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Neuroimaging predictors and biomarkers of rehabilitation gains after stroke

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Neuroimaging predictors and biomarkers of rehabilitation gains after stroke

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biomedical Sciences

by

Erin Burke Quinlan

Dissertation Committee:
Professor Steven C. Cramer, Chair
Professor Oswald Steward
Professor Craig E. Stark

2014
DEDICATION

To

my parents, stepparents, friends, family, in-laws, and, with endless gratitude, my husband.

This graduate school journey was possible with your support.

I also dedicate this to my grandparents. As members of The Greatest Generation, your examples of struggle, persistence, and love helped mold who I am today. I hope I've honored your legacies, making you proud.
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rehabilitation gains after stroke. Under review.


Publications in Preparation

Quinlan EB, Stewart JC, Dodakian L, See J, McKenzie A, Le V, Cramer SC. Imaging biomarkers of motor therapy after stroke differ according to stroke severity.

Fling BW, Quinlan EB, Le V, Cramer SC. A neural systems approach to measuring injury improves prediction of arm motor function in acute stroke.

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ABSTRACT OF THE DISSERTATION

Neuroimaging predictors and biomarkers of rehabilitation gains after stroke

By

Erin Burke Quinlan

Doctor of Philosophy in Biomedical Sciences

University of California, Irvine, 2014

Professor Steven C. Cramer, Chair

Stroke is a leading cause of long-term adult disability and many therapies are under study aiming to improve post-stroke motor function. Unfortunately, patient response to therapy is highly variable and the reasons for this are unknown. Clinical assessments are typically used to guide therapeutic decision-making after stroke. However, neuroimaging research over the last 15 years suggests that probes of neural injury and neural function provide crucial insight into post-stroke motor status. Limited research has examined how such measures could predict the likelihood of therapy-induced motor recovery and serve as biomarkers of treatment gains. Therefore, the current dissertation examined several neuroimaging measures of neural injury and neural function, as well as clinical and demographic variables, to 1) characterize our patient sample and understand the factors related to pre-therapy motor impairment and disability; 2) identify predictors of motor gains from a 3-week course of robotic arm therapy; and 3) elucidate potential biomarkers of motor gains from therapy. At baseline, reduced corticospinal tract (CST) integrity and neurophysiology (no motor evoked potential from transcranial magnetic stimulation) were correlated with greater pre-therapy motor impairment. Among less impaired patients, greater contralesional primary motor cortex (M1) and dorsal premotor cortex (PMd)
activation correlated with greater motor impairment. The factors related to greater disability were reduced CST integrity and poorer cognitive status. The baseline measures predictive of greater motor gains from therapy were smaller CST injury (percent lesion overlap) and greater interhemispheric functional connectivity. A notable finding was that predictors of gains varied according to lacunar stroke subtype: greater ipsilesional M1 activation and intrahemispheric functional connectivity predicted larger motor gains. Lastly, functional connectivity measures proved stronger biomarker candidates of treatment gains than changes in regional measures of motor cortex activation or CST integrity. Furthermore, biomarkers differed according to stroke severity. Among less impaired patients, reductions in intra- and interhemispheric functional connectivity with therapy correlated with greater motor gains whereas in more impaired patients decreases in interhemispheric functional connectivity correlated with smaller gains. The current findings illustrate that measures of neural injury and neural function provide great insight into motor status after stroke, the likelihood of gains from therapy, and the heterogeneity of patient response to therapy. Ultimately, these measures should be incorporated into clinical trials of restorative stroke therapies to stratify patients to appropriate therapies and guide therapeutic decision-making for maximal patient gains.
INTRODUCTION

Stroke is a leading cause of long-term adult disability in the United States [1], with over 7,000,000 stroke survivors living with deficits spanning many physical and cognitive domains. Approximately 50-75% of survivors experience long-term effects that greatly reduce quality of life [1, 2]. Due to the need for inpatient services, rehabilitation, and follow-up care, the projected combined direct and indirect costs of stroke are estimated at $71.6-184.1 billion [1]. Hence, there is a great need for novel ways to reduce disability and effectively allocate resources after stroke.

Although spontaneous recovery occurs after stroke, long-term functional recovery is often incomplete [3]. Spontaneous recovery reaches a plateau in 95% of patients 3 months after stroke [4], but functional gains can still be achieved with additional treatment. The administration of thrombolytic therapy, the only currently approved drug to treat acute stroke, is encumbered by the narrow 4-hour time window. As a result, only 5.2% of Americans receive tissue plasminogen activator (tPA) [5] and many still experience long-term disability. While tPA aims to salvage brain tissue threatened by an ischemic insult, restorative therapies can improve outcome through the promotion of neural plasticity in surviving tissue [6, 7].

While many restorative therapies are under study, including growth factors, cell-based therapies, and robotic devices, a key question is how to stratify patients to the appropriate therapies. Stroke is a very heterogeneous disease, considering the size and location of injury differ greatly across patients, which translates to substantial variability between patients and their response to therapy. A major hurdle to restorative therapy implementation, particularly in clinical trials, is the vast heterogeneity of stroke; the high inter-subject variance means reduced study power and increased cost. This is articulated by Bath et al: “In stroke trials, the impact of covariates such as age and severity on outcome is typically much larger than the treatment effect
that is being measured” [8]. The ability to assign the right patients to the right therapies would maximize treatment effects, for example, by confirming the presence of a therapy’s biological target. Because cellular and molecular measurements are generally inaccessible for study in humans, a number of neuroimaging methods have been examined to better understand, predict, and guide post-stroke restorative therapies.

The studies comprising this dissertation sought to understand which neural factors are important to therapy-induced motor recovery and provide insight into patient heterogeneity. Specifically, structural and functional magnetic resonance imaging (MRI) measures of the motor system were used to characterize an individual’s remaining neural resource before beginning a three-week course of standardized robotic hand therapy (i.e., identifying neural correlates of motor status and predictors of therapy gains), as well as characterize changes in these measures occurring in parallel with therapy (i.e., identify potential biomarkers of therapy effects) (for study design, see Figure 1). Prior research has shown that behavioral [9-11], neurophysiological [12], clinical [13, 14], and genetic factors [15, 16] are also related to post-stroke outcome. Therefore, a comprehensive multimodal approach was employed to best understand post-stroke motor status and motor gains from therapy.

The first aim examined which measure(s) were related to patients’ pre-therapy arm motor status and disability during the early behavioral plateau phase (Chapter 4). The second aim examined which combination of measures most strongly predicted motor gains from robotic therapy (i.e., explained the greatest variance in treatment gains (Chapter 5)). The third and final aim examined how changes in MRI measures of cortical activation, functional connectivity, and corticospinal tract integrity correlate with therapy-driven motor gains and could thus represent potential biomarkers of treatment effects (Chapter 6). Together, the comprehensive approach
provides evidence that measures of motor system injury and cortical function provide the greatest insight into pre-therapy arm motor status (outperforming traditional prognostic measures such as behavior and infarct volume); the potential for achieving motor gains from a restorative therapy; and the mechanisms likely supporting motor gains. Importantly, they also explain some of the patient heterogeneity routinely observed in motor recovery. The data resulting from this doctoral work strongly suggest that measures of neural injury and neural function should be incorporated into clinical decision-making after stroke in order to optimize treatments and maximize patient outcomes.

Figure 1. Study design.
CHAPTER 1

Neuroplasticity and recovery after stroke

Ischemic stroke is the result of insufficient blood flow caused by the blockage of an artery in the brain. Subsequent to the vascular occlusion, the cascade of excitotoxic and inflammatory responses lead to cell death around the occluded blood vessel, generating the ischemic core. Unless threatened brain tissue is reperfused via administration of tissue plasminogen activator (tPA) in the early hours after the ischemic insult, this vulnerable tissue, the ischemic penumbra, will also die. Due to the greater percentage of vascular occlusions occurring in the branches of the middle cerebral artery, the most often affected neural systems include, but are not limited to, the language, sensory, and motor systems. The research comprising this dissertation was focused on the latter. Amazingly, the ischemic brain undergoes significant structural and functional changes, both with and without the administration of stroke therapies, which mitigate damage and support motor recovery. The first part of this chapter will address the neuroplasticity processes implicated in spontaneous stroke recovery while the second part addresses treatment- and experience-induced plasticity and recovery.

Part I: Spontaneous stroke recovery

In the early days of modern neuroscience, the definition of neuroplasticity acknowledged that the brain could create new, or modify existing, synaptic connections in response to stimuli and experience [17, 18]. However, neuroplasticity principles also hold after both neural injury and post-injury experience [19, 20]. Indeed, the post-stroke ischemic brain is Janus-faced, with both injurious and restorative processes occurring simultaneously [21]. Ultimately, the relative balance of these processes results in the final ischemic infarct and subsequent deficits across
potentially numerous functional domains of the central nervous system. After stroke in humans, motor recovery occurs over the first few months even in the absence of therapy, a phase referred to as spontaneous recovery [22]. Animal models of ischemic stroke have provided great insight into the cellular and synaptic events following ischemic brain injury and their role in recovery.

After stroke, a number of plasticity-related processes occur both near the injury and at remote areas connected to the site of injury [23, 24]. Such changes include alterations in neuronal excitability [21, 25, 26], neurogenesis [27], and synaptogenesis [28]. For example, increased neurogenesis occurs in the ipsilesional subgranular zone of the dentate gyrus [29, 30], the subventricular zones of the lateral ventricles [31], and the damaged striatum [30]. In animal models [32], as well as humans [27, 33], neuroblasts have been shown to migrate toward the damaged cortex cued by signals in the post-stroke environment [32, 34, 35]. This may be observed at the macrostructural level, as ischemic injury induces changes in functional representations of the paretic hand in non-human primates [36, 37] that correlate with recovery of skilled dexterous movements [38].

Post-stroke plasticity is not relegated to the cortex, however, as white matter also undergoes remodeling. After ischemic injury, axonal sprouting occurs near the infarct due to the permissive environment created by the increased expression of growth-promoting molecules and the reduction of growth-inhibiting molecules [35]. New projections may also come from the unlesioned hemisphere, innervating areas deafferented by the stroke [39]. Importantly, these changes are not solely epiphenomenon to stroke but are supportive of behavioral recovery [40, 41].

Research conducted in animal models of stroke provides us with elegant evidence of the structural and functional changes occurring after ischemic brain injury and, therefore, the
potential mechanisms responsible for patients’ impairment at the end of the spontaneous recovery phase.

**Part II: Restorative therapy-induced stroke recovery**

Although motor recovery reaches a plateau after the spontaneous recovery phase, additional gains are possible with administration of restorative therapies. Unlike tPA, which indirectly improves motor function by reperfusing threatened tissue, restorative therapies aim to improve motor function through the promotion of neuroplasticity in surviving neural resource. Restorative therapies found to improve motor function in animal stroke models include small molecules [42-46], growth factors [47-49], cell-based therapies [39, 50-53], and cortical stimulation[54, 55]. Some of these therapies have been explored in human trials but with varying degrees of success [56-64]. As pointed out by Kahle and Bix: "[m]any stroke treatments have benefitted or seemingly cured stroke in animal models, but have ultimately failed in their translation to clinical trials" [65].

**Part IIa: Robotics as a therapy to improve stroke recovery:** The difficulty in translating the promising findings from preclinical studies to humans [66, 67] has left rehabilitative interventions as the therapeutic gold standard for improving motor function after stroke. In a 2009 review by Langhorne et al, interventions based on high-intensity, repetitive task-specific practice were the most promising in improving upper limb function [68]. Robotics is an example of such an activity-based therapy that has increasingly been tested in the context of stroke.

