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Predictors and Biomarkers of Treatment Gains in a Clinical Stroke Trial Targeting the Lower Extremity

Erin Burke, BS; Bruce H. Dobkin, MD; Elizabeth A. Noser, MD; Lori A. Enney, BS;
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Background and Purpose—Behavioral measures are often used to distinguish subgroups of patients with stroke (eg, to predict treatment gains, stratify clinical trial enrollees, or select rehabilitation therapy). In studies of the upper extremity, measures of brain function using functional magnetic resonance imaging (fMRI) have also been found useful, but this approach has not been examined for the lower extremity. The current study hypothesized that an fMRI-based measure of cortical function would significantly improve prediction of treatment-induced lower extremity behavioral gains. Biomarkers of treatment gains were also explored.

Methods—Patients with hemiparesis 1 to 12 months after stroke were enrolled in a double-blind, placebo-controlled, randomized clinical trial of ropinirole+physical therapy versus placebo+physical therapy, results of which have previously been reported (NCT00221390).¹⁵ Primary end point was change in gait velocity. Enrollees underwent baseline multimodal assessment that included 19 measures spanning 5 assessment categories (medical history, impairment, disability, brain injury, and brain function), and also underwent reassessment 3 weeks after end of therapy.

Results—In bivariate analysis, 8 baseline measures belonging to 4 categories (medical history, impairment, disability, and brain function) significantly predicted change in gait velocity. Prediction was strongest, however, using a multivariate model containing 2 measures (leg Fugl–Meyer score and fMRI activation volume within ipsilesional foot sensorimotor cortex). Increased activation volume within bilateral foot primary sensorimotor cortex correlated positively with treatment-induced leg motor gains.

Conclusions—A multimodal model incorporating behavioral and fMRI measures best predicted treatment-induced changes in gait velocity in a clinical trial setting. Results also suggest potential use of fMRI measures as biomarkers of treatment gains. (*Stroke*. 2014;45:00-00.)

Key Words: biomarkers ■ neuroimaging ■ neuroplasticity ■ stroke

Difficulty with lower extremity control is a major contributor to poststroke disability, with one third to two thirds of survivors having gait difficulties.^{1,2} Several restorative therapies have the potential to improve behavioral outcomes after stroke.³ However, the heterogeneous nature of stroke is an obstacle to effective clinical implementation. For example, differences in age, injury, and behavioral deficits can each increase intersubject variability in treatment response.

Improved methods are needed to identify distinct patient subgroups, such as those who will respond to a poststroke therapy from those who will not. Numerous measures have been used to predict poststroke outcomes, such as gait, particularly behavior and other clinical measures.^{4,5} Studies of the upper extremity have emphasized the use of a multimodal approach, whereby different forms of assessment, including

measures of cortical function or neurophysiology, are combined to best predict treatment response.^{6,7} To date, this approach has not been examined for the lower extremity. The main goal of the current study was to examine a multimodal set of measures, including measures of cortical function, to identify the best approach for predicting response to treatment targeting the lower extremity in the setting of chronic stroke. A better understanding of predictors could inform clinical trial design (eg, in relation to entry criteria or patient stratification).⁸

A secondary study goal was to explore potential biomarkers of treatment effect. The molecular and cellular events underlying treatment-induced behavioral gains are difficult to measure directly in humans, but methods, such as functional magnetic resonance imaging (fMRI), can provide insights, albeit indirectly, into treatment effects.⁹ A core

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feature of a valid biomarker is that it changes in parallel with treatment-induced behavioral gains. Several studies have described changes in cortical activation that correlate with motor gains from therapies targeting upper extremity function.^{10–14} Limited data exist, however, in relation to treatments targeting the lower extremity after stroke.

