RESEARCH ARTICLE

Predictors and brain connectivity changes associated with arm motor function improvement from intensive robotic practice in chronic stroke [version 1; referees: 3 approved with reservations]

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Abstract

Background and Purpose: The brain changes that underlie therapy-induced improvement in motor function after stroke remain obscure. This study sought to demonstrate the feasibility and utility of measuring motor system physiology in a clinical trial of intensive upper extremity rehabilitation in chronic stroke-related hemiparesis.

Methods: This was a substudy of two multi-center clinical trials of intensive robotic arm therapy in chronic, significantly hemiparetic, stroke patients. Transcranial magnetic stimulation was used to measure motor cortical output to the biceps and extensor digitorum communus muscles. Magnetic resonance imaging (MRI) was used to determine the cortical anatomy, as well as to measure fractional anisotropy, and blood oxygenation (BOLD) during an eyes-closed rest state. Region-of-interest time-series correlation analysis was performed on the BOLD signal to determine interregional connectivity. Functional status was measured with the upper extremity Fugl-Meyer and Wolf Motor Function Test.

Results: Motor evoked potential (MEP) presence was associated with better functional outcomes, but the effect was not significant when considering baseline impairment. Affected side internal capsule fractional anisotropy was associated with better function at baseline. Affected side primary motor cortex (M1) activity became more correlated with other frontal motor regions after treatment. Resting state connectivity between affected hemisphere M1 and dorsal premotor area (PMAd) predicted recovery.

Conclusions: Presence of motor evoked potentials in the affected

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motor cortex and its functional connectivity with PMAd may be useful in predicting recovery. Functional connectivity in the motor network shows a trends towards increasing after intensive robotic or non-robotic arm therapy. Clinical Trial Registration URL: http://www.clinicaltrials.gov. Unique identifiers: CT00372411 & NCT00333983.

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Introduction

The development of new methods for rehabilitation of deficits after stroke has enabled research into the brain mechanisms of improved function after such therapy. This has been accomplished in Constraint Induced Therapy[1,2], Bilateral Arm Training[3] and in one form of robotic hand training[4]. Some common themes in these studies include: 1. Changes in motor task-related brain activation after therapy (although both positive and negative changes have been reported) and, 2. Expansion of shrunken motor maps as measured by transcranial magnetic stimulation (TMS)[5,6]. However, there remain ambiguities and even controversies regarding the effects of repetitive task practice on brain activity and whether modern, well-defined therapeutic methods differ in their brain effects.

Robotic rehabilitation has certain mechanistic advantages over other therapeutic methods[7]. Robotic therapy is a better option for more severely affected stroke patients who may not be able to practice certain movements without external assistance. In such patients the mechanisms of recovery may be qualitatively different and who have the most to gain with improved understanding of the mechanisms of improvement of any particular therapy. In addition, although robotic therapy is well defined by the algorithms it uses for training, the therapy is flexible enough to train patients in various types of movements.

We had the opportunity to perform a multi-center investigation of the brain mechanisms underlying robotic rehabilitation by studying a subset of participants in two multi-center VA studies that compared robotic rehabilitation to both an intensity-matched non-robotic therapy regiment and usual care. The hypotheses for this study related to both prognosis (e.g. greater cortical motor excitability and reduction in transcallosal inhibition will predict greater functional improvement) and treatment effects (e.g. intensive rehabilitation will more effectively increase the ability to activate multiple muscles through motor cortical activity, partly through reduced interhemispheric inhibition to the affected motor cortex.) It was also an opportunity to test a connectivity-based approach that has shown promise in studies of recovery of function[8,9].

Methods

Clinical trial

This was a substudy of two multi-center clinical trials whose methods and results have been published[10–12]. It was originally intended to enroll approximately 40 participants across four sites but due to regulatory and staffing at issues at two sites, 13 subjects across two sites were enrolled. Briefly, all participants were chronic hemiparetic stroke patients with a significant degree of impairment (Upper Extremity Fugl-Meyer scale 7–38.) Figure 1 shows the basic design of the substudy, with TMS and magnetic resonance imaging (MRI) measures bracketing a 6–12 week intervention. Clinical Trial Registration URL: http://www.clinicaltrials.gov. Unique identifiers: NCT00372411 & NCT00333983.

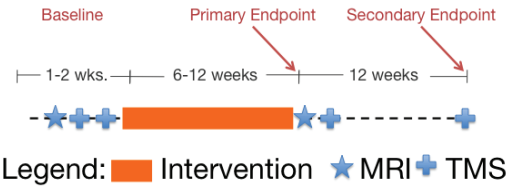

Figure 1. Study Design. The timeline of baseline measures and the therapy interventions are shown graphically. MRI and TMS measurements were performed before or after the intervention.

