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# The Effect of Contralesional cTBS on Motor Network Activity and Connectivity in Stroke Patients Assessed with fMRI and Dynamic Causal Modeling


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Scientific Report Major Internship (51 EC)
Master Neuroscience & Cognition

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06-02-2022


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## Abstract

Hemiparesis of the upper limb is the most common cause of disability after stroke. The exact mechanism underlying spontaneous upper limb recovery is still unclear, and more effective therapies for optimizing stroke rehabilitation are required. Following stroke, excessive inhibition from the contralesional (CL) primary motor cortex (M1) may additionally impair activation in the ipsilesional (IL) M1. This in turn may contribute to motor impairment. Continuous theta-burst stimulation (cTBS) applied over the CL M1 is believed to suppress its activation and as a result may improve motor function. The effective connectivity between both M1s can be estimated using functional magnetic resonance imaging (fMRI) and dynamic causal modeling (DCM). This study aimed to investigate the effect of cTBS applied over the CL M1 on bilateral M1 activation and effective connectivity between the M1s, and whether this correlated with motor function. Subacute stroke patients received real or sham cTBS treatment, and underwent fMRI scanning one week after treatment, and three and six months after stroke onset. Additionally, motor function tests were performed. M1 activation was assessed by the percent signal change and changes in effective connectivity were estimated by DCM of the motor network. No significant effects of real cTBS treatment on both CL M1 and IL M1 activation were found, although a trend for IL M1 activation was visible. In addition, IL M1 activation correlated positively with motor function, indicating that this might play an important role in stroke recovery. No differences in effective connectivity between bilateral M1s were found, although variability between patients was high. Taken together, more research is needed to reveal the exact role of effective connectivity between bilateral M1s in stroke recovery, and to establish the effect of cTBS applied over the CL M1 on bilateral M1 activation and effective connectivity.

## Layman's Summary

Een beroerte, ook wel herseninfarct of hersenbloeding genoemd, wordt veroorzaakt door een blokkade of het scheuren van een bloedvat in de hersenen. Als gevolg krijgt een deel van de hersenen dat bijvoorbeeld verantwoordelijk is voor het bewegen van de arm, geen bloed. Veel beroertepatiënten krijgen als gevolg hiervan last van verlamming van de arm, en sommigen houden zelfs chronisch problemen met het bewegen van de arm en hand. Het onderliggende proces dat leidt tot verlies van armfunctie is nog onduidelijk. Het is belangrijk om hierachter te komen, zodat nieuwe therapieën die het herstel van de armfunctie verbeteren en versnellen, ontwikkeld kunnen worden.

MRI-scans van het hoofd hebben aangetoond dat de primaire motor cortex (M1) een belangrijke rol speelt. Na een beroerte blijkt de hersenhelft waar de beroerte plaatsvond (de aangedane M1) minder actief is en dat de andere hersenhelft (de niet-aangedane M1) juist overactief is. Omdat de aangedane en niet-aangedane M1 een connectie met elkaar hebben, wordt gedacht dat de verschillen in activiteit aan elkaar gerelateerd zijn. De overactiviteit van de niet-aangedane M1 leidt waarschijnlijk tot het *overrulen* van de aangedane M1, waardoor deze minder actief is. De connectie tussen beide M1s kan worden vastgesteld met een techniek die *dynamic causal modeling* heet. Hierbij worden de MRI-beelden gebruikt om een inschatting te maken hoe deze connectie eruitziet.

Transcraniële magnetische stimulatie (TMS) wordt gezien als een mogelijke nieuwe therapie om armfunctie te verbeteren. TMS is een apparaat waarmee via de schedel een reeks magnetische pulsen af wordt gegeven. Hierdoor kunnen onderliggende hersengebieden geremd of geactiveerd worden. Door het TMS-apparaat precies op de plek boven de niet-aangedane M1 te plaatsen en dit gebied te remmen, hopen we dat deze minder actief wordt, met als gevolg dat de aangedane M1 juist actiever wordt. Hopelijk leidt dit tot verbetering van de armfunctie.

Het doel van deze studie was om het effect van TMS te bekijken op 1) de activatie van de aangedane en niet-aangedane M1, en 2) de connectie tussen beide M1s. Ook hebben we onderzocht of de activiteit van en connectie tussen beide M1s een relatie heeft met hoe goed de functie van de arm herstelt. Om de functie van de arm te bepalen voerden de patiënten verschillende testjes uit, waarbij ze de arm moesten bewegen.

De resultaten toonden geen significant effect aan van de TMS-behandeling op de activatie van de aangedane en niet-aangedane M1. Wel bleken patiënten met een hogere activatie van de aangedane M1, over het algemeen een betere armfunctie te hebben. Dit geeft aan dat het hebben van een actieve aangedane M1 na de beroerte wellicht een belangrijke rol speelt bij het herstel van de arm. We vonden geen significant verschil in de connectie tussen de aangedane en niet-aangedane M1, maar de verschillen tussen patiënten waren ook groot. Hierdoor is het lastig om hierover een duidelijke conclusie te trekken.

Door meer onderzoeken te doen naar het effect van TMS op de activatie en connectie van de M1s, en armfunctie, zal duidelijk worden of TMS een succesvolle therapie kan zijn voor beroertepatiënten met verlies van armfunctie.

## Introduction

Stroke is the second leading cause of long-term disability among adults worldwide (1). Hemiparesis of the contralateral upper limb is the most common type of disability after stroke, with more than 80% of stroke patients experiencing this condition acutely and more than 40% chronically (2). The exact mechanism underlying spontaneous upper limb recovery is still unclear, and a better understanding of this is needed to be able to develop more effective therapies, which are essential for optimizing stroke rehabilitation.

Stroke is associated with abnormalities in neural activity within the bilateral motor areas (3–5). A decreased activation of the ipsilesional (IL) primary motor cortex (M1) compared to healthy controls is commonly seen (6,7). Normalization of IL M1 activation is associated with improved motor performance (6,8). An increase in contralesional (CL) M1 activation may be an additional result of stroke (4,6–8), which in some patients correlates with severe motor impairments (7,9). Due to interhemispheric connectivity, a decreased IL M1 and increased CL M1 activation may be related to each other, as proposed in the interhemispheric imbalance model (3,8). The interhemispheric imbalance model assumes that 'overactivation' of the CL M1 leads to excessive inhibition of the IL M1, which results in its reduced activity. Since restoration of the interhemispheric imbalance is associated with promotion of motor recovery, it is an interesting target for new therapies (3,10,11).

A promising option to modulate the interhemispheric balance, and as a result potentially improve upper limb function, is a non-invasive neuromodulation therapy called transcranial magnetic stimulation (TMS) (3,12). Through a TMS-coil that is placed on to the scalp, an electrical field is generated in the stimulated area through electromagnetic induction (6,7). In this way, the cortex can be stimulated or inhibited. There are mainly two forms used to inhibit the CL M1: low frequency inhibitory repetitive TMS (rTMS) and continuous theta-burst stimulation (cTBS) (13,14). It is suggested that low frequency rTMS can suppress the excitability of the CL M1, and therefore can reduce overactivation (4,5). Thereby, it might be possible to indirectly enhance IL M1 activation (15). Even more important, it is expected to have a positive effect on motor recovery (16,17). In a big review about the efficacy of TMS, level A evidence (definite efficacy) was found for inhibitory rTMS applied over the CL M1 for hand motor recovery in the post-acute stage of stroke (18).

The influence that one brain region exerts over another is defined as the effective connectivity (10,19). Effective connectivity can therefore be excitatory (positive) or inhibitory (negative). Using functional magnetic resonance imaging (fMRI) data, the effective connectivity between different brain regions can be estimated by the concept of dynamic causal modeling (DCM) (20). DCM of motor network activation in stroke patients suggests that interhemispheric coupling between both M1s is disturbed. Increased negative connectivity i.e., increased inhibition from CL M1 towards IL M1 correlated with poorer motor performance (18,20). In addition, decreased inhibition from IL M1 towards CL M1 has been seen in stroke patients, which correlated with stronger functional impairments (22). Only one study has been conducted so far in which DCM is used to investigate the potential of inhibitory rTMS applied over the CL M1 to modulate the effective connectivity between bilateral motor areas (10). It was found that the motor function of the impaired arm was significantly improved after stimulation. In addition, this improvement significantly correlated with a decrease of negative connectivity from CL M1 towards IL M1 (10). However, more studies with a bigger cohort are needed to confirm these findings.

The Brain Stimulation for Arm Recovery after Stroke (B-STARS) study was a randomized sham-controlled trial in which the effect of cTBS applied over the CL M1 on motor function of the upper limb in 60 patients with subacute stroke (within three weeks) was investigated, with a follow-up period of a year (13). This study is part of the B-STARS trial and aimed to investigate i) the potential of cTBS applied over the CL M1 to modulate the blood-oxygen-level-dependent (BOLD) signal i.e., activation in the CL M1 and IL M1, as assessed with task-based fMRI; ii) the potential of cTBS applied over the CL M1 to modulate the effective connectivity between bilateral M1s, as estimated with DCM of task-based fMRI data; and iii) whether M1 activation and effective connectivity from CL M1 towards IL M1 correlate with upper limb function in stroke patients, as indicated by their scores on the Fugl-Meyer (FM) assessment. This study can provide a better understanding of the neurophysiology of stroke recovery and can give more insight about the applicability of cTBS as a therapy for upper limb impairment after stroke.

## Methods

### *Study design*

The B-STARS trial was a prospective, randomized, double-blind sham-controlled clinical trial (13). Starting maximally 21 days after stroke onset, all patients received 10 days of real cTBS or sham cTBS over a period of two weeks at De Hoogstraat (13). Stratified to the severity of their arm paresis, patients were randomly assigned to receive either real cTBS or sham cTBS treatment. Approval of the B-STARS trial has been given by the Medical Research Ethics Committee of the UMCU. Detailed criteria for stratification can be found in the B-STARS protocol (13). Patients were tested at the start of the study (baseline; T0), at the last day of cTBS treatment (T1), at 1 week (T2) and 1 month (T3) after cTBS treatment, and at 3 months (T4), 6 months (T5) and 1 year after stroke onset (T6) (Fig. 2) (13). Patients who agreed to undergoing MRI scans were scanned at T2, T4, T5 and/or T6. The scans made at T2, T4, and T5 were taken into analysis. The scans made at T6 were excluded, because not all patients finished T6 yet, and thus these data were not complete.

