Improving motor cortico-thalamic communication after stroke using real-time fMRI connectivity neurofeedback

Supplementary Material

Participants

The inclusion criteria for chronic stroke patients included: a) stroke > 6 months prior to participation; b) first-time stroke patient; c) age > 21 years old; d) moderate to severe hemiplegia (unable to extend fingers); e) safe for the MRI environment (assessed with MRI screening form); f) normal or corrected-to normal vision, g) normal audition; h) no history of seizures. Exclusion criteria included: a) greater than mild aphasia (unable to understand or communicate clearly); b) history of seizures or epilepsy; c) women who are pregnant or breastfeeding. All participants were right handed prior to their stroke. See **Supplementary Figure 1** for lesion locations.

Methods

Scanning parameters

Scanning was conducted on a 3T Siemens Prisma scanner**, and scanning order for each day can be found in Supplementary Figure 2**. High-resolution T1-weighted anatomical scans were collected in the sagittal plane (TR = 1950 ms, TE = 2.26 ms, voxel size: 1.0 x 1.0 x 1.0 mm^3, flip angle: 9°, 176 volumes). Echo-planar images were collected for both feedback and resting state runs. **Functional localizer and neurofeedback** runs had a duration of **217.5 seconds** (TR = 1500 ms, TE = 23 ms, matrix size: 64x64, flip angle: 90°, 1**45** volumes **total with first 10 volumes discarded**). Each volume consisted of 27 slices (voxel size: 3.28 x 3.28 x 5.0 mm^3)

