological deficits. FFT and BBW tests were performed on d0 before surgery, and on d7 and d22. The results showed that CIMT promoted the improvement of rats' movement, grasping, and coordination ability.

Micro-PET/CT imaging On d7 and d22 after cerebral ischemia, micro-PET/CT imaging was performed using the Siemens Inveon MM PET/CT scanner (SIEMENS, USA) with a lutetium oxyorthosilicate (LSO) crystal detector. ^{18}FDG was provided by the PET Center, Huashan Hospital of Fudan University. Before micro PET scan, 1.0 mCi of pyrogen-free ^{18}FDG was injected through the tail vein, and then the rats were returned to the cage for about 40 minutes so that the tracer could be completely absorbed and metabolized[16]. The rats were anesthetized with 10% chloral hydrate (350 mg/kg, intraperitoneum), and then were placed in the micro PET scanner in the prone position. After a 5-minute CT scan for positioning and attenuation correction, a 15-minute dynamic PET image acquisition was performed. The acquisition mode was emission acquisition with an energy window of 350–650 kV and a time window of 3.432 ns. The collected original data were divided into single frames, and the image reconstruction method was used to obtain a multiple planar reconstruction (MPR) image with a pixel size of $0.776\times0.776\times0.796$ mm^3 and an image matrix size of $128\times128\times159$. Each PET image was spatially normalized into the space of an ^{18}FDG template using the brain normalization function in the PMOD v3.3 (PMOD Technologies, Zurich, Switzerland)[17]. In the Fusion module, PET images of rats were overlapped with PET modules in the system, and standardized processing was performed. The standardized regions of interest (ROI) in the template were superimposed on the transformed PET images, and the standardized uptake values (SUVs) of brain regions were obtained for homogenization. This was done through the PMOD software package in conjunction with the W. Schiffer rat brain template and atlas.

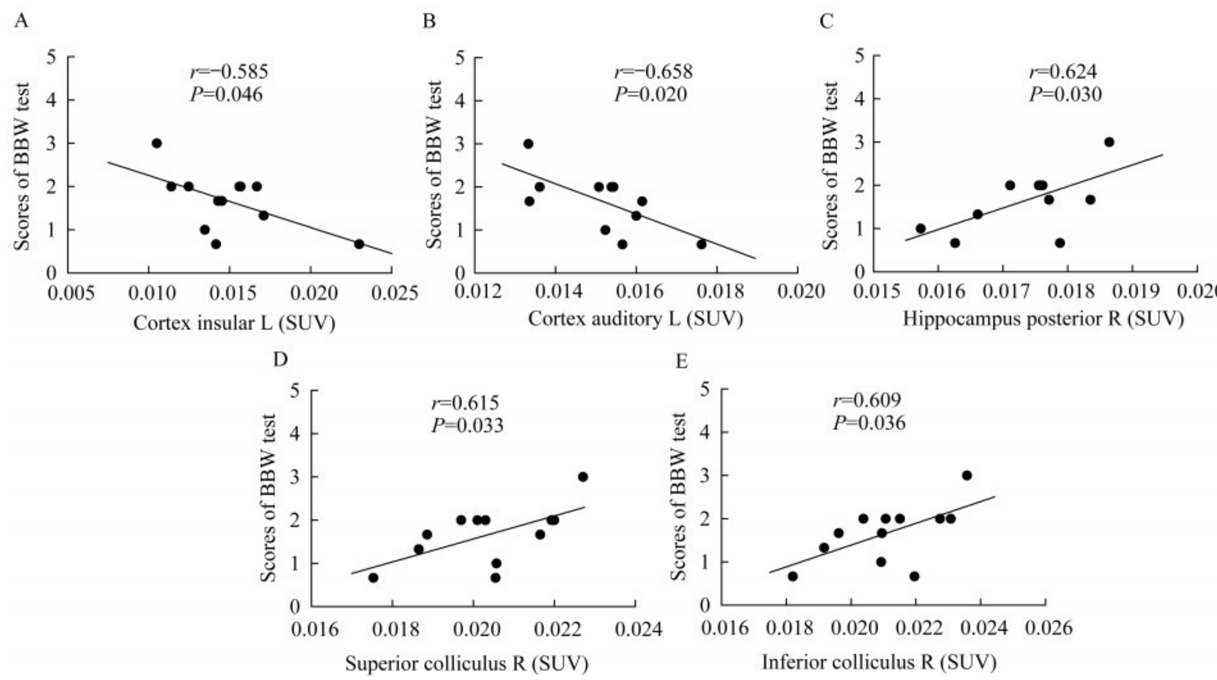
Statistical analysis Statistical analysis was performed using the SPSS 19.0 (IBM SPSS statistics, IBM Corp., USA). Data analysis was performed using the GraphPad Prism 5.0 (LA Jolla, CA, USA). Data were expressed in $\bar{x}\pm s$. Pearson correlation coefficient (r) was used to analyze the correlation between behavioral score and glucose metabolism level in local brain region. $P<0.05$ indicated that the difference was statistically significant.