Robotic devices are a rehabilitation double-threat. They are desirable standalone treatment candidates as well as adjunct therapies because of their consistent, precise, repetitive, high-
intensity performance – they are able to generate a higher number of movements than many comparison therapies [69, 70]. A number of clinical research studies have found that robot-assisted therapy reduces impairment and improves function of the paretic arm in the chronic phase [71-74], outperforming standard arm therapies [75]. However, patients with moderate-to-severe impairment may need robotic therapy for longer periods in order to significantly improve paretic arm function [76]. Previous work in the lab found a 3-week course of standardized robotic therapy led to significant gains on impairment and functional scales in thirteen chronic stroke patients [77]. The rationale for the active assistance (i.e., the robot supplements patients' efforts to move) provided in this therapy includes somatosensory stimulation, reestablishing a more normal pattern of motor output, and more intensive practice [78]. Functional reorganization within the motor system, namely increased activation [77] and increased excitability [79] within ipsilesional sensorimotor cortex, as well as reduced interhemispheric connectivity [80], are proposed mechanisms underlying the beneficial effects of robotic therapy.

The robotic intervention employed in this research was a 3-week course of standardized robotic therapy using the Hand Wrist Assistive Rehabilitation Device (HWARD), as published previously [77]. In addition to the previously stated benefits of robotic therapy, the HWARD therapy also included a virtual reality computer interface and the use of real-world objects for the added benefits of attentional valence and purposeful context, respectively[81-85].

Part IIb: Treatment- and experience-induced neuroplasticity: Many of the same plasticity processes that occur during spontaneous recovery are induced in response to motor learning and rehabilitative training [86]. This experience-dependent plasticity includes synaptogenesis, dendritic branching, altered astrocyte morphology, increases in cortical thickness, and functional
reorganization within the cortex. For instance, motor learning increases the number of synapses in layers 2/3 [87] and 5 [88] of motor cortex, a phenomenon that precedes motor map organization and occurs in the later, as opposed to early, phase of learning [89]. Motor training on a skilled reaching task also induces dendritic arborization in layer 5 pyramidal motor neurons in the peri-infarct cortex [90] as well as the contralesional motor cortex [91]. The neuronal activity and synaptogenesis that occurs with learning are likely associated with remodeling of astrocyte processes as well [92]. Furthermore, the dendritic, astrocytic, and synaptic changes resulting from the increased movement-dependent neuronal activity are possible substrates for increased motor cortex thickness observed with skill learning in rats [93]. Alterations in white matter microstructure have also been observed that may be due to increased myelination in the white matter underlying motor cortex [94]. Motor rehabilitation or task training that engages the participation of cortical regions across the motor network requires effective signal transmission between those regions. Hence, changes in white matter might occur to facilitate the speed and efficiency of those connections, resulting in improved behavior. Finally, intracortical microstimulation in primates undergoing rehabilitative training shows reorganization of the paretic hand’s movement representation into neighboring peri-infarct cortical areas [95, 96]; this suggests a functional plasticity mechanism for improved hand function with motor therapy. Many of these mechanisms supporting functional recovery are not transient, as they persist beyond the initial treatment exposure.
Although preclinical studies have provided important information as to the mechanisms of post-stroke recovery, the advent of neuroimaging enables scientists to study potential recovery mechanisms in vivo in humans. Despite the neurobiological relevance and elegant study design of animal stroke models, they cannot recreate the complex biological conditions of stroke in humans. This may limit the translation of resultant findings to humans. Not only does stroke affect white matter significantly more than in animals, but stroke in humans is often the result of various comorbidities that often are not incorporated into preclinical models. Another important benefit of neuroimaging is the ability to study the structural and functional mechanisms of stroke recovery at the systems level, as motor behaviors arise from the interplay of regions across the motor system.

Incorporating measures of residual motor system injury and function will improve upon using traditional prognostic measures such as pre-treatment behavioral status and infarct volume. Scores on impairment scales such as the NIH Stroke Scale (NIHSS) and Fugl-Meyer scale (FM) have been good indicators of likely improvement, particularly initial impairment in the acute phase. Infarct volume, a measure of global neural injury, was one of the first neuroimaging measures used to predict stroke outcome but has had moderate success. MRI brain mapping techniques such as transcranial magnetic stimulation (TMS), diffusion tensor imaging (DTI) and functional MRI (fMRI) have moved the neuroimaging field beyond global measures of injury, as they are able to probe the structural and functional integrity of the motor system after stroke:
**Transcranial magnetic stimulation**: Transcranial magnetic stimulation is a tool widely used to assess neurophysiology via principles of cortical excitability and corticospinal motor output. The TMS coil placed on an individual's skull (over the cortex of interest) first emits a brief magnetic pulse perpendicular to the coil that then generates an electric current in the brain perpendicular to the magnetic pulse. The result of this electromagnetic induction is stimulation of the underlying cortex [107]. Depolarization of the cortical (motor) neurons produces descending action potentials in the pyramidal tract which, after synaptic relays in the brain and spinal cord, elicit contraction in the contralateral limb muscles. The strength of these motor evoked potentials (MEP) can be measured using electromyography (EMG) leads placed on the target muscles [107, 108]. The neural mechanisms of the corticospinal propagation of action potentials and excitatory postsynaptic currents are proposed to be the early direct (D) and late indirect (I) waves depending on the stimulation intensity [109]. TMS generates D waves through direct stimulation of pyramidal neurons (with higher intensities) whereas I waves (lower intensities) are likely generated via stimulation of cortico-cortical connections and interneurons that are transsynaptically connected to pyramidal neurons [110].

There are many TMS-related metrics that can be used to assess corticospinal integrity: the stimulation intensity (motor threshold) required to elicit an MEP; the presence, amplitude, and latency of MEPS; an input/output recruitment curve (generated by averaging 10 pulses at 4 stimulation intensities, plotting the MEP amplitude as a function of the intensity level, fitting it to a linear model, and calculating the slope); and the representational map of the sites that elicited a qualified response in the target muscle [12]. However, in stroke, it is not uncommon to fail to elicit an MEP, never mind be able to generate the other measures. Therefore, the current work focused on the presence or absence of an MEP.
**Diffusion tensor imaging:** Preclinical research has provided crucial information regarding post-stroke changes in white matter structure and metabolism. However, using histological [111, 112] or neuroanatomical tracing [113, 114] methods to identify axonal degeneration or growth is impractical in clinical stroke research. Also, the inherent tissue composition differences in rodents versus humans is large: it is estimated that white matter accounts for approximately 10% of total brain volume in rats but nearly 2/3 of the total brain volume in humans [115]. Due to this natural variation, strokes involve more white matter in humans than in rodent models of stroke; small subcortical white matter lesions account for at least 25% of strokes [116]. This might complicate the translation of findings from animal stroke models to patients with lacunar lesions.

Diffusion tensor imaging is a means of noninvasively examining white matter integrity throughout the brain *in vivo*, providing insight into structural alterations [117, 118] after stroke and their relationship with impairment.

Diffusion tensor imaging provides quantitative information about the cellular microstructure of white matter through the reconstruction of white matter tracts on the basis of random water diffusion [119-121]. The diffusion tensor is the primary orientation of water diffusion in each voxel, representing the local fiber orientation [122]. Water diffusion is considered “isotropic” if it is able to move freely and equally in all directions, and is “anisotropic” if its movement is restricted due to structures such as myelin sheaths, cell membranes, and axon tracts. In white matter axonal bundles, water diffusion moves freely along the long axis of the axons and has restricted movement in the perpendicular plane [123]. This relative difference in diffusion leads to higher anisotropic diffusion, expressed as fractional anisotropy (FA). Fractional anisotropy values range between 0 and 1: a value near 1 is indicative of greater anisotropic diffusion (e.g., intact corticospinal tract (CST)) whereas values close to 0 indicate greater isotropic diffusion due
to white matter damage. A colorized FA orientation map [124] that aligns with axonal bundles eases identification of desired axonal tracts such as the CST and, importantly, corresponds with postmortem histology [122]. In recent years, studies have correlated FA with behaviors such as reaction time [125], bimanual coordination [126], reading ability [127], and executive dysfunction [128]. DTI has also been used to evaluate white matter changes within the motor system both in ischemia models [129-131] and patients (see below).

After stroke, the decline of white matter integrity within the ipsilesional CST correlates with motor impairment. Just as the cortex is sensitive to ischemia, white matter is as well although it has a higher ischemic threshold [132, 133]. Damage caused by ischemia leads to decreases in axial diffusivity (diffusion along the primary axonal axis; thought to reflect axonal damage [134]) while radial diffusivity increases (diffusion average of the two perpendicular axes; thought to reflect demyelination [135]) which, ultimately, is reflected by a decrease in FA (the relative diffusion difference of the three axes) [136, 137]. In cross-sectional studies, FA within the CST has helped explain the degree of motor impairment and muscle weakness in patients with chronic stroke [138-141]. Longitudinal studies can capture in vivo changes in diffusivity/anisotropy measures corresponding to axonal injury and demyelination [118, 142, 143] and possibly remyelination [144].

Diffusion tensor tractography (DTT) is another tool that can be used to assess injury to the CST after stroke. The diffusion and orientation data derived from DTI can be used to map neuronal fiber tracks of interest in vivo [145]. Although probabilistic DTT has been used in stroke [146, 147], a major hurdle to performing white matter tractography in stroke patients, particularly in a heterogeneous sample, is the lack of tracks within large infarct cores. A way to circumvent this issue has been devised such that CSTs are generated in healthy controls. Then,
patients’ infarcts can be overlapped with the healthy CST to calculate the degree of injury to the CST, or the lesion load. Lesion load positively correlates with motor impairment [148, 149] and is even predictive of motor gains from therapy [150]. It has recently been suggested that such healthy template tracts could be used to calculate tract-specific FA values for patients with similar accuracy to FA values extracted from tracts generated in stroke patients [151].

Diffusion tensor imaging is not without its limitations. Although changes in DTI metrics of diffusivity and anisotropy have been correlated with histological myelin and axonal changes, the cause for decreases in FA may not solely be due to injury. Extensive fiber crossing is not considered in the diffusion tensor calculation, so a voxel in a region with many crossing fibers might appear as a voxel with low FA [152]. Therefore, decreases in anisotropy may not be due to loss of axons or myelin but rather the reorganization of fibers [122]. However, in predominantly longitudinal axon bundles such as in the CST, this poses less of a problem and, therefore, was not a major concern in the current work.

Functional magnetic resonance imaging -- cortical activation: Functional MRI indirectly measures neuronal activity based on the relative ratio of deoxygenated to oxygenated hemoglobin, or the blood oxygenation level-dependent (BOLD) contrast [153]. Oxygenated blood is diamagnetic and repelled by magnetic fields. Deoxygenated blood is paramagnetic and, therefore, becomes magnetized by the fields of the MR scanner thereby decreasing the MRI signal [154]. When a population of neurons is active, cerebral blood flow increases to that region supplying more oxygenated blood than is used by the active neurons [155, 156]. This results in a local decrease of deoxygenated blood and a subsequent increase in fMRI signal [157]. Simultaneous recordings of BOLD fMRI responses and intracortical local field potentials (LFPs) in the non-human primate visual cortex confirmed that the increases in BOLD signal are
representative of increased neuronal activity [158, 159]. However, fMRI cannot differentiate between neuromodulatory versus function-specific processing or between excitation versus inhibition [160]. Functional MRI has very high spatial resolution, allowing researchers to visualize the brain regions activated in response to a task, to observe how those activation patterns change in response to neural injury, and how they may change with a therapeutic intervention.

There are some important points to consider in designing and interpreting fMRI studies in patients with stroke. First, clinical stroke populations tend to be older. Natural aging could lead to alterations in neurovascular coupling and, therefore, changes in the BOLD signal [161-163]. Second, under pathophysiological conditions as such stroke neurovascular coupling could be altered. But a longitudinal study using BOLD fMRI and electrophysiological recordings suggests that neurovascular coupling is preserved [164]. When implemented and interpreted appropriately, fMRI is a powerful tool to probe motor cortex function and identify neural correlates of motor behavior.