Transcranial magnetic stimulation (TMS)

Stimulation of the motor cortex responsible for upper-extremity impairment was performed using a MagStim 200 or 200^2 Magnetic Stimulator (MagStim Ltd., Wales, UK and 70 mm D double circular coil. Motor evoked potentials (MEP) were recorded unilaterally by surface electrodes fixed over the biceps and extensor digitorum communus (EDC) muscles in bipolar montage with 3 cm spacing. Responses were amplified by a battery-powered surface electromiography (EMG) integrated electrode and amplifier (B&L Engineering, Tustin, CA or DelSys, Boston, MA), and fed into a personal computer through a multifunctional I/O board and LabView acquisition/analysis software (National Instruments, Austin, TX). A 100 ms period after stimulus was examined with time window adjusted to capture only the MEP. Amplitudes were measured peak-to-peak. Bandpass was 30–1000 Hz and digitization 2000 Hz.

The target muscle was ensured to be at rest during the entire procedure, through audio and visual monitoring of muscle activity. Motor threshold was determined using International Federation of Clinical Neurophysiology criteria[13] except that a 25 μV limit was used because of the bipolar montage. The coil was localized on the frontoparietal region contralateral to the target muscle in the examined limb and moved until each muscle's hot-spot, where the response threshold was the lowest, was found. Exact position of stimulation was recorded using a stereotactic system (BrainSight, Rogue Research, Montreal, QC, Canada) and guided by a 1 cm Cartesian coordinate system projected onto the subject's own MRI. If a different hotspot was found on a subsequent visit, threshold and recruitment curves were obtained at both the original and new hotspot, but the original hotspot data were used for group analysis.

Recruitment curves were measured by stimulating at a range of intensities, from 10% below threshold, increasing in increments of 10% of threshold until the response plateaued or the maximum output of the stimulator was reached. Ten stimuli at each intensity were delivered.

Ipsilateral silent period

The ipsilateral silent period[14] was measured by stimulation of the unaffected (defined here as contralesional) cortex and voluntary activation of the affected arm. The maximum force that the subject could sustain in each target muscle was determined. For the biceps the weight was placed on the wrist and for the extensor digitorum communis (EDC) on the proximal interphalangial joints. The coil was placed over the hand knob (hand representation within M1) of the unaffected hemisphere. After the subject stabilized the weight against gravity, a TMS pulse 100% of the maximal stimulator output was delivered. The subject was allowed to rest for several seconds and the procedure was repeated two further times. The EMG signal was integrated, and a ratio of post-relative to pre-stimulation activity was computed, making the appropriate adjustment for the length of period.

Magnetic resonance imaging (MRI)

Anatomical and Functional Magnetic Resonance Imaging was performed at each center on a Tim Trio 3T scanner (Siemens AG, Erlangen, Germany) equipped with an 8-channel receive-only head coil. Anatomical Imaging: These consisted of a high-resolution three-dimensional sagittal T1-weighted magnetization-prepared rapid gradient echo (MP-RAGE) image, and oblique proton density and T2-weighted images acquired with 2 mm slice thickness. Diffusion-tensor images were acquired using a single-shot echo-planar technique and 65 directions. A b value of 1000 s/mm^2 was used with an average of six images acquired to increase signal-to-noise ratio. A fractional anisotropy (FA) map was created from these data. A 5 mm radius spherical Region of Interest (ROI) was centered on the posterior limb of the left and right internal capsules (IC) on the FA images and the mean, standard deviation, and ratio (affected/unaffected) were computed.

Functional Imaging. Two eyes-closed rest scans were obtained, each with 128 coronal blood oxygenation-level dependent (BOLD) weighted volumes (echo planar imaging; 3 sec TR, 30 ms TE, 4 mm slice thickness with no gap, flip angle = 90°, 36 axial slices, 1.8 × 1.8 mm^2 inplane resolution, FOV = 23 cm.) These were separated in time by at least 5 minutes. A tape and cushion technique was employed to reduce head motion and remind the subjects of the need to keep their head as still as possible. We examined head motion parameters within the analysis and rejected runs with absolute head movement greater than 2 voxels. Images were corrected for head motion by realignment and Independent Component Analysis (ICA) was used to remove movement related signal[15].

Region of Interest (ROI) resting state correlation analysis. ROI based analysis was performed without spatial normalization in AFNI[16] and MATLAB (MathWorks Inc., Natick, MA). All of a participant's resting state scans were corrected for slice timing and spatially registered to the first resting sate scan from their first session. The structural image was skull-stripped and also spatially registered to the subject's first functional scan. A 6-mm FWHM Gaussian blur was then applied to all spatially registered EPI scans. Nine ROIs were selected manually identifying the following anatomical landmarks: medial part of the precentral gyrus, postcentral gyrus, cerebellar hemispheres, supramarginal gyrus, supplementary motor area (caudal supplementary motor area between medial precentral gyrus and a coronal plane through the anterior commisure[17] and superior, middle, and inferior frontal gyri, Figure 2. Pairwise ROI correlations were computed on the time series for each ROI and Z-transformed.