with Anterior Commissure (AC) and Posterior Commissure (PC) both aligned to the axial position. Resting state runs had a duration of 4.6 minutes (TR = 2300 ms, TE = 30 ms, matrix size: 64x64, slice thickness: 3 mm, spacing between slices: 3.6 mm^3, flip angle: 70°, 36 slices per volume, 120 volumes). Participants completed approximately 10 feedback runs in each session (average=**18±2.94, with differences in runs** due to fatigue). Overt movements were visually monitored, and runs were interrupted if overt movements were detected. No adverse effects were reported.

Real-time Data Analysis

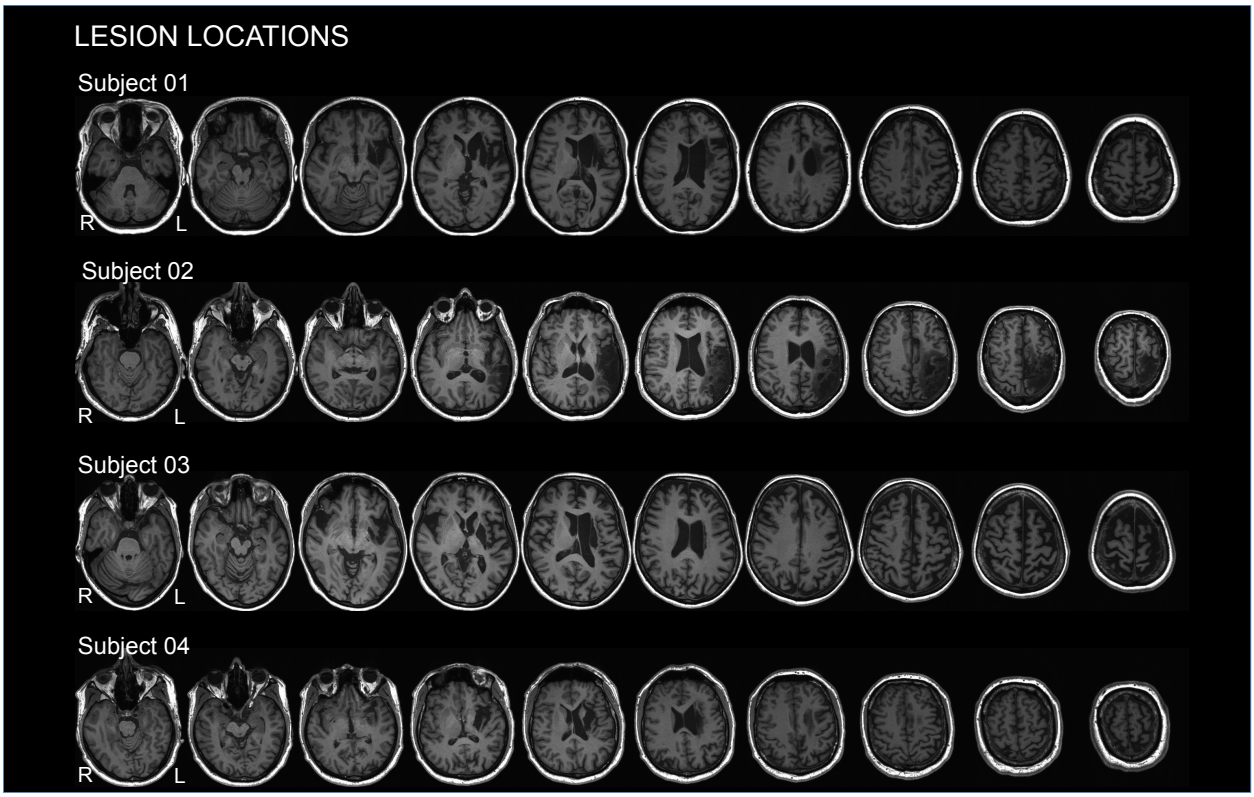
Volumes were acquired every 1.5 seconds and analyzed in Turbo- Brainvoyager (TBV) version 2.6 (Brain Innovation, Maastricht, The Netherlands; (Goebel, 2001)) and a custom Matlab toolbox developed in our lab to provide feedback in the form of a visual thermometer that grew as connectivity increased. TBV retrieved reconstructed fMRI images from the MRI reconstruction system via a LAN connection and performed online 3D motion correction. Timecourses from the ROIs drawn in TBV were extracted and a Pearson's linear correlation coefficient was derived from the time-series of the two ROIs using custom Matlab software. We used a sliding window of eight data points (i.e., the current time point and seven data points before the current data point), from each ROI, to compute the correlation coefficient. To provide a smooth feedback to the participants, the custom Matlab software provided an average of the last three feedback values as feedback to the participant. The Matlab software also controlled the visual stimulus presentation.