These issues were examined in the setting of a clinical trial (NCT00221390).¹⁵ Enrollees underwent multimodal evaluation at baseline that included 19 measures spanning 5 assessment categories (medical history, impairment, disability, brain injury, and brain function), and gait velocity was assessed at 3 weeks after therapy. The primary hypothesis of the current study was that an fMRI-based measure of cortical function would be an independent, significant predictor of change in gait velocity across the period of therapy, as has been described in therapeutic studies targeting the upper extremity.^{6,7}

Methods

Study Overview and Subjects

The clinical trial was a randomized, double-blind, placebo-controlled study that compared 9 weeks of ropinirole+physical therapy versus placebo+physical therapy in patients with chronic stroke. The primary end point was change in gait velocity from baseline to week 12, 3 weeks after end of therapy (Figure 1). Secondary end points included 2 measures of impairment related to the leg: change in leg Fugl–Meyer (FM) score and in gait endurance. Entry criteria included ischemic or hemorrhagic stroke 1 to 12 months before, age 18 to 80 years, and motor deficits that were neither mild (arm+leg FM motor score <84 of 100) nor severe (FM score >22). Exclusion criteria included gait difficulty that was neither mild (gait velocity >1 m/s) or severe (Functional Independence Measure ambulation score, <3). Patients took escalating doses of ropinirole versus placebo once daily for 9 weeks; starting at week 5, each also received 90 minutes of standardized physical therapy twice per week. Full clinical trial details have been published.¹⁵

Assessments

A battery of behavioral assessments was performed at baseline (Table 1), some of which (gait velocity, leg FM scale, gait endurance, Hamilton Depression score, Barthel Index, and Stroke Impact Scale 16) were repeated at the week-12 visit.

MRI Data Acquisition

Subjects without contraindication to MRI were scanned twice: (1) at baseline, before the first dose of drug and (2) at the post-therapy visit that occurred in week 12. Subjects were positioned in the scanner (1.5 T), knees flexed atop a pillow, with bilateral MRI-compatible ankle splints that went from tibia-to-toes and restricted the ankle to 10° dorsiflexion/plantarflexion while preventing lateral leg rotation. Scanning included a T1-weighted anatomic scan plus fMRI scan during which subjects executed 0.25-Hz ankle dorsiflexion/plantarflexion. Additional MRI acquisition details appear in the Methods in the online-only Data Supplement.

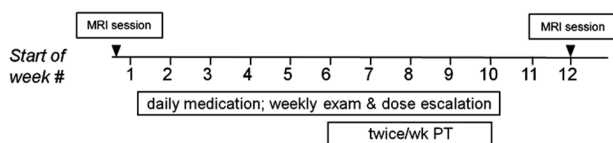


Figure 1. Study time course. After baseline assessments, subjects received 9 weeks of daily study medication (ropinirole or placebo), with 4 weeks of twice per week physical therapy (PT) added on week 5. Three weeks after end of therapy, magnetic resonance imaging (MRI) and final exams were performed.

Table 1. Baseline Assessments

	Baseline Value	Change From Baseline to Week 12
Medical history		
Age, y	61±14 (32–89)	...
Sex	23/10	...
Time after stroke, d	212±104 (71–437)	...
History of hypertension	21 (64%)	...
History of hyperlipidemia	24 (73%)	...
History of diabetes mellitus	5 (15%)	...
Impairment		
Gait velocity, m/s	0.52±0.33 (0.03–1.3)	0.22±0.21*
Gait endurance (number meters ≥6 min)	137±92 (8–284)	55±60*
Leg FM score	22±5 (14–34)	1.9±3.3*
Arm FM score	29±17 (7–61)	...
FIM ambulation score	5.3±1.2 (2–7)	...
Disability		
Modified Rankin score	0.18±0.46 (0–2)	...
Hamilton depression score	6±4	–0.4±4.8
Barthel Index	81±18	3.4±8.8*
SIS-16	57±10	5.8±8.2*
Brain injury		
Infarct volume, mL	34±61 (0.13–281)	...
% Corticospinal tract injury	49±39 (0–100)	...
Brain function		
Activation volume, ipsilesional foot primary sensorimotor cortex	333±347	54±239
Activation volume, contralesional foot primary sensorimotor cortex	212±193	46±232
Activation magnitude, ipsilesional foot primary sensorimotor cortex	0.18±0.32	0.07±0.23
Activation magnitude, contralesional foot primary sensorimotor cortex	0.15±0.29	0.12±0.23

FM indicates Fugl–Meyer; FIM, functional independence measure; and SIS, Stroke Impact Scale.