Statistical analysis

SAS (Cary, North Carolina) was used for all analysis. Pearson correlation was used to calculate recruitment curve slope and associations between variables. Mixed models (REML with compound symmetry) were used to analyze predictive factors such as presence of TMS responses and recruitment curve slope.

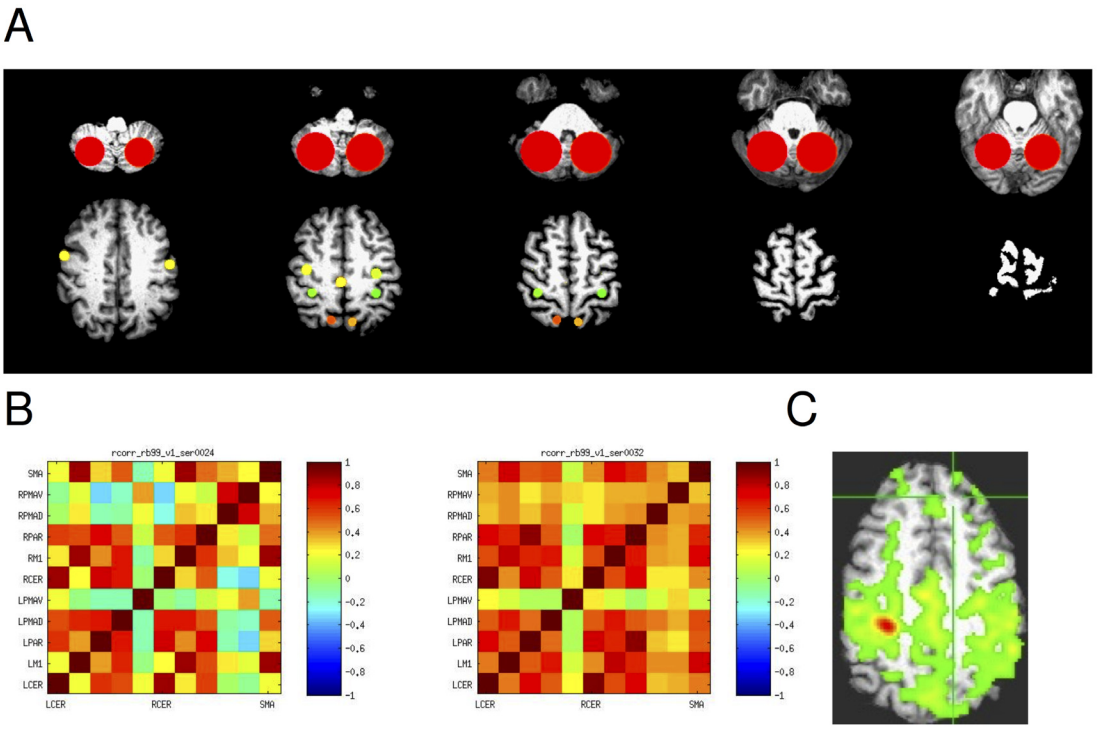

Figure 2. Resting state connectivity (correlations). A: The 11 regions of interest (ROIs) are shown on representative axial slices of an example brain MRI. The top slices show the cerebellar ROI, then the PMAv on the left bottom slice, and PMAd, M1, and superior parietal regions from anterior to posterior. The SMA is represented by a single midline ROI. **B**. Correlation matrix with correlations at baseline in two resting states scans in the same participant. **C**. Example correlation in a single slice with a affected side M1 ROI as the seed.

Results

Functional outcome and TMS

Fourteen subjects were enrolled between 2008 and 2010 at the Baltimore and North Florida/South Georgia VAMC. Thirteen were stroke patients, three of whom were randomized to intensive comparison therapy and ten of which were randomized to robot therapy, and one subject was a healthy control who received no therapy. The relationship between initial and follow-up Fugl-Meyer (FM) impairment score and presence of MEP is shown in Figure 3. While MEP were absent in most of the lower functioning participants initially, there were both low and high functioning participants with absent and present MEP. Controlling for the effects of baseline FM in a fixed effects analysis, presence of an MEP at baseline was associated with a mean 3.3 ± 6.2 S.E. (N.S.) higher change in FM across all post-baseline visits. (There were up to four post-baseline visits.) A biceps MEP was never present without an EDC MEP, but not *vice versa* and the predictive value of these two measures was approximately equal.

TMS: recruitment curves

One of the hypotheses regarding recruitment curves was that steeper recruitment curves would correlate with better function. However, there were no significant correlations between either EDC or biceps recruitment curves and function, within the population in which recruitment curves could be evaluated (N=6). Almost all participants had very shallow recruitment curves, with one moderately affected individual (FM=35) being the exception (Figure 4). There was a non-significant trend for higher recruitment curve slope at baseline correlating with functional improvement in a mixed effects model that controlled for baseline FM.