Cortical activation in motor areas observed with fMRI during movement of the paretic limb provides insight into post-stroke motor behavior. After stroke, moving the paretic limb consistently activates primary and secondary motor areas in both the ipsilesional (i.e., hemisphere contralateral to the paretic limb) and contralesional (i.e., hemisphere ipsilateral to the paretic limb) hemispheres – a pattern of activation not observed in healthy individuals [165]. This activation present in bilateral primary and secondary motor areas correlates with greater motor impairment [166-169]. However, a question arose resulting from these studies: how functionally relevant is the activation? Studies incorporating TMS have shown that 1) interfering with contralesional primary motor cortex (M1) and dorsal premotor cortex (PMd) during a motor
task using the paretic hand impairs performance in a manner that correlates with the degree of impairment [170, 171] and 2) contralesional PMd activation exhibits a facilitatory influence on ipsilesional sensorimotor cortex during paretic hand movement [172]. Despite its functional relevance, longitudinal decreases in contralesional motor cortex activation have been typically been associated with greater recovery after stroke [173-175] although interindividual variability has been observed [176]. Differences in motor impairment and injury to M1 or CST may also account for different patterns of reorganization during recovery [54, 177]. Therefore, a multimodal approach and heterogeneous patient sample, as used in the studies comprising this dissertation, are vital to try to best understand the neurobiological bases of recovery as well as interindividual variability.

**Functional magnetic resonance imaging -- cortical connectivity:** In recent years fMRI-based cortical connectivity has received much attention because it can provide insights into how functionally connected regions are, moving beyond the isolated nature of regional activation measures. Additional salience lies in the fact that regional activation may remain unchanged despite alterations at the network level [178]. With respect to neuroimaging, functional connectivity is defined as the correlations between spatially remote neurophysiological events [179] but the neurobiological events mediating the correlations are still under investigation.

At the neuronal level, functional connectivity refers to the synchronous firing of sets of neurons, whereas at the network level it is represented by oscillations of neural populations. It is believed that functional connections reflect activity of structurally connected neuroanatomical substrates, particularly for very strongly correlated oscillating neural signals, although functional connectivity may exist via indirect structural connections [180]. Simultaneous EEG/fMRI studies suggest that connectivity changes may be due to fluctuations in local cortical field potentials in
different power bands, often depending on the nature of imaging acquisition (i.e., active vs. resting state, visual vs. motor task) [181-184]. Dual BOLD/neurophysiology recording in anesthetized rats found that variations in BOLD signal correlation between bilateral sensorimotor cortices were strongly related to changes in beta and gamma power derived from local field potentials [185]; the beta band has particular relevance to the motor system [186, 187]. GABA neurons are believed to be a key mediator of neuronal synchronization and network oscillations [188] underlying observed changes in band power. In humans, levels of GABA within M1 are negatively correlated with interhemispheric M1-M1 functional connectivity and the application of transcranial direct current stimulation, thought to decrease M1 GABA levels, subsequently increases motor network functional connectivity [189]. Important for stroke recovery, changes in functional connectivity may be stable beyond the rehabilitation exposure. A study of spatial navigation learning in rats found greater correlation in the firing of prefrontal cortical neurons that stabilized and remained elevated after learning [190]. Although additional research is needed into the neurobiological and electrophysiological underpinnings of BOLD functional connectivity, the collective data to date do suggest a neural basis.
CHAPTER 3

Other variables related to post-stroke functional recovery

In addition to examining neuroimaging measures of brain injury and cortical function as probes of motor status before and after rehabilitation, measures of impairment, demographics/medical history, cognition/mood, and genetics that have also been related to outcome after stroke were evaluated.

Traditionally the most commonly used prognostic indicators of stroke recovery have been measures of impairment, both overall neurological impairment and motor impairment. Neurological impairment scales tend to be more global in that they assess impairment beyond the motor domain such as level of consciousness, speech/language, vision, and sensory function. Scores indicative of greater neurologic impairment have been correlated with reduced long-term functional recovery [13, 191, 192]. However, motor system-specific measures are currently used to guide post-stroke rehabilitative care because they give insight into the likelihood of predicting improvement in the upper extremity after stroke. While some studies have found a linear relationship [9, 70, 193], current work from our lab suggests the relationship may be second order [194] with patients at the mild and severe ends of the impairment spectrum improving the least.

Age is a demographic variable found to strongly predict stroke outcome when assessed in parallel with only other demographic or clinical features. For example, one of the earliest studies examining what factors influence stroke outcome found that patients under 65 had better outcomes than patients over the age of 65 [195], although not all studies specify a cutoff [191].
Age has also shown prognostic value in a multivariate model with motor impairment measures [193].

Post-stroke depression is present in at least one-third of stroke survivors and negatively impacts quality of life and motor recovery [196-199]. To this end, alleviating depression using antidepressants has been shown to improve motor recovery both in observational [200] and experimental [201] settings. Because of these findings it's also likely that similar scales related to hopelessness [202] and quality-of-life [203] may be related to post-stroke motor recovery as well.

Similarly, cognitive impairment may impede functional recovery after stroke. Cognitive deficits are observed in approximately half of all patients living with stroke. Interestingly impairment in the cognitive domain has been associated with disability and handicap in chronic stroke [204] and cognitive assessments attained early in the subacute phase are predictive of functional outcome a year later [205]. Although cognitive impairment has not been studied in terms of motor rehabilitation training, we hypothesize that greater cognitive impairment will negatively impact motor gains with rehabilitation [206].

Common stroke risk factors and stroke comorbidities such as diabetes, hypertension (high blood pressure), and hypercholesterolemia (high cholesterol) may also negatively impact motor recovery after stroke. The higher amounts of glucose circulating in the blood in individuals with diabetes means they are two to four times more likely to have a stroke because of the higher probability of fatty deposits or clots forming on blood vessel walls. Patients with diabetes have a greater prevalence of limb weakness [207] and disability [14] compared to patients without diabetes. Potential reasons for greater post-stroke motor impairment include reduced reparative vascular density [208], as assessed via stereology of 3D reconstructed vasculature in diabetic
versus control rats, and reduced cortical plasticity [209], as assessed via the relative reorganization of forelimb evoked cortical depolarizations in diabetic versus control mice. High blood pressure over time also increases one's chances of having a stroke. With the increased stress on blood vessel walls, they tend to thicken in a manner where pieces of buildup can break off and block blood flow or, conversely, the walls may weaken and lead to vessel walls breaking resulting in a hemorrhage. It's not clear how high blood pressure may be related to improvement with rehabilitation but a study of 252 patients with acute stroke found that greater recovery was associated with lack of pre-stroke high blood pressure as well as a drop in mean arterial pressure early after the stroke [210].

Naturally occurring genetic variations in the genes coding for brain-derived neurotrophic factor (BDNF: val<sup>66</sup>met polymorphism) and apolipoprotein E (ApoE: ApoE4 allele), were evaluated as exploratory measures related to upper extremity motor status. Brain-derived neurotrophic factor is an abundant growth factor in the brain, with a prominent role in neuroplasticity. Administration of purified BDNF onto primary cultured hippocampal neurons leads to increased amplitude and frequency of excitatory postsynaptic currents in both embryonic [211] and adult [212] hippocampal neurons that may occur via a glutamatergic mechanism [213]. In ischemic animal models, knocking down BDNF expression in the brain using antisense BDNF oligonucleotide reduces the beneficial effects of forelimb rehabilitation training [214] suggesting BDNF mediates post-stroke recovery. However, a naturally occurring polymorphism in the BDNF gene that decreases activity-dependent BDNF release might negatively impact post-stroke plasticity and improvement with motor rehabilitation as learned from studies of healthy adults. In this single nucleotide polymorphism (SNP), a methionine is substituted for a valine at codon 66 (val<sup>66</sup>met). Approximately 30-50% of the population is either heterozygous (val/met) or
homozygous (met/met) for this polymorphism [215]. A key study by Egan et al was the first to show that the BDNF val^66^met polymorphism reduces BDNF activity-dependent secretion in vitro in hippocampal neurons [216]. In humans with the val^66^met polymorphism they did not observe the characteristic reductions in hippocampal activation during a working memory task seen in patients without the polymorphism but, rather, bilateral hippocampal activation. Individuals homozygous for the polymorphism (i.e., met/met) had poorer performance on a test of verbal episodic memory than individuals with val/val or val/met genotypes. Important for the motor context of the current studies, the presence of the val^66^met polymorphism in healthy adults has been associated with reduced motor system activation with motor training and greater error on a motor learning task compared to subjects with the val/val polymorphism[217]. Studies using cortical stimulation methods such as TMS and transcranial direct current stimulation have shown that carriers of the Met allele have reduced cortical excitability [218] and smaller hand motor training-induced increases in cortical map size [219]. After subarachnoid hemorrhage, individuals with the SNP have poorer spontaneous recovery [15]. Combined, there is strong evidence that the BDNF val^66^met polymorphism may negatively influence chronic stroke motor recovery as well as motor cortex plasticity.

**Apolipoprotein E** has long been known to have a role in transporting cholesterol and other lipids through the bloodstream to their targets but also has strong links to neurodegenerative disease and plasticity. The gene coding for ApoE has 3 possible alleles: ε2, 3, and 4. The latter allele is strongly associated with the development of Alzheimer's disease [220, 221] but also with poorer outcome after head injury [222, 223] and subarachnoid hemorrhage [16, 224] Potential mechanisms may be impaired synaptic stability [225] and neuronal plasticity [226, 227]. In a clinical trials of 255 stroke patients, individuals with the ApoE4 allele had poorer recovery over
the first month and greater disability at 3 months [228]. While not expected to be key players in the aims of this work, the BDNF val<sup>66</sup>met polymorphism and ApoE4 allele were hypothesized to have additional explanatory value.
CHAPTER 4

A multimodal approach to understanding motor impairment and disability after stroke

Abstract

Many different measures are related to behavioral outcome after stroke. Preclinical studies emphasize the importance of brain injury and neural function. However, the measures most important to human outcomes remain uncertain, in part because studies often examine one measure at a time or enroll only mildly impaired patients. The current study addressed this by performing multimodal evaluation in a heterogeneous population. Patients (n=36) with stable arm paresis 3-6 months post-stroke were assessed across 6 categories of measures related to stroke outcome: demographics/medical history, cognitive/mood status, genetics, neurophysiology, brain injury, and cortical function. Multivariate modeling identified measures independently related to an impairment-based outcome (arm Fugl-Meyer motor score). Analyses were repeated (1) identifying measures related to disability (modified Rankin Scale score), describing independence in daily functions and (2) using only patients with mild deficits. Across patients, greater impairment was related to measures of injury (reduced corticospinal tract integrity) and neurophysiology (absence of motor evoked potential). In contrast, (1) greater disability was related to greater injury and poorer cognitive status (MMSE score), and (2) among patients with mild deficits, greater impairment was related to cortical function (greater contralesional motor/premotor cortex activation). Impairment after stroke is most related to injury and neurophysiology, consistent with preclinical studies. These relationships vary according to the patient subgroup or the behavioral endpoint studied. One potential implication of these results is that choice of biomarker or stratifying variable in a clinical stroke study might vary according to patient characteristics.
Introduction

Behavioral outcomes after stroke show substantial variation across patients. Many factors contribute to this, including differences between patients prior to stroke, in the stroke injury itself, and in post-stroke brain plasticity. Inter-subject variability in patients with stroke complicates efforts to evaluate new therapies, including efforts to stratify patients in clinical trials [229] or to develop reliable biomarkers [230].

Numerous measures have been found to correlate with outcome after stroke in humans. Examples include age [191], comorbidities such as diabetes [14], cognitive status [205], depression [199], and genetic variation [228], as well as neuroimaging measures such as infarct volume [102], corticospinal tract (CST) integrity [138], and cortical function [166]. However, the measures most critical to outcomes in patients with stroke remain uncertain, in part because few studies have examined multiple variables in parallel. A multimodal approach could help identify the factors most closely linked with behavioral outcome after stroke.

The current study adopted such a multimodal approach, with a focus on the motor system. In a cohort of patients who reached a plateau in behavioral recovery after stroke (defined as a change of no greater than 3 points on the Fugl-Meyer scale between baseline visits), variables in 6 categories were measured: demographics/history, cognitive/mood, genetic, neurophysiology, brain injury, and cortical function. The primary active study hypothesis, based on the preclinical literature [20, 131, 231], was that measures of neural injury and of neural function would have the strongest relationships with final level of motor impairment after stroke. The secondary independent measures were also evaluated in relation to baseline impairment but hypothesized to be less important to pre-therapy motor status. As per the evidence provided in Chapter 3, we hypothesized that older age, presence of diabetes mellitus, hypertension, hypercholesterolemia,
post-stroke depression, presence of the BDNF val^{66}met polymorphism and ApoE4 allele, would be related to poorer stroke recovery - that is, greater motor impairment.