Results

Correlation between the behavioral score of MCAO rats and the level of glucose metabolism in activated brain regions before CIMT (d7) Micro-PET/CT was performed in all the four groups. The Sham group was designed to eliminate the effect of plaster fixation method used in CIMT intervention on regional cerebral glucose metabolism. The

Normal group was to perform as control. Because these two groups of rats have no cerebral ischemia injury, micro-PET/CT results showed that there were no significant differences in local glucose metabolism and their behavior was also normal. Therefore, we only selected the CIMT and Sham groups for correlation analysis. The same was true for the following.

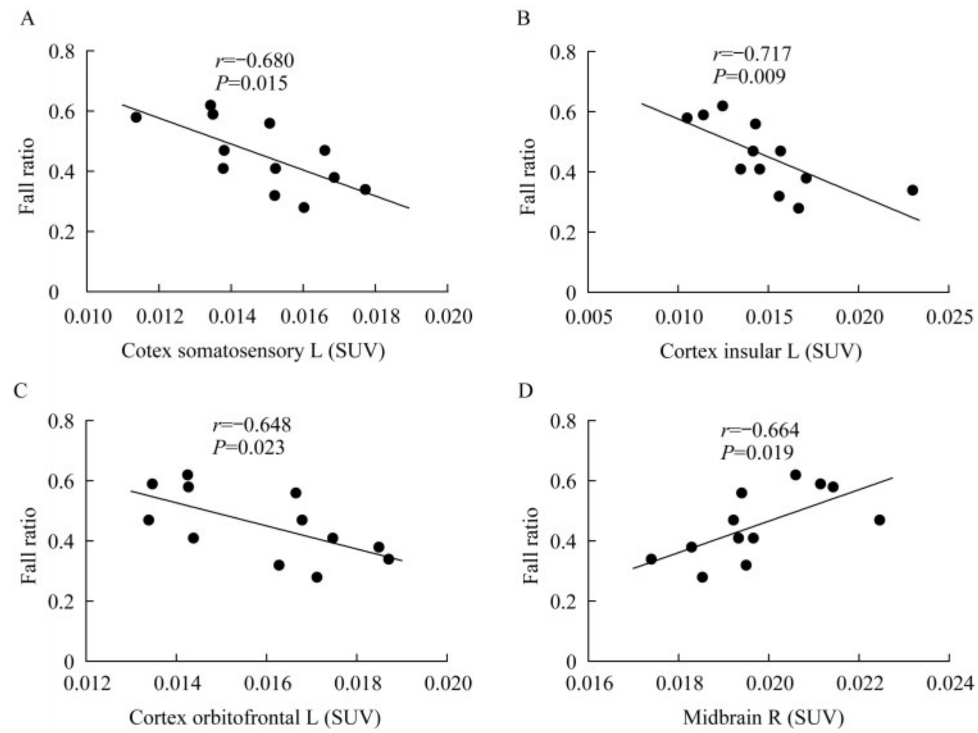
The results showed that the BBW score was negatively correlated with the level of glucose metabolism in the left insular cortex and the auditory cortex (r=−0.585, P=0.046; r=−0.658, P=0.020, respectively), and was positively correlated with the level of glucose metabolism in the right hippocampus, superior colliculus, and inferior colliculus (r=0.624, P=0.030; r=0.615, P=0.033; r=0.609, P=0.036, respectively). The results also showed that a higher glucose metabolism level in the left insular cortex and auditory cortex was associated with a lower balance beam score and better balance function in rats. Over-activation of the right hippocampus, superior colliculus, and inferior colliculus was detrimental to the recovery of balance function. It was observed that the recovery of balance function in MCAO model rats mainly depended on the activation of the affected brain region during the short-term natural recovery process (Fig 1).



