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Pre-therapy neural state of bilateral motor and premotor cortices predicts therapy gain after subcortical stroke – a pilot study

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Abstract

Objective—To examine whether neural state (proton magnetic resonance spectroscopy, ¹H-MRS) of spared motor and premotor cortices captured before a therapy predicts therapy-related motor gains in chronic subcortical stroke.

Design—Ten survivors, presenting chronic moderate upper extremity (UE) impairment, underwent ¹H-MRS, MRI, clinical, and kinematics assessments prior a four-week impairment-oriented training. Clinical/kinematic assessments were repeated after therapy and motor gain was defined as positive values of clinical UE/elbow motion changes and negative values of trunk motion changes. Candidate predictors were N-acetylaspartate-neuronal marker, Glutamate-Glutamine-indicator of glutamatergic neurotransmission, and myo-Inositol-glial marker, measured bilaterally within the UE territory in motor (M1) and premotor (premotor cortex-PM, supplementary motor area-SMA) cortices. Traditional predictors (age, stroke length, pre-therapy UE clinical impairment, infarct volume) were also investigated.

Results—Poor motor gain was associated with lower Glutamate-Glutamine levels in ipsilesional M1 and PM ($r=0.77$, $p=0.01$ and $r=0.78$, $p=0.008$ respectively), lower N-acetylaspartate in ipsilesional PM ($r=0.69$, $p=0.02$), higher Glutamate-Glutamine in contralesional M1 ($r=-0.68$,

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$p=0.03$), and lower Glutamate-Glutamine in contralesional SMA ($r=0.64$, $p=0.04$). These predictors outperformed myo-Inositol metrics and traditional predictors ($p\approx 0.05-1.0$).

Conclusion—Glutamatergic state of bilateral motor and premotor cortices and neuronal state of ipsilesional premotor cortex may be important for predicting motor outcome in the context of a restorative therapy.

Keywords

stroke; glutamatergic and neuronal state; motor and premotor cortices; therapy gain

1. Introduction

Despite remarkable advances in the field of stroke over the past several decades, commonly available rehabilitation therapies for upper extremity (UE) motor function are characterized by only modest efficacy (only 11.6% of the patients demonstrating complete functional recovery at six months post-stroke)¹. Thus, the restoration of UE function continues to be an unfulfilled goal for over seven million persons with stroke in the US². As stroke mortality decreases in parallel with advances in acute stroke care, it is expected that more patients with UE impairment will survive over the next decades. Such impairment poses barriers to independence and community integration and diminishes quality of life³. Given these facts, evidence-based improvements in stroke rehabilitation are of high priority.

More accurate models for predicting the patient's capacity for UE recovery would be helpful in developing and validating novel, effective restorative therapies. Such models would also enable clinicians to optimize rehabilitation, improve stroke care quality, better allocate the rehabilitation resources, and help patients to manage expectations. The current study has been focusing on the chronic phase of stroke (defined here as greater than six months after stroke) in which the extent of brain injury and the alternative cortical networks recruited in both injured (ipsilesional) and un-injured (contralesional) hemispheres to assist the paretic UE movement become established⁴. In this phase of stroke, numerous predictors (i.e., demographic⁵, genetic variation⁶, baseline clinical status⁵, neuropsychological status⁷, and metrics of brain injury^{7, 8}, functional integrity of motor and premotor cortices or cortical resources^{7, 8}) have been tried. Unfortunately, these predictors, either in isolation or in combination, have not provided useful models for health care management or rehabilitation service allocation⁹. A possible explanation for this shortfall is the preferential enrollment of patients with good baseline clinical status, leaving little opportunity for measurable improvement and limiting generalizability. Indeed, to estimate the cortical resources, the patients should have the ability to engage in a behavioral paradigm (task-related functional MRI studies), endure extended testing (resting-state fMRI) or exhibit motor evoked potentials in affected muscle (transcranial magnetic stimulation-TMS). Thus, the cortical resources have not been broadly explored in patients with severe UE paresis and/or limited capacity to extended testing. Proton magnetic resonance spectroscopy (¹H-MRS) is a safe, non-invasive tool that could be administered in these patients. Notably, this tool is uniquely able to measure neural state, by means of biomarkers related to neuronal and glial integrity, inflammation/necrosis, intra-cortical excitability/inhibition, and intracellular metabolism in discrete regions of the human brain¹⁰. Our prior stroke ¹H-MRS studies provide evidences

of functionally-relevant abnormalities in biomarkers specific to neuronal and glial integrity across ipsilesional motor and premotor cortices^{11, 12}. Of direct relevance to the current study, these data demonstrate that ¹H-MRS provides probes of stroke-related remote cortical changes that span on microscopic cellular level of analysis. Thus, ¹H-MRS measures may add, without question, new insights into the pathophysiology of motor recovery after stroke.

The aim of the current study was to investigate for the first time whether pre-therapy neural state of the motor (M1) and premotor (premotor cortex-PM, supplementary motor area-SMA) cortices are predictive of therapy-related motor gain in a subcortical ischemic stroke sample evincing severe to mild UE impairment. Based on the aforementioned neural alterations reported in these areas¹¹ and the findings from fMRI/TMS studies suggesting that functional activation and excitability are critical to recovery potential and improvements with restorative therapies^{7, 8}, the first hypothesis was that altered neuronal-glial interactions and lower intra-cortical excitability (as evidenced by lower levels of a neuronal biomarker, N-acetylaspartate-NAA, higher levels of a glial biomarker, myo-Inositol-mIn, and lower levels of a glutamatergic neurotransmission biomarker, glutamate-glutamine complex or Glx, respectively) within the ipsilesional cortices would be associated with poor motor gain. A different pattern would be expected for the contralesional cortices; since there are no significant neuronal-glial alterations in these areas in chronic stroke¹¹, no correlations would be expected between the levels of neuronal/glial biomarkers and motor gain; based on maladaptive relevance of hyperexcitability in the contralesional hemisphere¹³, an inverse relationship would be expected between the levels of glutamatergic neurotransmission biomarker and motor gain. In addition to the primary hypothesis concerning remote neural state, several traditional predictors of motor recovery after stroke, such as patient's age, time post-injury, infarct volume, and pre-therapy UE clinical impairment, were also examined.