Two exploratory hypotheses were also examined. The first was that the correlates of motor outcome after stroke vary in relation to severity of the deficits. The heterogeneity of stroke in humans suggests that differences may exist in the biology of recovery across patient subgroups. A better understanding of biological differences in stroke subgroups might inform 1) stratification approaches in clinical trials of restorative therapies after stroke, and 2) the extent to which published reports generalize, given that many prior studies have focused on patients with mild deficits [232]. The second hypothesis was that the correlates of motor outcome after stroke vary across dimensions of the World Health Organization International Classification of Functioning, Disability, and Health (WHO ICF) [233]. Specifically, correlates of impairment (loss of body functions and structures) are hypothesized to differ from correlates of disability (activities limitations). Less is known about the neurobiological basis of post-stroke disability, as compared to impairment, although such information is important given that measures of disability are directly linked with patient functional status [234], and that such measures have only a limited relationship with level of impairment.

**Methods**

**Patients**

Forty-one patients with stable motor deficits early after stroke were recruited. Inclusion and exclusion criteria appear in Table 4.1. Of the 41 enrollees, 4 could not complete MRI due to claustrophobia/anxiety and 1 was found ineligible after baseline assessments, leaving 36 patients. All patients provided informed consent. The study was approved by the UC Irvine Institutional Review Board.
Demographics/history

Medical history was obtained, and hospitalization records reviewed.

Cognitive/mood status

A single rater performed all behavioral assessments, which included Mini Mental State Exam (MMSE) and Geriatric Depression Scale [235].

At this exam, impairment was measured using the arm motor Fugl-Meyer scale (FM) [236], and disability, using the modified Rankin Scale (mRS) [237]. Stable arm motor status was operationally defined by obtaining a second baseline FM score 7-21 days after the first; stability was present if the second FM score was within 3 points of the first. Handedness was determined using the Edinburgh Handedness Inventory [238].

Table 4.1. Inclusion and exclusion criteria.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tr>
<td>Age ≥ 18 years</td>
<td>Contraindication to MRI</td>
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<tr>
<td>Diagnosis of stroke 11-26 weeks prior</td>
<td>Severe cognitive impairment</td>
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<tr>
<td>Residual arm motor deficit (ARAT&lt;52 or 9-hole peg test score &gt; 25% longer than with unaffected hand)</td>
<td>Concurrent diagnosis affecting arm/hand function</td>
</tr>
<tr>
<td>Preserved voluntary movements in distal upper extremity (≥5 degrees range of motion in affected index metacarpophalangeal joint or wrist)</td>
<td>Arm motor status not at stable plateau</td>
</tr>
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</table>

Genetics

A blood sample was obtained, and presence of the brain derived neurotrophic factor (BDNF) val66met polymorphism and the ApoE4 allele were each determined, as described previously [228].
Neurophysiology

Patients underwent transcranial magnetic stimulation (TMS) of ipsilesional motor cortex. Motor evoked potential (MEP) amplitudes were measured in the paretic first dorsal interosseous muscle at rest [219]. In sum, the site of lowest motor threshold (LMT) in the ipsilesional hemisphere that elicited a suprathreshold response of 0.5 μV [239] in the stroke-affected first dorsal interosseus (FDI) muscle at rest was identified. If an LMT was found, TMS was applied at four stimulus intensities (90%, 110%, 130%, 150%). If an MEP was detectable, reflecting neurophysiological integrity, latency to MEP was calculated (measured in ms, determined at 110% of resting motor threshold, and reflecting speed of motor system conduction), as well as the input/output recruitment curve (which finds the slope of the plot looking at MEP in relation to the 4 TMS stimulation levels, and reflects recruitment and reserve of stimulation targets [239, 240]). Presence of a TMS-elicited MEP was also categorized as present or absent. If no MEP greater than 0.5μV was elicited in and around motor cortex after four stimulations at the maximum output of the TMS coil then MEP was defined as absent. For maximal safety, subjects did not undergo TMS if contraindicated, e.g., due to calvarial defect or usage of certain medications [219, 241]. Due to these criteria, 16 subjects did not undergo TMS.

Brain injury

Image acquisition: MRI images were acquired using a 3.0T Philips Achieva system. Imaging included both anatomical (high-resolution T1-weighted images, T2-FLAIR, and diffusion tensor imaging (DTI)) and functional MRI (fMRI). For the T1-weighted image, parameters included repetition time (TR) = 8.5 ms, echo time (TE) = 3.9 ms, slices =150, voxel size = 1 x 1 x 1 mm³. For the T2-FLAIR image, parameters included (TR = 11000 ms, TE = 125 ms, slices = 31 slices, voxel size = .58 x .58 x 5 mm³). One set of diffusion-weighted images was
acquired using 32 directions; b-value 1000 smm$^2$; 60 slices; and voxel size = 1.75 x 1.75 x 2 mm$^3$.

*Image analysis* was performed blinded to clinical data. In sum, three classes of brain injury metrics were extracted: (1) total brain injury (infarct volume); (2) gray matter injury (to primary motor cortex (M1), to dorsal premotor cortex (PMd), and total cortical injury); and (3) white matter injury (overlap with corticospinal tract (CST), and per DTI fractional anisotropy (FA) values within ipsilesional cerebral peduncle).

*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, as informed by the T2-FLAIR image. 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. 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; intraclass correlation coefficient = 0.98) with this method, in a separate analysis of 10 subjects who were 3-6 months post-stroke.

*Gray matter injury:* To determine the contribution of gray matter motor system injury to behavioral gains, each patient's baseline T1-weighted image was inspected to evaluate stroke-related injury to cortex of M1 (i.e., precentral gyrus), cortex of PMd, and the entire cerebral cortex. The regions of interest (ROI) for M1 and for PMd were drawn on the 1x1x1mm MNI T1 template in FSL. The M1 ROI consisted of the posterior bank of the precentral gyrus, whereas the PMd ROI consisted of the posterior bank of the middle frontal gyrus anterior to the precentral
sulcus. The cerebral cortex ROI was generated by segmenting the same MNI template using the FAST module and isolating the segmented deep gray matter. Each patient's stroke mask was transformed into MNI space using the same template. Those two images were then multiplied to generate an overlap image. The number of voxels of the infarct that overlapped with the ROIs was counted. Injury was measured both as a continuous variable (i.e., the number of damaged voxels within the ROI) and as a dichotomous variable (the ROI was injured or not).

*Corticospinal tract injury:* Using the diffusion-weighted images, white matter integrity within the CST was quantified as mean FA [152] within a peduncular ROI, using FSL (www.fmrib.ox.ac.uk/fsl). Diffusion data was corrected for eddy currents and head motion using a 3D affine registration. Fractional anisotropy maps were then generated by fitting a diffusion tensor model at each voxel (DTIFIT module in FSL). An ROI was then drawn on the axial slice that showed the greatest cross-sectional area of the cerebral peduncle (CP) [242, 243]. The colorized FA image [124] was used to guide ROI drawing, ensuring ROIs did not extend into the substantia nigra. The region of the CP was selected for this measure because of its large content of descending motor fibers and because it was located remotely from all but one of the subjects' stroke lesions.

A second method was used to measure corticospinal tract injury: the amount of overlap in MNI stereotaxic space between each subject's infarct and the normal M1 corticospinal tract [149, 150, 244]. The normal tract was generated using diffusion tensor tractography in 17 healthy controls as described previously [150]. In sum, in these 17 subjects, 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 and a second seed ROI was placed in the cerebral peduncles. Tractography was initiated from a mask of the precentral gyrus 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 CST was divided into 16 separate longitudinal subsections. The binary stroke mask was overlapped onto each CST subsection. For each subject with stroke, a CST subsection was classified as injured if more than 5% of that subsection overlapped with the binary stroke mask. 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.

Cortical function

*Image acquisition:* Three runs of blood oxygenation level-dependent (BOLD) images were acquired for functional MRI (fMRI) using the following parameters: TR = 2000 ms, TE = 30 ms, 31 slices with thickness 4 mm and 1 mm interslice gap. Each of the three fMRI runs was 96 seconds (48 brain volumes), during which subjects viewed a video that guided the paretic hand to alternate between 24 seconds of grasp-release movements and 24 seconds of rest. An investigator observed movements during scanning to ensure compliance. Three measures of brain function were extracted from fMRI images: (1) activation beta (contrast) estimate and (2) activation volume, each measured in right and in left M1 and PMd; and (3) activation laterality index (LI) for M1 and PMd.

Functional data from the three BOLD fMRI runs were preprocessed using SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). Preprocessing steps included realignment to the first image, coregistration to the mean EPI image, normalization to the standard MNI EPI
template, and spatial smoothing (FWHM = 8 mm). Data were visually inspected for head movement after the realignment step. Data were rejected for subjects with >2mm head displacement, and as a result fMRI data for 7 subjects were excluded leaving 29 patients with complete fMRI data.

For statistical analysis, the fMRI data were modeled as a boxcar convolved with a hemodynamic response. A high-pass filter of 128 seconds was used to remove low signal changes. Functional run data were inspected for outliers due to excessive head motion (>1mm translation or >0.2 radians rotation between each volume) and signal noise (Z>3 from the mean image intensity) using the Artifact Detection Tool toolbox (http://www.nitrc.org/projects/artifact_detect). Any outliers were deweighted during statistical analysis. Single-subject t-maps (task versus rest) were generated using p < 0.001 uncorrected. Using the Marsbar toolbox [245], right and left ROIs were created within M1 and PMd, as well as a midline supplementary motor area (SMA) ROI, based on coordinates reported in a meta-analysis by Mayka et al. [246]. Peak beta contrast estimates and activation volumes were extracted in SPM8 using small volume correction. If no suprathreshold clusters were detected at p<0.001 uncorrected, small volume correction was evaluated at p<0.01.

**Statistical analysis**

Statistical analyses were conducted using JMP software (v.8.0.2, SAS Institute, Inc., Cary, NC).

*Correlates of impairment (loss of body functions and structures):* The above metrics were examined as independent variables in relation to an impairment-based dependent measure, arm motor FM score. First, bivariate screening examined the relationship that each independent
variable had with the FM score, using two-tailed alpha=0.05. When possible, parametric methods were used, with non-normally distributed variables transformed to a normal distribution, else non-parametric methods were used. Second, multivariate modeling was used. For each of the 6 categories of measurement, if at least one independent variable showed a significant bivariate relationship, the variable with the strongest correlation in that category was advanced into a stepwise forward multivariate model (using p=0.1 to enter, p= 0.15 to leave).

Correlates of impairment among patients with mild deficits: Analyses were repeated examining only those patients in the top quartile of FM scores (FM>47).

Correlates of disability (activities limitations): Analyses were repeated using a disability-based dependent measure, mRS score, which was dichotomized as none-slight disability (mRS score=0-2) or moderate-severe disability (mRS score>2).

Results

Patients

Motor impairment was on average moderate-severe (FM=35.0±14.5, mean±SD), with values spanning a wide range (FM scores 14-60). A wide range of disability was also present (mRS scores 1-4), with scores being 0-2 in 69% of patients and >2 in 31% of patients. One patient was excluded from DTI analysis because the stroke directly injured the region of interest within the cerebral peduncle (leaving n=35 for DTI analyses), seven patients were excluded from fMRI analyses due to excessive task-related head motion during scanning (leaving n=29 for fMRI analyses), and a contraindication to TMS (generally medication-related) was present in 16 patients (leaving 20 for neurophysiology analyses).

Demographics/history: Features for the 36 patients are presented in Table 4.2.
Cognitive/mood status: Overall, patients were not cognitively impaired or depressed (Table 3.2).

Genetics: Genotype frequencies were in Hardy-Weinberg equilibrium. The BDNF val<sup>66</sup>met polymorphism and ApoE4 allele were present in 31% and 22% of patients, respectively.