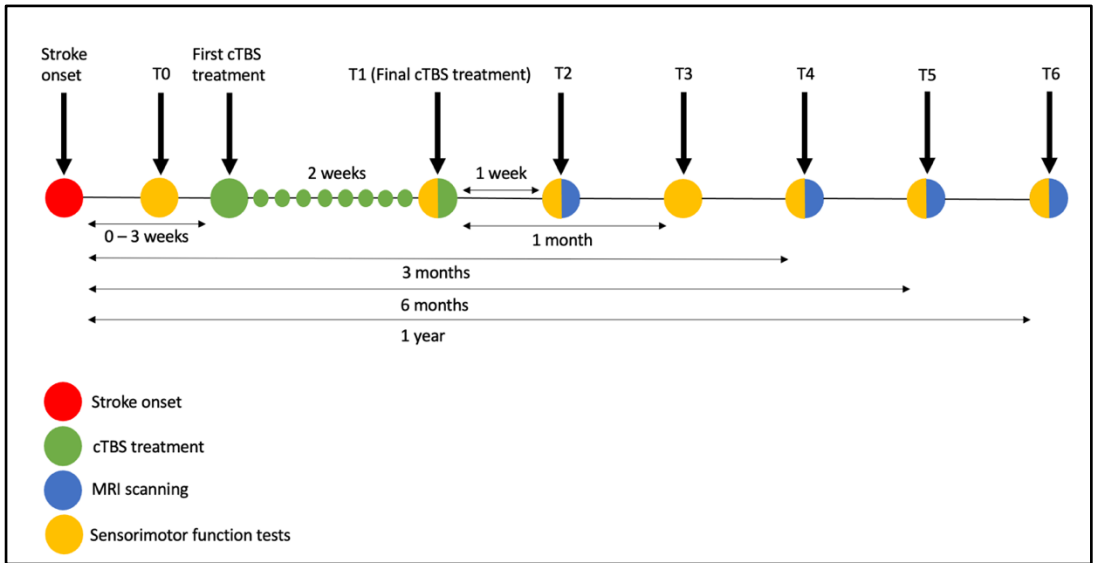


**Figure 2:** Overview of the experimental design of the B-STARS trial. Within three weeks after stroke onset (red circle), patients received 10 days of cTBS treatment (green circles) over a period of two weeks. Patients were tested seven times in total (T0-T6) and had to perform several sensorimotor function tests at all follow-up measurements (yellow circles). Optionally, patients underwent MRI scanning at T2, T4, T5 and T6 (blue circles). Created in Microsoft Powerpoint.

### *Participants*

60 patients were recruited from the University Medical Center Utrecht (UMCU) and rehabilitation center De Hoogstraat in Utrecht. Patients were selected according to the following inclusion criteria: i) age ≥18 years; ii) first-ever unilateral ischemic stroke; iii) a Motricity Index shoulder abduction score of ≥9; iv) admission to De Hoogstraat within the first 21 days after stroke onset. Patients were excluded if they had i) severe medical history; ii) history of epilepsy; iii) (almost) normal hand function, as indicated by a Motricity Index hand score of 33; iv) severe cognitive deficits e.g., deficits in communication, memory or understanding, that hinders participation to this study (as determined by rehabilitation physician), and v) contraindications for the TMS treatment (13). All patients provided written informed consent. Optionally, patients could undergo MRI scans.

*Intervention*

Details of the cTBS treatment can be found in the B-STARS protocol (13).

*Motor function test*

All patients performed the FM assessment, at all follow-up measurements (Fig. 2).

*Image acquisition*

A 3D anatomical T1-weighted image was acquired for each patient. The following imaging parameters were used: TR = 8.25 ms, TE = 3.78 ms, flip angle = 8°, voxel size = 0.47x0.47x1.00 $mm^3$, field of view (FOV) = 512x512, scan duration = 2.9 min. Functional MR images were acquired on a 3 Tesla whole-body scanner (Philips Achieva, Best, The Netherlands). A fast field echo-planar imaging (FFE-EPI) sequence was used, with the following imaging parameters: TR = 1000 ms, TE = 25.0 ms, flip angle = 65°, voxel size of 2.3x2.3x2.5 $mm^3$, FOV = 96x96 mm. An fMRI block design was employed in which patients were visually instructed to perform movements with the affected or unaffected hand using a monitor inside the MRI scanner room. Hand movements consisted of flexion and extension of the fingers. Blocks of unaffected and affected hand movement were interchanged. Each block lasted 20 seconds and was followed by 14-16 seconds of rest. In total, there were six motor task blocks for left hand movement, and five for right hand movement. Patients wore data gloves (5DT, Pretoria, South Africa), which measured movement of the fingers to record the task performance.

*Image preprocessing*

For the analyses, the SPM software package (SPM12; https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and custom MATLAB scripts were used. First, EPI volumes were realigned and coregistered with the anatomical image for each session. Then, all volumes were spatially normalized to the standard template of the Montreal Neurological Institute (MNI) and smoothed using an isotropic kernel of 8 mm full width at half maximum (FWHM). Images from patients with left-sided lesions were flipped about the midsagittal plane. In this way, the affected hemisphere corresponded to the right side of the brain in all patients. A generalized linear model (GLM) analysis was performed. Affected and unaffected hand movement were used as regressors of interest and constructed as boxcar vectors convolved with a canonical hemodynamic response function. To exclude variance due to head movements, motion parameters were added as covariates. For each patient, linear T-contrast images were computed for the conditions "affected hand vs. baseline", "unaffected hand vs. baseline", and "hand movement vs. baseline", and an F-contrast image was computed for the condition "effects of interest". Voxels were identified as significant on the single subject level at a threshold of T = 3.11 (P<0.001, uncorrected). After preprocessing, the movement regressors, coregistration and fMRI activation maps of the conditions "affected hand vs. baseline" and "unaffected hand vs. baseline" were checked. In addition, using the data collected from the hand gloves the patients wore, the task performance was checked.

*Task-based fMRI activation IL M1 and CL M1*

Using the following anatomical constraints: M1 was located at the "hand knob" of the precentral gyrus, masks for both IL M1 and CL M1 were constructed. The mean percentage change in BOLD signal in these regions of interest (ROIs) was computed from the beta maps obtained from the GLM.

*Dynamic causal modeling*

In DCM, the brain is treated as an input-state-output system. Using known inputs e.g., a specific task, this system can be perturbed, and measured responses are used to estimate various parameters that reflect the changes in effective connectivity between different brain areas (20). DCM allows the estimation of i) endogenous coupling parameters that are task-independent, which in this case reflect the effective connectivity when the hands are not moved; ii) parameters for changes in effective connectivity between two regions caused by the task i.e., affected or unaffected hand movement, and iii) parameters that represent the direct experimental, sensory input to the system that drives regional neuronal activity (10,20,21). DCM is a hypothesis-driven approach, using biophysically validated hemodynamic models to decompose the measured data into the underlying neuronal signal which represents the effective connectivity, and hemodynamic effects (20,21). DCM was used to estimate effective connectivity between cortical motor areas using the MRI scans made 1 week after cTBS (T2), 3 months after stroke onset (T4) and 6 months after stroke onset (T5). As the cortical motor system comprises three core areas, the following ROIs were included: the IL M1, supplementary motor cortex (SMA) and premotor cortex (PMC), and the CL M1, SMA and PMC (10). Because DCM requires a sensory input region, and patients were informed via a visual cue to move their hands, both V5s were also added (20). This decision was made after the fMRI activation maps of all patients were checked, which revealed that V5 showed the highest activation within the visual cortex, in contrast to V1.

*ROI extraction*

An essential first step in DCM is to define all eight ROIs in the fMRI activation maps of each patient (20,23). Each ROI was defined according to the next local maximum in relation to the group coordinates. Since local activation maxima may vary across individuals, comparability was ensured by selecting coordinates using anatomical masks for each ROI. Additional to the M1 masks, the following anatomical constrains were used to create the other masks: PMC in lateral precentral cortex/sulcus at the level of the inferior frontal sulcus (corresponding to the superior ventral PMC), SMA in the dorsal medial wall within the interhemispheric fissure, and V5 at the occipitotemporal junction (23). Coordinates were determined in the baseline contrasts for each patient i.e., CL M1, SMA, and PMC in contrast "unaffected hand vs. baseline" and IL M1, SMA, and PMC in contrast "affected hand vs. baseline" ($p<0.001$, uncorrected). The coordinates for both V5s were determined in the contrast "hand movement vs. baseline", which included both affected and unaffected hand movement, to assure that the peak voxel was active in both conditions. Since some patients underwent multiple scanning sessions and local activation maxima may also vary between sessions, a dispersion of coordinates up to 8 mm (equivalent to the smoothing kernel), was allowed (21). Taken together, the timeseries of all ROIs were extracted in an 8-mm sphere region from the "effects of interest" F-contrast ($p<0.001$, uncorrected), for each patient. In seven patients, one or multiple ROIs showed no activation within the 8-mm sphere at $p<0.001$ at T2, T4 or T5. In this case, the statistical threshold was lowered to $p<0.05$ uncorrected for that specific ROI.

*Model definition*

Standard, deterministic bilinear one-state DCM was used. Connections between the ROIs were based on prior knowledge about anatomy (24). Multiple papers in which DCM was used to estimate effective connectivity between bilateral motor areas in stroke patients or

healthy participants, used the same endogenous model (10,21–23). Therefore, we decided to use this fully connected model as well (Fig. 4). The endogenous connectivity model represents the intrinsic neural coupling between the ROIs in absence of hand movements.