A: Balance beam score and left insular cortex. B: Balance beam score and left auditory cortex. C: Balance beam score and right posterior abdominal hippocampus. D: Balance beam score and right superior colliculus. E: Balance beam score and right inferior colliculus. The balance beam score was negatively correlated with the glucose metabolism level in the left insular cortex and the auditory cortex (both $P<0.05$), and positively correlated with the glucose metabolism level in the right posterior abdominal hippocampus, superior colliculus, and inferior colliculus (all $P<0.05$). L: Ipsilateral; R: Contralateral; SUV: Standardized uptake value.

Fig 1 Correlation analysis diagram for balance beam score and glucose metabolism level in related activated brain regions on d7

The results showed that the fall ratio was negatively correlated with the glucose metabolism level in the left somatosensory cortex, insular cortex, and orbitofrontal cortex (r=−0.680, P=0.015; r=−0.717, P=0.009; r=−0.648, P=0.023, respectively) and was positively correlated with the glucose metabolism level in the right midbrain (r=0.664, P=0.019). The results also showed that rats with a higher level of glucose metabolism in the left somatosensory cortex, insular cortex, and orbitofrontal cortex had a lower fall ratio and better fine grasping function. Over-activation of the right midbrain was detrimental to behavior. During the short-term natural recovery process 7 days after operation, the recovery of fine function in rats with cerebral ischemia was related to the activation of the affected brain region (Fig 2).