Neurophysiology: The 20 patients able to undergo TMS did not significantly differ from the 16 patients who could not in terms of age, FM score, or time post-stroke. An MEP could be elicited in only 5 of the 20 patients, and so TMS findings are presented dichotomously (MEP present/absent), as insufficient data were available to analyze the continuous TMS measure MEP amplitude.

Table 4.2. Patient characteristics.

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<tr>
<td>Age (years)</td>
<td>58.4 ± 13.8 (21 - 86)</td>
</tr>
<tr>
<td>Gender</td>
<td>10 F/ 26 M</td>
</tr>
<tr>
<td>Time post-stroke (months)</td>
<td>4.4 ± 1.1 (2.5 - 6.0)</td>
</tr>
<tr>
<td>Handedness</td>
<td>2 L/ 34 R</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>10 Y/ 26 N</td>
</tr>
<tr>
<td>Hypertension</td>
<td>18 Y/ 18 N</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>16 Y/ 20 N</td>
</tr>
<tr>
<td>Fugl-Meyer arm motor score</td>
<td>35.0 ± 14.5 (14-60)</td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>28 [25-30]</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>2.5 [1.25-4.75]</td>
</tr>
<tr>
<td>BDNF val&lt;sup&gt;66&lt;/sup&gt;met polymorphism present</td>
<td>11 Y/ 25 N</td>
</tr>
<tr>
<td>ApoE4 allele present</td>
<td>8 Y/ 28 N</td>
</tr>
<tr>
<td>Side of stroke</td>
<td>19 L/ 17 R</td>
</tr>
<tr>
<td>Infarct volume (cc)</td>
<td>31.7 ± 48.6 (0.5 - 178)</td>
</tr>
</tbody>
</table>

* For the 36 individuals with stroke, values presented are mean ± SD (range) or median [IQR]. For the FM scale, normal score is 66; for MMSE, 30; for both, higher score is better. For the Geriatric Depression Scale, normal score is 0 and lower score is better.
**Brain injury:** Infarct volumes were moderate on average (31.7±48.6 cc) and spanned a wide range (0.5-178 cc). The infarcts involved M1 in 42%, PMd in 33%, and any cortical gray matter in 72% of patients (among whom volume of cortical injury averaged 26.8±37.2 cc). Extensive CST injury was present by both methods. First, lesion-CST overlap was 51.6±32%, indicating substantial CST injury, with the full range of values (0%-100%) present. Second, DTI-derived values for mean FA within ipsilesional cerebral peduncle were lower compared to contralesional peduncle (0.37±0.12 vs. 0.56±0.10, p<0.0001), indicating reduced ipsilesional CST integrity.

**Cortical function:** Paretic hand movement was generally associated with bilateral motor system activation. Ipsilesionally, significant activation was present within M1 in 93%, and within PMd in 90%, of patients; contralesionally, M1 activation was present in 90%, and PMd in 100% of patients. Functional activation in ipsilesional M1 was larger than in contralesional M1 (p<0.004) or ipsilesional PMd (p<0.002), whether examining beta estimates or activation volumes. Overall, activation was lateralized towards the ipsilesional hemisphere for M1 (LI=0.41±0.85) and contralesional hemisphere for PMd (LI=-0.19±0.87).

**Correlates of impairment**

In 4 of the 6 assessment categories (demographics/medical history, cognitive/mood, neurophysiology, and brain injury), bivariate screening found a single independent variable to be significantly associated with FM score (Table 4.3): greater impairment (lower FM score) was associated with presence of hypertension, poorer cognitive status, lower CST integrity (Figure 4.1A), and absence of MEP (Figure 4.1B). In 2 of the 6 categories (genetics and cortical function), no independent variable showed a significant bivariate relationship with impairment (negative data can be found in Appendix 1); the latter remained true when fMRI analyses were repeated excluding the 12 patients with direct stroke-related injury to M1 and/or PMd.
The 4 independent variables identified in bivariate screening were advanced into a multivariate model, where 2 of these (MEP absent and lower CST integrity by DTI) survived as significant predictors of greater impairment in the final model ($r^2=0.71$, $p<0.0001$). Note that these two independent variables were not correlated ($p>0.1$).

**Correlates of impairment among patients with mild deficits**

Analyses were repeated examining only the 9 patients in the top quartile of FM scores (FM score $>47$). In bivariate screening, only one category had an independent variable significantly related to FM score, cortical function, where 3 instances were present: greater impairment was associated with a higher beta estimate in contralesional M1 ($p=0.017$), higher beta estimate in contralesional PMd ($p=0.02$), and greater lateralization of PMd activation towards the contralesional hemisphere ($p=0.0496$). Because all significant independent variables in bivariate analyses were from the same category, multivariate modeling was not pursued.
Table 4.3. Bivariate correlations of variables with motor impairment and disability.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Correlation with greater impairment (lower FM score)</th>
<th>Correlation with greater disability (higher mRS score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics/medical history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.005</td>
<td>0.08</td>
</tr>
<tr>
<td>Time post-stroke</td>
<td>0.25</td>
<td>0.14</td>
</tr>
<tr>
<td>Gender</td>
<td>0.08</td>
<td>0.66</td>
</tr>
<tr>
<td>Diabetes mellitus (Y/N)</td>
<td>0.15</td>
<td>0.41</td>
</tr>
<tr>
<td>Hypertension (Y/N)</td>
<td>-0.49</td>
<td>0.0032</td>
</tr>
<tr>
<td>Hypercholesterolemia (Y/N)</td>
<td>-0.16</td>
<td>0.34</td>
</tr>
<tr>
<td>Cognitive/mood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>-0.34</td>
<td>0.048</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>0.11</td>
<td>0.51</td>
</tr>
<tr>
<td>Genetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF val&lt;sup&gt;66&lt;/sup&gt; met polymorphism present (Y/N)</td>
<td>-0.08</td>
<td>0.57</td>
</tr>
<tr>
<td>ApoE4 allele present (Y/N)</td>
<td>-0.09</td>
<td>0.55</td>
</tr>
<tr>
<td>Neurophysiology (n=20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of motor evoked potential (Y/N)</td>
<td>-0.74</td>
<td>0.003</td>
</tr>
<tr>
<td>Brain injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarct volume (cc)</td>
<td>0.27</td>
<td>0.12</td>
</tr>
<tr>
<td>M1 injury (Y/N)</td>
<td>0.26</td>
<td>0.14</td>
</tr>
<tr>
<td>M1 injury (cc)</td>
<td>0.30</td>
<td>0.08</td>
</tr>
<tr>
<td>PMd injury (Y/N)</td>
<td>0.19</td>
<td>0.25</td>
</tr>
<tr>
<td>PMd injury (cc)</td>
<td>0.18</td>
<td>0.30</td>
</tr>
<tr>
<td>Total cortical injury (cc)</td>
<td>0.26</td>
<td>0.12</td>
</tr>
<tr>
<td>Corticospinal tract integrity (ipsilesional FA)</td>
<td>-0.60</td>
<td>0.0001</td>
</tr>
<tr>
<td>Percent injury to CST (lesion overlap with CST)</td>
<td>0.27</td>
<td>0.11</td>
</tr>
<tr>
<td>Cortical function (n=29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilesional M1 activation - beta estimate</td>
<td>-0.01</td>
<td>0.94</td>
</tr>
<tr>
<td>Ipsilesional PMd activation - beta estimate</td>
<td>-0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Contralesional M1 activation - beta estimate</td>
<td>0.30</td>
<td>0.12</td>
</tr>
<tr>
<td>Contralesional PMd activation - beta estimate</td>
<td>0.04</td>
<td>0.82</td>
</tr>
<tr>
<td>Ipsilesional M1 activation volume</td>
<td>-0.10</td>
<td>0.60</td>
</tr>
<tr>
<td>Ipsilesional PMd activation volume</td>
<td>-0.23</td>
<td>0.22</td>
</tr>
<tr>
<td>Contralesional M1 activation volume</td>
<td>0.16</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------</td>
<td>-----</td>
</tr>
<tr>
<td>Contralesional PMd activation volume</td>
<td>-0.02</td>
<td>0.93</td>
</tr>
<tr>
<td>Activation laterality in M1</td>
<td>-0.18</td>
<td>0.39</td>
</tr>
<tr>
<td>Activation laterality in PMd</td>
<td>-0.30</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Correlation between independent variable and outcome measure ($^a P<0.05$, $^b P<0.01$, $^c P<0.001$).

M1=primary motor cortex, PMd=dorsal premotor cortex, FA=fractional anisotropy.
Figure 4.1. Across all patients, the strongest bivariate relationships found with greater impairment (lower FM score) were [A] a measure of injury, lower CST integrity by FA ($r=0.60$, $p=0.0001$); and [B] a measure of neurophysiology, presence of MEP ($r=0.74$, $p=0.003$). These two measures remained significant in multivariate modeling.
Correlates of disability (activities limitations)

When the 6 categories of independent variables were examined in relation to disability (mRS score), rather than impairment, a different pattern of findings emerged (Table 4.3). Bivariate screening identified independent variables significantly associated with mRS score in three categories: demographics/medical history (one instance), cognitive/mood (one instance), and brain injury (three instances). Specifically, greater disability (higher mRS score) was associated with presence of diabetes mellitus, poorer cognitive status (lower MMSE score), larger infarct volume, presence of M1 injury, larger cortical injury, and lower CST integrity by DTI.

The most significant independent variable from each of the 3 categories identified in bivariate screening was advanced into a multivariate model. Two of these variables, poorer cognitive status and lower CST integrity, survived as significant predictors of greater disability in the final model ($r^2=0.42$, $p=0.0001$). Note that these two independent variables were not correlated ($p>0.1$).

Discussion

Stroke is a very heterogeneous disease, and so not surprisingly numerous measures have been found to be associated with behavioral outcome. The current study used a multimodal approach to determine which measures are most strongly related to outcome by examining six categories in parallel. Greater impairment was most strongly related to measures of injury (lower CST integrity) and neurophysiological status (absent MEP), a pattern overall consistent with preclinical studies. Results differed when examining only patients with mild deficits, where measures of cortical function were most important, or when examining correlates of disability, where poorer cognitive status emerged as important. The correlates of outcome after stroke vary according to the patient subgroup or behavioral endpoint studied, a finding that may be important
to many aspects of clinical trial design such as choice of entry criteria, stratifying variable, or biomarker.

Motor impairment was most strongly related to injury and neurophysiology. The injury measure, CST integrity using the DTI-based measure FA, reflects reduction in the directionality of water diffusion after stroke [122]. The correlation between lower CST integrity (lower FA) and poorer motor outcome (lower FM score) is consistent with prior studies [138, 247]. The neurophysiology measure, loss of MEP, reflects reduced function across motor cortex, CST, and motor unit [12]. The correlation between absent MEP and poorer motor outcome after stroke has also been reported [248]. The finding that CST integrity and neurophysiological status are the two factors mostly strongly related to motor impairment after stroke is in direct agreement with a study by Stinear et al [249] that examined 4 of the 6 current measurement categories in 21 patients with chronic stroke. Measures of brain injury and of neurophysiology provide complementary insights after stroke: CST integrity by DTI was not correlated with neurophysiological status in the current cohort, or in other populations [250, 251]; both measures remained significant in the multivariate model; and the multivariate model explained substantially more variance in motor impairment ($r^2=0.72$) than did either measure alone (DTI: $r^2=0.31$; neurophysiology: $r^2=0.55$). The current results are concordant with findings in the preclinical literature [20, 131], which emphasize that behavioral outcome after stroke is related to both extent of stroke injury and degree of residual function.

