Supplementary Material

Cortical excitability and resting motor threshold

Single pulse transcranial magnetic stimulation (TMS) was performed using a Magstim Super Rapid^2 stimulator (The Magstim Co. Ltd, Whitland, United Kingdom) and a Magstim 70mm figure-of-eight Alpha Film Coil inducing a biphasic waveform.

TMS was applied over the ipsilesional primary motor cortex (M1). The 'motor hotspot' for ipsilesional M1 was defined as the coil position eliciting motor evoked potentials (MEP) of the highest amplitude of the first interosseous (FDI) muscle of the paretic hand in response to a TMS pulse applied tangentially to the skull in a 45° posterior-anterior direction. This orientation induces a perpendicular current at neurons in the anterior wall of the central sulcus (equivalent to the posterior wall of the precentral gyrus, Brodmann area 4 (Geyer *et al.*, 1996)) and activates the corticospinal system preferentially trans-synaptically (Di Lazzaro *et al.*, 2008).

The position of the coil was tracked and recorded using a neuronavigation system (BrainSight V.2.0.7; Rogue Research Ltd; Montreal, Canada) throughout the whole experiment. For neuronavigation, the head of each participant was co-registered with the participant's anatomical MR image via anatomical landmarks.

MEP recordings were obtained by Ag/AgCl surface electrodes (Tyco Healthcare) in a belly-to-tendon montage with the active electrode placed over the muscle belly and the reference electrode placed on the metacarpophalangeal joint of the index finger. The EMG signal was sampled at 1kHz, amplified, filtered (0.5Hz high pass and 30–300Hz bandpass), and digitized using a Powerlab 26T device and the LabChart software package version 8.0 (AD Instruments, Sydney, Australia). The resting motor threshold (RMT) was defined using an algorithm provided by the TMS Motor Threshold Assessment Tool (MTAT) 2.0 (http://www.clinicalresearcher.org/software.html) (Awiszus, 2003). This software makes use of a maximum-likelihood procedure for

reliably estimating the 50μV RMT in twelve steps via investigator-provided answers about positive or negative MEP responses.

Data analysis

Local mean field power

The Local Mean Field Power (LMFP) was computed as the square root of squared TEPs averaged across channels of the region of interest (ROI) closest to the site of stimulation (FC3-FC1-C3-C1-Cz/FC4-FC2-C4-C2-Cz for M1) (Casarotto *et al.*, 2012). Then, we applied bootstrap statistics on LMFP time-series at the single-subject level (number of permutations=1000, alpha<0.01) to estimate the LMFP values significantly different from the baseline (-300ms to -50ms) (Fecchio *et al.*, 2017; Rosanova *et al.*, 2012). **We calculated the integral of significant activations averaged between +10ms to +100ms and +100ms to +200ms post-stimulus. The reason for these two intervals was based on the of a simple positive TMS-EEG response in stroke patients (Fig. 1 and 2)**

The numbers of deflections of TEP response

Since the deflections of the TMS-evoked potentials are attributed to slow and fast excitatory and inhibitory post-synaptic potentials and the complexity of these deflections could be altered dependent on the state of the brain or in neurological disorders (Massimini *et al.*, 2005; Rosanova *et al.*, 2012), we considered the number of deflections as a simple reference contrasting fast-frequency recurrent waves in healthy controls and low-frequency and simple waves in altered neural networks. **Therefore, we plotted the averaged TEP at the electrodes under the coil (C3/C4) and counted all significant deflections in the first 200ms post-stimulus. To assess the threshold for significance, a bootstrap method was applied (number of permutations=1000, alpha-level<0.001) on the pre-stimulus activity (-300ms to -50ms). Activity above that threshold was considered significant.**

Natural frequency analysis

Spectral features were evaluated by computing the event-related spectral perturbation (ERSP) after time-frequency decomposition using Morlet wavelet transform (3.5 cycles). This procedure was implemented by using the EEGLAB function *newtimef* (Delorme and Makeig, 2004). Absolute spectra normalization was applied at the single trial level first performing a full epoch length single trial correction (Grandchamp and Delorme, 2011) and subsequently by pre-stimulus baseline correction (-500ms to -100ms) on the resulting ERSP averaged across all trials. Finally, only significant ERSP values surviving bootstrap-based statistics (number of permutations=1000, alpha-level<0.05,) with respect to baseline were considered for further analyses. We averaged the ERSP in the 5-50Hz frequency range and in a time window between 20ms-200ms post-stimulus to minimize the effects of possible artifacts occurring at the time of stimulation (Rosanova *et al.*, 2009).

K-means cluster

We used a k-means cluster analysis to group the ARAT scores and the grip force indices at the early subacute stage into three clusters, reflecting mild (cluster centers of 45.9 in the ARAT and 79.8% relative grip strength), moderate (cluster centers of 37.0 in the ARAT and 44.8% relative grip strength) and severe motor impairment (cluster centers of 1.1 in the ARAT and 3.4% relative grip strength). Accordingly, fifteen patients were assigned to the severely affected patient group, seven to moderate, and six patients were grouped as mildly impaired.

PCA

Using principal component analysis (PCA), we computed a motor composite score from the (i) ARAT scores of the affected hand, (ii) the relative grip force, and (iii) the Motricity Index scores of the affected arm assessed in the first session, i.e., on average

6.7±2.5 days, after stroke. This PCA yielded a one-factor solution with the first component explaining 96.6% of the variance (factor loadings: ARAT 0.98, grip force 0.98, Motricity Index 0.99) and was then used as the "motor composite score".

In addition, we computed a composite recovery score from the z-standardized difference relative differences scores of the three motor parameters of interest. The first component explained 78.8% of the variance (factor loadings: ARAT 0.95, grip force 0.87, Motricity Index 0.85).

Voxel lesion symptom mapping

For all stroke patients, magnetic resonance images using standard sequences (DWI, T2-weighted, T2*-weighted, FLAIR, and time-of-flight angiography sequence) were acquired in a clinical routine setting within the first two days after stroke (1.5T MRI scanner; Philips, Guildford, Great Britain). Based on the diffusion-weighted images (DWI) (TR=3900ms, TE=95ms, FOV=230mm, 22 axial slices, voxel size=1.8x2.99x6 mm^3), individual lesion maps were created using the software MRIcron (https://www.nitrc.org/projects/mricron). After interactive delineation of individual lesion volumes, lesion maps and DWI images were co-registered to the individual FLAIR/T2-weighted images and subsequently normalized to the T2-weighted MNI-template implemented in Statistical Parametric Mapping (SPM8, http://www.fil.icon.ucl.ac.uk/). Lesions located in the right hemisphere (n=15) were flipped along the mid-sagittal plane. **We conducted a voxel lesion symptom mapping (VLSM) using the non-parametric mapping (NPM) software (Rorden *et al.*, 2007).** Only voxels that were damaged in at least 10% of the patients were included in the analysis. We corrected for multiple comparisons using the non-parametric permutation tests (2000 permutations) recommended for medium sample sizes (Medina *et al.*, 2010).

Cortical excitability for MEP positive patients

When excluding the sixteen MEP negative patients, RMT did not differ between patients and the healthy controls (55.0% maximum stimulator output (MSO)±11.2% SD), neither in the early days after stroke (55.8% MSO ± 12.3% SD, p=0.64) nor after three months (60.4% MSO±11.1% SD, p=0.25). Moreover, RMT did not significantly differ between the first and second sessions (p=0.19).

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