Post-hoc Data Analysis

In order to quantify the level of rtfMRI control across sessions, correlation scores from each run were extracted **(see Supplementary Figure 3 for all runs for each individual)**. To assess learning of rtfMRI control, the average level of rtfMRI control during the 'active blocks' of the first three ('beginning of training') and last three runs' ('end of training') were compared using a paired Student's t-test **(see Supplementary Figure 2 for details about scanning order)**. A correlation was then calculated between the change in correlation scores (post-pre) and participants' level of motor impairment as assessed by the Fugl-Meyer Upper Extremity score. **In addition, a linear regression was applied to the full runs per individual (Supplementary Figure 3). We then tested for significance of these training effects over time (e.g., slope across runs) using a permutation test on the ordering of the runs. We generated 10,000 permutations of the orders of the runs and linear regression was performed. A pairwise t-test was then performed on the slopes of the generated permutations of runs and the original sequence of runs.**

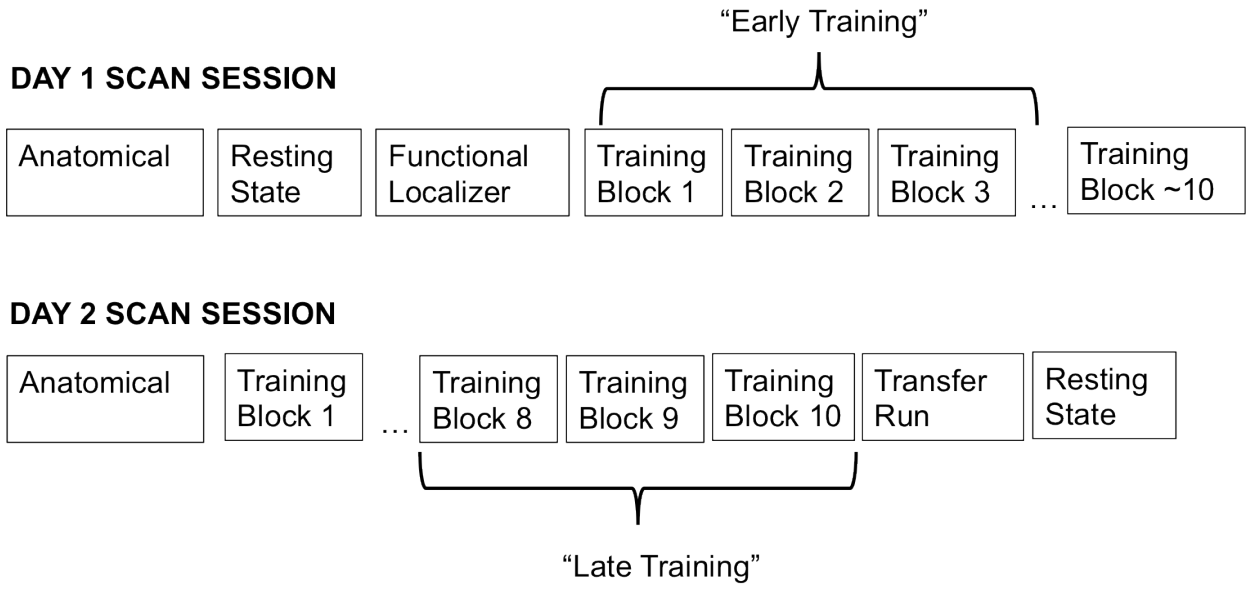
Post-hoc whole brain neuroimaging analyses were performed using the Analysis of Functional NeuroImages (AFNI) software package (Cox, 1996). The following preprocessing steps were performed for both feedback and resting **state** runs: 3dDespike was used to remove large movement fluctuations; the EPI volumes were co-registered with the anatomical T1-weighted scan to the first volume; spatial blurring was done with a 6-mm (full width at half maximum) Gaussian smoothing kernel; linear regression was used to remove motion in excess of 2 mm (6 motion parameters); spatial normalization to the MNI anatomical template was performed (included in AFNI as the MNI_TT27 template) for the purpose of higher-level comparisons. For **both feedback and resting-state** runs, **we calculated a** between-day comparison for each participant using a voxel-based Student's t-test. **This provided us with whole-brain changes**

for resting state before and after neurofeedback, and for neurofeedback training runs during the early and late stages of training (e.g., the first two neurofeedback runs on the first day, compared to the last two neurofeedback runs on the second day). These analyses were run for each subject and at the group level across all four subjects. To compare connectivity results across subjects, **we analyzed both the connectivity between our two regions of interest as well as the whole brain connectivity using a seed-based analysis in each ROI. Regions** of interest for the left (ipsilesional) primary motor cortex and thalamus were derived from the AFNI TTatlas+tlrc (from the Talairach Daemon database). BOLD signal timecourses were extracted from each ROI and used as regressors to examine whole-brain connectivity both during task and during rest. **An example of the whole-brain connectivity during task (Supplementary Figure 4) and during resting state (Supplementary Figure 5) are provided, showing the connectivity for the primary motor cortex (shown in red) and thalamus (shown in blue) and their overlap (shown in purple).** Correlations between the two ROI timecourses were also calculated using AFNI 1dCorrelate. A cluster-corrected threshold, determined by Monte Carlo simulations implemented in AFNI's AlphaSim, was set at a minimum cluster size of 55 voxels to reach a cluster level significance of $p < 0.05$ (family-wise error, FWE).