Materials and methods

Stroke survivors' enrolment

Written informed consent in accordance with the Institutional Review Board, University of Kansas Medical Center, was obtained from all participants prior to enrollment.

Note that the stroke survivors studied here were part of a large group who participated in earlier overarching study describing remote neurochemical changes arising from stroke and their functional relevance. The cross-sectional component of this study revealed, as stated above, significant alterations of neuronal-glial interactions in ipsilesional M1, which were related to the proximal and distal clinical motor impairment of the UE¹¹ and to the activation pattern of the same area, as measured by fMRI¹². Similar neuronal-glial alterations were reported in the ipsilesional premotor areas, and these alterations were specifically related to the proximal UE motor impairment¹¹. Ten patients completed the longitudinal (interventional) component of this study and were studied as follows. They were selected if they have had a single symptomatic subcortical ischemic stroke without cortical involvement (confirmed on T2-weighted images – see below) occurring at least six months prior to study enrollment, presented UE motor deficits (UE Fugl-Meyer test¹⁴, FM less than 66), and had adequate comprehension (able to follow simple 3-step commands, e.g. point to the ceiling, floor, and face in this order). Patients were also required to have no visual attention deficits

(Cancellation test), apraxia (clinical observation of the use of scissors to cut paper and making coffee), other neurological or psychiatric diseases, substance/alcohol abuse or dependence, or MRI contraindications. There were no gender or handedness exclusions.

To evaluate the pre-stroke ^1H -MRS and kinematics (see below) metrics, we also included 16 age/sex/academic performance-matched healthy controls with normal T2-weighted images, and with no history of neurological, psychiatric or orthopedic disease, head trauma, or substance abuse.

Study protocol

Pre-therapy assessment of ^1H -MRS metrics was obtained within 24-hours prior therapy – a four-week impairment-oriented motor training. Candidate predictors included biomarkers of neuronal-glial interactions and glutamatergic state. Traditional stroke predictors included brain injury metrics (infarct volume) and pre-therapy demographics/clinical status (age, time post-stroke, UE impairment). UE clinical impairment was again evaluated within 24-hours after motor training. To identify the therapy-related changes in movement pattern, we also investigated kinematics of UE and trunk before and after training. Motor gain (or motor recovery) was defined as positive values of changes in clinical scores and UE kinematics (reflective of improved UE motor control) and negative values of changes in trunk kinematics (decreased behavioral compensation)^{15, 16}.

Pre-therapy brain injury assessment

MRI acquisition: MRI studies were performed on a Siemens 3T Allegra MR system. The details of MR scans have been described previously¹¹. Specifically, two structural data sets were acquired: T₁-weighted (MPRAGE, TR=2300ms, TE=3ms, FOV=240mm, matrix=256x256, resolution=1x1x1mm³) and T2-weighted (TR=4800ms; TE1/TE2=18/106ms; FOV=240mm; matrix=256x256; slice thickness=5mm, no gap) images, and used to confirm lesion location, exclude other pathological conditions, extract brain injury metrics, and estimate the brain tissue volume in spectroscopic voxels.

MRI analysis: The brain injury metric extracted was the infarct volume. The white matter changes (hyperintensities, WMH) were also quantified.

Infarct volume: A stroke mask was created on each patient's MRI by manually tracing each lesion on slice-by-slice basis in the axial view of T2-weighted images in MedINRIA software (Medical Image Navigation and Research Tool by INRIA, Cedex, France). Lesion was defined as tissue with abnormal high signal intensity on each image (Figure 1A) and as subcortical if they are situated for 50% or more at the subcortical level¹⁷. Infarct volume was calculated using MIPAV (<http://mipav.cit.nih.gov/>). Intra-rater reliability was verified by repeated drawing (two times) of three lesions by one investigator (SCC) and the inter-rater reliability by comparing the drawings of two investigators (CMC and SCC) of three lesions.

WMH was assessed on T2-weighted and were rated according to the Fazekas visual rating scale¹⁸. Shortly, this is a scale grading WMH according to severity: 0 - none or a single

punctate lesion, 1 - multiple punctate lesions, 2 - early confluency of lesions, and 3 - large confluent lesions. The scores 0 and 1 are considered normal in the elderly.

Pre-therapy motor and premotor cortices neurochemistry assessment

¹H-MRS acquisition: Immediately after the structural MRI acquisition was completed, ¹H-MRS imaging (¹H-MRSI) was performed. The volume of interest was placed in the frontal and parietal lobes in both hemispheres, including three regions of interest (ROI: M1, PM, and SMA cortices) per hemisphere. ¹H-MRSI was performed with point-resolved spectroscopy sequence with water suppression (PRESS, TR=1500ms, TE=30ms, FOV=160mm, matrix size=16x16, thickness=15mm). Scalp lipid artifact was minimized with eight outer voxel suppression bands. Shimming was performed using field-map based automatic shimming routines followed by manual adjustments, resulting in full width at half maximum of water signals below 20 Hz¹¹. Importantly, this approach did not require the patient's engagement in any behavioral paradigm or the presence of motor evoked potentials in affected muscle, and took about 15 min.

¹H-MRS analysis: Analysis methods for ¹H-MRS data have been detailed previously¹¹. Briefly, LCModel¹⁹ was used to quantify concentrations of three neurochemicals most likely associated with neural plasticity¹⁰: NAA, mIn, and Glx - considered here as candidate predictors. The hand/UE territory was identified in each ROI based on anatomical landmarks: the omega shape or hand knob on the anterior bank of the central sulcus for M1, the anterior half of precentral gyrus adjacent to the hand knob for PM, and about one centimeter rostral to the paracentral sulcus for SMA (<http://neuro.imm.dtu.dk/services/jerne/ninf/voi.html>).