In this study, three variables that have previously been correlated with motor impairment did not exhibit such a relationship. First, infarct volume did not correlate with motor impairment, as has been described in prior studies [252, 253], possibly due to differences in study populations or procedures, and moreover did correlate with disability. A global measure of injury such as infarct
volume does not provide any information about the lesion location, and so might not be expected to provide precise insight into the functional state of any one neural system such as the motor system, but would be expected to correlate with global outcome measures. On the other hand, a motor-system specific injury measure such as CST integrity does provide specific insights into injury location, and would be expected to correlate with motor status (FM score), as per Figure 3.1 as well as previously published reports [149, 150, 254]. Together, the current constellation of findings support the idea that a global measure of injury (infarct volume) correlates more strongly with a global outcome measure (mRS score), while a neural system-specific measure of injury (CST integrity) correlates more strongly with an outcome measure specific to that system (arm motor FM score) [255]. Second, patients' depression scores also did not correlate with motor impairment. Prior studies have shown that post-stroke depression negatively impacts outcome [196, 200]. However, the primary reason depression was not related to motor impairment is likely because the patient sample herein was not, on average, depressed (median GDS = 2.5, normal = 0). Also, the demands placed upon a patient to enroll then participate in the study may have introduced an ascertainment bias against subjects suffering from depression. Third, regional measures of motor cortex activation were not correlated with motor impairment in all 29 patients, nor when the 12 patients with ipsilesional M1/PMd damage were excluded (to see if the latter patients were obscuring a relationship). In cross-sectional neuroimaging studies of stroke recovery greater magnitude and larger volume of activation in primary and secondary motor areas correlate with greater post-stroke motor impairment [166, 171]. One possibility is that we were underpowered to detect a relationship (see Appendix 4). However, we did detect a relationship between cortical activation and motor impairment in the smaller, less impaired cohort suggesting it was not due to low power.
The current results emphasize that the measures most related to behavioral outcome after stroke vary across patient subgroups. When analyzing only those patients with mild deficits, greater impairment correlated with measures of cortical function (larger contralesional activation), in contrast with results when analyzing all patients, where impairment was related to injury and neurophysiology rather than cortical function. Given that greater motor impairment after stroke has a well-established relationship with increased activation in contralesional motor areas [166, 175], why in the current study did this relationship only emerge in the subgroup with mild motor impairment? The answer may rest with the fact that many previous studies have preferentially enrolled populations at the mild end of the impairment spectrum [232] or with small infarcts [256, 257]. This divergence of findings across patient subgroups suggests that restorative stroke trials might benefit from use of sliding outcomes [236], whereby the definition of treatment success differs across patient subgroups. Furthermore, the current results suggest that in restorative stroke trials, a singular approach to choosing stratification variables [258] or biomarkers [230] may be unwise.

The factors most strongly related to outcome also varied across WHO ICF dimensions. Using disability as the dependent measure rather than impairment resulted in a different constellation of findings: greater disability was associated with poorer cognitive status and reduced CST integrity. The association between greater disability and poorer cognitive status by MMSE is consistent with prior studies [259]. The current findings extend this observation and support recent models suggesting that functional status after stroke results from an interaction between cognitive status and motor system injury [260]. Multivariate modeling explained much less variance in disability ($r^2=0.42$) than in impairment ($r^2=0.71$), emphasizing that numerous complex factors influence disability after stroke. Finally, the incomplete correspondence between
variables in the disability model and the impairment model is consistent with the fact that there is not a 1:1 relationship across WHO ICF dimensions [233].

Several limitations exist with the current study. The central results reflect correlation rather than causation, and the potential impact of mediating variables is difficult to estimate. Limited statistical power might have affected some analyses such as genetics, where very large sample sizes are customarily used to clearly identify relationships (see Appendix 4 for power calculations and the 95% confidence interval). The BDNF val66met polymorphism and the ApoE4 allele have each been shown to correlate with stroke recovery [16, 214, 228], and each is also linked to important plasticity-related processes [216, 225, 261]. A cross-sectional study, such as the current one, measures final outcome whereas a longitudinal study captures the actual extent of recovery and so might better elucidate behavioral relationships with these plasticity-related genotypes. It is also noted that safety precautions precluded data collection in some patients, leading to a small subset of patients with neurophysiology data.

The current findings indicate that tissue injury and residual function are each related to motor outcome after stroke. Importantly, these relationships vary when examining different patient subgroups or when using an outcome measure from a different WHO ICF dimension. These findings may be instructive for the design of restorative stroke trials. Results also support the value of a multimodal approach for understanding outcomes after stroke [262]. Finally, the current findings support a body of data [258] suggesting that a measure of injury to a specific neural system (e.g., CST integrity) might be useful for distinguishing patient subgroups on a biological basis.
CHAPTER 5

Neural function, injury, and stroke subtype predict treatment-induced behavioral gains after stroke

Abstract

The effect of a restorative therapy given after stroke is highly variable, emphasizing the need for improved methods to predict the response of individual patients. Preclinical studies suggest that 3 types of measurements are useful in this regard: neural function, injury, and clinical status. The current study hypothesized that a multivariate approach that incorporates all 3 measurement types would have the greatest predictive value. Patients 3-6 months post-stroke underwent a multimodal battery of baseline assessments prior to receiving a 3-week course of standardized robotic therapy targeting the upper extremity. A secondary hypothesis explored the effect of stroke subtype (lacunar versus non-lacunar). Candidate predictors spanned 7 baseline assessment categories covering various measures of neural injury (brain injury), neural function (cortical function and cortical connectivity), and clinical status (demographics/medical history, cognitive/mood, impairment), plus genetics. Across all 29 patients (8 females; mean age 57±14 years), bivariate screening found significant predictors of treatment gains in three categories, brain injury, cortical function, and cortical connectivity, with less corticospinal tract (CST) injury, greater ipsilesional motor cortex (M1) activation, and greater interhemispheric M1-M1 connectivity each predicting larger treatment gains. These three variables were advanced into a multivariate model, where CST injury and M1-M1 connectivity survived as significant ($r^2=0.44$, $p=0.002$). Results were independently confirmed using an alternate statistical approach (Lasso regression). Findings varied according to stroke subtype: for patients with lacunar stroke (n=8),
treatment gains were best predicted by a measure of intrahemispheric connectivity, whereas in patients with non-lacunar stroke (n=21) gains again were predicted by corticospinal tract injury and interhemispheric connectivity. The results indicate that measures of neural injury and neural function are each useful for predicting gains from rehabilitative training, emphasize the value of a multimodal approach, and suggest that lesion-specific strategies may be useful to optimize gains from a restorative therapy.

**Introduction**

Stroke remains a leading cause of adult disability in the United States [1]. A number of different therapeutic strategies exist to improve patient outcomes after stroke. Acute reperfusion therapies aim to limit acute injury but currently can be offered to only a small fraction of patients [5]. A second strategy focuses on restorative therapies, which do not limit extent of stroke injury but instead aim to promote favorable neuroplasticity and thereby reduce disability [7, 263]. Restorative therapies do not share the narrow treatment time window present with acute reperfusion strategies, suggesting the potential to access a higher fraction of patients with stroke [264].

Stroke is a very heterogeneous diagnosis, which translates to substantial variability between patients in their response to therapy. This extends to clinical trials, where higher inter-subject variance means reduced study power and increased cost; on this, Bath et al noted: “In stroke trials, the impact of covariates such as age and severity on outcome is typically much larger than the treatment effect that is being measured [8].” This issue has undergone considerable discussion in relation to acute stroke trials, where acute CT and MRI techniques are under study to improve prediction as to which patients are most likely to benefit from reperfusion therapy.
The issue offers a similar challenge in the setting of restorative therapies, as the best measures for predicting treatment response remain undetermined [266].

Efforts to identify predictors of response to a restorative treatment in humans are informed by preclinical studies [67]. In studies of animals with experimental stroke, behavioral improvement following administration of a restorative therapy has been linked to a number of growth-related events in the brain, including axonal outgrowth, increased dendritic branching, and release of growth factors [263, 267, 268]. Such findings have been consistent and robust across numerous classes of restorative therapies, including amphetamine [45], inosine [269], atorvastatin [270], neurotrophic factors [47, 271, 272], drugs that reduce inhibitory signals [273], brain stimulation [274, 275], stem cells [51, 276, 277], environmental enrichment [91, 278], and intensive skill training [88, 95, 279]. Animal studies have also identified a number of measures that predict response to a restorative therapy. Among restorative therapies targeting the motor system after stroke, predictive measures can be grouped into three categories: (1) neural injury, such as infarct topography [280, 281] and infarct volume [280-282]; (2) neural function, such as motor cortex integrity [20, 54]; and (3) clinical status, such as baseline behavioral assessments [54], age [267, 283, 284], and vascular risk factors [267].

Predictors of response to a restorative therapy after stroke have also been identified in human subjects. These can also be grouped into measures of: (1) neural injury, such as extent of injury to white or gray matter [150, 249, 258, 285, 286]; (2) neural function, such as functional activation [287-290], functional connectivity [80, 291], and neurophysiological status [285, 290]; and (3) clinical measures such as demographics [292] or baseline behavioral status [249, 288, 293, 294]. Genetic variation may also be important for predicting response to a restorative therapy but limited data exist [228, 295, 296]. Furthermore, stroke subtype is important to many
aspects of clinical decision-making after stroke [297, 298], but this issue has received limited attention in regard to gains from a restorative therapy.

The number and diversity of predictive measures reflects the complexity of neural repair after stroke. Preclinical studies emphasize that training-induced behavioral gains after stroke depend on CNS plasticity, which is influenced by degree of neural injury and neural function [54, 284]. Optimal prediction of response to a restorative therapy may therefore require a multivariate approach that assesses both injury and function [230, 299]. However, only a few human studies have taken this approach [249, 258, 288, 290, 300], and none has taken a multivariate approach that included measures of neural injury, functional activation, functional connectivity, and genetics, along with clinical and demographic measures, in a heterogeneous stroke population.

The current study addressed these issues, with the primary hypothesis being that pre-therapy measures of neural injury, neural function, and clinical status together would have greater power for predicting response to a restorative therapy as compared to any single measure. As per the evidence provided in Chapter 3, we hypothesized that older age, presence of diabetes mellitus, hypertension, hypercholesterolemia, post-stroke depression, presence of the BDNF val^{66}met polymorphism and ApoE4 allele, would be related to smaller motor gains from robotic therapy. An additional hypothesis examined was that the best predictors of treatment gains would differ according to stroke subtype; lacunar vs. non-lacunar strokes were compared given substantial differences in pathophysiology and outcomes [301-307].
Methods

Patients

Forty-one chronic stroke patients gave informed consent to be part of a longitudinal study of standardized robotic-assisted hand therapy (clinicaltrials.gov ID# NCT01244243). Inclusion and exclusion criteria can be found in Table 4.1. They focused on capturing a population at a specific time point in stroke recovery (close to the time when spontaneous motor recovery is complete [4, 308]), with confirmed plateau in arm motor status, and within a prescribed range of motor deficits. In one patient, baseline MRI revealed an incidental finding that met exclusion criteria, rendering the patient ineligible for the study. The 40 eligible patients were studied as below.

Baseline assessments were organized into 7 categories: 1) demographics/medical history; 2) cognitive/mood; 3) genetics; 4) impairment; 5) brain injury; 6) cortical function; and 7) cortical connectivity. A complete list of the baseline assessments can be found in Table 5.1.

Robotic Therapy

Patients underwent twelve treatment sessions of robotic hand therapy (Figure 5.1) over three weeks: 2 hours/day, 4 days/week for a total of 24 hours. All patients completed at least 11 of 12 robotic therapy sessions. Therapy consisted of repeated grasp-release ('close' and 'open') movements of the affected hand and wrist using a pneumatically-actuated robotic device that has been described previously [77]. The average number of daily repetitions across each of the twelve sessions was 954 for the fingers; 2,579 for the thumb; and 1,298 for the wrist.
Demographics/medical history

Medical history was obtained and hospitalization records were reviewed.

Cognitive/mood

A single rater performed all behavioral assessments, which included the Mini Mental State Exam and Geriatric Depression Scale.

Impairment

Patients underwent behavioral assessments before and 1-month after a 3-week course of standardized robotic hand therapy. Baseline Fugl-Meyer (FM) & Action Research Arm Test (ARAT) assessments were performed twice before beginning therapy (once at baseline screening and again 1-3 weeks later) to ensure stability of motor status (e.g., the second FM score was required to be within 3 points of the first score), and averaged for each patient.
**Genetics**

A blood sample was obtained at the second baseline visit. Presence of the brain derived neurotrophic factor (BDNF) val^{66}met polymorphism and the apolipoprotein E4 (ApoE4) allele were determined as described previously [228].