*Change over time was significant ($P<0.04$ to $P<0.0001$); change in contralesional activation magnitude showed a trend ($P=0.056$). Values are mean±SD (range); activation volume=8mm³ voxels; activation magnitude=task-related percentage signal change.

Image Processing and Analysis

The fMRI images were analyzed using SPM2. For each subject, the first 2 volumes were removed because of tissue nonsaturation. Remaining images were realigned, coregistered to the volumetric scan, spatially normalized, transformed into Montreal Neurological Institute (MNI) stereotaxic space, and spatially smoothed (full width at half maximum=8 mm). Images at rest were contrasted with images during attempted foot movement, using measures of head motion as covariates, to create a contrast image for each subject. Scans with excess head movement were discarded.

Two regions of interest were drawn in MNI stereotaxic space, representing the foot area of primary sensorimotor cortex in the right and left hemispheres (Figure 1D of Cramer et al¹⁶). Two measures of brain function were extracted from each subject's fMRI activation map: activation volume, determined on each brain side at threshold $Z=3$ (approximately $P<0.001$ uncorrected for multiple comparisons,

and activation magnitude, determined on each brain side as the task-related signal change using MarsBaR. These calculations were performed twice for each subject (on the baseline and on the week-12 fMRI scan).

In addition to fMRI, imaging analyses also included 2 measures of brain injury: infarct volume and percentage injury to the corticospinal tract. Infarct volume was calculated by outlining by hand each subject's infarct on the T1-weighted MRI. Corticospinal tract injury was evaluated as the amount of overlap between each subject's infarct with an M1 corticospinal tract in MNI stereotaxic space derived from diffusion tensor imaging tractography in healthy control subjects (additional MRI imaging analysis details appear in the Methods in the online-only Data Supplement).

Statistics

Statistical analyses used JMP-8 software (SAS Institute, Cary, NC) were 2-tailed and used $\alpha=0.05$. Normally distributed data, and data that could be transformed to a normal distribution, were analyzed using parametric statistics, otherwise nonparametric statistics were

Table 2. Bivariate Predictors of Change in Gait Velocity From Baseline to Week 12

Predictive Variable	n	r	P Value
Medical history			
Age	33	0.04	0.84
Time after stroke	33	-0.52	0.002*
No. of outside physical therapy sessions	33	0.12	0.49
No. of all outside therapy sessions	33	0.34	0.054
Impairment			
Gait velocity	33	0.27	0.13
Gait endurance	33	0.46	0.007*
Leg FM score	33	0.46	0.007*
Arm FM score	33	0.43	0.01*
FIM ambulation score	33	0.47	0.006*
Disability			
Modified Rankin score	33	0.05	0.80
Hamilton depression score	33	0.15	0.40
Barthel Index	33	0.51	0.003*
SIS-16 score	33	0.36	0.042*
Brain injury			
Infarct volume	24	0.02	0.92
% corticospinal tract injury	24	-0.06	0.80
Brain function			
Activation volume, ipsilesional foot primary sensorimotor cortex	20	0.54	0.01*
Activation volume, contralesional foot primary sensorimotor cortex	20	0.34	0.15
Activation magnitude, ipsilesional foot primary sensorimotor cortex	20	0.34	0.15
Activation magnitude, contralesional foot primary sensorimotor cortex	20	0.34	0.15

Correlation between 19 independent variables measured at baseline and the primary study outcome measure, change in gait velocity. For normally distributed variables, r is the Pearson correlation coefficient; for non-normally distributed, r is Spearman's ρ . FM indicates Fugl-Meyer; FIM, functional independence measure; and SIS, Stroke Impact Scale.

*Indicates $P<0.05$.

used. The clinical trial found that the 2 treatment groups showed no significant differences in change for the primary or secondary behavioral end points or in the time \times group interaction term,¹⁵ and so the 2 arms were combined for current analyses.