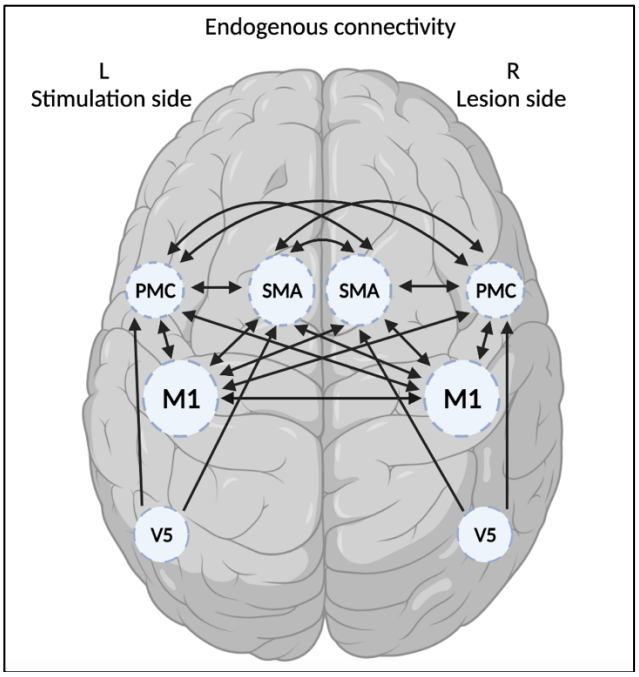


**Figure 4:** The endogenous connectivity model, including the eight ROIs: left premotor cortex (PMC), supplementary motor area (SMA), primary motor cortex (M1) and V5, and right PMC, SMA, M1 and V5. Connections between V5 and contralateral SMA and PMC are not shown for simplicity of the model. Scans from patients with left-sided lesions were flipped at the midsagittal plane. L = left, R = right. Created in Biorender.com.

Since the endogenous model is a fully connected model and hand movement does not utilize all endogenous connections, different task-dependent modulation models were constructed. When using DCM, it is important to define and motivate the model space (i.e., the set of specified models) carefully (24). Therefore, we took a close look at the model space of previous published papers in which DCM was used to estimate effective connectivity between bilateral motor areas. Grefkes et al. (10) and Rehme et al. (21) stood out since they both i) included (sub)acute stroke patients; ii) used the same endogenous connectivity model (except for connections between both M1 and contralateral PMC, which were absent in Grefkes et al. (10)); and iii) gave a clear explanation about the choice for the task-dependent models. Besides, in Grefkes et al. (10), inhibitory rTMS was applied over the CL M1, making it comparable to this study. Therefore, both winning and second-best models of Grefkes et al. (10) and Rehme et al. (21) were chosen, resulting in four task-dependent modulation models (Supplementary Fig. 1).

*Bayesian model selection*

Once the four models were specified and estimated for all patients at T2, T4 and T5, Bayesian model selection (BMS) was applied (21,24,25). BMS is a statistical procedure that is based on computing the model evidence i.e., the probability of your data, given a certain model. A model scores high when its complexion is minimal, but at the same time explains the observed data as accurately as possible (24). There are two options to identify which of the four models is the best at group level: applying fixed-effects (FFX) analysis, done by Grefkes et al. (10), or applying random-effects (RFX) analysis, done by Rehme et al. (21) (24,25). When

the studied population is heterogeneous or when studying extensive pathophysiological mechanisms, which means that the model structure is likely to differ between participants, it is preferred to use RFX analysis (24,25). Given that stroke is a heterogeneous disease with a complex pathophysiology, and the papers we followed had different winning models, model estimation using BMS RFX analysis was applied to determine the best model given the observed data (21,26). Eventually model fitting was diagnosed manually, by checking their explained variance. Coupling parameters of the winning model were acquired, and the parameters representing effective connectivity from CL M1 towards IL M1 when the affected hand was moved, and from IL M1 towards CL M1 when the unaffected hand was moved, were used for statistical analysis.

*Statistical analysis*

Statistical analysis was performed in SPSS (version 27.0.0.0 for Windows) and graphs were created in Graphpad Prism (version 9.3.0 for MacOS). To analyze the effect of cTBS treatment (sham or real) and time (T2, T4, and T5) on both IL M1 and CL M1 activation, two-way Analyses of Variance (ANOVA) without repeated measures were performed. If a significant effect of treatment was found ($p<0.05$), independent sample t-tests were performed to test for significant differences in IL M1 and CL M1 activation between real and sham cTBS treatment groups at different timepoints. The relationship between M1 activation and motor function was investigated by computing Pearson's correlation analyses between IL M1 or CL M1 activation and FM scores at T2, T4, and T5 individually, and for all three time points combined. The significance threshold was set at $p<0.05$ two-tailed. Multiple comparisons correction was performed using the Bonferroni approach. To investigate whether IL M1 or CL M1 activation predicts the functional outcome, a Pearson's correlation between M1 activation at T2 and FM score at T5 was computed for both hemispheres.

To test for significant differences at T2, T4, and T5 between effective connectivity from CL M1 towards IL M1 when the affected hand was moved, and from IL M1 towards CL M1 when the unaffected hand was moved, paired samples t-tests were performed. The significance threshold was set at $p<0.05$ two-tailed. The relationship between effective connectivity from CL M1 towards IL M1 and motor function was investigated by computing Pearson's correlations analyses between coupling parameters of this connection and the FM scores at T2, T4, and T5 individually, and for all three time points combined. The significance threshold was set at $p<0.05$ two-tailed. Again, multiple comparisons correction was performed using the Bonferroni approach. To investigate whether the effective connectivity from CL M1 towards IL M1 predicts the functional outcome, a Pearson's correlation between these coupling parameters at T2 and FM score at T5 was computed.

## Results

*Patient selection*

Of all 60 patients included in the B-STARS trial, 48 agreed to undergo additional MRI scanning (Fig. 3). As two patients only underwent MRI scanning at T6, these were excluded. 25 patients were allocated to the sham cTBS treatment group, and 21 patients were allocated to the real cTBS treatment group. At T2, T4 and T5 patients were again asked whether they approved with undergoing the MRI scan. Since some patients refused, or because of other (medical) reasons, not all patients underwent all four MRI scans (Fig. 3). Eventually 13 scans were excluded due to excessive head movement (ranging from 4.0-8.0 mm, as indicated by the movement regressors), excessive mirror movement (as indicated by the task performance of both hands), or because of other reasons (e.g., missing, or unreliable glove data, corrupt scan files, scan missing part of cortex). This led to the analysis of 62 scans of in total 42 patients, of which the clinical details can be found in Table 1.

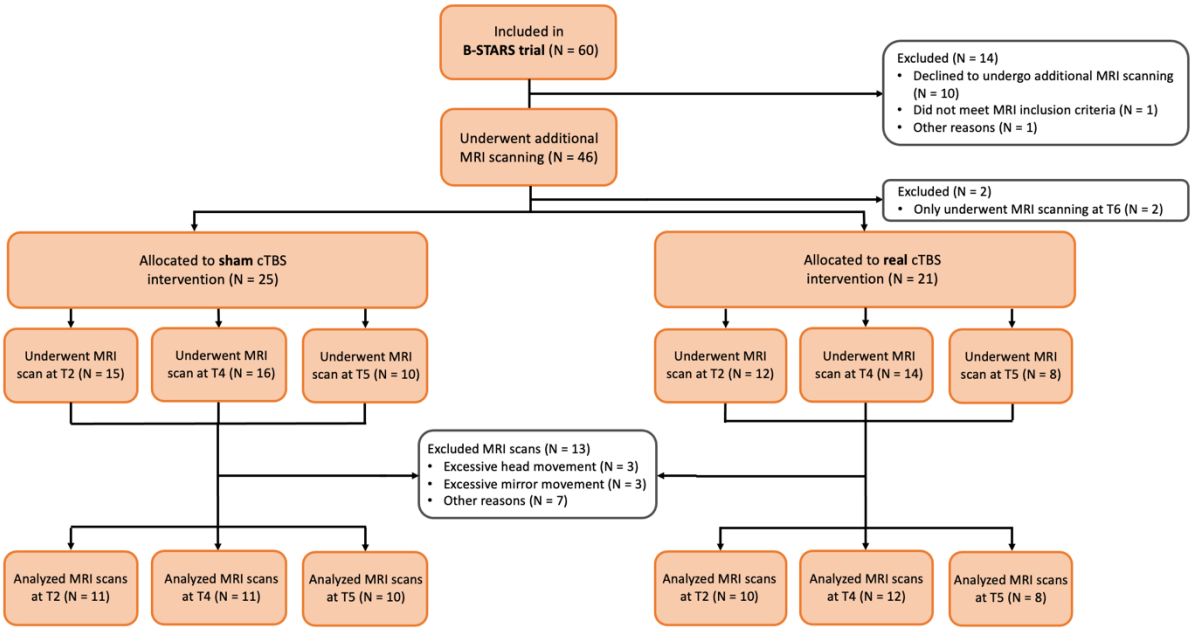


**Figure 3:** Flow chart of this study. Of the 60 patients included in the B-STARS trial, 46 underwent additional MRI scanning. 25 patients were allocated to the sham cTBS intervention, and 21 were allocated to the real cTBS intervention. After exclusion of 13 MRI scans, a total of 62 MRI scans were taken into analysis. T2 = 1 week after cTBS, T4 = 3 months after stroke onset, T5 = 6 months after stroke onset. Created in Microsoft Powerpoint.