Silent periods

A long-lasting stimulus artifact contaminated too many cases to allow group analysis. One example of change in silent period is noted in Figure 5. In this case the subject had an increase in voluntary activity after the intervention, despite the same amount of force requirement, and demonstrated a clearer iSP only after the intervention. However, in most other subjects, there was no visible iSP.

RSC analysis

Predictive measures. Resting state analysis resulted in a correlation matrix for the chosen ROI. While there was not an age-matched control population, there were clear asymmetries in the correlation matrices, as within hemisphere connections were decreased on the affected side as compared to the unaffected, but with exceptions such as the parietal area (data not shown). We were particularly interested in exploring the changes of correlation of the affected side motor cortex (M1) with other brain areas. Correlation of the affected M1 with all frontal lobe motor regions increased over the course of treatment, but there was no change in correlation of M1 with the cerebellum (Figure 6). The change in the unaffected M1, SMA and the unaffected side superior parietal area were most striking.

Correlative measures. Improvement in Fugl-Meyer correlated with a trend towards reduction in two pairwise correlations. Greater FM increase was associated with decrease in affected M1-unaffected M1 connectivity ($r^2 = 0.31$, $p = 0.07$), and unaffected M1-affected superior parietal area ($r^2 = 0.34$, $p = 0.06$.) All other changes in functional connectivity measures correlated less well with changes in connectivity.

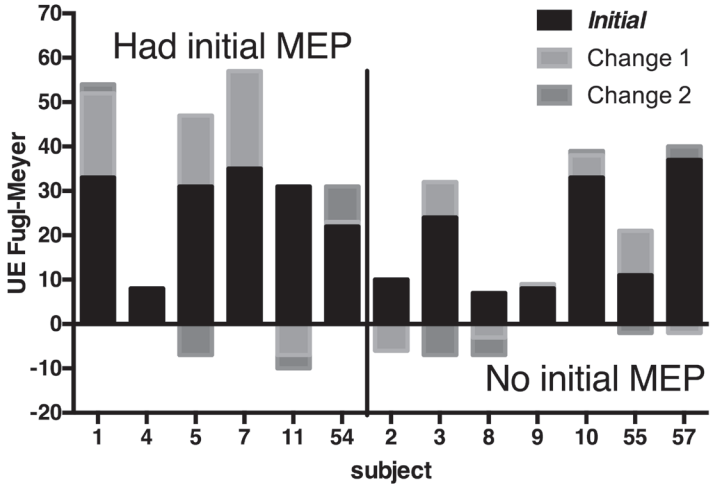

Figure 3. Clinical Outcomes. The baseline and change in Fugl-Meyer score are shown for each participant, grouped by whether there was initial motor evoked potential as measured by TMS. Change 1 is across the intervention; Change 2 is between the end of the intervention and the last outcome measurement (12 weeks). Negative changes are always shown below the baseline.