**Brain injury**

*Image acquisition:* Magnetic resonance imaging was performed at the second baseline visit and acquired using a 3.0T Philips Achieva system. Anatomical imaging consisted of a high-resolution T1-weighted image, T2-FLAIR, and diffusion tensor imaging (DTI) as described in Chapter 4.

*Image analysis:* Image analysis was performed blinded to clinical data. Three classes of brain injury metrics were extracted: (1) total brain injury (infarct volume); (2) gray matter injury (to primary motor cortex (M1), dorsal premotor cortex (PMd), and total cortical injury); and (3) white matter injury (percent lesion overlap with corticospinal tract (CST), or CST integrity within ipsilesional cerebral peduncle using DTI).

*Infarct volume:* Infarct volume was calculated as described in Chapter 4.

*Gray matter injury:* Gray matter injury to M1 (i.e., precentral gyrus), PMd cortex, and the entire cerebral cortex was calculated as described in Chapter 4.

*White matter (CST) injury:* White matter integrity calculated from DTI and the percent of overlap between patients' lesions and the corticospinal tract were calculated as described in Chapter 4.
Cortical function

*Image acquisition:* Three runs of blood oxygenation level-dependent (BOLD) images were acquired for fMRI as described in Chapter 4.

*Image analysis:* Two measures of brain function were extracted from fMRI images: (1) activation beta (contrast) estimate and (2) activation volume, each measured in four ROIs, right and left M1 and PMd as described in Chapter 4.

Cortical connectivity

Functional connectivity was assessed as the temporal correlation of the hemodynamic response between regions. After the fMRI data were preprocessed in SPM8, intra- and inter-hemispheric functional connectivity metrics were calculated using the Conn toolbox [309]. The time courses were filtered between 0.008 and 0.13 to minimize low-frequency drift and high-frequency noise. Within-subject realignment parameters, outliers (as identified in above paragraph), and main session effects were included as first-level covariates. The three connections evaluated for functional connectivity were ipsilesional M1-ipsilesional PMd (iM1-iPMd), ipsilesional M1-contralesional M1 (iM1-cM1), and ipsilesional M1-contralesional PMd (iM1-cPMd). The Fisher-transformed correlation coefficients were extracted for each connection in all patients.

Statistical analyses

*Predictors of treatment-induced behavioral gains - bivariate screening:* Bivariate screening was performed to determine the strongest predictors of motor gains. The primary outcome measure was arm motor gains from baseline to one month post-therapy, defined by combining change measures of impairment (change in upper extremity FM from baseline to one month post-
therapy) and of function (change in ARAT from baseline to one month post-therapy) via principal component analysis (PCA) [166]; only the first component was used, as this accounted for 84% of the variance in these two behavioral outcomes. To characterize baseline arm motor status, a separate PCA was performed on baseline mean FM and ARAT scores, where the first component accounted for 98% of the variance.

Bivariate screening determined the significance of the relationship between each baseline measure and the dependent measures (treatment-induced arm motor gains). Parametric statistical methods were used for measures that were normally distributed or could be transformed to a normal distribution otherwise non-parametric methods were used. Continuous variables were evaluated using Pearson's correlation coefficient or Spearman's rank order, while categorical variables were evaluated using Student's t-test or the Wilcoxon signed-rank test. All analyses were two-tailed with alpha=0.05 and used JMP-8 software (SAS Institute, Inc., Cary, NC). For each of the seven categories, results of this bivariate screening determined whether any baseline measure survived as a predictor of treatment gains and would be advanced to multivariate modeling.

Secondary statistical analysis used a principal component of our main predictor variables of interest [166]. Several permutations of PCA were generated from the brain injury and function categories (the only categories with significant variables from bivariate analyses). Also, motor gains were evaluated as the percent of the change in FM score.

**Predictors of treatment-induced behavioral gains - multivariate modeling:** The primary predictive model used a forward stepwise multivariate linear regression approach (0.1 to enter, 0.15 to leave the model), advancing the most significant predictors identified in bivariate screening. The strongest bivariate predictor within each of the 7 categories was advanced as long
as bivariate screening showed \( p < 0.1 \). If a category had more than one variable with \( p < 0.1 \) during bivariate screening, only the variable with the strongest correlation coefficient was advanced into the stepwise forward model. In order to understand whether results vary according to stroke subtype, the above analyses were repeated separately for the 8 patients with a lacunar infarct (defined as an infarct that is subcortical and has infarct volume \(< 4 \text{cc} \) [310]) and for the 21 patients with a non-lacunar infarct.

*Predictors of treatment-induced behavioral gains - Lasso regression method:* To confirm findings from bivariate screening, a secondary approach to predictive modeling used a penalized regression approach. A very large number of variables has been reported to influence stroke outcomes [15, 16, 191, 195, 200]. The statistical question is therefore high-dimensional, suggesting the utility of a secondary approach that used a "penalized regression" (or "regularization" [311]) method for predictive modeling, which (a) makes conclusions based on joint consideration of all variables simultaneously, and (b) reduces overfitting (i.e. the situation in which a model performs well on the current dataset but does not generalize well to new datasets). In particular, Lasso (least absolute shrinkage and selection operator [312]) was chosen as the penalized regression model because it selects a subset of useful predictors from a total pool of candidate predictors and, unlike linear regression, minimizes the influence of outliers. Specifically, a group Lasso model was used, which is similar to the standard Lasso regression but takes the grouping of variables (i.e., categories) into account [313]. The Lasso procedure requires a tuning parameter, lambda, which was chosen in a standard way through (five-fold) cross-validation. All numerical variables were standardized before running the Lasso model.
Results

Patient characteristics

Data from 29 patients were available for analysis. Of the 40 patients enrolled, four could not complete MRI due to claustrophobia and 7 patients were excluded due to excessive head motion during fMRI scanning. These 29 patients did not differ significantly from the other eleven patients in age or baseline FM (p>0.1). The stroke was ischemic in 27 patients and hemorrhagic in 2.

Demographics/medical history: Across all patients at baseline (Table 5.1) they were, on average, 4.3 months post-stroke and 56.5 years of age. All but two were right-handed.

Cognitive/mood: Overall, patients were not cognitively impaired nor were they clinically depressed.

Genetics: The genotypic frequencies for the BDNF val^66met polymorphism and the ApoE4 allele were in Hardy-Weinberg equilibrium, and these polymorphisms were present in 28% and 14% of patients, respectively.

Impairment: Overall, patients had mild global impairment and moderate to severe motor impairment in the upper extremity.
Table 5.1. Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Non-lacunar</th>
<th>Lacunar</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>29</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td><strong>Demographics/medical history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.5 (13.9)</td>
<td>55.3 (13.9)</td>
<td>55.3 (5.4)</td>
</tr>
<tr>
<td>Time post-stroke (months)</td>
<td>4.3 (1.0)</td>
<td>4.2 (1.0)</td>
<td>4.5 (0.9)</td>
</tr>
<tr>
<td>Gender</td>
<td>8 F/ 21 M</td>
<td>7 F/ 14 M</td>
<td>1 F/ 7 M</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8 Y/ 21 N</td>
<td>6 Y/ 15 N</td>
<td>2 Y/ 6 N</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 Y/ 15 N</td>
<td>8 Y/ 13 N</td>
<td>6 Y/ 2 N</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>12 Y/ 17 N</td>
<td>6 Y/ 15 N</td>
<td>6 Y/ 2 N</td>
</tr>
<tr>
<td><strong>Cognitive/mood</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geriatric Depression Scale (normal = 0)</td>
<td>3.0 [1.5-4.5]</td>
<td>2 [0.5-4]</td>
<td>3 [2-6.75]</td>
</tr>
<tr>
<td><strong>Impairment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIH Stroke Scale (normal = 0)</td>
<td>4.2 (1.8)</td>
<td>4.4 (1.7)</td>
<td>3.5 (1.9)</td>
</tr>
<tr>
<td>Fugl-Meyer Scale (normal = 66)</td>
<td>36.3 (14.8)</td>
<td>35.7 (16.1)</td>
<td>37.7 (11.7)</td>
</tr>
<tr>
<td>Action Research Arm Test (normal = 57)</td>
<td>26.2 (18.8)</td>
<td>25.7 (20.2)</td>
<td>27.5 (15.9)</td>
</tr>
<tr>
<td>Nottingham Sensory Assessment (normal = 17)</td>
<td>13.5 (4.3)</td>
<td>12.2 (4.4)</td>
<td>16.9 (0.4)</td>
</tr>
<tr>
<td><strong>Genetics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF val&lt;sup&gt;66&lt;/sup&gt;met polymorphism present</td>
<td>8 Y/ 21 N</td>
<td>5 Y/ 16 N</td>
<td>3 Y/ 5 N</td>
</tr>
<tr>
<td>ApoE4 allele present</td>
<td>4 Y/ 25 N</td>
<td>2 Y/ 19N</td>
<td>2 Y/ 6 N</td>
</tr>
<tr>
<td><strong>Brain injury</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarct volume (cc)</td>
<td>32.6 (48.4)</td>
<td>44.6 (52.3)</td>
<td>1.2 (0.6)</td>
</tr>
<tr>
<td>Precentral gyrus injury?</td>
<td>14 Y/ 15 N</td>
<td>14 Y/ 7 N</td>
<td>0 Y/ 8 N</td>
</tr>
<tr>
<td>Precentral gyrus injury (cc)</td>
<td>0.61 (1.1)</td>
<td>0.85 (1.2)</td>
<td>0</td>
</tr>
<tr>
<td>Cortical PMd injury?</td>
<td>10 Y/ 19 N</td>
<td>10 Y/ 11 N</td>
<td>0 Y/ 8 N</td>
</tr>
<tr>
<td>Cortical PMd injury (cc)</td>
<td>0.25 (0.52)</td>
<td>0.34 (0.58)</td>
<td>0</td>
</tr>
<tr>
<td>Cortical injury?</td>
<td>21 Y/ 8 N</td>
<td>21 Y/ 0 N</td>
<td>0 Y/ 8 N</td>
</tr>
<tr>
<td>Total cortical injury (cc)</td>
<td>19.8 (33.6)</td>
<td>27.3 (69.9)</td>
<td>0</td>
</tr>
<tr>
<td>Corticospinal tract integrity (DTI FA)</td>
<td>0.39 (0.12)</td>
<td>0.39 (0.13)</td>
<td>0.36 (0.08)</td>
</tr>
<tr>
<td>Percent injury to CST</td>
<td>54.5 (32.6)</td>
<td>61.2 (34.2)</td>
<td>35.2 (18.0)</td>
</tr>
<tr>
<td><strong>Cortical function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Values presented are mean ± SD (range) or median [IQR]. M1 = primary motor cortex, PMd = dorsal premotor cortex, iM1 = ipsilesional M1, cM1 = contralesional M1, iPMd = ipsilesional PMd, cPMd = contralesional PMd.

<table>
<thead>
<tr>
<th></th>
<th>iM1 activation - contrast estimate</th>
<th>iPMd activation - contrast estimate</th>
<th>cM1 activation - contrast estimate</th>
<th>cPMd activation - contrast estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.9 (1.9)</td>
<td>2.6 (1.9)</td>
<td>3.7 (1.7)</td>
<td>2.3 (1.0)</td>
</tr>
<tr>
<td>iM1 activation volume</td>
<td>67 (42)</td>
<td>64 (44)</td>
<td>72 (38)</td>
<td></td>
</tr>
<tr>
<td>iPMd activation volume</td>
<td>43 (46)</td>
<td>40 (46)</td>
<td>49 (47)</td>
<td></td>
</tr>
<tr>
<td>cM1 activation volume</td>
<td>39 (41)</td>
<td>38 (42)</td>
<td>42 (42)</td>
<td></td>
</tr>
<tr>
<td>cPMd activation volume</td>
<td>49 (42)</td>
<td>45 (44)</td>
<td>45 (41)</td>
<td></td>
</tr>
</tbody>
</table>

**Cortical connectivity**

<table>
<thead>
<tr>
<th></th>
<th>iM1-iPMd correlation coefficient</th>
<th>iM1-cM1 correlation coefficient</th>
<th>iM1-cPMd correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.31 (0.32)</td>
<td>0.28 (0.30)</td>
<td>0.38 (0.38)</td>
</tr>
<tr>
<td></td>
<td>0.13 (0.30)</td>
<td>0.14 (0.30)</td>
<td>0.13 (0.31)</td>
</tr>
<tr>
<td></td>
<td>0.10 (0.21)</td>
<td>0.11 (0.19)</td>
<td>0.07 (0.26)</td>
</tr>
</tbody>
</table>

* Brain injury: Infarct volumes were moderate overall. The stroke affected the left hemisphere in 55% of patients; the dominant hemisphere, in 48%. Approximately one-third of patients had injury to PMd cortex and 48% of patients had injury to M1; among these patients, the infarct overlap was greater with cortical M1 than with PMd. The extent of CST injury, as determined by lesion overlap, showed substantial variability across patients (55±33%, range 0-100%). Similar results were obtained when determining extent of CST injury using DTI, with mean FA in the affected cerebral peduncle being 0.38±0.12, significantly (p<0.0001) lower than unaffected cerebral peduncle values (0.55±0.1), indicating reduced integrity within the ipsilesional CST.