**Table 1:** Clinical details at baseline for all included patients in sham and real cTBS treatment groups. ARAT = Action Research Arm test, FM = Fugl-Meyer assessment.

| | Sham cTBS (N = 23) | Real cTBS (N = 19) |
|---|---|---|
| **Gender**, n women (%) | 7 (30.4) | 8 (42.1) |
| **Age in years**, mean (SD) | 61.7 (12.3) | 55.8 (12.2) |
| **Start cTBS intervention**, mean days after stroke onset (SD) | 15.1 (4.5) | 13.6 (4.7) |
| **Ischemic/Hemorrhagic**, n ischemic/n observed (%) | 19 (82.6) | 17 (89.5) |

| | | |
|---|---|---|
| **Stroke location**, n (%) | | |
| Subcortical | 10 (43.5) | 9 (47.4) |
| Cortical | 1 (4.3) | - |
| Brainstem | 3 (13) | 3 (15.8) |
| Mixed | 7 (30.4) | 7 (36.8) |
| Unknown | 2 (8.7) | - |
| **Impaired hemisphere**, n right (%) | 14 (60.9) | 10 (52.6) |
| **Impaired hemisphere dominant**, n (%) | 16 (69.6) | 9 (50) |
| **Baseline ARAT**, mean (SD) | 13.1 (16) | 13.7 (17.8) |
| **Baseline FM**, mean (SD) | 27.7 (18.4) | 27.3 (19.2) |

*M1 activation results*

*Task-based fMRI activation in the IL M1 when the affected hand was moved*

IL M1 activation was slightly higher in the real cTBS treatment group compared to the sham cTBS treatment group at T2, T4, and T5 (Fig. 4). A two-way ANOVA without repeated measures revealed there was a non-significant effect of treatment (sham or real) on IL M1 activation *(F(1,56) = 2.32, p = 0.13)*. In addition, there was no statistically significant effect of time (T2, T4, T5) *(F(2,56) = 0.47, p = 0.63)* or the interaction between treatment and time *(F(2,56) = 0.03, p = 0.97)* on IL M1 activation. Since the two-way ANOVA did not reveal significant effects, independent sample t-tests were not performed.

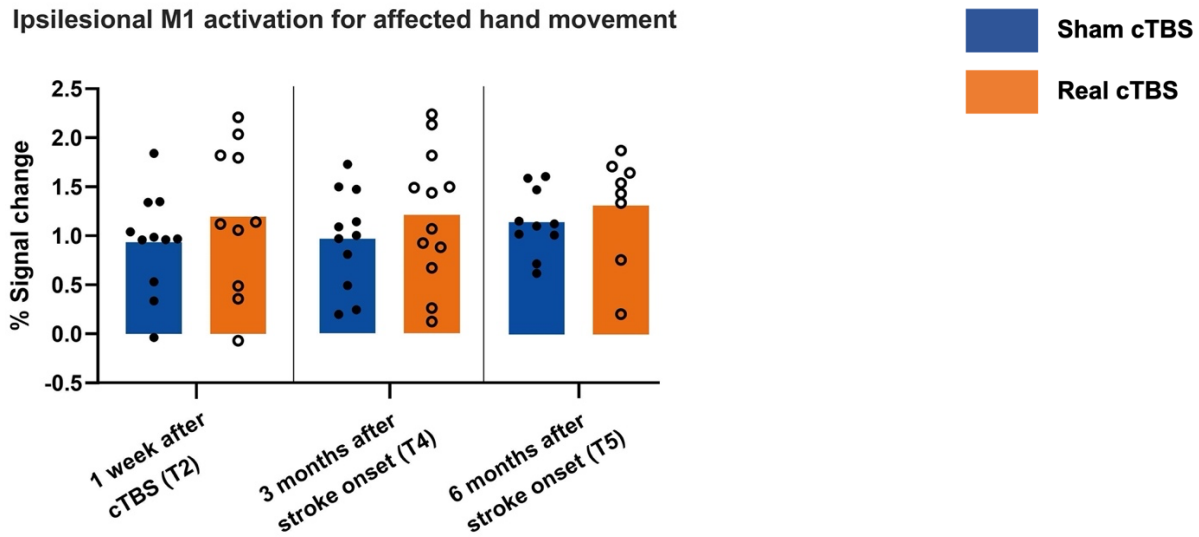


**Figure 4:** Graph represents the percent signal change in the ipsilesional M1 when the affected hand is moved 1 week after cTBS (T2), 3 months after stroke onset (T4), and 6 months after stroke onset (T5). Both sham cTBS (blue) and real cTBS (orange) treatment groups are shown. Individual data points are scattered.

*Task-based fMRI activation in the CL M1 when the affected hand was moved*

CL M1 activation was slightly lower in the real cTBS treatment group compared to the sham cTBS treatment group at T2, and T4. At T5 it was slightly higher in the real cTBS treatment group compared to the sham cTBS treatment group. (Fig. 5). A two-way ANOVA without repeated measures revealed there was no statistically significant effect of treatment (sham or real) *(F(1,56) = 1.12, p = 0.29)*, time (T2, T4, T5) *(F(2,56) = 0.95, p = 0.39)* or interaction between treatment and time *(F(2,56) = 1.21, p = 0.30)* on CL M1 activation. Since

the two-way ANOVA did not reveal significant effects, independent sample t-tests were not performed.

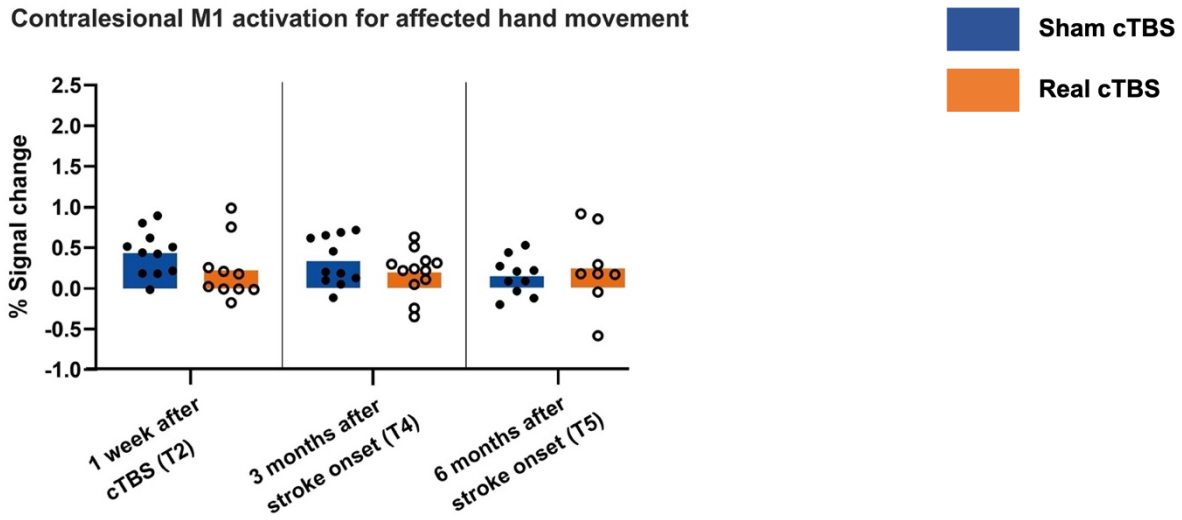


**Figure 5:** Graph represents the percent signal change in the contralesional M1 when the affected hand is moved 1 week after cTBS (T2), 3 months after stroke onset (T4), and 6 months after stroke onset (T5). Both sham cTBS (blue) and real cTBS (orange) treatment groups are shown. Individual data points are scattered.

*Correlation between IL M1 activation and FM scores*

Pearson's correlation analysis showed there was a significant positive correlation between IL M1 activation and FM scores at T2, T4, and T5 *(R = 0.45, p = 0.0002)* (Fig. 6). In addition, a significant positive correlation between IL M1 activation and FM score at T4 was found *(R = 0.58, p = 0.0034)* (Supplementary Fig. 2). There was no statistically significant correlation between IL M1 activation and FM score at T2 *(R = 0.38, p = 0.10)*, and at T5 *(R = 0.26, p = 0.31)*, and between IL M1 activation at T2 and FM score at T5 *(R = 0.30, p = 0.27)* (Supplementary Fig. 2).

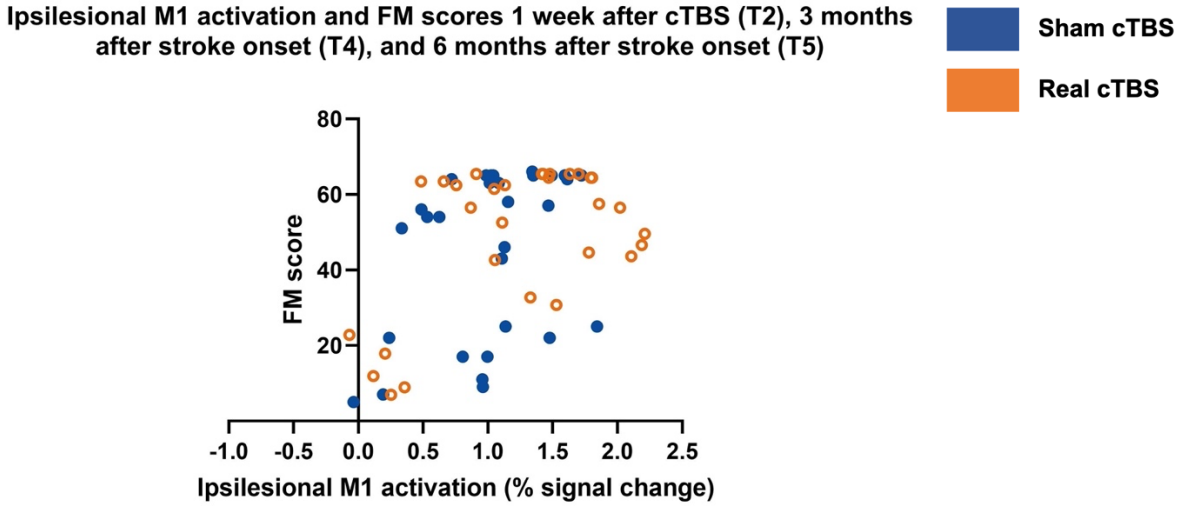


**Figure 6:** Ipsilesional M1 activation (% signal change) significantly correlated positively *(R = 0.45, p = 0.0002)* with Fugl-Meyer (FM) score 1 week after cTBS (T2), 3 months after stroke onset (T4), and 6 months after stroke onset (T5) (Pearson's correlation). Individual data points of both sham cTBS (blue) and real cTBS (orange) treatment groups are shown.

*Correlation between CL M1 activation and FM scores*

Pearson's correlation analyses showed there was a non-significant correlation between CL M1 activation and FM scores at T2, T4, and T5 *(R = 0.09, p = 0.47)* (Supplementary Fig. 3). In addition, there was no statistically significant correlation between CL M1 activation and FM score at T2 *(R = -0.02, p = 0.10)*, at T4 *(R = 0.20, p = 0.36)*, and at T5 *(R = 0.23, p = 0.36)*, and between CL M1 activation at T2 and FM score at T5 *(R = 0.03, p = 0.53)* (Supplementary Fig. 3).