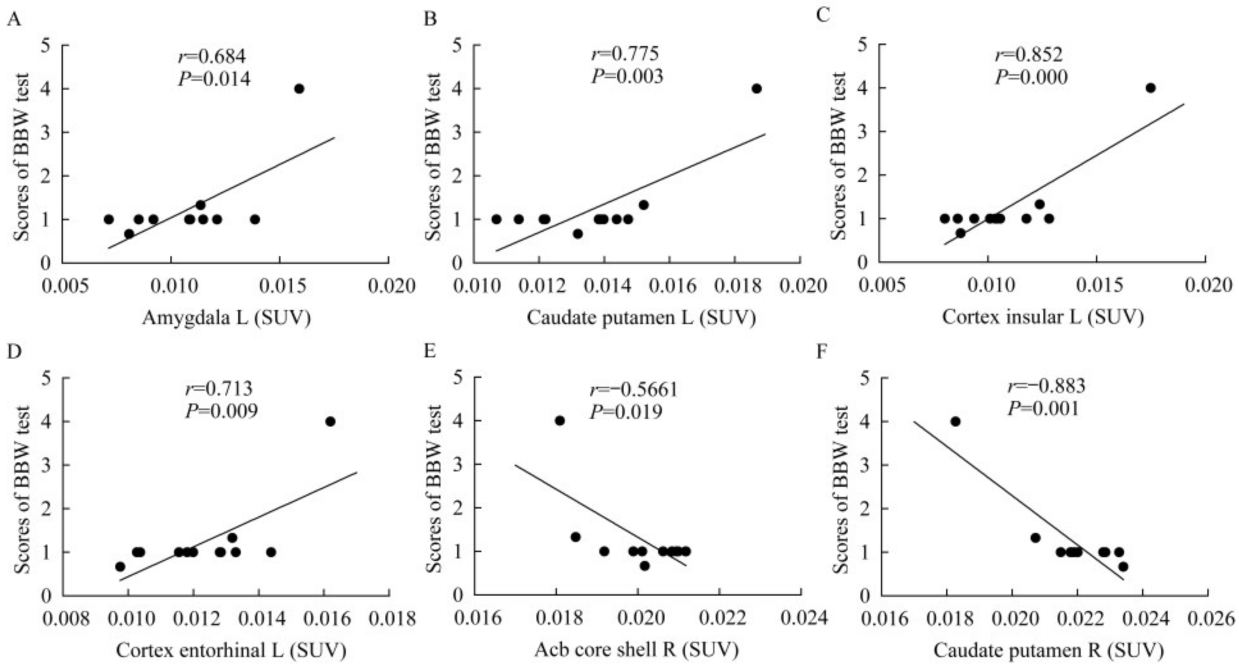


A: Fall ratio and left somatosensory cortex. B: Fall ratio and left insular cortex. C: Fall ratio and left orbitofrontal cortex. D: Fall ratio and right midbrain. The fall ratio was negatively correlated with the level of glucose metabolism in the left somatosensory cortex, insular cortex, and orbitofrontal cortex (all $P<0.05$), and positively correlated with the level of glucose metabolism in the right midbrain ($P<0.05$). L: Ipsilateral; R: Contralateral; SUV: Standardized intake value.

Fig 2 Correlation analysis between the fall ratio and the glucose metabolism level in related activated brain regions on d7

Correlation between the behavioral score of MCAO rats and the level of glucose metabolism in activated brain regions after CIMT (d22) The results showed that the BBW score was positively correlated with the level of glucose metabolism in the left amygdala, caudate putamen, insular cortex, and entorhinal cortex ($r=0.684$, $P=0.014$; $r=0.775$, $P=0.003$; $r=0.852$, $P=0.000$; $r=0.713$, $P=0.009$, respectively), and was negatively correlated with the level of glucose metabolism in the Acb core shell and the caudate putamen in the contralesional hemisphere ($r=-0.661$, $P=0.019$; $r=-0.883$, $P<0.001$). The results suggested that a lower level of glucose metabolism in the ipsilateral amygdala, caudate putamen, insular cortex, and entorhinal cortex was associated with a higher level of glucose metabolism in the contralesional Acb core shell and caudate putamen, a lower balance beam score and better balance function in rats with cerebral ischemia. After CIMT intervention, the improvement of balance function in the ischemia group was related to the activation of bilateral brain regions. This suggested that the activation of both brain regions may play an effective role in the recovery 22 days after surgery. Compared with the Control group, CIMT promoted the recovery of motor function in rats with cerebral ischemia mainly through activation of Acb core shell in the contralesional hemisphere (Fig 3).

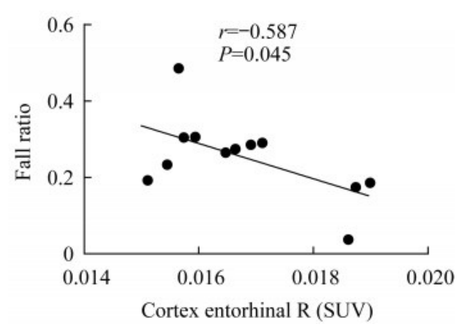
The results showed that the fall ratio was negatively correlated with the level of glucose metabolism in the right entorhinal cortex ($r=-0.587$, $P=0.045$), which indicated that over-activation of the



A-F: Balance beam score and left amygdala, left caudate putamen, left insular cortex, left entorhinal cortex, right Acb core shell, and right caudate putamen, respectively. The balance beam score was positively correlated with glucose metabolism in the amygdala, putamen, insular cortex, and entorhinal cortex in the left brain regions (all $P<0.05$), and negatively correlated with glucose metabolism in Acb core shell and putamen in the right brain regions (both $P<0.05$). L: Ipsilateral; R: Contralateral; SUV: standardized intake value.

Fig 3 Correlation analysis between balance beam score and glucose metabolism level in related activated brain regions on d22

right entorhinal cortex contributed to the decrease in the fall ratio, thus promoting the improvement of the fine gripping function, but there was no significant statistical difference between the groups. However, the glucose metabolism level in the experimental group (0.017 ± 0.001) was higher than that in the Control group (0.016 ± 0.001), suggesting that the higher the glucose metabolism level in the right entorhinal cortex, the lower the fall ratio and the better the fine gripping function of rats with cerebral ischemia would be (Fig 4).



Step error rate was negatively correlated with the glucose metabolism level in the right entorhinal cortex ($P<0.05$). R: Contralateral.