Neurochemicals' concentrations were corrected for partial brain tissue volume within each spectroscopic voxel using in-house software written in Matlab software (2013a Mathworks, Natick, Mass). Brain tissue volume was calculated using T1-weighted images segmented to grey matter, white matter, and cerebrospinal fluid (SPM8, Wellcome Trust Center for Neuroimaging, University College London, London, UK). Neurochemicals with Cramér-Rao lower bounds values less than 15% and voxels with a signal-to-noise ratio greater than 10 were included in the analysis (Figure 1B). The corrected concentration was then converted into molar concentrations (millimoles per kilogram wet weight brain tissue)¹¹. About three spectroscopic voxels were selected in each ROI and mean ($\pm$ SD) values were obtained for each neurochemical per ROI.

Therapy: Impairment-oriented motor training—This motor training was built on our and others prior studies^{15, 20}. Specifically, stroke survivors underwent twelve practice sessions (90 repetitions/day, 3 days/week, a total of 1080 repetitions) over four consecutive weeks. Therapy consisted of repeated reach-to-grasp task with the paretic UE. This task was selected because it is familiar to participants, has a great chance of generalization, and is of high ecological relevance. Further, this task should be re-acquired during recovery from stroke. From the motor control perspective, this task involves the hand control and coordinated motions between the shoulder and elbow motions to reach the target with a very

limited trunk recruitment¹⁵. Yet, after stroke, the contribution of the trunk could be considerable in accomplishment of this action¹⁵ (see below).

Participant was seated at about 15 cm distance in front of a table (Figure 2A). At the initial position, the participant rested the paretic UE on the table. A cylindrical object was placed within a comfortable range for grasping (about 90% arm's length, vertically oriented) on a platform located in front of the patient. Nine cylindrical objects of varying diameters (4, 6, and 8 cm diameter – similar diameters to those of routinely used objects, i.e., can, cup, and jar) and weights (50, 100, and 150 grams) were used. Objects were presented in a random order. The variable practice was chosen based on the large advantages of this type of practice relative to constant practice on performance and retention²¹. In response to an auditory signal, the participant reached and grasped the object with the whole hand and transported it to the initial position at a comfortable self-selected speed. The patient had no UE or trunk restrictions during movement. Visual feedback about elbow extension motion was provided by an electrogoniometer (Exos, Inc., Woburn, MA, USA) placed on the lateral epicondyle and displayed on a computer screen at the end of the task. Mean elbow extension from the healthy control group was presented as a target goal on the same screen. To minimize dependence on feedback, the feedback frequency was decreased throughout the practice session (for the first 30 trials, feedback was provided every trial, for the second 30 trials, every 2nd trial, and for the last 30 trials, every 5th trial). All participants completed the training sessions.

Pre- and post-therapy clinical evaluation—A single clinical assessor rated each patient using the FM, with higher scores reflecting greater function (total score, normal=66). In addition to the FM total score, we also selected FM components specifically dealing with proximal movements, including active shoulder and elbow movements in and out of synergy (proximal subscore, normal=30), and distal movements, including hand and wrist movements (distal subscore, normal=24).

Pre- and post-therapy movement kinematics assessment—The clinical test used here (FM) is mainly focus on task accomplishment and is not qualitatively sensitive enough to discriminate how the task is achieved (see review²²). Kinematic analysis may overcome this limitation²³. As stated above, by using this analysis, we strived to differentiate between true motor recovery - restitution of the original UE function and behavioral compensation - a mechanism to facilitate UE function by compensating for the distal UE weakness^{15, 16}. The latter has been widely studied by us^{15, 16} and mainly includes the use of new degrees of freedom, i.e., trunk, during motor tasks executed with the paretic UE. For example, during reaching, the patients increased the trunk forward displacement to compensate for lack of or diminished active range of elbow extension to bring the hand to the target¹⁶. Here, we assessed the UE and trunk kinematics using a reach-to-grasp task, a similar task to the trained task.

The workstation included a 4cm diameter cylinder placed on a platform located in front of the participant. This cylinder was aligned with the midline of the trunk and located at 90% of extended arm's length and at the participant's shoulder height. At the initial position, the participant had the arm to be trained rested on the table located ipsilaterally (elbow

angle=90deg, measured by means of goniometer). The contralateral arm rested alongside the body. We instructed participants to reach and grasp the cylinder in response to an auditory tone and hold the hand in the final position, until a second tone signaled the end of the trial. There were no restrictions of trunk or UE movements or feedback provided during data recording.

Prior to recording, participants practiced the task five times. Then, 20 movements performed with full vision were recorded by using motion analysis system (VICON, Oxford Metrics). Five reflective balls were positioned on the participant's UE (#1-ulnar process; #2-lateral epicondyle; acromion processes of #3-ipsilateral and #4-contralateral shoulder) and sternum (#5). Each movement was recorded for 3–6 sec at a sampling rate of 100 Hz. The vectors joining the reflective balls #1, #2, and #3 were used to compute the elbow motion and the displacement in the sagittal plane of the #5 was used to define trunk motion. Specifically, the elbow extension (deg) and anterior trunk displacement (cm) were calculated as the differences between the final and the initial positions of the corresponding vectors.

Statistical analysis

The participant characteristics were summarized by frequencies and proportions for qualitative covariates (stroke side, medication) and by means and SD for ¹H-MRS predictors (levels of NAA, mIn and Glx in each ROI), traditional predictors (age, time post-stroke, log-transformed infarct volume), clinical (FM) and kinematics (elbow and trunk motions) metrics.

The distribution of normality for predictors and clinic/kinematic metrics was evaluated using Shapiro-Wilk test. All, but three (log-transformed infarct volume, $p=0.03$, time after stroke, $p=0.001$, trunk motion, $p=0.01$), were normally distributed. T-test (or the Wilcoxon rank sum test if the data were not normally distributed) was employed to assess the reliability of infarct volume and WMH measurements and the changes in the clinical/kinematic metrics from pre- to post-therapy. ANOVA was performed to assess between-group differences in each ¹H-MRS biomarker after adjusting for hemisphere.