## *DCM results*

*BMS RFX analysis*

Model 2 containing a fully connected matrix for movements of the affected or unaffected hand showed the best model fit across sessions (Fig. 7; Supplementary Fig. 1). The expected posterior probabilities of each model i.e., how likely it is that this specific model generated the data of a randomly chosen patient, and the expected exceedance probabilities of each model i.e., how likely it is that this specific model is more likely than any other model, given the data of all patients included (24), are shown in Supplementary Fig. 4. Taken together, model 2 is the most likely generative model for the participants included from the respective population. Given the observed data, model 2 had a probability of 33-60% (Supplementary Fig. 4).

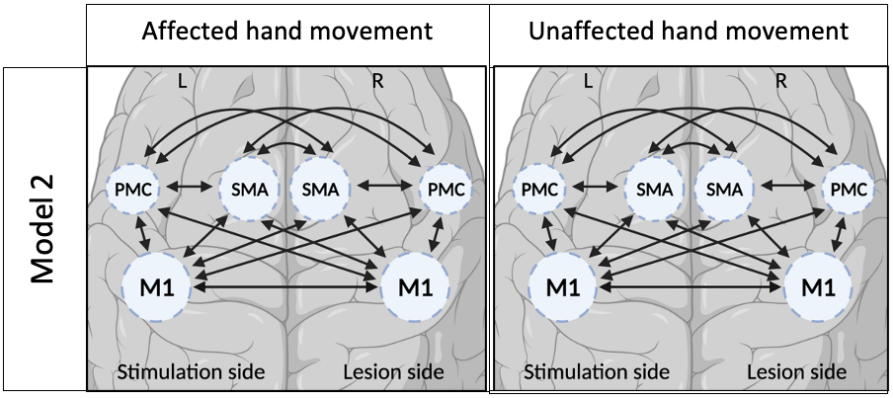


**Figure 7:** Task-dependent modulation model 2 was the winning model. PMC = premotor cortex, SMA = supplementary motor cortex, M1 = primary motor cortex, L = left, R = right. Created in Biorender.com

*Effective connectivity between bilateral M1s*

The effective connectivity from CL M1 towards IL M1 when the affected hand was moved showed a high variability in coupling strength (ranging from roughly -0.75 to roughly +0.75) at T2, as well as at T4, and T5 (Fig. 7). This was also the case for the effective connectivity from IL M1 towards CL M1 when the unaffected hand was moved (ranging from -0.65 to +1.25). At T4, effective connectivity from IL M1 towards CL M1 when the unaffected hand was moved seemed to be more positive compared to the effective connectivity from CL M1 towards IL M1 when the affected hand was moved, in the real cTBS treatment group (Fig. 7B). However, paired samples t-tests between the coupling parameters at T2, T4, and T5 showed there were no significant differences between effective connectivity from CL M1 towards IL M1 when the affected hand was moved, and from IL M1 towards CL M1 when the unaffected hand was moved *(resp. p = 0.18, p = 0.58, and p = 0.74)*.

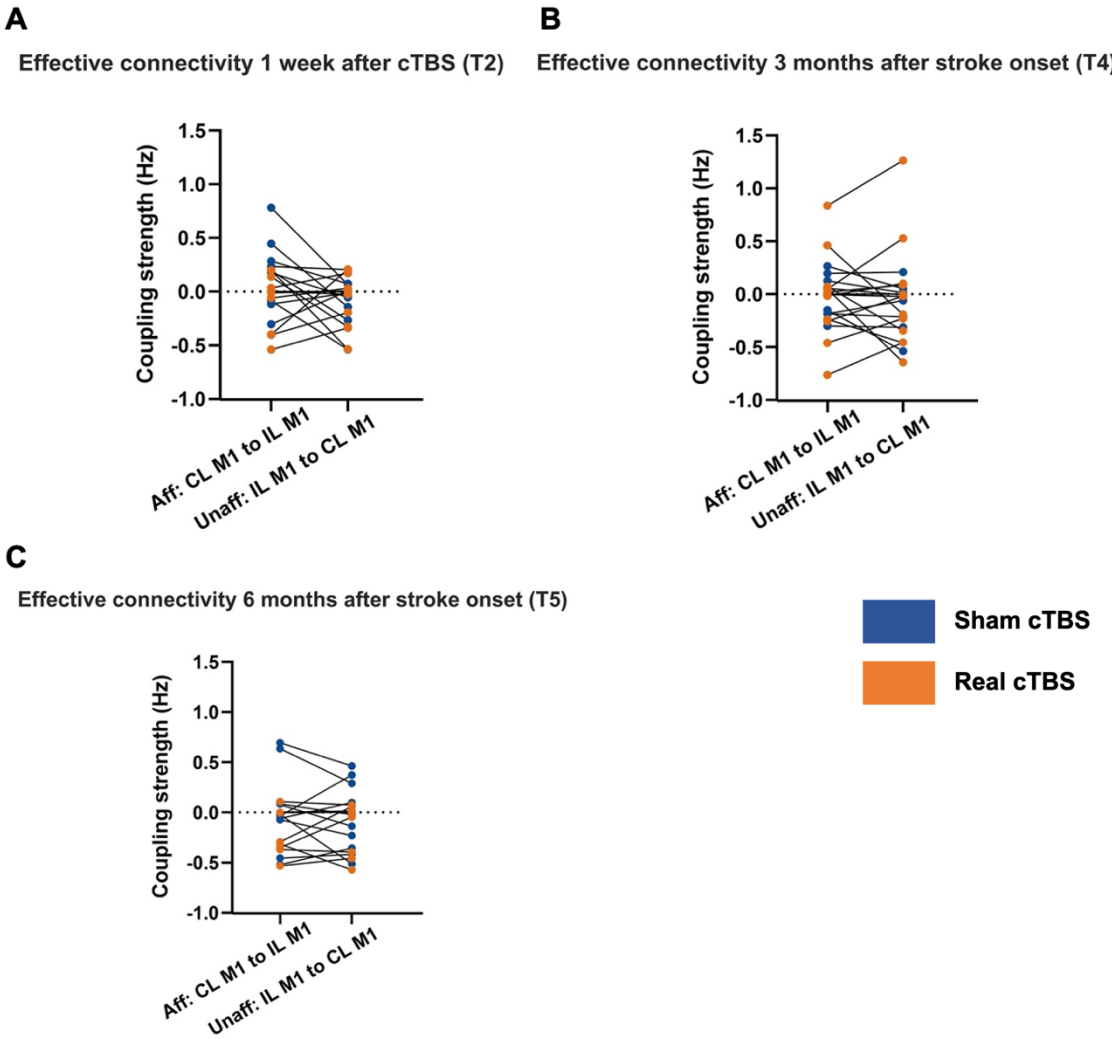


**Figure 7:** Individual parameters (coupling strength (Hz)) reflecting the effective connectivity from contralesional (CL) towards ipsilesional (IL) M1 when the affected (aff) hand is moved, and from IL towards CL M1 when the unaffected (unaff) hand is moved, (A) 1 week after cTBS (T2), (B) 3 months after stroke onset (T4), and (C) 6 months after stroke onset (T5). Individual datapoints are shown and connected for every patient. Both sham cTBS (blue) and real cTBS (orange) treatment groups are shown.

*Correlation effective connectivity from CL M1 towards IL M1 and FM scores*

Pearson's correlation analyses showed there was a significant negative correlation between effective connectivity from CL M1 towards IL M1 when the affected and was moved and FM scores at T2, T4, and T5 *(R = -0.27, p = 0.03)* (Fig. 8). After a Bonferroni correction, there was a non-significant negative correlation between effective connectivity from CL M1 towards IL M1 and FM score at T5 *(R = -0.47, p = 0.049)* (Supplementary Fig. 5). At T2 and T4 this correlation was also non-significant *(resp. R = -0.21, p = 0.38; and R = -0.19, p = 0.38)* (Supplementary Fig. 5). This was also the case for the effective connectivity at T2, and FM score at T5 *(R = -0.18, p = 0.51)* (Supplementary Fig. 5).

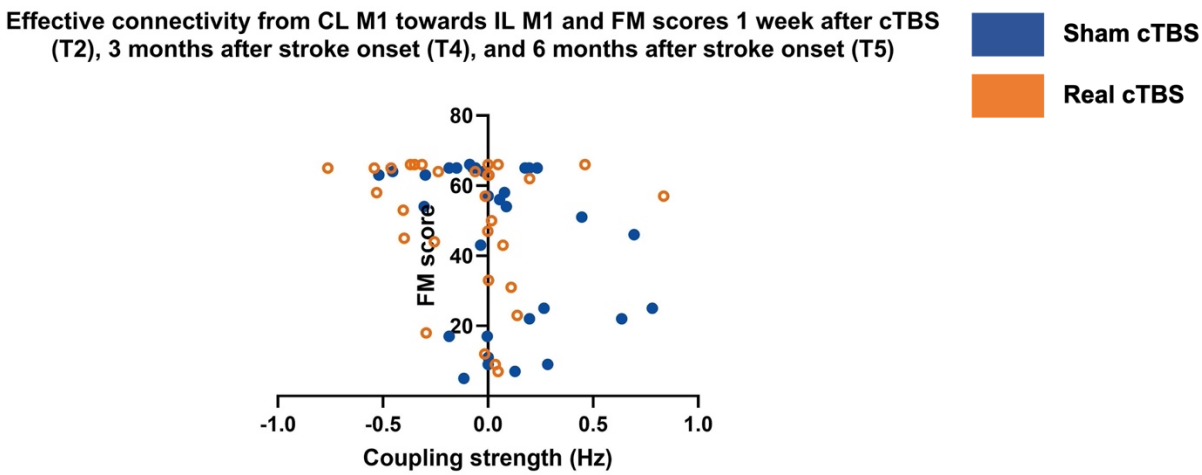


**Figure 8:** Effective connectivity from contralesional (CL) towards ipsilesional (IL) M1 when the affected hand was moved (coupling strength (Hz)) significantly correlated negatively *(R = -0.27, p = 0.03)* with Fugl-Meyer (FM) score 1 week after cTBS (T2), 3 months after stroke onset (T4), and 6 months after stroke onset (T5) (Pearson's correlation). Individual data points of both sham cTBS (blue) and real cTBS (orange) treatment groups are shown.