Fig 4 Correlation analysis between step rate and glucose metabolism level in the right entorhinal cortex on d22

Discussion

Micro-PET uses radionuclide-labeled molecules for *in vivo* imaging and can provide a noninvasive, dynamic, and quantitative view of neuronal activity at the molecular level. It has become an important research method in the field of neurofunctional imaging. The basic principle of micro PET is roughly the same as that of clinical PET[18]. At present, the most widely used molecule for micro-PET, ^{18}F-FDG, is an isomer of glucose[19] that can directly reflect the metabolic changes of neurons in the brain and can be applied to the study of the central reorganization mechanism. Traditional electrophysiological or molecular biological methods can selectively visualize functional changes in only a few brain regions, while PET technology can show the metabolic changes in the entire brain. Micro-PET can clearly display the glucose metabolism activity at different levels in different brain regions and the metabolism in brain regions can be observed from the coronal plane, horizontal plane, and sagittal plane, providing direct evidence for the activation of brain functional areas after CIMT. The plasticity of neuronal synapses depends on energy metabolism. Glucose metabolism is the main energy metabolism mode in the brain and the level of glucose metabolism is closely related to the activity of neurons[20-21]. Therefore, glucose metabolism in the brain reflects brain function activity to a certain extent.

In our previous study[11], we reported the behavioral performance and changes in cerebral glucose metabolism after CIMT treatment in a rat model of cerebral ischemia. We found that CIMT improved the behavior of rats with cerebral ischemia. Furthermore, rats in the CIMT group showed changes in glucose metabolism at specific regions in the ipsilateral and contralateral hemispheres after 2 weeks of intervention. Upper limb injury is one of the most common disabilities experienced by survivors of cerebral ischemia. Clinical and experimental studies have shown that during the CIMT intervention, due to the inhibition of the healthy limbs, the use and high-intensity autonomous movement of the affected limbs can promote the recovery of motor function and improve motor disorders after cerebral ischemia[22-24]. Based on our previous research, we further explored the correlation between behavioral changes and glucose metabolism levels in specific brain regions in rats with cerebral ischemia in this study and provided evidence for the mechanism underlying motor function improvements in rats by CIMT.

In this study, during the natural recovery process 7 days after surgery, the BBW score was negatively correlated with glucose metabolism in the left insular cortex and auditory cortex, and positively correlated with glucose metabolism in the right posterior hippocampus, superior colliculus, and inferior colliculus. The results showed that higher glucose metabolism levels in the left insular cortex and auditory cortex were associated with lower balance beam scores and better balance function in rats. Over-activation of the right hippocampus, superior colliculus, and inferior colliculus was detrimental to the recovery of balance function (Fig 1). The fall ratio was negatively correlated with glucose metabolism in the somatosensory cortex, insular cortex, and orbitofrontal cortex in the left cerebral region and positively correlated with glucose metabolism in the midbrain of the right cerebral region. The results suggested that higher levels of glucose metabolism in the left somatosensory cortex, insular cortex, and orbitofrontal cortex were associated with lower fall ratio, and better fine grasping function in rats. Excessive activation of the right midbrain was detrimental to behavior (Fig 2). These findings suggested that the balance beam score for rats in the ischemic group was lower than that in the Sham and Normal groups 7 days after surgery and glucose metabolism was reduced in the surgery-related brain regions in the left cerebral cortex. Due to the lack of behavioral and PET/CT scan results 24 hours after surgery, it was impossible to compare the values before and after surgery. However, it was not difficult to speculate that the behavioral scores of rats 24 hours after surgery and the level of glucose metabolism in operation-related areas of the affected brain region may be lower. Therefore, during the first 7 days of natural recovery after MCAO, the recovery of balance function in rats with cerebral ischemia mainly played a role through the activation of the insular cortex and visual cortex (visual compensation) on the affected side. The improvement of the fine grasping function may mainly depend on the increase of the metabolic level in the somatosensory cortex, insular cortex, and orbitofrontal cortex. In conclusion, the motor function recovery in MCAO model rats during the first 7 days of natural recovery mainly depended on the activation of the affected cortex.