Spearman Rank Order correlation test was employed to determine the relationships between predictors (¹H-MRS and traditional) and therapy-related changes in clinical/kinematic metrics ($FM=FM_{\text{post}}-FM_{\text{pre}}$, elbow, trunk). Significant level was considered at $p<0.05$ (SPSS 23.0, SPSS Inc. Chicago, IL).

Results

Participant characteristics

Participant's demographics/medical history/present medical status—Ten chronic (time post-onset, mean $\pm$ SD, 32.9 $\pm$ 7.7months) stroke survivors (8 male - 80% male, age 58.7 $\pm$ 6.8yrs) participated (Table 1). Eight of 10 patients (80%) presented with right hemiparesis. All were premorbid right handed. Patients were not apraxic, cognitively impaired, or clinically depressed, and they were not receiving current inpatient/outpatient treatment or spasticity medication. Patients were on anti-hypertensive (90%), cholesterol-lowering (50%), and/or antiplatelet (90%) therapy.

Control group provided an adequate comparison group with no differences in potential confounding demographic influences: age (58.7 ± 6.8 years in stroke vs. 49.4 ± 14.7 years in controls, $p > 0.05$), sex (80% vs. 75% male) or years of education (12.9 ± 2.2 vs. 14.7 ± 3.2 years, $p > 0.05$).

Pre-therapy UE impairment and movement pattern—Overall, our stroke survivors had moderate UE motor impairment (pre-therapy FM, 35.6 ± 18.6 points). Accordingly to the clinical subdivision suggested by Fugl-Meyer et al.¹⁴, three had mild UE weakness (gross and fine movements with subtle deficits, FM from 57 to 64), six moderate (gross and some fine movements, 23 to 50), and one patient had severe weakness (gross motor function only, 10; Table 1).

While all participants completed the reach component of the task (with or without compensatory trunk use), the severely to moderately impaired patients did not have sufficient motor control to perform the whole action - some grasped the objects from the top while some were not able to transport the objects to the initial position. Thus, the kinematic metrics presented and discussed here are primarily related to the reach component of the task. As expected²³, stroke survivors had significantly lower active range of elbow extension and greater trunk anterior displacement compared to controls ($35.5 \pm 20.0^\circ$ in stroke vs. $57.9 \pm 5.2^\circ$ in controls, $t = -3.5$, $p = 0.006$, and 12.5 ± 12.8 cm vs. 2.9 ± 1.1 cm, $z = -2.1$, $p = 0.03$ respectively).

Pre-therapy MRI findings—Review of T1- and T2-weighted images showed that participants had sustained a single cerebral infarction. No participant had lesions involving the M1, PM, or SMA. The infarct volume varied from 0.5 to 25.3 cm^3 with an average of $8.1 \pm 10.1 \text{ cm}^3$. Both intra- and inter-rater reproducibility of infarct volume measurements were high (Wilcoxon rank sum test, $p < 0.001$ and $p < 0.01$ respectively). Fazekas scores varied between 0 and 1, reflective of no lesion or punctate lesions, which are considered normal in the elderly. Similarly, a high agreement regarding WMH was found between the two evaluators (paired t-test, $p < 0.001$ for both intra- and inter-rater analyses).

Pre-therapy ^1H -MRS findings—Brain tissue volumes were similar between stroke and control groups (Table 2), suggesting that neurochemicals presented here have been corrected for a similar brain tissue volume regardless the group. As we previously shown¹¹, the stroke survivors displayed significantly altered neuronal-glial interactions ipsilesionally (lower levels of NAA in both M1 and SMA, higher levels of mIn in PM), no contralesional neuronal-glial alterations, and no glutamatergic alterations in either ipsi- or contralesional hemisphere (Table 2).

Therapy gain

Stroke survivors showed significant clinical gain after four weeks of training (FM total, $t = -5.1$, $p = 0.000$). Similar to findings reported from studies involving a similar training protocol¹⁵, the magnitude of FM ranged from 1 to 9 points with an overall mean increase of 3.9 ± 2.4 points. A higher number of patients improved the proximal FM subscores (70%) compared to those who improved the distal subscores (40%, Figure 2B). This could be

explained, at least partially, by the feedback provided during the training. Yet, considering the lack of consensus on the best measurements of improvement to be used as definitive outcomes in stroke research and the fact that FM subscores have not been validated before, we used FM total for the analyses below.

Patients also improved the active range of elbow extension (from $35.5 \pm 20.0^\circ$ to $41.5 \pm 17.6^\circ$, $\Delta = 6.1 \pm 4.1^\circ$, $t = -4.7$, $p = 0.001$) and used less trunk (from $12.5 \pm 12.8\text{cm}$ to $8.5 \pm 8.7\text{cm}$, $\Delta = -4.5 \pm 4.9\text{cm}$, $z = -2.29$, $p = 0.02$). All, but one, patients increased the elbow motion and decreased the trunk motion with training.

The correlation coefficients between the $^1\text{H-MRS}$ biomarkers, traditional predictors and motor gain (FM total, elbow, trunk) are presented in Table 3.

Depressed excitability in ipsilesional M1 was associated to poor motor gain

As predicted, the levels of Glx in M1 were positively correlated with both FM total ($r = 0.77$, $p = 0.01$) and elbow ($r = 0.77$, $p = 0.01$; Table 3 and Figure 3A left and central panels). This finding is consistent with the view that the level of the ipsilesional M1 excitability may be related to UE motor recovery^{7, 8, 24}. Importantly, these relationships persisted after controlling for age ($r = 0.79$, $p = 0.007$ and $r = 0.86$, $p = 0.001$ respectively) or time post-stroke ($r = 0.79$, $p = 0.006$ and $r = 0.80$, $p = 0.005$).

Depressed excitability and neuronal metabolism in ipsilesional PM were associated with poor motor gain

Again, as expected, the levels of Glx and NAA in PM were positively correlated with FM total ($r = 0.78$, $p = 0.008$ and $r = 0.69$, $p = 0.002$ respectively; Table 3 and Figure 3A right panel). These data provide further support for the potential role of the ipsilesional PM in UE motor recovery in the context of a restorative therapy (see review²⁵). Notably, age and time post-stroke have no substantial effect on these relationships (age, $r = 0.62$, $p = 0.05$ and $r = 0.76$, $p = 0.01$ respectively; time post-stroke, $r = 0.71$, $p = 0.02$ and $r = 0.82$, $p = 0.003$).

