Supplementary Material

Animals

Monkeys were housed in the *** indoors individually at a constant temperature of 24°C on a 12-h light/dark cycle by professional breeders. All monkeys were fed twice a day and had free access to clean drinking water. Intravenous 0.9% saline was administered throughout the operation in case of fluids lost. The monkeys were placed on a warm operating table, and a craniotomy was performed from the left frontotemporal pterion. Then the left middle cerebral artery (MCA) was gradually exposed, and the distal M1 branch of MCA was occluded with bipolar electrocoagulation. Penicillin (0.4 million IU, intramuscular, per day) was administered to prevent infection, and tramadol (50-100mg, intramuscular, per day) was administered to relieve pain during the 3 days after the surgery. 25ml of 20% mannitol was administered intravenously daily to relieve cerebral edema, for monkeys with severe consciousness deficits, mannitol was administered twice a day.

Skeletal Muscle Coordination Assessment

The scale is scored from 0 to 18, where the higher the score the poorer the skeletal muscle coordination and the poorer the motor function. A score of 0 is considered normal walking and a score of 18 shows a complete absence of movement, suggesting a loss of motor function.

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Primate Rankin Scale (pRS) Score

The pRS scores include 0-5 points. 0 points indicated no symptoms, 1 point indicated no significant disability, 2 points indicated slight disability, 3 points indicate moderate disability, 4 points indicate moderately severe disability, and 5 points indicated severe disability or death.

Behavior Assessment

Hill Task unit consists of two staircases each with 5 compartments in the forward direction, while the staircases in the Valley Task unit are in the reverse direction. A small piece of carrot or apple, about $14\times14\times14$ mm in size, was placed on each staircase. The monkeys were allowed to retrieve all the food within 5 minutes and the time spent retrieving each side of the stairs was recorded by observers. Only successful retrieval of food through the square holes on both sides of the Hill Task device or through the round hole in the middle of the Valley Task device using the correct hand was recorded. If the monkey could not successfully retrieve the food after several attempts for a total time of more than 5 minutes, the time was recorded for that side of the hand as three times the average of the corresponding hand before surgery.

MEP Amplitude and Latency

The relationship between stimulus intensity and MEP amplitude is in

the form of an S-shaped curve, generally referred to as the "stimulus-response curve" or "recruitment curve". [1] The initial segment of this curve was relatively flat, with a near-linear increase in MEP amplitude at stimulus intensities between 120% and 140% of RMT, while at higher stimulus intensities the curve approached a plateau, at which point the MEP amplitude did not increase further even when the stimulus intensity was increased. In many studies, only a single intensity MEP was assessed due to experimental limitations, rather than assessing the entire stimulus-response curve. In that case, TMS intensity is usually set to 115%-125% of the individual RMT to ensure that the experiment detects the MEP size in the rising phase of the stimulus-response curve. However, in clinical diagnosis, the primary goal of TMS-MEP is to elicit the maximal response of motor cortex. Therefore, the intensity of TMS should be high enough. In our study MEP with multiple stimulus intensities was performed. According to the International Federation of Clinical Neurophysiology guidelines, TMS stimulation intensity is usually expressed as a single motor threshold or as a percentage of the maximum stimulator output (MSO).[1] The stimulus intensity was gradually increased from RMT until the MEP no longer increased, at which point the maximum MEP amplitude was obtained. In the TMS-MEP on motor cortex, the range of stimulus intensities where maximum MEP amplitude was between 60% MSO and 80% MSO in our

experiment. Thus, the magnetic stimulation output was set to 60-80% MSO to obtain the stable and maximum MEP amplitude. It is known that the variability of the MEP amplitude excited by 100% RMT TMS was small when the muscle was completely relaxed, generally 0-0.5mV in absolute value.[1] Therefore, the maximum amplitude in the pre-stimulation period generally did not exceed 0.5mV in absolute value when excited by 100% RMT. In general, the muscles in sedated monkeys were presumed to be relaxed, yet further confirmation by electromyography would have been preferable. Since electromyography could not be performed due to the instrumentation limitations, no pre-stimulus muscle activities were confirmed by baseline mean amplitude before the stimulation. And it was possible to judge whether a waveform was an artifact based on the location, shape, etc. Due to the numerous factors influencing the flatness of the MEP baseline, slight fluctuations in the baseline have little effect on the assessment of MEP parameters when the animal's muscles stayed relaxed.