The results of this study suggested that 22 days after surgery, lower levels of glucose metabolism in the left amygdala, caudate putamen, insular cortex, and entorhinal cortex were related to higher glucose metabolism levels in the right Acb core shell and caudate putamen, lower balance beam scores, and better balance function in rats with cerebral ischemia (Fig 3). These results indicated that both cerebral hemispheres were involved in the recovery of balance function in cerebral ischemia rats, and the activation of the right Acb core shell may play a major role. Compared to d7, glucose metabolism in the left caudate putamen and entorhinal cortex decreased at d22 in the Control group, while metabolism in the right caudate putamen increased. This indicated that the recovery of balance function during the natural recovery process after 22 days of MCAO in rats with cerebral ischemia was related to the activation of the bilateral cerebral hemispheres, which mainly played a role through activation of the contralateral basal ganglia. The fall ratio was negatively correlated with glucose metabolism in the right entorhinal cortex (Fig 4). There was no significant difference in the glucose metabolism in the right entorhinal cortex between the two groups, and there was an increasing trend in the CIMT group compared with the Control group. In conclusion, the improvement of fine motor function in rats with cerebral ischemia by CIMT may be related to the increase of glucose metabolism in the right entorhinal cortex. This conclusion needed to be verified with a larger sample size.

The Acb, located in the ventral striatum, is the main brain region that controls motivation and reward and is also involved in the regulation of exercise fatigue[25]. Acb dysfunction involves mental diseases such as depression, obsessive-compulsive disorder, or neurological diseases as well as obesity, drug abuse, addiction, etc. Interestingly, a similar behavioral pattern can be observed in healthy adults who receive a motivational stimulus. Functional magnetic resonance imaging (fMRI) confirms that the ventral striatum is responsible for encoding expectations and drives motor/cognitive behavior to improve behavioral performance[26]. Animal studies have shown that the Acb plays an important role in the acquisition and expression of incentive significance[27]. Restoration of motor function and the ability to perform daily activities after stroke can be used as a reward or intrinsic motivation for therapeutic exercise. Thus, the Acb helps to promote participation and reprogramming responses during motor recovery after stroke. This conclusion has also been confirmed in related studies by our research group[28].

In the process of rehabilitation after stroke, bilateral neurons in the central nervous system are recruited to the neural network that innervates the paralyzed upper limb. An fMRI study of stroke patients with good recovery supported a model of enhanced bilateral hemispheric motor reproduction after subcortical stroke, confirming the possibility of functional integration of these regions into the reconstructed hand functional network[29]. Our previous study suggested that CIMT significantly improved the walking ability of the rats and stimulated more neurons to enter the neural network of the paralyzed forelimb in the motor cortex and red nucleus on the healthy side, and that the motor cortex and red nucleus on the healthy side may play a more important role in structural restructuring than the corresponding motor cortex on the affected side[30]. The results of this study also confirmed the activation of bilateral cerebral hemispheres in the promotion of motor recovery in cerebral ischemia rats by CIMT, which may occur mainly through the activation of the basal ganglia. Basal ganglia are subcortical structures involved in regulating motor sequencing, motor skills, and complex actions[31], and the specific molecular mechanism for their activation by CIMT needs to be further studied.

There are several limitations to this study as follows: (1) Lack of evaluation time points, lack of behavioral scores and PET/CT scan results 24 h or 48 h after cerebral ischemia, mainly due to the severe impairment of motor function in rats during the acute period after MCAO and the inability to complete behavioral evaluation or PET/CT scans, leading to increased mortality. (2) Lack of follow-up after treatment to investigate the long-term effects of CIMT on cerebral glucose metabolism in rats with cerebral ischemia in order to observe the dynamic changes and rules for the activation of brain regions promoted by CIMT after cerebral ischemia and further explore the mechanism of action for CIMT. (3) The sample size is small, mainly due to the high cost of micro-PET scanning.

During the natural recovery 7 days after cerebral ischemia, the recovery of motor function mainly depended on the improvement of glucose metabolism in the cortex on the affected side, while the activation of the brain region on the healthy side may be detrimental to the recovery of motor function.