Hyperexcitability in contralesional M1 was associated with poor motor gain

Consistent with the maladaptive relevance of the contralesional M1 changes in excitability¹³, the levels of Glx in M1 were negatively related to trunk ($r = -0.68$, $p = 0.03$, Table 3 and Figure 3B left panel). This suggests that patients with hyperexcitable contralesional M1 increased the use of the compensatory strategies available rather than improving their paretic UE. This relationship persisted after controlling for age ($r = -0.62$, $p = 0.05$) or time post-stroke ($r = -0.65$, $p = 0.04$).

Depressed excitability in contralesional SMA was associated with poor motor gain

Contrary to our expectations, the levels of Glx in contralesional SMA were positively associated with FM ($r = 0.64$, $p = 0.04$, Table 3 and Figure 3B right panel). This finding broadly agrees with the quite selective nature for the contralesional brain regions engaged in stroke recovery. Age had no substantial effect on this relationship ($r = 0.62$, $p = 0.05$) but time post-stroke had ($r = 0.47$, $p = 0.2$).

Others ¹H-MRS biomarkers and traditional predictors did not predict therapy gain

NAA levels in ipsilesional M1, ipsilesional SMA, and contralesional ROIs and mIn levels in all ROIs have non-significant predictive value (p varied between 0.05 to 1.0, Table 3). When each of traditional predictors has been examined, all had relatively low and non-significant predictive value of therapy-related clinical or kinematic change (p varied between 0.05 to 1.0, Table 3).

¹H-MRS biomarkers were not significantly correlated with traditional predictors

Importantly, none of the ¹H-MRS biomarkers had significant relationships with the traditional predictors (Table 4).

Discussion

To our knowledge, this is the first demonstration that pre-therapy ¹H-MRS biomarkers of neural state of the motor and premotor cortices might be worthwhile for predicting therapy-related motor gain in chronic subcortical stroke. Although in a very small sample ($n=10$), the results confirm previously described neuroimaging/neurophysiological studies^{7, 8}, demonstrating the role of these areas in predicting therapy gain after stroke. Specifically, this study revealed four main results. First, consistent with our predictions, the pre-therapy biomarkers of intracortical excitability (Glx) in the ipsilesional M1 and PM and of neuronal metabolic integrity (NAA) in the ipsilesional PM were associated positively with the magnitude of motor gain. Second, as predicted, the biomarkers of intracortical excitability in the contralesional M1 were negatively associated with motor gain. Third, contrary to our predictions, the biomarkers of intracortical excitability in the contralesional SMA were positively related to motor gain. Importantly, no such relationships were detected in other candidate predictors (glial biomarkers) assessed in all areas, indicating that these effects are quite selective in nature. Fourth, the ¹H-MRS biomarkers reported above outperformed the traditional predictors of stroke recovery.

Depressed excitability in ipsilesional M1 and PM was associated with poor motor gain

As predicted, the pre-therapy levels of Glx (glutamate-glutamine) in the ipsilesional M1 and PM were significantly correlated with therapy-related gain (Table 3, Figure 3A). Specifically, participants with lower cortical Glx levels had poorer change in clinical scores/kinematic metrics. Lower Glx levels are thought to be indicative of decreased intracortical excitability²⁶. This finding is in line with the overwhelming evidence from human studies (see review²⁷) demonstrating increased tonic neuronal inhibition ipsilesionally after stroke. For instance, excessive inhibitory drive exercised by contralesional motor areas on the ipsilesional homotopic areas could be responsible for such reduction in ipsilesional excitability²⁸. In line with the results of current study, a series of TMS studies have demonstrated that the increase of the excitability of the ipsilesional M1, directly or by reducing excessive inter-hemispheric inhibition from the contralesional hemisphere, facilitates motor recovery²⁴. This is also consistent with evidence showing that an increase in excitability is required for use-dependent plasticity or motor learning in these patients²⁹. In summary, the lower levels of Glx in these areas are likely to be reflective of decreased

(suppressed) ipsilesional intracortical excitatory activity, which in turn, induces a less suitable environment for use-dependent neural plasticity.

Depression of the neuronal metabolism in ipsilesional PM was associated with poor motor gain

Consistent with our predictions, the level of NAA in the ipsilesional PM had a predictive value (Table 3, Figure 3A). Yet, contrary to our predictions, the level of NAA in the ipsilesional M1 did not reach statistical significance. A possible explanation for this is the small sample size; perhaps a larger sample would reach statistical significance. An alternative explanation could be the enrollment of patients with motor deficits spanning a relatively wide range (70% of participants were moderately to severely impaired and 30% had mild impairment). Specifically, in those with more severe impairment, the M1 output is likely to be damaged while the PM output is expected to have a higher probability to survive, making this area (and its corticospinal/reticulospinal pathways) an alternative cortical substrate in face of the injury to the original output (see review²⁵). The current results of the positive relationship between the levels of NAA in the ipsilesional PM and the therapy gain confirm the potential role of this area in recovery of movement. Specifically, lower levels of NAA might reflect neuronal metabolic dysfunction¹¹. This is in line with previous reports demonstrating morphological and metabolic cell body changes after axonal injury (in our case, at the subcortical level) and/or metabolic abnormalities associated with diaschisis³⁰. Indeed, dysfunctional neurons seem to be associated with less synthesis of brain-derived neurotrophic factor and neurotransmission related-proteins, which in turn, might impact use-dependent plasticity³¹. Thus, it appears that patients with more dysfunctional neurons in the ipsilesional PM would have low reserve to improve behavioral outcome in response to therapy, by having less potential to increase the drive to spared corticospinal or other pathways (see above) to boost of the output projecting to the paretic UE.