Primary analyses examined predictors of behavioral gains. The dependent variable was the clinical trial's primary outcome measure, change in gait velocity. First, 19 baseline measures from 5 categories (medical history, impairment, disability, brain injury, and brain function) were screened as predictors in bivariate analyses. Next, those baseline measures with $P<0.10$ in bivariate analyses were advanced into a forward stepwise multivariate model ($P<0.1$ to enter; $P<0.15$ to leave). Because of high collinearity among baseline impairment measures, only the one showing the strongest correlation with change in gait velocity (leg FM) was entered into the model; the same was true for disability measures (where Barthel Index showed the strongest correlation and was entered into the model).

Separate, exploratory analyses assessed the performance of 4 fMRI-based measures as biomarkers of behavioral gains. These 4 were change in ipsilesional and contralesional activation volume and change in ipsilesional and contralesional activation magnitude. Performance of each biomarker candidate was evaluated based on the strength of its correlation with behavioral change during the same period. Behavioral change was examined with each measure of leg impairment (gait velocity, gait endurance, and leg FM score) in this exploratory analysis, using $\alpha=0.0042$ based on a Bonferroni correction for 12 comparisons.

Results

Subjects and Clinical Trial Overview

A total of 33 subjects were enrolled (Table 1). All subjects had complete data except for MRI measures: MRI was not performed at the University of Texas (4 subjects) and could not be completed (eg, because of claustrophobia) in 5 subjects at baseline and 7 at week 12. In addition, 4 baseline and 3 week-12 fMRI scans were excluded because of excess head movement. This left 24 anatomic MRI and 20 fMRI scans at baseline plus 22 anatomic and 19 fMRI scans at week-12. During fMRI scanning, all subjects attempted movement as requested.

Enrollees on average were 7 months after stroke, had moderate impairment, and moderate size infarcts. Ipsilesional activation was greater than contralesional activation within foot primary sensorimotor cortex, in both volume and magnitude. Gait velocity, and most of the secondary behavioral measures, showed significant improvement from baseline to week 12 (Table 1).

Predicting Behavioral Gains

In bivariate analyses, 8 of the 19 baseline measures were found to predict change in gait velocity from baseline to week 12 (Table 2). These predictors included assessments from 4 categories (medical history, impairment, disability, and brain function) but not from the fifth category (brain injury). Data for one of these measures, activation volume within ipsilesional foot primary sensorimotor cortex, are presented in Figure 2.

These predictors were entered into a forward stepwise multivariate model. The final model found that change in gait velocity was predicted ($r^2=0.63$; $P=0.0002$) by 2 baseline measures: one based on behavior (leg FM score, $P=0.002$) and one based on brain function (fMRI activation volume within ipsilesional foot primary sensorimotor cortex, $P=0.03$).