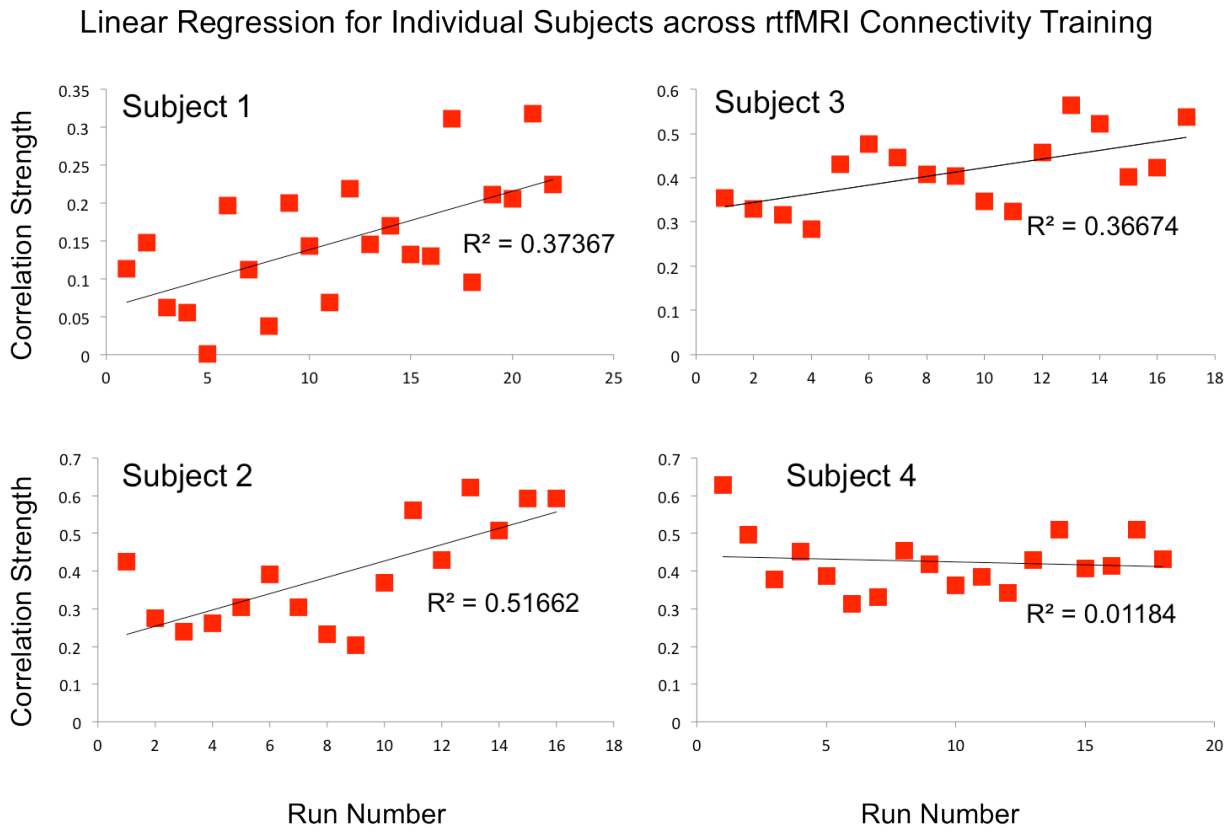
Supplementary Figure 1.

Lesion locations of each of the participants shown in horizontal slices. Subjects 01 and 02 have cortical and subcortical lesions, while subjects 03 and 04 have only subcortical lesions. All subjects have lesions comprising portions of the thalamus, caudate, putamen, and internal capsule. Subject 01's lesion extends into the premotor region, while Subject 02's lesion is the largest, extending into the premotor, motor, and parietal regions.



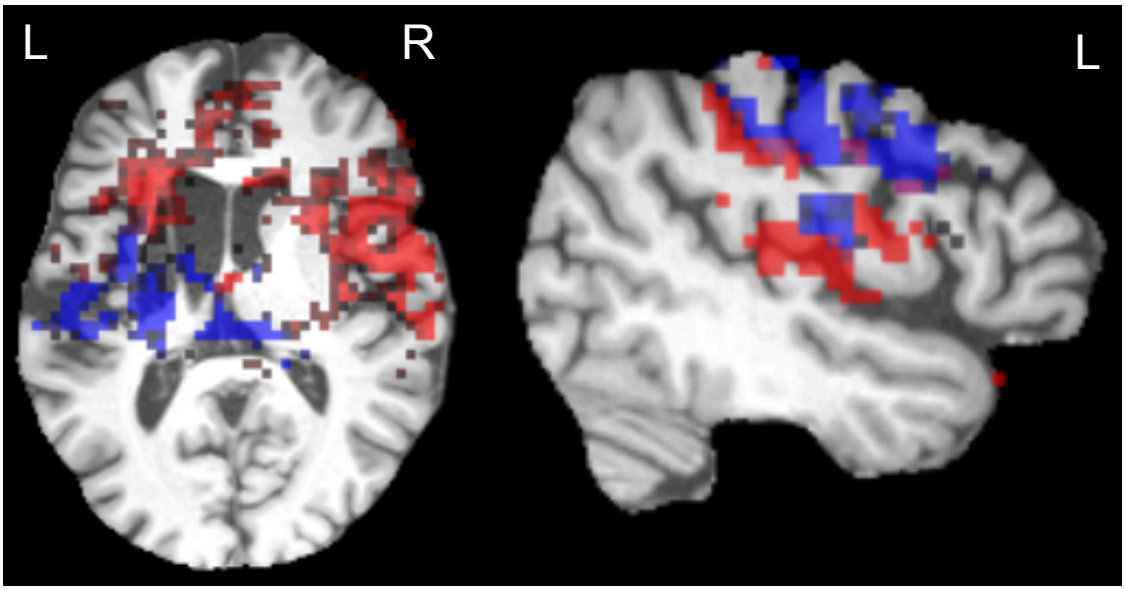
Supplementary Figure 2.