Depressed excitability in contralesional M1 was associated with greater motor gain

As hypothesized, the levels of Glx in contralesional M1 were negatively related to the therapy gain (Table 3, Figure 3B). While the ipsilesional M1 is undoubtedly critical for recovery, the role of the contralesional M1 is still inconclusive (see review²⁸). Being spared by ischemic events and based on its role in high-level motor control and possible role in the control of the ipsilateral hand movements, this area could be particularly well suited to contribute to UE performance after stroke²⁸. Evidence from human studies²⁸ demonstrating structural and functional reorganization within contralesional M1 has made this argument even more compelling. Indeed, an abnormally increased excitatory neural activity and activation have been reported in contralesional M1 after stroke. Some investigations contend that these changes have maladaptive relevance¹³. Our finding supports this hypothesis. Specifically, lower intra-cortical excitability in this area is likely to be associated with greater decrease in behavioral compensation that, in turn, could be interpreted as greater motor recovery. Notably, the contralesional M1 biomarkers did not show significant correlations with clinical gain. This is not surprising because, as stated above, clinical testing is often not qualitatively sensitive enough to discriminate between true recovery (adaptive) and compensation (maladaptive strategy at the behavioral level)²². Thus, the trunk metrics

reported here allowed us not only to understand the movement strategy employed by patient to improve its function but also to differentiate between adaptive vs. maladaptive plasticity at the neural level (i.e., contralesional M1).

Depressed excitability in contralesional SMA was associated with poor motor gain

Contrary to our expectations, the level of Glx in the contralesional SMA was positively correlated with clinical gain (Table 3, Figure 3B), suggesting an adaptive role in mediating motor recovery, at least as ¹H-MRS metrics could discern. These findings broadly emphasize that the reorganizational changes in contralesional hemisphere are quite selective in nature for the brain regions engaged in stroke recovery. Further, the effects of the time post-stroke on the role of this area in stroke recovery corroborates well with prior animal studies showing a temporal disparity between neurophysiological/neuroanatomical changes in SMA and behavioral changes during spontaneous recovery³². Precisely, this result further supports the possible critical engagement of this area in recovery processes during the late phases of stroke. Knowing how and when a specific cortical resource (i.e., M1, PM, SMA) contributes to recovery after stroke is crucial to tailor new interventions, i.e., non-invasive brain stimulation, and more studies are needed to clarify this.

Taken together, these findings suggest that training-induced motor gain is related to the level of excitatory activity measured pre-therapy in two ipsilesional and two contralesional cortical areas and to the neuronal integrity in one ipsilesional area. As stated above, these areas may serve as potential anatomic substrate for this process. We therefore speculate that ¹H-MRS biomarkers could be indicators of brain tissue state that reflects the cerebral resource. Indeed, without a sufficient cerebral resource, the therapy may not help. We may speculate that there might be a critical molecular/cellular level of the cerebral reserve related to the ability of the patient to respond to a motor therapy. This critical level might be defined by a bimodal distribution of the excitability state in both ipsilesional and contralesional motor/premotor cortices and neuronal state of ipsilesional premotor cortex, but studies in larger populations are necessary for the development of this concept. The notion of a critical level being related to response to a therapy may have prognostic value: patients falling below this level (less excitable/more dysfunctional neurons) may be non-responders while patients above this level may retain the ability to respond to a therapy. Therefore, such biomarker could serve for patient's selection in stroke recovery/rehabilitation trials. Further, identification of the remaining cerebral resources that could support the paretic UE function may become essential in development of targeted, individualized therapies, i.e., non-invasive brain stimulation. As the restorative therapies proceed after stroke, such biomarkers could be also used to reveal the progress of therapy and to guide quantitative therapy choices: pushing the potential for gains by continuing a therapy until no further neurochemical changes in regions of interest occur, ensuring that the reorganizational potential of these regions has peaked, at least as ¹H-MRS can discern. However, we recommend caution in interpreting these results until more work is completed. If evidence continues to mount that ¹H-MRS metrics add value to stroke prediction, support will be also needed to insure MR Spectroscopy is accessible in a standardized fashion across hospitals.

It is important to note that the predictive value of ^1H -MRS biomarkers was selective to Glx and NAA and not evident for mIn (Table 3). This is consistent with the hypothesis that after years and even decades after injury, there is a low probability to find gliosis/demyelination or inflammation processes in these territories³³. Alternatively, it may be that glial marker (in isolation) is not sensitive enough to provide information about the remaining cerebral resources needed for the therapy to help.

Certain ^1H -MRS predictors outperformed traditional predictors of stroke recovery

Certain ^1H -MRS biomarkers outperformed the traditional predictors of therapy-related behavioral gains after stroke such as age, time post-stroke, pre-therapy clinical status, or infarct volume (see Table 3). The current results regarding the age of patient, time post-stroke, and infarct volume are largely consistent with prior studies (see review⁵) reporting inconclusive evidence of association between these variables and motor recovery. Our current results regarding the pre-therapy clinical impairment (as measured by FM total) are in contrast with a recent meta-analysis, reporting the important value of baseline clinical assessments for response to restorative therapies⁵. This could be explained by the low predictive value of the baseline clinical impairment in those with more severe hemiparesis³⁴. Specifically, in a population with a baseline clinical severity (FM=32.5±25.3points) similar to our sample (FM=35.6±18.6points), the clinical impairment at 24 to 72 hours post-infarct was a strong predictor of recovery at three to six months post-infarct when those with severe impairment were removed³⁴. Overall, our results confirm prior studies^{5, 8} showing that traditional predictors alone have minimal to moderate association with behavioral gains from restorative therapies in chronic patients. We therefore advocate that the measures of the microscopic cellular level of spared motor/premotor cortices - a level which is not detected by the currently in use imaging or clinical approaches - may improve prediction models by providing information about the remaining cerebral resources required to boost activity associated with recovering UE movement in response to a restorative therapy.