During the natural recovery 22 days after surgery, both cerebral hemispheres were involved in the recovery of balance function in rats with cerebral ischemia; the recovery of motor function promoted by CIMT was also related to the activation of bilateral cerebral hemispheres, and there was no obvious bias. However, compared with the Control group, further improvements in the balance function promoted by CIMT were mainly related to the activation of the right Acb core shell in rats with cerebral ischemia.

In conclusion, compulsory exercise training mainly promotes the recovery of balance function in rats with cerebral ischemia through the activation of the Acb core shell on the contralesional hemisphere. CIMT promotes the recovery of the fine griping function, which may be related to the increase of glucose metabolism in the right entorhinal cortex.

Authors' Contributions LI Ying-ying designed the study, performed the experiments, collected and analyzed the data, prepared the manuscript. HUA Yan and YU Ke-wei acquired and analyzed the data. BAO Wei-qi performed micro-PET/CT scaning and image reconstruction. WANG Yu-yuan and HU Jian acquired the data and performed the literature survey. HU Shi-hong revised the manuscript. BAI Yu-long designed and supervised the study, and interpreted the data in the experiment.

Conflicts of Interest The authors declare no conflict of interest.

References

[1] MURRAY CJ, LOPEZ AD. Mortality by cause for eight regions of the world: global burden of disease study[J]. *Lancet*, 1997, 349(9061): 1269-1276.

[2] HOYER EH, CELNIK PA, Understanding and enhancing motor recovery after stroke using transcranial magnetic stimulation[J]. *Restor Neurol Neurosci*, 2011, 29(6): 395-409.

[3] GROTTA JC, NOSER EA, RO T, *et al*. Constraint-induced movement therapy[J]. *Stroke*, 2004, 35(11): 2699-2701.

[4] TAUB E, USWATTE G, BOWMAN MH, *et al*. Constraint-induced movement therapy combined with conventional neurorehabilitation techniques in chronic stroke patients with plegic hands: a case series[J]. *Arch Phys Med Rehabil*, 2013, 94(1): 86-94.

[5] TREGER I, AIDINOF L, LEHRER H, *et al*. Modified constraint-induced movement therapy improved upper limb function in subacute poststroke patients: a small-scale clinical trial[J]. *Top Stroke Rehabil*, 2012, 19(4): 287-293.

[6] MACLELLAN CL, GRANS J, ADAMS K, *et al*. Combined use of a cytoprotectant and rehabilitation therapy after severe intracerebral hemorrhage in rats[J]. *Brain Res*, 2005, 1063(1): 40-47.

[7] ZHAO CS, WANG J, ZhAO SS, *et al*. Constraint-induced movement therapy enhanced neurogenesis and behavioral recovery after stroke in adult rats[J]. *Tohoku J Exp Med*, 2009, 218(4): 301 -308.

[8] ZHAO S, ZHAO M, XIAO T, *et al*. Constraint-induced movement therapy overcomes the intrinsic axonal growth-inhibitory signals in stroke rats [J]. *Stroke*, 2013, 44(6): 1698-1705.

[9] OPATZ J, KURY P, SCHIWY N, *et al*. SDF-1 stimulates neurite growth on inhibitory CNS myelin [J]. *Mol Cell Neurosci*, 2009, 40(2): 293-300.

[10] WOLF SL, THOMPSON PA, WINSTEIN CJ, *et al*. The EXCITE stroke trial: comparing early and delayed constraint-induced movement therapy [J]. *Stroke*, 2010, 41(10): 2309- 2315.

[11] LI YY, ZHANG B, YU KW, *et al*. Effects of constraint-induced movement therapy on brain glucose metabolism in

a rat model of cerebral ischemia: a micro PET/CT study [J].*Int J Neurosci*,2018,128(8):736-745.

[12] NATIONAL RESEARCH COUNCIL (US) COMMITTEE FOR THE UPDATE OF THE GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS.Guide for the Care and Use of Laboratory Animals [M].8th ed. Washington (DC): National Academies Press (US),2011.

[13] ZHANG B, HE Q, LI YY, *et al*. Constraint-induced movement therapy promotes motor function recovery and downregulates phosphorylated extracellular regulated protein kinase expression in ischemic brain tissue of rats[J]. *Neural Regen Res*,2015,10(12):2004-2010.