## Discussion

In this unique randomized sham-controlled double-blinded study, where patients were followed up several times from the subacute to chronic phase post-stroke, the first goal was to investigate the effect of cTBS applied over the CL M1 i) on the BOLD response i.e., activation in both the IL M1 and CL M1, and ii) on the effective connectivity between bilateral M1s in stroke patients. Finally, we assessed whether M1 activation and effective connectivity from CL M1 towards IL M1 correlated with motor function of the arm.

### *IL M1 activation and its relationship with upper limb function*

IL M1 activation was higher in the real cTBS compared to the sham cTBS treatment group at all time points, although the differences between the groups and different time points were not significant. However, the clearly visible trend is in line with another study that reported a significantly increased IL M1 activation after application of inhibitory rTMS over the CL M1 (15). There are also studies that - similar to this study - reported no statistically significant increase in IL M1 activation after application of inhibitory rTMS over the CL M1, of which Du et al. (27) had a similar research design to this study (4,27).

A significant positive correlation was found between IL M1 activation and FM scores at T2, T4, and T5, meaning that patients with better motor outcome showed a higher activation within the IL M1. This is in line with a recent meta-analysis that included 24 neuroimaging studies, which concluded that higher activation values in the IL M1 were found in patients with good outcome, compared to those with a poor outcome (6). The finding of a significant positive correlation between IL M1 activation and FM score at T4 is consistent with the findings of Du et al. (27). Here, a significant positive correlation between IL M1 activation and motor function 3 months post-treatment was found too (27). Taken together, it can be suggested that the restitution of IL M1 activation to 'normal' levels might play a critical role in recovery of motor function.

It is important to mention that the sample size for Du et al. (27) was rather small, which is also the case for our study for the individual time points. This could be a likely explanation for why we did not find a significant effect of cTBS applied over the CL M1 on IL M1 activation at any individual time point, but also why we did not find a significant correlation between IL M1 activation and FM score at T2, or T5 individually.

### *CL M1 activation and its relationship with upper limb function*

CL M1 activation was lower in the real cTBS compared to the sham cTBS treatment at T2, and at T4, although the differences between the groups and different time points were not significant. This is in contrast with two other studies, which both found a statistically significant reduction in CL M1 activation after application of inhibitory rTMS over the CL M1 compared to sham stimulation (4,27). A possible explanation for this is the fact that in both studies patients underwent MRI scanning immediately after or within 24 hours after the rTMS treatment, which makes the chances of finding a significant effect of rTMS higher. However, our results may then point to the possibility that the effects of real cTBS treatment on CL M1 activation only last for a short duration. This is in line with literature, as the direct effect of cTBS on excitability of the motor cortex, and thus probably also on BOLD-activation, is thought to last for one hour (28).

As we did not find significant correlations between CL M1 activation and FM scores, activation of the CL M1 seems not to be directly associated with motor function. This makes

sense, as the corticospinal tract crosses over and thus the CL M1 is not directly involved in the making of movements of the impaired upper limb.

*Effective connectivity between bilateral M1s and its relationship with upper limb function*

As effective connectivity from CL M1 towards IL M1 when the affected hand was moved, and from IL M1 towards CL M1 when the unaffected hand was moved, both point to the – at that moment – driving hemisphere, it was expected to be quite similar in healthy people. However, increased negative connectivity from CL M1 towards IL M1 has been seen in stroke patients when the affected hand was moved, which means we expected to see differences between these two connections (10,19). However, we were not able to identify a difference between effective connectivity from CL M1 towards IL M1 when the affected hand was moved, and from IL M1 towards CL M1 when the unaffected hand was moved. Nevertheless, it is important to mention that coupling parameters showed high variance at all time points. It is possible that estimation of the models ended in a local maximum rather than a global maximum, which could have resulted in small errors in the estimation of the coupling parameters. Therefore, it is harder to draw conclusions out of these data. Second, as already mentioned, the fact that we did not find significant results is possibly due to the small sample size.

The significantly negative correlation between effective connectivity from CL M1 towards IL M1 and FM score at T2, T4, and T5, indicates that the more negative i.e., inhibiting the effective connectivity from CL M1 towards IL M1 is, the higher the FM score and thus the better the motor function. Grefkes et al. (19) found exactly the opposite: the stronger the inhibition from CL M1 towards IL M1, the lower the motor performance of the impaired hand. However, this was measured by the frequency of movements made by the impaired hand, and not by FM score, as was done in this study. Rehme et al. (21) found that stroke patients who developed a more negative effective connectivity from CL M1 towards IL M1 over time, showed a relatively poor outcome compared to patients with positive effective connectivity from CL M1 towards IL M1. It would therefore be interesting to investigate whether changes in coupling strength parameters of this connection between T2, T4, and T5 correlate with the FM score.

*Limitations*

First, the statistical approaches used in this study were not ideal. Due to missing data, a two-way ANOVA with repeated measures could not be performed. Instead, an ordinary two-way ANOVA without repeated measures was performed to analyze the effect of cTBS treatment and time on both IL M1 and CL M1 activation. However, a linear mixed model analysis could increase statistical power, as this can analyze repeated measures data and can handle missing data (29). The Pearson's correlation analyses used to investigate the relationship between M1 activation or effective connectivity and FM scores at all time points combined, should be interpreted with caution, as this might have led to overinflation of statistical power.

Second, DCM itself has some limitations. It is important to keep in mind that DCM uses the data to model, and thus estimate, the effective connectivity. Therefore, certain assumptions are made, and for instance some biophysiological and neurovascular processes are neglected in DCM, as revealed by Daunizeau et al. (30) which provided a critical review about DCM. However, new, and improved DCM features have become available. For example, a new form of second level analysis, named Parametric Empirical Bayes (PEB), has been introduced (31). This method does not only take the expected values of the coupling parameters into account,

but also their covariance, in contrast to the conventional FFX or RFX approach (31,32). This may result in more reliable parameters, and thus results. Overall, DCM still can be improved, but its usage has been validated by multiple studies and it is still the predominant analysis used to estimate effective connectivity between different brain areas (19,25,32).

*Future recommendations*

For future research, patients should preferably undergo MRI scanning before cTBS treatment. As a result, it would be possible to compare the actual change in M1 activation and effective connectivity prior and after cTBS treatment for each individual patient. Since cTBS treatment already started within three weeks after stroke in this study, it was practically impossible to do an MRI scan in advance. In addition, patients are most vulnerable shortly after stroke onset, which also played a role.

Second, universal DCM guidelines should be developed. When these are available, it will be easier to replicate DCM studies and to compare them to each other. Because only Grefkes et al. (10) had a comparable research design to this study, a lot is still unclear about the outcome measures. In other words, more randomized sham-controlled trials in which the effect of contralesional cTBS on effective connectivity within the motor network in stroke patients is estimated using DCM are needed, to reveal the exact role effective connectivity plays in stroke recovery and to confirm or reject previously concluded results.

*Conclusion*

This unique randomized sham-controlled study with multiple follow-up measurements, has shown promising results regarding cTBS applied over the CL M1 and its effect on M1 activation and motor function. Possibly due to sample size, no significant effect of cTBS applied over the CL M1 was found on IL M1 activation, although a trend was clearly visible. Literature has shown the direct effect of cTBS on the CL M1 seems to be short term, which is possibly the reason why we did not find a significant effect. Additionally, IL M1 activation seems to play an important role in stroke recovery, as shown by its correlation with motor function. Taken together, this study has shown that cTBS applied over the CL M1 might be able to increase IL M1 activation in the longer term, and possibly as a result improve motor function, but more studies with a bigger cohort are needed to confirm this.

No differences between effective connectivity from CL M1 towards IL M1 for affected hand movement, and from IL M1 towards CL M1 for unaffected hand movement were found, which is probably due to high variability between patients and small sample size. Furthermore, a reduction in effective connectivity from CL M1 towards IL M1 was associated with improved function of the affected hand, which is in contrast with previous literature, which however was limited. In conclusion, more research is needed using preferably universal DCM guidelines, to reveal the exact role effective connectivity between bilateral M1s plays in stroke recovery.

## Acknowledgements

First, I want to thank my daily supervisor Jord Vink for his guidance and support during my internship. I am grateful for everything he taught me about TMS, MATLAB, and SPM, and all the valuable feedback he provided me with. I also enjoyed all the interesting and funny conversations we had. Second, I am very grateful I got to be part of the BMRIS group, under guidance of Prof. Dr. Rick Dijkhuizen. I would like to thank him for always being interested in my project, and for the meetings in which he shared his ideas and provided me with valuable feedback. I want to thank the other members of the BMRIS group for their interesting presentations during the lab meetings. Finally, I would like to thank my fellow students for supporting me while working with patients for the first time, and most of all for making my time during this internship extra fun and enjoyable: Camille Biemans, Marius Sauca, Hosna Anwar, and Marit de Jong.