Limitations and strengths

This study is limited by small sample size. However, one strength of this study was the use of restrictive inclusion criteria and a controlled motor learning paradigm to reduce heterogeneity, so the current findings are relevant to this particular stroke type and location, as well as for this type of training. All patients were able to communicate and understand simple commands, limiting the generalizability to those with severe receptive aphasia or cognitive impairment. Given that the patients were in the chronic stage of stroke, the current results might not be applicable to acute or sub-acute stages. The meaningful clinical improvement (which would be >4 points on the FM scale) associated with the motor training paradigm used in the current study was not discussed here, as the study was not designed to evaluate the overall behavioral effects of this training. We did not record whether the study participants have gone through similar therapeutic interventions prior to starting participation in this study. Thus, we cannot rule out that their capacity for improvement has been already achieved prior to participation. However, it is not surprisingly that some patients improved more than others - not all stroke survivors retain the capacity to respond to therapy. During the kinematic assessment, the forearm rotation may contribute to elbow extension calculation. This possible elbow extension error has not been evaluated here. The

pre-stroke hand dominance has been reported to impact motor recovery³⁵ and should be also taken into account in future studies. As stated above, a multimodal approach, i.e., including metrics of neural injury and cerebral resources, is likely to be the best approach to predict behavioral gains from a restorative therapies in chronic stroke^{7, 8}. Unfortunately, the sample size in the current study was insufficient to investigate such combinations.

Conclusions

The preliminary data of the current study suggest that ¹H-MRS measures of structurally intact motor and premotor areas in both ipsi- and contralesional hemispheres might be useful to predict behavioral gains from a restorative therapy in chronic stroke. Further study to confirm whether these findings may be generalized to the stroke survivor population as a whole in a larger sample is warranted. Although kinematic analysis is time-consuming, we also advocate that when a therapy appears to enhance recovery, the use of a combined movement kinematics-neuroimaging approach may facilitate distinctions between recovery and compensation at both neural and behavioral levels.

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A. T1-weighted MRI - infarct mask **B.** LCModel output **Figure 1.**

(**A**) Manual tracing of brain lesion in a patient with infarct in middle cerebral artery, striato-capsular distribution (MedINRIA). R, right, L, left. (**B**) LCModel output from one spectroscopic voxel in the UE territory in ipsilesional M1. The output consisted in the acquired ^1H -MRS data, estimated spectrum, and underlying baseline. The acquired ^1H -MRS data show distinct peaks corresponding to N-acetylaspartate (NAA, at 2.02 ppm), glutamate-glutamine (Glx, 2.05–2.50 ppm), and myo-Inositol (mIn, 3.56 ppm) and a signal-to-noise ratio (S/N) of 12. Cramér-Rao lower bounds (CRB) were less than 15 for all neurochemicals in this voxel. ppm, parts per million.

A. Impairment-oriented training experimental set-up

B. Fugl-Meyer subscores prior to and immediately after therapy

Figure 2.

(A) Impairment-oriented training consisted of repeated reach-to-grasp task with the paretic UE (1080 repetitions over four weeks) focusing the participant's attention on a specific component of motor impairment, i.e., decreased elbow extension. Seated participant had the paretic arm rested on the table placed in front of the body. The contralateral arm rested alongside the body. The seat height was adjusted according to the participant's lower leg length so the feet could rest comfortably on the floor and the table height was set to coincide with the mid-point between the inferior margin of the sternum and the umbilicus. The participant reached and grasped an object, placed on a platform located in front and at shoulder height and on median line, with the whole hand and transported it to the initial position at a comfortable self-selected speed. The participant had no arm or trunk restrictions during movement. Visual feedback about their elbow extension (reported to a "healthy" movement for patients) was provided with faded frequency throughout the practice session. (B) Individual proximal and distal FM subscores before (black symbols) and after (white symbols) therapy in stroke survivors. Healthy values for FM proximal is 30 and for distal is 24.

Figure 3.

Correlations (Spearman coefficients- r , p -values) between ¹H-MRS biomarkers (N-acetylaspartate-NAA, black symbols, glutamate-glutamine-Glx, grey symbols) in ipsilesional (primary motor cortex-M1, premotor cortex-PM, **A**) and contralateral (M1, supplementary motor area-SMA, **B**) cortices and therapy-related changes in clinical scores (FM, points) and kinematic metrics (Elbow extension, deg; Trunk, cm); mM/kg, millimol/kilogram.

Table 1

Demographic and clinical data in stroke survivors.

Age /Sex	Time post-onset	Site of lesion	Pre-therapy Movement Pattern		
			FM	Elbow extension	Trunk displacement
57/M	6	L MCA territory, corona radiata	64	60.5	2.1
48/F	11	L MCA territory, striato-capsular distribution	61	63.3	3.3
61/F	27	L MCA territory, striato-capsular distribution	57	60.2	2.2
45/M	27	R MCA territory, striato-capsular distribution	36	41.5	6.2
63/F	48	L BA territory, pons	31	25.5	12.7
61/M	15	L BA territory, pons	25	28.7	14.5
59/M	144	R MCA territory, Basal ganglia	25	32.9	3.6
58/M	27	L MCA territory, striato-capsular distribution	24	17.1	24.3
63/M	16	L MCA territory, striato-capsular distribution	23	15.1	13.1
61/M	24	L MCA territory, striato-capsular distribution	10	9.9	43.1

Age, years; Time post-onset, months; FM, Fugl-Meyer test (maximal score=66); Elbow extension, degree; Trunk displacement, cm; M, male, F, female; L, left, R, right; MCA, middle cerebral artery; BA, basilar artery

Table 2

Between-group comparisons of ^1H -MRS biomarkers (millimoles/kilogram, mean (SD)) and brain tissue composition (%) for each region of interest.