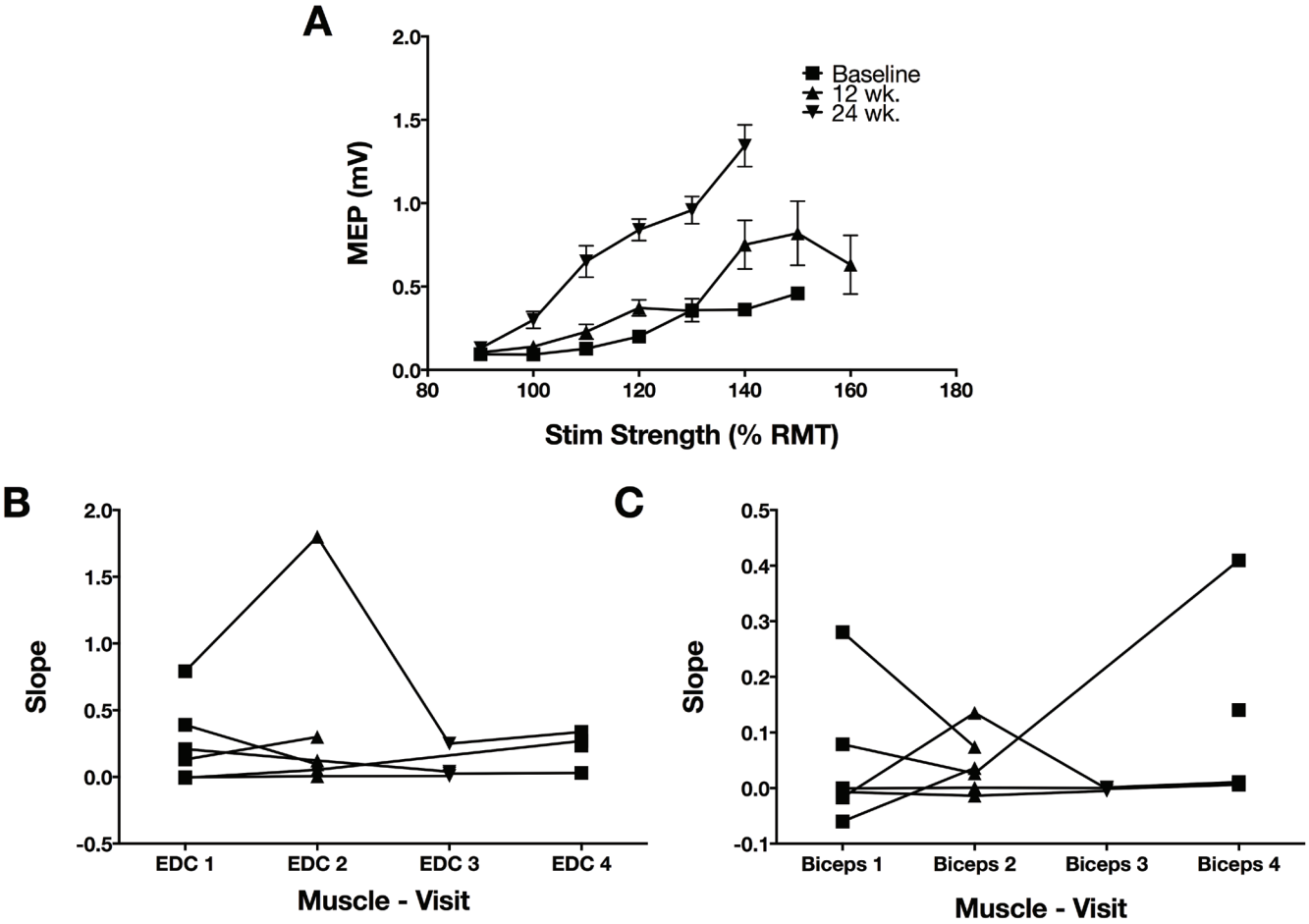

Figure 4. Recruitment curves on the affected upper extremity. A. Single participant (#1) recruitment curves in the EDC and the second baseline and two follow-up measurements. Stimulation strength is indicated on the x-axis as a percentage of resting motor threshold. **B**. The slope of the recruitment curve between 100 and 120% of resting motor threshold stimulation strength was extracted from each recruitment curve of each participant that had measurable recruitment curves that could be measured on the affected side EDC. The first two measurements are both baseline periods. Changes in the recruitment curve slope were not significant. **C**. Recruitment curve slope in biceps (otherwise, as in **B**).

MRI: Fractional anistropy of the corticospinal tract

FA of the affected internal capsule was correlated with baseline motor ability ($r^2 = 0.48$, $p < 0.01$.) but did not predict motor recovery, although the trend was for greater FA to be associated with better recovery.

There were no significant differences in any outcome measure for robotic vs non-robotic comparison therapy.

Dataset 1. Raw data for predictors and brain connectivity changes associated with arm and motor function improvement from intensive robotic practice in cronic strocke

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Descriptions of each dataset are provided in the readme file.

Discussion

The purpose of this study was to obtain feasibility data on the use of TMS and MRI to provide predictive and mechanistic information about the motor functional response to intensive arm rehabilitation. It was not expected to provide definitive results in a field that has been marked by inconsistency. Some of the lessons learned in this study are that the lack of TMS responses in a majority of the moderate-to-severe population limits the utility of TMS for measuring change, although when MEP are present this predicts a better response to intervention, as has been demonstrated previously[24]. The ipsilateral silent period, a measure of transcallosal inhibition that can be performed even when MEP cannot be elicited, has limitations as well, and was not useful in this particular study, partly for technical reasons. MRI measures of resting state connectivity were more revealing, demonstrating both the deficits and changes with therapy, although in a purely exploratory manner.