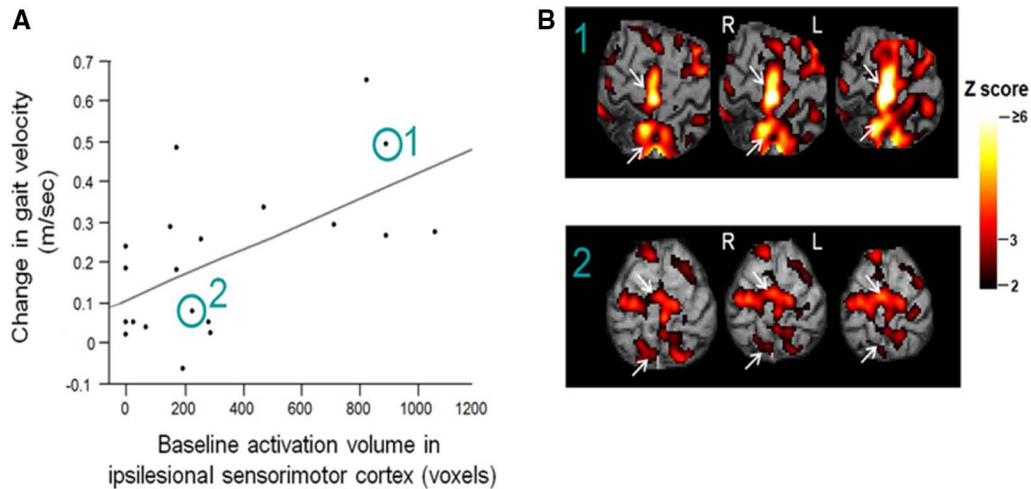


Figure 2. **A**, Activation volume in ipsilesional foot primary sensorimotor cortex at baseline predicts treatment-related gains in gait velocity ($r=0.54$; $P=0.01$). Activation volume=8 mm³ voxels, gait velocity=m/s. **B**, The functional magnetic resonance imaging images (slices at Montreal Neurological Institute (MNI) $Z=+62$, $+64$, and $+66$) from 2 representative subjects, both with stroke in right hemisphere. White arrows bracket ipsilesional foot primary sensorimotor cortex.

Performance of fMRI Measures as Biomarkers of Behavioral Gains

The evolution of 4 fMRI measures from baseline to week 12 was evaluated in relation to behavioral gains during the same time interval. Although on average none of these fMRI changes were significant (Table 1), 2 correlated with change in behavior: change in ipsilesional activation volume correlated with change in leg FM score ($P=0.04$), and change in contralesional activation volume correlated with change in leg FM score ($P=0.0043$), as well as change in gait endurance ($P=0.05$). Each showed an increase in activation volume over time that correlated positively with extent of motor improvement, but did not survive formal Bonferroni correction ($\alpha=0.0042$). Change in activation magnitude did not correlate with change in any behavioral measure. For no fMRI measure did change over time correlate with change in gait velocity.

Correlates of fMRI Brain Activation

To aid interpretation of these results, behavioral-fMRI correlations were examined at baseline and at week 12. At baseline, none of the 3 measures of leg impairment (gait velocity, gait endurance, and leg FM score) correlated ($P>0.05$) with any fMRI measure. At week 12, however, all 3 behavioral measures showed a significant positive relationship with ipsilesional and with contralesional activation volume. Specifically, week-12 gait velocity correlated with ipsilesional ($\rho=0.55$; $P=0.02$) and contralesional ($\rho=0.63$; $P=0.004$) activation volume, as did gait endurance ($\rho=0.48$; $P=0.04$ and $\rho=0.59$; $P=0.01$, respectively), and leg FM score ($\rho=0.66$, $P=0.002$ and $\rho=0.46$, $P=0.046$, respectively). No week-12 behavioral measure correlated with activation magnitude, on either brain side.

Discussion

There is wide variability in the degree of benefit that patients with stroke derive from therapy. Predictors and biomarkers of treatment effect might, therefore, be useful to define therapy plans for individual patients. Many different measures have

been found to predict treatment gains; however, few studies have examined multiple predictors in parallel. This suggests the need for a multimodal approach that directly compares multiple measures. Such an approach has been found useful for predicting gains from therapy targeting the upper extremity^{6,7} but has not been examined for the lower extremity, for which the optimal approach to predicting outcomes may be different given fundamental differences in neural organization.^{17,18} Of 19 candidate predictor measures spanning 5 assessment categories examined, measures of impairment and brain function best predicted change in gait velocity. This study also explored the use of lower extremity-based fMRI measures as biomarkers of treatment effect and found that behavioral gains may be related to activation volume increases in both hemispheres. These findings might inform design of studies of restorative therapies targeting the lower limb after stroke.

Change in gait velocity across treatment was best predicted by a multimodal model that incorporated baseline measures of cortical function (greater ipsilesional foot sensorimotor cortex fMRI activation volume) and behavior (less lower extremity impairment). Consistent with previous reports,^{19–22} bivariate analyses found that many different types of baseline measures significantly predicted treatment gains (Table 2), including measures of medical history, impairment, disability, and brain function. However, previous studies predicting lower extremity treatment gains did not include an fMRI measure of cortical function. When such a measure was added to the multivariate model, it emerged as an independent and significant predictor, a result concordant with previous studies that measured cortical function using evoked potentials.^{23,24} The finding that an fMRI measure of cortical function combined with a measure of impairment best predicts treatment gains precisely echoes the findings of 1 previous therapeutic study that targeted upper extremity,⁷ but not a second study,⁶ the latter possibly reflecting the use of different injury measures or testing procedures. The current results support the use of a multimodal approach²⁵ for predicting treatment gains, including a measure of cortical function,^{6,26} and extend this approach to therapies that target the lower extremity.