	NAA	mIn	Glx	Brain tissue
Left vs. Ipsilesional hemisphere				
M1				
Healthy	11.2 (1.7)	4.9 (0.8)	12.4 (2.5)	89.4 (6.5)
Stroke	9.7 (0.9)	5.5 (0.9)	10.9 (2.2)	88.8 (6.0)
$F_{1,26}$ p-value	5.9, 0.02*	2.6, 0.1	2.1, 0.1	0.06, 0.8
PM				
Healthy	11.9 (2.2)	5.4 (0.9)	13.5 (3.8)	89.3 (8.0)
Stroke	10.8 (1.8)	6.4 (1.4)	12.5 (2.5)	86.3 (7.5)
$F_{1,26}$ p-value	1.8, 0.2	4.5, 0.04*	0.3, 0.6	0.9, 0.3
SMA				
Healthy	12.5 (2.1)	6.7 (1.6)	14.7 (2.3)	77.6 (4.3)
Stroke	10.6 (1.5)	6.6 (1.4)	15.2 (3.5)	80.6 (3.9)
$F_{1,26}$ p-value	5.6, 0.03*	0.02, 0.9	0.2, 0.6	3.1, 0.09
Right vs. Contralesional hemisphere				
M1				
Healthy	11.6 (2.0)	5.1 (1.2)	11.2 (2.6)	86.2 (4.9)
Stroke	10.5 (1.1)	5.4 (0.9)	11.7 (2.6)	84.0 (8.4)
$F_{1,26}$ p-value	2.4, 0.1	0.5, 0.5	0.3, 0.6	0.7, 0.4
PM				
Healthy	12.2 (2.0)	5.8 (1.1)	12.8 (2.7)	84.9 (6.4)
Stroke	11.7 (1.8)	6.0 (1.5)	14.3 (2.9)	85.6 (10.1)
$F_{1,26}$ p-value	0.4, 0.5	0.2, 0.7	1.7, 0.2	0.05, 0.8
SMA				
Healthy	11.1 (2.2)	6.2 (1.5)	13.2 (3.0)	85.1 (5.8)
Stroke	10.6 (1.2)	6.3 (1.2)	14.0 (3.0)	80.5 (5.4)
$F_{1,26}$ p-value	0.5, 0.5	0.04, 0.8	0.5, 0.5	4.1, 0.06

* Significant difference; M1, primary motor cortex; PM, premotor area; SMA, supplementary motor area; NAA, N-acetylaspartate, Glx, glutamate-glutamine, mIn, myo-Inositol.

Table 3

Bivariate correlation (r/p =value) between ^1H -MRS biomarkers, traditional predictors, and the therapy-related change () in FM, elbow extension, and trunk anterior displacement.

Predictors	FM	Elbow	Trunk
^1H-MRS biomarkers			
Ipsilesional hemisphere			
M1			
NAA	0.49/0.2	0.26/0.5	0.16/0.6
Glx	0.77/0.01 *	0.77/0.01 *	-0.33/0.3
mIn	-0.42/0.2	0.06/0.9	-0.33/0.3
PM			
NAA	0.69/0.02 *	0.33/0.3	0.21/0.5
Glx	0.78/0.008 *	0.39/0.2	0.05/0.9
mIn	0.14/0.7	-0.17/0.6	-0.12/0.7
SMA			
NAA	0.13/0.7	0.15/0.7	-0.35/0.3
Glx	0.27/0.4	0.43/0.2	-0.60/0.05
mIn	0.16/0.7	0.29/0.4	-0.58/0.1
Contralesional hemisphere			
M1			
NAA	0.23/0.5	0.35/0.3	-0.43/0.2
Glx	0.17/0.6	0.33/0.3	-0.68/0.03 *
mIn	-0.42/0.2	0.01/1.0	-0.47/0.2
PM			
NAA	0.48/0.1	0.19/0.6	-0.21/0.5
Glx	0.23/0.5	0.18/0.6	-0.56/0.1
mIn	0.15/0.7	0.01/1.0	-0.42/0.2
SMA			
NAA	-0.14/0.7	-0.12/0.7	0.37/0.3
Glx	0.64/0.04 *	0.48/0.2	0.10/0.8
mIn	0.43/0.2	0.47/0.2	-0.01/1.0
Traditional predictors			
Age	0.61/0.06	0.17/0.6	-0.03/0.9
Time post-stroke	0.32/0.4	0.40/0.2	-0.11/0.8
Infarct volume	-0.55/0.09	-0.63/0.05	-0.05/0.9
Pre-therapy FM	-0.29/0.4	-0.55/0.09	0.60/0.07

* significant correlation; M1, primary motor cortex; PM, premotor area; SMA, supplementary motor area; NAA, N-acetylaspartate, Glx, glutamate-glutamine, mIn, myo-Inositol; FM, Fugl-Meyer test

Table 4

Bivariate correlations (r/p-value) between traditional predictors and ¹H-MRS biomarkers (Glx/NAA).

¹ H-MRS biomarkers	Age	Time post-stroke	Pre-therapy FM	Infarct volume
Ipsilesional hemisphere				
M1				
NAA	-0.34/0.4	-0.27/0.4	0.19/0.6	0.01/1.0
Glx	0.39/0.3	0.35/0.3	-0.54/0.1	-0.29/0.4
PM				
NAA	0.37/0.3	-0.02/1.0	-0.22/0.5	-0.33/0.3
Glx	0.64/0.06	0.29/0.4	-0.49/0.1	-0.16/0.7
SMA				
NAA	-0.05/0.9	-0.39/0.3	0.24/0.5	-0.17/0.6
Glx	0.01/1.0	-0.01/1.0	-0.21/0.6	-0.27/0.4
Contralesional hemisphere				
M1				
NAA	0.38/0.3	-0.07/0.8	-0.22/0.5	-0.33/0.3
Glx	0.33/0.3	0.02/1.0	-0.49/0.1	-0.16/0.7
PM				
NAA	0.55/0.09	-0.44/0.2	-0.06/0.9	0.11/0.7
Glx	0.32/0.4	-0.22/0.5	-0.21/0.5	0.03/0.9
SMA				
NAA	-0.05/0.9	-0.39/0.3	0.24/0.5	-0.17/0.6
Glx	0.0/1.0	-0.01/1.0	-0.21/0.6	-0.27/0.4

M1, primary motor cortex; PM, premotor area; SMA, supplementary motor area; NAA, N-acetylaspartate, Glx, glutamate-glutamine; FM, Fugl-Meyer test