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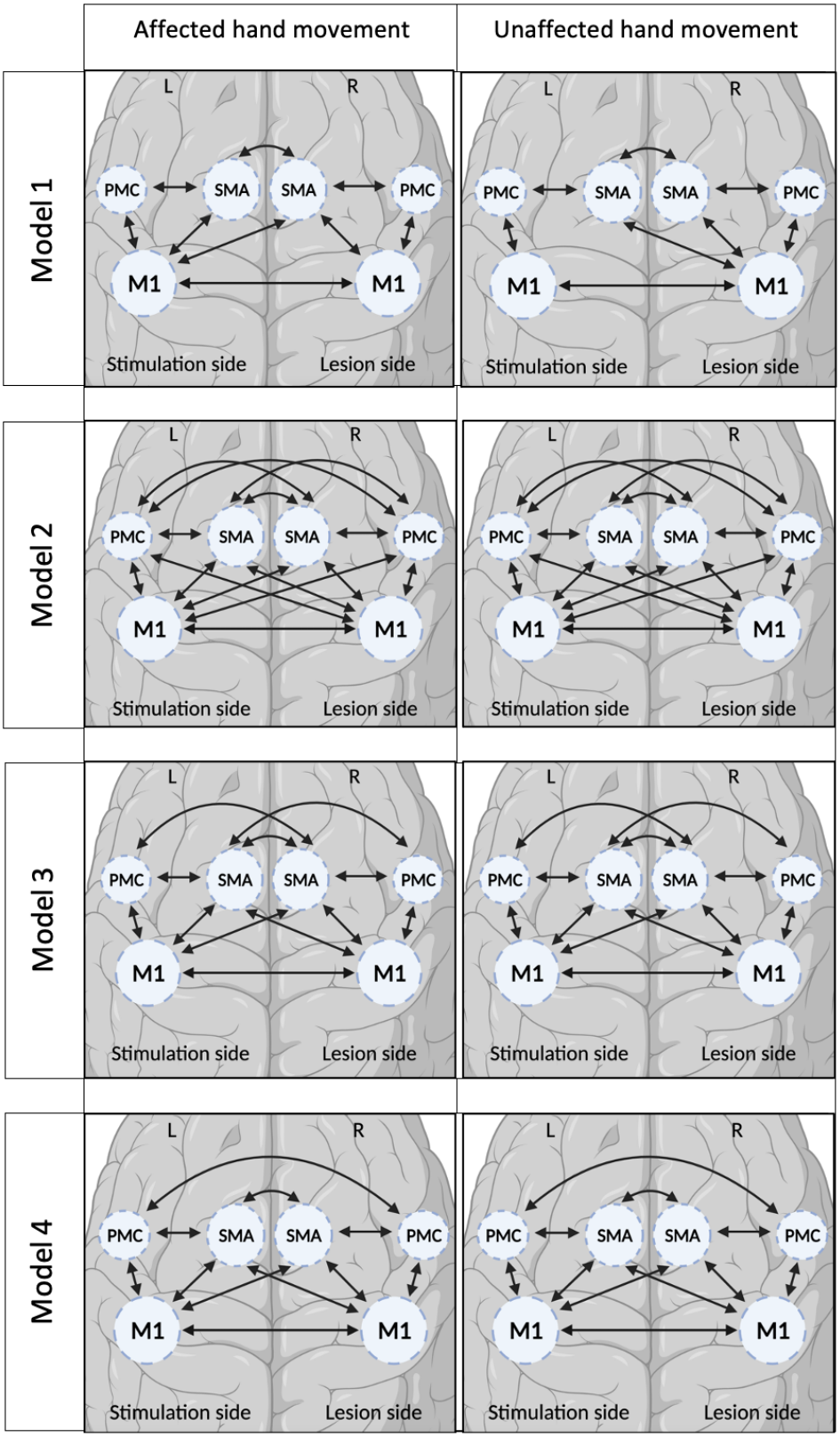
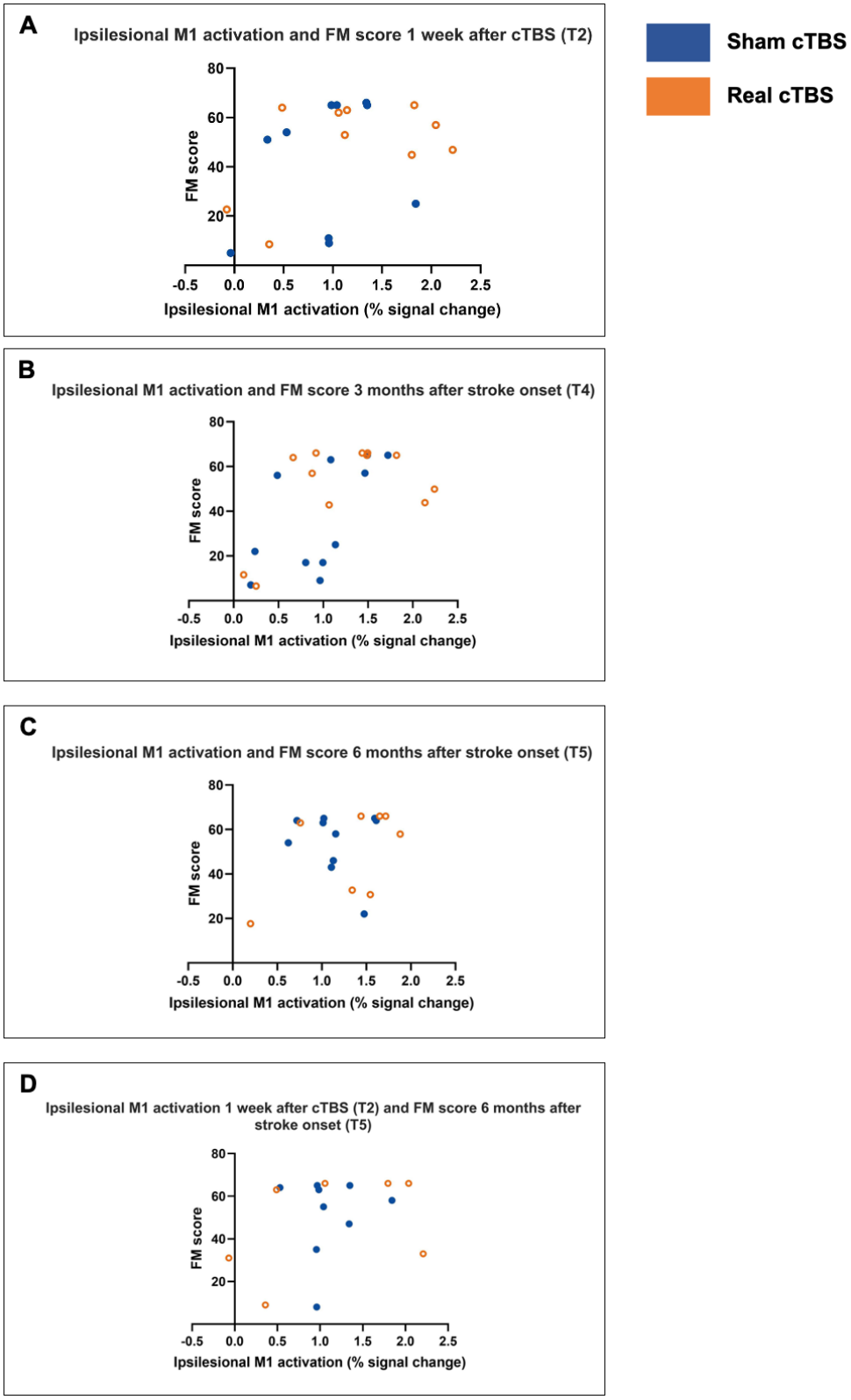
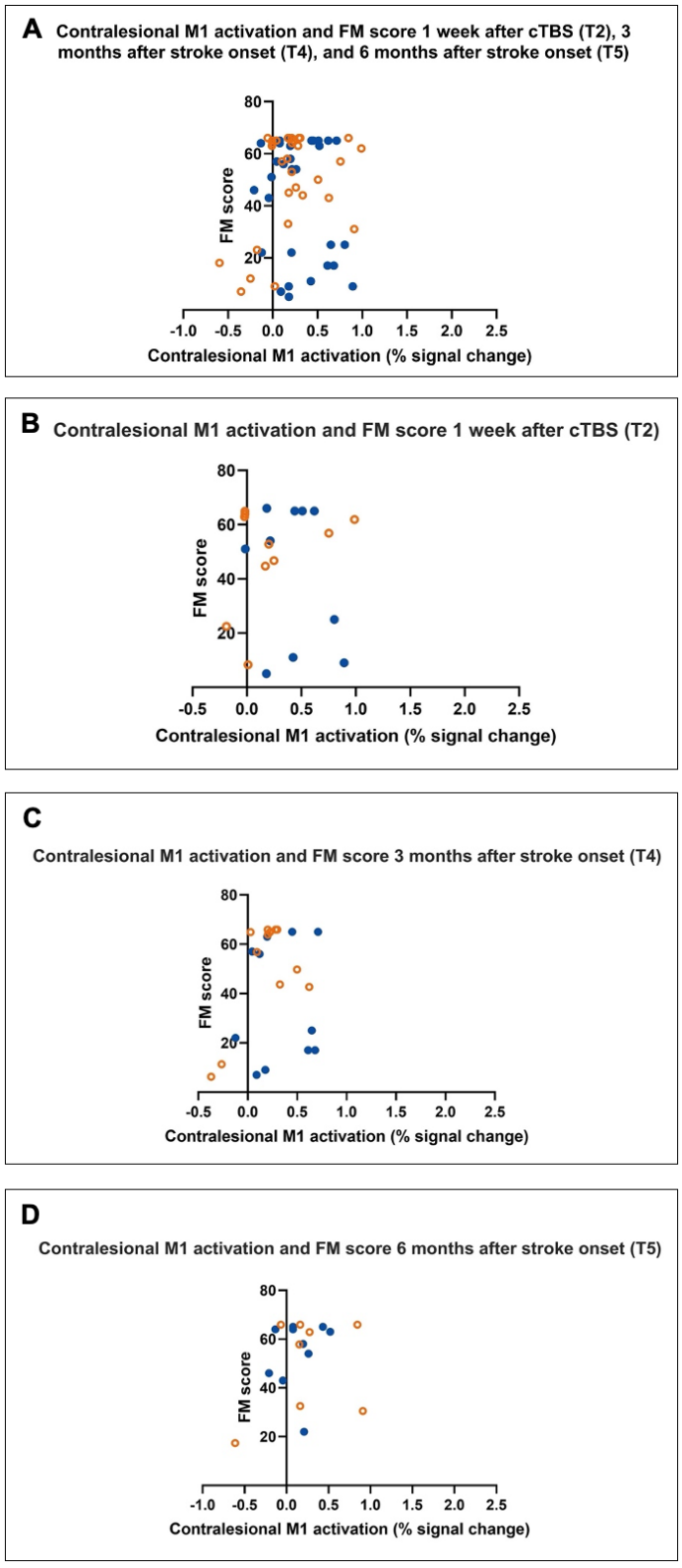
## Supplementary figures

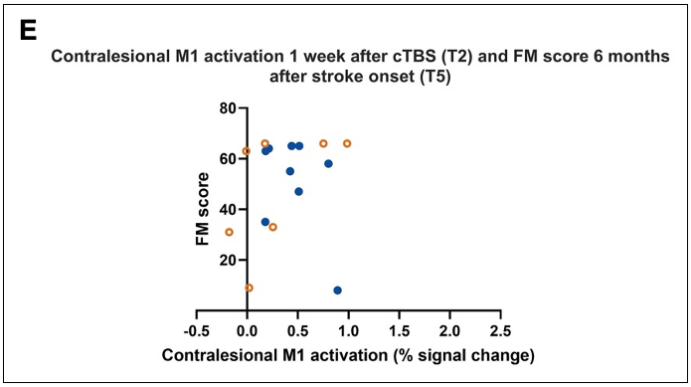



**Supplementary Figure 1:** The four task-dependent modulation models. Model 1 was the winning model from Grefkes et al. (10), model 2 was identical to the endogenous connectivity model, which was also the winning model from Rehme et al. (21) , model 3 was the second-best model from Rehme et al. (21), and model 4 was the second-best model from Grefkes et al. (10). Connections between V5 and ipsi- and contralateral SMA and PMC are not shown for simplicity of the model. L = left, R = right. Created in Biorender.com.