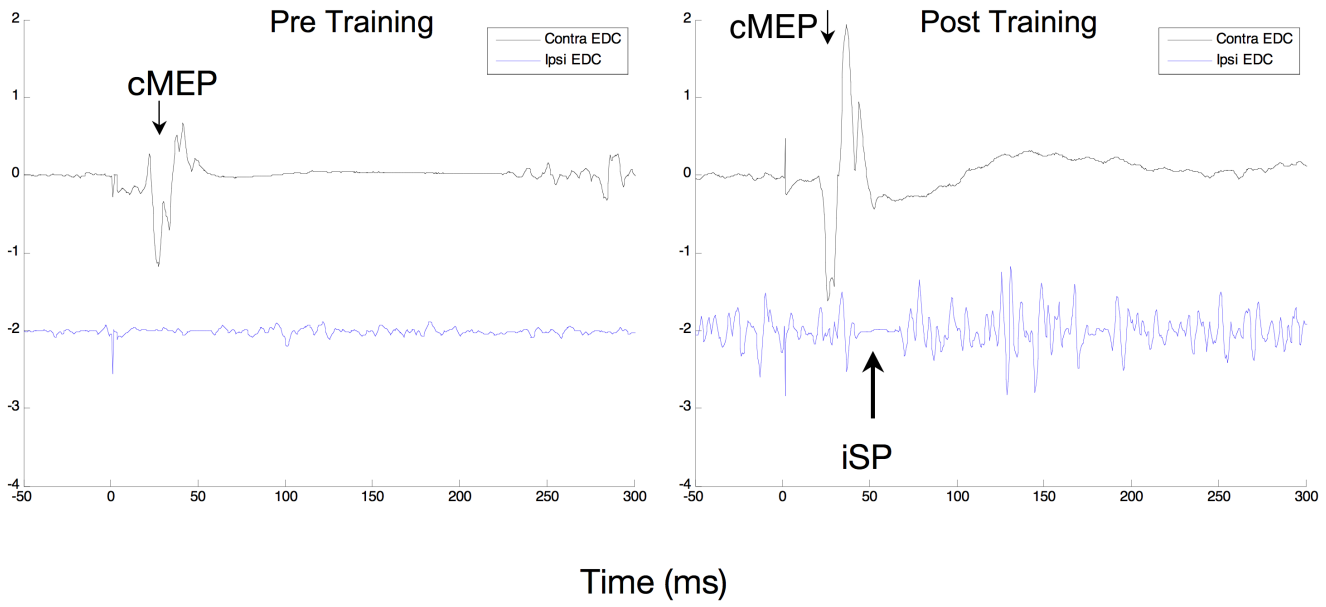

Figure 5. Ipsilateral Silent Period. An example of ipsilateral silent period measured at baseline (left) and immediately after the 12 weeks intervention (right) in participant RB5. EMG is measured in mV in the EDC muscle contralateral to the TMS stimulator in the upper trace and ipsilateral in the lower trace, which is offset by 2 mV. Before the intervention there is little activation of the muscle and also no apparent silent period. There is much more activation of the muscle after the intervention and also a visible short ipsilateral silent period.

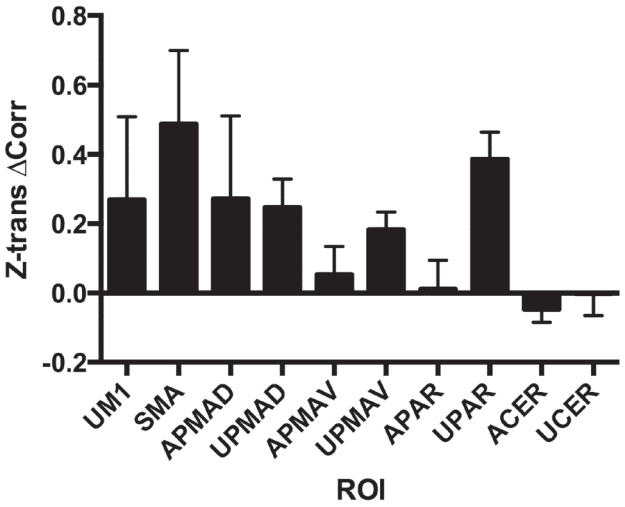

Figure 6. Change in connectivity of AM1. The mean change and S.D. of the Z-transformed correlation coefficient of the affected primary motor cortex (AM1) with each of the other regions is shown. Note that all regions showed an increase in connectivity except the parietal regions. Region names include 'A' for affected side hemisphere (the side opposite to the affected hemisphere in the case of the cerebellum, 'CER') and 'U' for unaffected.

Silent period and recovery of cortical control

While there were technical limitations to use of silent periods, as shown in Figure 5, the silent period could become more apparent after therapy. Since the appearance of a silent period depends on cortical activation of an affected muscle, a silent period could appear to be absent if there is little such cortical activation. Increased corticomotor effectiveness can thus result in the appearance of a silent period, and give a misleading impression that more intercortical inhibition is related to better function. The role of intercortical inhibition in shaping motor function is complex, and interpretation of tests that require activity in interhemispheric networks needs to be sophisticated.

Role of superior parietal cortex in recovery and compensation

In the resting state connectivity analysis, all connectivity with the affected motor cortex was negatively correlated with impairment. The two exceptions were the superior parietal area, in which increased connectivity was correlated with impairment. There have been a number of reports of the role of the superior parietal area in recovery of function after stroke[18–22]. However, its correlation here would suggest an association with more impairment and a role in compensation in only the more severely affected individuals.

Table 1. Demographic information of study participants who completed the study. Subject numbers started at 1 in Baltimore, and at 50 in Florida. All had anterior circulation ischemic strokes except for #1 who had a thalamic hemorrhage. Therapy assignment included either robotic or intensive comparison (comp.) therapy. When length of therapy was 6 weeks, there was no therapy with the wrist robot, only planar and vertical robots.