Scanning sessions for Day 1 versus Day 2. Day 1 consisted of an anatomical run, a resting state run, a functional localizer, and then neurofeedback training runs. The first three neurofeedback training runs were analyzed as "early training." Day 2 consisted of an anatomical run, then neurofeedback training runs. The last three neurofeedback training runs were analyzed as "late training." These were followed by a transfer run with no feedback, and a resting state run.



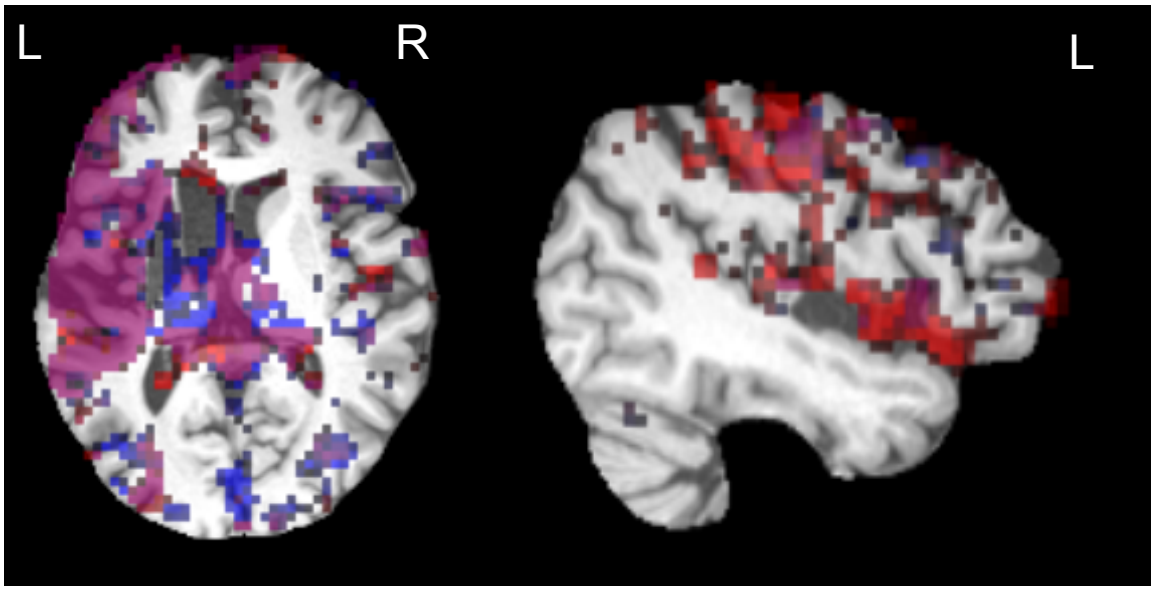
Supplementary Figure 3.

Linear regression for individual subjects across all rtfMRI connectivity training runs, with run number on the X-axis and correlation strength on the Y-axis. Subjects 1, 2, and 3 all show increases in connectivity strength with training. Subject 4 does not, but started with a relatively high correlation to begin with and then maintained it at a moderate-to-high level (~0.45-0.5).



Supplementary Figure 4.

An exemplary figure of a whole-brain correlation during late training from an ROI in the thalamus (blue) and primary motor cortex (red), as well as the overlap between the two ROIs correlated activity



Supplementary Figure 5.

An exemplary figure of a whole-brain correlation during resting state from an ROI in the thalamus (blue) and primary motor cortex (red), as well as the overlap between the two ROIs correlated activity (purple).

Supplementary References

RW Cox. AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research* 1996;29:162-173.