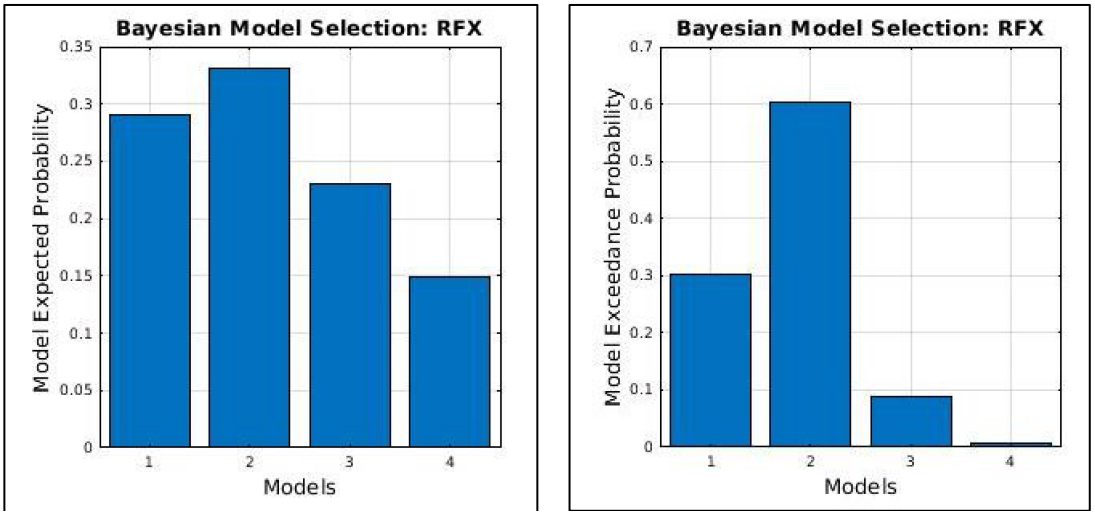



**Supplementary Figure 2:** Pearson's correlation of ipsilesional M1 activation (% signal change) and Fugl-Meyer (FM) score (A) 1 one week after cTBS (T2) *(R = 0.38, p = 0.10);* (B) 3 months after stroke onset (T4) *(R = 0.58, p = 0.0034*)*; and (C) 6 months after stroke onset (T5) *(R = 0.26, p = 0.31)*. (D) Pearson's correlation of ipsilesional M1 activation 1 week after cTBS (T2) and FM score 6 months after stroke onset (T5) *(R = 0.30, p = 0.27)*. Individual data points of both sham cTBS (blue) and real cTBS (orange) treatment groups are shown.

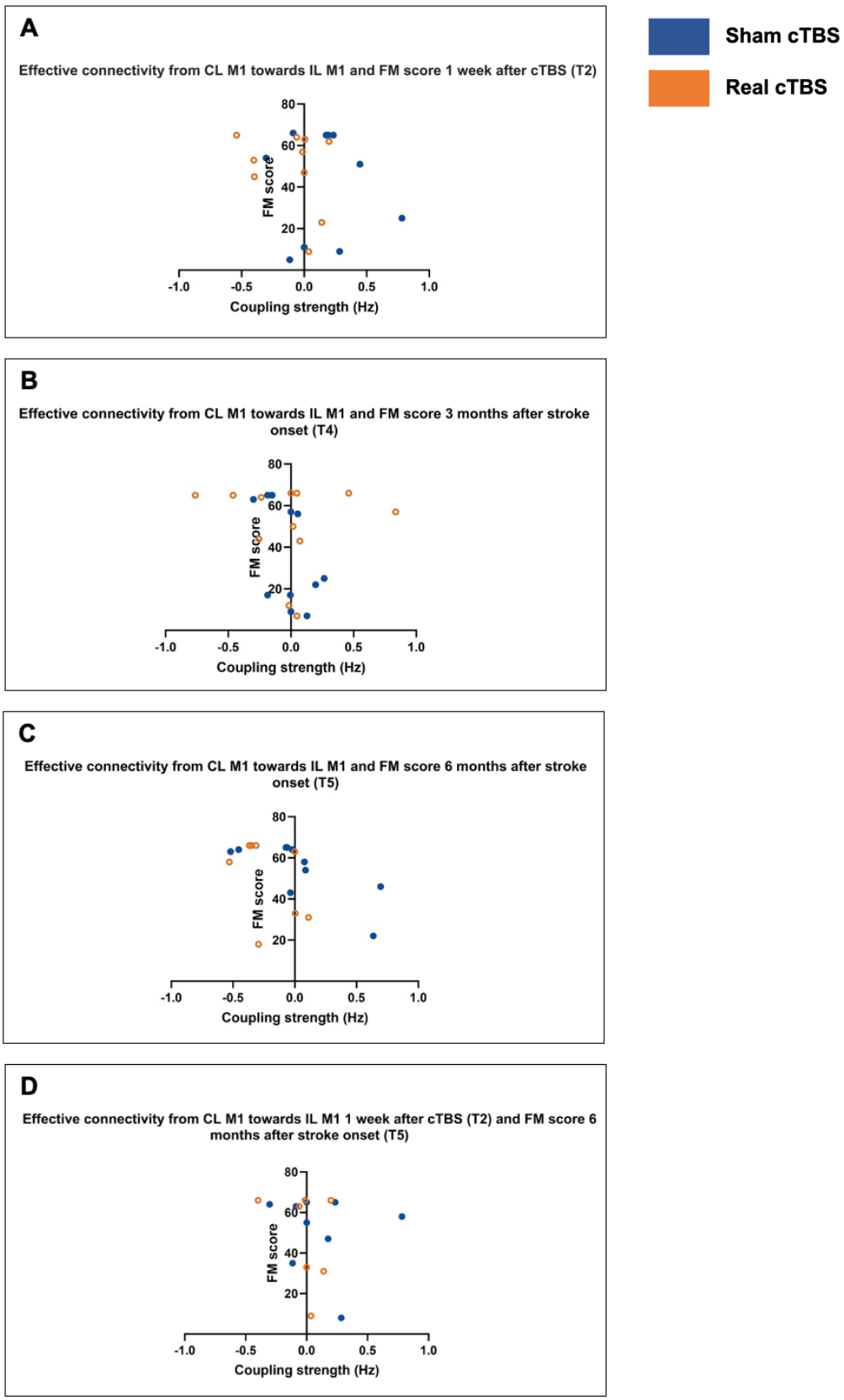

A Contralesional M1 activation and FM score 1 week after cTBS (T2), 3 months after stroke onset (T4), and 6 months after stroke onset (T5)
FM score
Contralesional M1 activation (% signal change)
B Contralesional M1 activation and FM score 1 week after cTBS (T2)
FM score
Contralesional M1 activation (% signal change)
C
Contralesional M1 activation and FM score 3 months after stroke onset (T4)
FM score
Contralesional M1 activation (% signal change)
D
Contralesional M1 activation and FM score 6 months after stroke onset (T5)
FM score
Contralesional M1 activation (% signal change)
Sham cTBS
Real cTBS




**Supplementary Figure 3:** Pearson's correlation of contralesional M1 activation (% signal change) and Fugl-Meyer (FM) score (A) 1 week after cTBS (T2), 3 months after stroke onset (T4), and 6 months after stroke onset (T5) combined *(R = 0.09, p = 0.47)*; (B) at T2 *(R = -0.02, p = 0.92)*; (C) at T4 *(R = 0.20, p = 0.36)*; and (D) at T5 *(R = 0.23, p = 0.36)*. (E) Pearson's correlation of contralesional M1 activation 1 week after cTBS (T2) and FM score 6 months after stroke onset (T5) *(R = 0.03, p = 0.53)*. Individual data points of both sham cTBS (blue) and real cTBS (orange) treatment groups are shown.




**Supplementary Figure 4:** Bayesian model selection random-effects analysis (RFX). The left figure shows the expected posterior exceedance probabilities for each of the four models. The right figure shows the exceedance probabilities for each of the four models. According to both figures, model 2 is the best fit given the data. Figures obtained from SPM12.




**Supplementary Figure 5:** Pearson's correlation of effective connectivity from contralesional (CL) towards ipsilesional (IL) M1 (coupling strength (Hz)) and Fugl-Meyer (FM) score (A) 1 one week after cTBS (T2) *(R = -0.21, p = 0.38)*; (B) 3 months after stroke onset (T4) *(R = -0.19, p = 0.38)*; and (C) 6 months after stroke onset (T5) *(R = -0.47, p = 0.049)*. (D) Pearson's correlation of effective connectivity from CL towards IL M1 1 week after cTBS (T2) and FM score 6 months after stroke onset (T5) *(R = -0.18, p = 0.51)*. Individual data points of both sham cTBS (blue) and real cTBS treatment groups (orange) are shown.