The current study also explored whether changes in fMRI measures over time correlated with change in behavioral measures and, therefore, have potential use as biomarkers of treatment effect. The change in activation volume within foot primary sensorimotor cortex of each hemisphere was related to certain behavioral gains, suggesting that these fMRI measures may provide insights into neural-mediated behavioral gains²⁷ (eg, change in contralesional activation volume correlated with change in leg FM score; $P=0.0043$). However, although previous reports support current findings (eg, increased ipsilesional^{28–30} or bilateral³¹ activation paralleling behavioral improvement with gait training), these findings did not survive formal Bonferroni correction ($\alpha=0.0042$). Additional studies of cortical function are needed to understand whether such measures may be useful as biomarkers of lower extremity treatment effects better.

Interestingly, sensorimotor cortex activation did not correlate with behavioral status before therapy but did at week 12. Thus, at baseline, no fMRI measure correlated with gait velocity but after therapy, all 3 behavioral measures correlated significantly with ipsilesional and with contralesional sensorimotor cortex activation volume. Brain activation was not tightly linked with behavior at baseline but became so with the motor system plasticity stimulated by 9 weeks of therapy. A previous cross-sectional fMRI study of subjects with chronic stroke found contralesional activation during paretic foot movement to have a negative correlation with lower limb function,³² in contrast to the positive correlation identified at week 12 in the current study; these divergent results might reflect details of that study,³² such as stroke topography (subcortical only), greater time poststroke (37 months), and choice of fMRI metrics.

The current findings provide useful insight into predictors and biomarkers of treatment effect in studies targeting the lower extremity in patients with chronic stroke. Results may be useful for the development of entry criteria and stratification measures in clinical trials. Addition of an interim fMRI study acquired after initiating therapy might improve prediction of treatment gains—determining whether treatment is engaging sensorimotor pathways and inducing cortical reorganization could improve prediction.³³ Measures of injury did not achieve significance in the current study, but this might, in part, reflect the specific patterns of injury present in the current cohort because lesion characteristics influence cortical plasticity³⁴ and response to treatments targeting the lower extremity.³⁵ One weakness of the current study is the absence of neurophysiology measures in the trial protocol. Heterogeneity of enrollee time after stroke might confound data interpretation although the effect of this issue may be limited because the earliest a subject was enrolled was 71 days after stroke (Table 1), and the first dose of study medication was given in the chronic phase (ie, ≥ 3 months after stroke) in all but 2 subjects. Subjects averaged 212 days after stroke at enrollment, potentially limiting the direct relevance of current findings to stroke rehabilitation care, most of which is administered in the first month after stroke. However, many studies,³⁶ in addition to the present study, have reported that treatment initiated in the chronic phase after stroke can improve motor status.

In current practice, behavioral assessments are mainly used to distinguish subgroups of patients with stroke (eg, to guide rehabilitation therapy, stratify clinical trial enrollees, or predict treatment gains). Consistent with this approach, the current study found that leg FM score alone was a significant predictor of change in gait velocity ($r=0.46$). However, leg FM score together with ipsilesional fMRI activation volume in a multivariate model predicted change in gait velocity more precisely ($r^2=0.63$, thus $r=0.79$), suggesting that the combination of these 2 baseline measures reflect the capacity to achieve gains in motor control for walking, resulting in higher speed. The current results suggest that a multivariate approach that adds a measure of brain activation to behavioral assessments substantially improves the ability to predict treatment gains.