Subject	Age	Gender	Arm Aff.	Group	Duration	FM baseline	FM 6 or 12 wk
1	44	M	L	Robot	12	33	53
2	64	M	R	Comp.	12	10	4
3	61	M	L	Comp.	12	24	32
4	54	M	R	Comp.	12	8	8
5	81	M	R	Comp.	12	30	47
7	48	M	L	Robot	12	35	57
8	69	M	R	Robot	12	7	4
9	58	M	L	Comp.	6	8	9
10	51	M	R	Robot	6	33	38
11	52	M	R	Robot	6	31	24
54	45	F	R	Comp.	12	22	23
55	71	M	R	Robot	12	11	21
57	70	M	R	Comp.	12	37	35

Changes in connectivity

The correlation matrix for even a small number of ROIs is a large amount of data and causes a multiple comparison problem. We focused on connectivity with the affected motor cortex as being most relevant to recovery of function. Out of the ten other regions, correlation with eight of them increased over the course of therapy. The largest increases were in the contralesional superior parietal area, ipsilesional dorsal premotor area, and supplementary motor area. These regions have strong bilateral connections and are good candidates for brain regions that would be engaged by the practice involving visual motor feedback and proximal arm movements. There were no significant associations between change in interregional correlation and a change in motor function. The best correlation with recovery was in connections of the unaffected M1 with two areas in the affected hemisphere: affected M1 and affected superior parietal area. The fact that this was a negative correlation suggests that intensive unimanual therapy may be decreasing the importance of the unaffected primary motor area in movement of the affected side. But other changes, not measured in this study, may be related to recovery of function, and the measured network changes, whether or not they are a significant effect of the intervention, may not be necessary for recovery or may represent compensatory changes. Likely because of the relatively small size of the study, were not able to find significant differences between such changes in the two types of treatment, if any differences exist. It would be interesting to speculate that the superior parietal activity would be more involved in robotic rehabilitation, with its visuomotor component and SMA in the intensive comparison treatment that involved more self-initiated activity.

Conclusions

Measurement of brain changes related to motor recovery in moderate-to-severely affected stroke patients is complicated by difficulties in measuring brain function noninvasively. But our study showed that simple MEP presence might be useful in predicting response to rehabilitation in chronic stroke, while resting state connectivity appears to be responsive to treatment, with increase in affected primary motor cortical connectivity to other frontal motor areas. Motor cortical functional connectivity with the superior parietal cortex may be marker for compensatory changes that do not respond to affected side intensive practice.

Data availability

F1000Research: Dataset 1. Raw data for predictors and brain connectivity changes associated with arm and motor function

improvement from intensive robotic practice in cronic strocke, 10.5256/f1000research.8603.d133175[25]

Author contributions

GFW and ACL conceived of the study, SRR and RPG did MRI protocol design and analysis, PDG and SY statistical analysis, GFW, LGR, LMJL and LGR performed TMS studies.

Competing interests

No competing interests were disclosed.

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Data Source

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Current Referee Status: ? ? ?

Version 1

Referee Report 24 October 2016

doi:10.5256/f1000research.9257.r16609

? **Sean Dukelow**, **Jennifer Semrau**
Department of Clinical Neurosciences, University of Calgary, Calgary, AB, Canada

The authors present an interesting paper that examines neurophysiologic measurements in 13 chronic stroke survivors who completed robotic therapy or intensive interventions lasting either 6 weeks or 12 weeks. The study explores the use and predictive capabilities of MEPs, FA, and resting state fMRI. Further there is some discussion of the ipsilateral silent period but this was difficult to obtain in a number of subjects for technical reasons. Although we are enthusiastic about the study, we have some concerns over the manuscript in its existing format and put forward a number of questions/suggestions for the authors below.

- The authors used a number of different measures – it would have been nice to see a hypothesis associated with each of these measures.
- Could the authors please be more clear on the rehabilitation intervention? We recognize that they do cite the trials from which the data was taken, but even a line or two discussing what went on in the robot vs the comp. groups would be helpful. It would also be helpful to demarcate which individual received what therapy in Figure 3.
- The authors state that the ROI analysis for the resting state fMRI was performed without normalization. Those unfamiliar with this type of analysis may not understand why normalization is not required. Please add a sentence or two to provide justification.
- How often did the authors note a change in the motor hotspot using TMS?
- Why use a 5mm spherical region of interest for the PLIC as the PLIC is not a spherical structure? Some explanation of the reasoning for this would be helpful.
- What was the role of the single control subject who is mentioned in the methods? Please clarify.
- Exactly how many subjects did the authors find/not find an ipsilateral silent period in?
- Re: Fractional Anisotropy (FA): R-squared = 0.48 is reported for the relationship of FA to baseline motor ability. It would be nice to see this in a scatter plot as the relationship is quite strong.
- The manuscript might benefit from some discussion of why steeper MEP recruitment does not lead to better function.

- The authors mention the technical limitations of using silent periods. For readers who are not familiar with this technique, a sentence or two briefly describing what those limitations are would be helpful. Further, the discussion of the importance of the silent period relative to what is known in the literature would be helpful.

- We would like to see slightly more discussion about the limitations of an n=13 sample size. For instance, the results that those subjects with an MEP at baseline tended to do better appears to be driven by 3 subjects based on figure 3.