[14] ZHANG Q,WU Y,SHA HY,*et al*.Early exercise affects mitochondrial transcription factors expression after cerebral ischemia in rats[J].*Int J Mol Sci*,2012,13(2):1670-1679.

[15] ZHANG AJ, BAI YL, HU YS, *et al*. The effects of exercise intensity on p-NR_2B expression in cerebral ischemic rats [J].*Can J Neurol Sci*,2012,39(5):613-618.

[16] MATSUMURA A,MIZOKAWA S,TANAKA M,*et al*. Assessment of micro PET performance in analyzing the rat brain under different types of anesthesia: comparison between quantitative data obtained with micro PET and *ex vivo* autoradiography [J].*Neuroimage*,2003,20(4):2040-2050.

[17] PARTHOENS J, VERHAEGHE J, WYCKHUYS T, *et al*. Small-animal repetitive transcranial magnetic stimulation combined with [^{18}F] -FDG microPET to quantify the neuromodulation effect in the rat brain [J]. *Neuroscience*,2014,275(5):436-443.

[18] TAKASAWA M,BEECH JS,FRYER T D,*et al*.Single-subject statistical mapping of acute brain hypoxia in the rat following middle cerebral artery occlusion: a micro PET study [J].*Exp Neurol*,2011,229(2):251-258.

[19] KORNBLUM HI,ARAUJO DM,ANNALA AJ,*et al*.*In vivo* imaging of neuronal activation and plasticity in the rat brain by high resolution positron emission tomography (microPET)[J].*Nat Biotechnol*,2000,18(6):655-660.

[20] BRUEHL C, WITTE OW. Cellular activity underlying altered brain metabolism during focal epileptic activity[J]. *Ann Neurol*,1995,38(3):414-420.

[21] CHUGANI HT, HOVDA DA, VILLABLANCA JR, *et al*. Metabolic maturation of the brain: a study of local cerebral glucose utilization in the developing cat[J].*J Cereb Blood Flow Metab*,1991,11(1):35-47.

[22] ZHAO CS,WANG J,ZHAO SS,*et al*.Constraint-induced movement therapy enhanced neurogenesis and behavioral recovery after stroke in adult rats [J].*Tohoku J Exp Med*, 2009,218(4):301-308.

[23] LANG KC,THOMPSON PA,WOLF SL.The EXCITE Trial: reacquiring upper-extremity task performance with early versus late delivery of constraint therapy [J]. *Neurorehabil Neural Repair*,2013,27(7):654-663.

[24] HIDAKA Y, HAN CE, WOLF SL, *et al*. Use it and improve it or lose it: interactions between arm function and use in humans post-stroke [J].*PLoS Comput Biol*,2012,8(2):e1002343.

[25] SCHULTZ W.Neuronal reward and decision signals: from theories to data [J].*Physiol Rev*,2015,95(3):853-951.

[26] SCHMIDT L, LEBRETON M, CLERY-MELIN ML, *et al*. Neural mechanisms underlying motivation of mental versus physical effort [J]. *PLoS Biol*, 2012, 10 (2) : e1001266.

[27] PECINA S,BERRIDGE KC.Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness[J].*J Neurosci*,2005,25(50): 11777-11786.

[28] HUA Y, LI C, HU J, *et al*. Fluoxetine adjunct to therapeutic exercise promotes motor recovery in rats with cerebral ischemia: roles of nucleus accumbens [J]. *Brain Res Bull*,2019,153:1-7.

[29] RIECKER,A,GRÖSCHEL K,ACKERMANN H,*et al*. The role of the unaffected hemisphere in motor recovery after stroke[J].*Hum Brain Mapp*,2010,31(7):1017-1029.

[30] LIU PL, LI C, ZHANG B, *et al*. Constraint induced movement therapy promotes contralesional-oriented structural and bihemispheric functional neuroplasticity after stroke[J].*Brain Res Bull*,2019,50:201-206.

[31] RIVA D, TADDEI M, BULGHERONI S. The neuropsychology of basal ganglia [J]. *Eur J Paediatr Neurol*,2018,22(2):321-326.

(收稿日期:2021-10-13; 编辑:张秀峰)