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Disclosures

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Stroke

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SUPPLEMENTAL MATERIAL

Predictors and biomarkers of treatment gains in a clinical stroke trial targeting the lower extremity

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MRI data acquisition

Scanning began with a T1-weighted, high resolution (1 mm³ voxels) volumetric anatomical scan covering the entire brain, which was followed by a fMRI scan that alternated 30 seconds of rest with 30 seconds of 0.25 Hz paretic ankle dorsiflexion/plantarflexion movement, i.e., 10 degrees ankle dorsiflexion then 10 degrees plantarflexion every 4 seconds. Movements were paced by an auditory metronome. Blood oxygenation level dependent (BOLD) scanning parameters included: 25 axial slices, 4mm thick with a 1mm interslice gap, TR = 2500 msec, TE = 40 msec, 110 volumes over 4 min 35 sec.

A member of the study team observed study subjects during fMRI scanning. Five subjects made additional movements (one moved the stroke-affected proximal leg, two had mirror movements in the non-affected ankle, and two had both stroke-affected proximal leg movement and mirror movements), and two subjects had no visible movements. Note that change in gait velocity from baseline to post-treatment did not vary significantly in relation to these findings, i.e., was not significantly different in those subjects who showed proximal movements, showed mirror movements, or who had no visible movements.

Brain injury

Infarct volume: Using the MRI image analysis program MRICron (<http://www.mccauslandcenter.sc.edu/mricro/mricron>), each subject's infarct was outlined by hand on the T1-weighted MRI image. The T1 parameters included repetition time (TR) = 13 ms, echo time (TE) = 4.47 ms, 128 slices, and voxel size = 1 x 1 x 1 mm³. All areas of injured tissue (i.e., the infarct core and surrounding diffuse white matter injury) were included. When multiple spatially separate foci of injury were present, they were all summed into a single stroke mask. The resulting stroke masks were binarized and then spatially transformed into MNI standard stereotaxic space using FSL. With this method, in 10 subjects who were 3-6 months post-stroke and who were enrolled in the robotic therapy study, we have found good intra-rater reliability (Pearson's $r = 0.998$, $p < 0.0001$; Intraclass Correlation Coefficient = 0.998) and inter-rater reliability ($r = 0.994$, $p < 0.0001$; ICC = 0.98).

Percent corticospinal tract injury: Corticospinal tract injury was evaluated as the amount of overlap between each subject's infarct with a normal M1 corticospinal tract¹⁻³ in MNI stereotaxic space. The normal tract was generated using diffusion tensor tractography in 17 healthy controls (9 females, 8 males; mean age = 29.8 +/- 2.5 SEM) in a manner similar to earlier work¹. T1-weighted MPRAGE images were acquired using a 3T Achieva scanner using the following parameters (TR = 8.4 ms, TE = 3.7 ms, flip angle = 8°, 190 axial slices, 1-mm isotropic voxels, no interslice gap, SENSE factor 2.4). Diffusion tensor images were acquired using an echo planar sequence with the following parameters: TR = 11194 ms, TE = 55 ms, 60 axial slices, acquisition matrix = 112 mm × 110 mm (FOV = 224 mm), 64 diffusion directions with a b value of 800 seconds/mm². After DTI images were corrected for eddy current distortions and head motion artifacts, FSL's BEDPOSTX program was used to generate probability distributions of diffusion parameters at each voxel, including modeling for diffusion of crossing fibers along two directions. Seed regions for tractography were placed in the precentral gyrus (obtained from cortical masks generated using FreeSurfer) and a second seed ROI was placed in the cerebral

peduncles (outlined on cross section at $z = -16\text{mm}$ of normalized T1 images for each subject). Tractography was initiated from the PCG mask using the CP as a waypoint mask. The resulting tracts were transformed into MNI space, binarized, and summed to create a group corticospinal tract. This tract was then thresholded to include only voxels in which at least 6 of the subjects were included. To simulate damage to groups of axons, the tract was divided into 16 separate longitudinal subsections. The binary stroke mask was overlapped onto the subsections. A subsection was classified as damaged if more than 5% of the subsection was compromised. The percentage of CST injury was calculated from the summed number of damaged subsections divided by the total number of subsections, which was then converted to a percentage.

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