Animal Sacrifice and Histopathology

Intramuscular ketamine overdoses were given to anesthetize the monkeys (n=2) at 12 weeks after MCAO. Saline cardiac perfusion was performed after the pain reflexes had completely disappeared, followed by 4% paraformaldehyde perfusion. The skin and subcutaneous tissues of both upper limbs were incised layer by layer, and the bilateral median nerves

were removed and soaked in 4% paraformaldehyde for post-fixation. After 24-48 hours of fixation, dehydration and paraffin embedding were performed and cut into paraffin sections of 3μm thickness. The slices were stained with a HE staining kit (Servicebio, G1003) and a Luxol Fast Blue staining kit (Servicebio, G1030) according to the manufacturer's instructions. Morphological evaluation of the median nerve was performed according to LuxolFastBlue (LFB) staining, and the evaluation criteria were: grade 0: normal; grade 1: structural disorder of nerve fibers; grade 2: obvious vacuole formation; grade 3: disappearance of myelinated nerve fibers.

Double-labeling Immunofluorescence

Cross sections (3- μ m thick) were cut, and every 20th cross section was selected for fluorescence staining, for a total of 3 sections. Areas where corresponding fluorescence could be detected were defined as regions of interest. A negative control was placed on each section without the adding of the corresponding primary antibody. For double-labeled immunofluorescence staining, paraffin sections were incubated overnight at 4℃ with the following primary antibodies after dewaxing to water, antigen repair and blocking steps: rabbit anti- myelin basic protein (MBP; myelin marker, 1:200, Cell Signaling Technology, #78896) and mouse monoclonal anti-neurofilament 200 antibody (NF-200; 1:100, Sigma-Aldrich, N5389). Sections were then incubated with the following

fluorescent secondary antibodies for 1 hour at room temperature shielded from light: Alexa Fluor 555-conjugated goat anti-mouse IgG and Alexa Fluor 488-conjugated goat anti rabbit IgG (1:200, Cell Signaling Technology). Fluorescence images were acquired by a Leica DMi8 fluorescence confocal microscope.

Statistical Analysis

All data were first tested for normal distribution by the Shapiro-Wilk method. Data that did not meet the normal distribution were analyzed with the paired Wilcoxon test. Data collected at different time points for the same indicator that matched the normal distribution, equal variances and Mauchly's test of sphericity were analyzed with repeated measures ANOVA. The pRS and SMCS scores at multiple time points were analyzed by one-way repeated measures ANOVA. MEP parameters between the affected and unaffected side at multiple time points were analyzed by two-way repeated measures ANOVA. The MFI data of bilateral median nerve sections was tested for normality with Shapiro-Wilk test. Then paired t-test was applied if they met the normality distribution, otherwise non-parametric test (paired Wilcoxon test) was conducted. Correlation analysis was performed between the Hill and Valley Task, pRS and SMCS dataset with parameters of TMS-MEP/median nerve magnetic stimulation MEP, respectively. Pearson correlation test was used for data conforming to normal

136 distribution, and non-normal distribution data was analyzed with
137 Spearman correlation test. The relationships between these datasets were
138 then explored with automatic linear regression modeling. The Hill and
139 Valley Task, pRS and SMCS dataset were modeled separately, with
140 predictors RMT, RMTlat, latency, and amplitude of TMS-MEP and
141 median nerve MEP.

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