Minor Concerns:

- Many abbreviations go undefined in the paper:

1. Last paragraph of introduction: It would be appreciated if the authors provided the references for the two multicenter VA studies that they discuss. They should also define VA for readers.

2. Figure 2 – the authors need to state what the abbreviations are in the text for the correlation matrices.

3. Figure 6 – Please label your abbreviations.

- Dataset 1 label: Please check your spelling “cronic strocke”

- In the PDF version of the manuscript, the quality of the correlation matrices in Figure 2B appears to be low resolution.

- Introduction: “In such patients the mechanisms of recovery may be qualitatively different and who have the most to grain with improved understanding of the mechanisms…” – this sentence appears to be incomplete.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Referee Report 19 September 2016

doi:10.5256/f1000research.9257.r15975

? **Rudiger Seitz**
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The authors present a well-designed, multimodal study on the effect of robotic practice in 13 chronic stroke patients using transcranial magnetic stimulation (TMS), high resolution magnetic resonance imaging (MRI), diffusion tensor imaging and resting state BOLD imaging. Investigated variables were motor evoked potentials (MEPs) on the affected side, silent period after TMS of the contralesional motor cortex, fractional anisotropy (FA) of the ipsilesional internal capsule, and functional connectivity of motor cortex in the affected hemisphere using anatomically based regions of interest (ROIs). The main results were that MEPs were associated with better functional outcome, FA of internal capsule was associated with better function at baseline, and BOLD in motor cortex was more correlated with other motor areas after training of which resting connectivity between motor cortex and dorsal premotor cortex predicted

recovery. There are some issues that need clarification.

- Table 1 shows that 6 patients received robot training, while 7 received intensive comparison therapy. At no other instance it is said what intensive comparison treatment is. In fact, the entire manuscript including the title argues for robot training and presents the data as if all patients received robot training. It is not stated if the two treatments resulted in the same or different motor function. Further, a formal comparison of the two groups also concerning the studied variables is lacking.

- Moreover, patient 1 differed from the other patients as he was the only one with an intracerebral hemorrhage and a subcortical location of the lesion. It should be added how many of the other patients also had subcortical and cortical infarct lesions, respectively.

- Were the imaging data analyzed in one centre or in the participating centres? Were the ROIs drawn by one of the authors or by different authors? What was the interobserver reliability?

- How many MRI slices did the ROIs listed in the methods include?

- What is meant with "predictive values of the MEP measures were approximately equal (page 5)"? Please, be specific and provide the data.

- The increase of connectivity with the superior parietal area is noteworthy, since this is a brain area with profound somatosensory function. The authors should provide information about the sensory deficits of their patients.

- Figure 4 should provide information about which patients are presented in parts B and C.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response (*Member of the F1000 Faculty*) 21 Sep 2016

George Wittenberg, Department of Neurology, University of Maryland, USA

Thank you for the careful reading of this manuscript. We should be able to respond to all of the comments directly in the manuscript. But to immediately answer two of the comments:

- The intensive comparison therapy was described in other papers, and we will make that more clear and add a summary. We in no way wanted to give the impression that any changes were specifically related to the type of intensive therapy. That was a possibility, of course, but we did not find that to be true, and had too small a sample to have the power to do so unless it was a truly dramatic difference.
- All MRI analysis was done at one center and ROI drawn by the first author. It was a simple, consistent approach to the data.

Competing Interests: No competing interests.

Referee Report 12 September 2016

doi:10.5256/f1000research.9257.r15971

? **Argye E. Hillis**
John Hopkins School of Medicine, Baltimore, MD, USA

This is a well-written and informative substudy of a thoughtfully designed clinical trial to improve motor function. The authors report imaging predictors of recovery before and after controlling for initial severity.

Main criticism:

- It was not clear that resting state sequences masked infarct. How many patients had infarct in M1. It would be unsurprising that there would be lower connectivity between M1 on the affected side and other areas if it is partially infarcted, and that would predict recovery.

- Conclusions about M1 connectivity predicting recovery are too strong, since the results were not statistically significant, but only showed a trend (e.g. p = 0.06). Furthermore, it seems that they did not control for multiple comparisons, so these could have been found just by chance.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response (*Member of the F1000 Faculty*) 13 Sep 2016

George Wittenberg, Department of Neurology, University of Maryland, USA

The resting state analysis was a very simple ROI based one. The ROI were registered by hand to M1 and if it was infarcted, the ROI included the infarct. In the the group, the strokes were predominantly subcortical and the analysis measured changes in connectivity, so this method was appropriate.

The comment about the limitations are well-taken. We also introduced confusion on many levels by labeling two paragraphs "predictive" and "correlative". The predictive section presents data on longitudinal changes but doesn't state statistics, although it shows S.E in the graph. It also perpetuates a common misuse of the term "predictive." In fact, we did not find RSC data that predicted response to the intervention, although that was a goal of the study. This can be corrected in a version after other reviews.

Competing Interests: No competing interests.