* Cortical function: Patients varied in the quality of performance of requested movements during the active blocks of fMRI scan acquisition, with full range of motion present in 38%, partial movement in 51%, and no visible movement (due to weakness) in 11%; in addition, 24%
of patients had at least one mirror movement in the non-affected hand. However, none of the fMRI activation measures differed significantly according to presence vs. absence of these observations.

Unilateral grasp/release movement of the paretic hand was associated with prominent bilateral activation within M1 and PMd. Activation contrast estimates within ipsilesional M1 were larger than within contralesional M1 (p=0.0002). Activation volumes were larger within ipsilesional M1 than within contralesional M1 (p=0.005), as well as within ipsilesional PMd (p=0.002), and contralesional PMd (p=0.03).

Magnetic resonance angiograms were available in 23 patients and disclosed significant ipsilesional disease of the internal carotid artery or middle cerebral artery in 26% of patients; none of the fMRI activation parameters differed significantly according to presence vs. absence of these cerebrovascular observations.

*Cortical connectivity:* During unilateral grasp/release of the paretic hand, functional connections were present in all three ipsilesional M1 connections (iM1-iPMd, iM1-cM1, iM1-cPMd). Functional connectivity was significantly greater in iM1-iPMd than iM1-cM1 (p=0.04) or iM1-cPMd (0.003). There was no significant difference between iM1-cM1 and iM1-cPMd connectivity (p=0.60). Note that correlation coefficients were not significantly correlated with any measure of *cortical function*, with the sole exception being iM1-cPMd functional connectivity and ipsilesional M1 activation volume (p=0.005), nor did any correlation coefficient differ according to stroke subtype (iM1-iPMd: p=0.41; iM1-cM1: p=0.77; iM1-cPMd: p=0.85).
Treatment-induced behavioral gains

Patients showed significant gains across the 3 weeks of robotic therapy, with improvements from baseline to 1 month post-therapy in both primary endpoints (FM: 3.7 points, p<0.0001; ARAT: 4.1 points, p=0.002).

Prediction of treatment-induced behavioral gains -- all patients

*Bivariate screening:* When each of the variables from the seven categories was examined in bivariate screening, four categories (*demographics, impairment, genetics, and cognitive/mood*) had no variables significantly predicting gains, while three categories (*brain injury, cortical function, and cortical connectivity*, see Table 5.2) had at least one variable that significantly predicted therapy-induced behavioral gains. The most significant bivariate predictor of gains from each of these three, respectively, was percentage CST injury (Figure 5.2A: r=-0.49, p=0.007), ipsilesional M1 activation contrast estimate (r=0.37, p=0.045), and iM1-cM1 functional connectivity (Figure 5.2B: r=0.45, p=0.01). The plots for the variables that did not predict therapy-induced behavioral gains can be found in Appendix 2.
Table 5.2. Bivariate correlations between baseline measures and arm motor gains with therapy.

<table>
<thead>
<tr>
<th>Baseline variable</th>
<th>Correlation with improvements in arm motor status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
</tr>
<tr>
<td><strong>Demographics/medical history</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.07</td>
</tr>
<tr>
<td>Time post-stroke</td>
<td>0.06</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>0.03</td>
</tr>
<tr>
<td>Diabetes mellitus (y/n)</td>
<td>-0.04</td>
</tr>
<tr>
<td>Hypertension (y/n)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hypercholesterolemia (y/n)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Cognitive/mood</strong></td>
<td></td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>-0.13</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Impairment</strong></td>
<td></td>
</tr>
<tr>
<td>NIH Stroke Scale</td>
<td>-0.26</td>
</tr>
<tr>
<td>Mean baseline Fugl-Meyer</td>
<td>-0.05</td>
</tr>
<tr>
<td>Mean baseline Action Research Arm Test</td>
<td>0.04</td>
</tr>
<tr>
<td>Nottingham Sensory Assessment</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Genetics</strong></td>
<td></td>
</tr>
<tr>
<td>BDNF val66met polymorphism present (y/n)</td>
<td>0.19</td>
</tr>
<tr>
<td>ApoE4 allele present (y/n)</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Brain injury</strong></td>
<td></td>
</tr>
<tr>
<td>Infarct volume (cc)</td>
<td>-0.42</td>
</tr>
<tr>
<td>Precentral gyrus injury (y/n)</td>
<td>-0.15</td>
</tr>
<tr>
<td>Precentral gyrus injury (cc)</td>
<td>-0.18</td>
</tr>
<tr>
<td>Cortical PMd injury (y/n)</td>
<td>-0.28</td>
</tr>
<tr>
<td>Cortical PMd injury (cc)</td>
<td>-0.36</td>
</tr>
<tr>
<td>Cortical injury (y/n)</td>
<td>-0.46</td>
</tr>
<tr>
<td>Total cortical injury (cc)</td>
<td>-0.41</td>
</tr>
<tr>
<td>Corticospinal tract integrity (DTI FA)</td>
<td>0.18</td>
</tr>
<tr>
<td>Percent injury to CST</td>
<td>-0.49</td>
</tr>
<tr>
<td><strong>Cortical function</strong></td>
<td></td>
</tr>
<tr>
<td>iM1 activation - contrast estimate</td>
<td>0.37</td>
</tr>
<tr>
<td>iPMd activation - contrast estimate</td>
<td>0.36</td>
</tr>
<tr>
<td>cM1 activation - contrast estimate</td>
<td>0.28</td>
</tr>
<tr>
<td>cPMd activation - contrast estimate</td>
<td>-0.01</td>
</tr>
<tr>
<td>iM1 activation volume</td>
<td>0.16</td>
</tr>
<tr>
<td>iPMd activation volume</td>
<td>0.02</td>
</tr>
<tr>
<td>cM1 activation volume</td>
<td>-0.01</td>
</tr>
<tr>
<td>cPMd activation volume</td>
<td>-0.10</td>
</tr>
<tr>
<td>Cortical connectivity</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---</td>
</tr>
<tr>
<td>iM1-iPMd connectivity</td>
<td>0.11</td>
</tr>
<tr>
<td>iM1-cM1 connectivity</td>
<td>0.45</td>
</tr>
<tr>
<td>iM1-cPMd connectivity</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**Figure 5.2.** Smaller injury to M1 CST and greater iM1-cM1 functional connectivity significantly predicted greater motor gains. The change in motor behavior was assessed as the principal component of the change in FM and ARAT scores from baseline to one-month post-therapy. To aid interpretation, the back-propagated change in FM score was included on the Y-axis. A) Percent injury to M1 CST: $r = -0.49$, $p = 0.007$. B) iM1-cM1 functional connectivity: $r = 0.45$, $p = 0.01$. 
**Multivariate modeling:** To generate a predictive model, the three variables that best correlated with change in behavior based on bivariate screening were entered into multivariate analyses. The resultant model predicted 44% of variance in therapy-induced motor gains (p=0.002; Table 4.3); a measure of brain injury (percent CST injury) and a measure of cortical connectivity (iM1-cM1 functional connectivity) remained significant in this model.

Generating principle components of the independent variables did not explain more variance in therapy-induced motor gains than found with bivariate screening (44%). The closest was a multivariate model created with percent CST injury and the principal component of ipsilesional M1 contrast estimate plus the interhemispheric M1-M1 correlation coefficient. The resultant model explained 43% of the variance in treatment gains (p=0.0006). In this case, the PC of the cortical function variables retained significance (p=0.006) but percent CST injury just missed (p=0.05). Evaluating the independent variables against the percent change rather than the PC in outcome measures did not improve upon the current results.

**Table 5.3.** Multivariate predictor model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Std error</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.269</td>
<td>0.890</td>
<td>--</td>
<td>0.765</td>
</tr>
<tr>
<td>Ipsilesional M1 activation - contrast estimate</td>
<td>0.276</td>
<td>0.161</td>
<td>-0.05 to 0.61</td>
<td>0.10</td>
</tr>
<tr>
<td>iM1-cM1 beta</td>
<td>2.57</td>
<td>1.005</td>
<td>0.49 to 4.64</td>
<td>0.017*</td>
</tr>
<tr>
<td>Percent CST injury</td>
<td>-0.020</td>
<td>0.010</td>
<td>-0.40 to -0.0001</td>
<td>0.049*</td>
</tr>
</tbody>
</table>
Lasso regression method: To independently verify the results from bivariate screening, Lasso regression was applied to the same 29 patients' data. This analysis identified the same 2 categories—brain injury and cortical connectivity—as having variables significantly predictive of treatment-induced behavioral gains. In the brain injury category, the selected variables were percent injury to the CST and cortical injury (yes/no). In the cortical connectivity category, the selected variable was the iM1-cM1 functional connectivity correlation coefficient. Estimated Lasso coefficients are provided in Figure 5.3.
Figure 5.3. Regression coefficients determined from the group Lasso regression method. The variables were standardized before the Lasso regression was applied. Variables identified as important for therapy-induced gains came from the cortical connectivity, cortical function, and brain injury categories.
Prediction of treatment-induced behavioral gains -- stroke subtype

In order to understand how differences in stroke pathophysiology influence prediction of treatment response, the above analyses were repeated examining only the subgroup of patients with a lacunar infarct (n=8). In these patients, baseline measures were overall similar to those found in the subgroup of 21 patients with a non-lacunar infarct (Table 5.1), except that patients with lacunar infarct had higher prevalence of hypercholesterolemia (p=0.03), less severe sensory deficits (higher Nottingham Sensory Assessment scores, p=0.001), less severe injury by several measures such as extent of CST injury by lesion overlap (p=0.04). Patients with a lacunar infarct had a greater treatment response compared to patients with a non-lacunar infarct (5.8±3.2 vs. 2.8±3.4 for FM, p=0.02; 8.8±7.0 vs. 2.3±5.1 for ARAT, p=0.02).

Examining the interaction between stroke subtype and the candidate predictor variables identified significant differences in only two categories: cortical function (ipsilesional M1 activation contrast estimate: p=0.057, Figure 5.4B) and cortical connectivity (iM1-iPMd correlation coefficient: p=0.004, Figure 5.4B). Evaluating these relationships solely in patient with lacunar infarct identified significant bivariate relationships: ipsilesional M1 activation contrast estimate, r=0.79, p=0.02, Figure 5.5A; iM1-iPMd correlation coefficient, r=0.81, p=0.02, Figure 5.5B. Because of colinearity between ipsilesional M1 contrast estimate and the iM1-iPMd correlation coefficient (r=0.61, p=0.02), a multivariate model was not pursued for the lacunar subtype. These findings contrast with results of bivariate screening in the 21 patients with a non-lacunar infarct (Figure 5.6), among whom the significant predictors of motor gains were the same as in the full cohort of 29 subjects: percent CST injury (r=-0.51, p=0.02) and iM1-cM1 functional connectivity (r=0.56, p=0.009). There was no correlation between percent CST injury and iM1-cM1 functional connectivity.
It is worth noting that adding the 3 patients with small subcortical strokes (5.1±0.5 cc) to the n=8 lacunar subgroup (1.2±0.6 cc) did not improve the fit of the predictor relationships in Figure 4.4 (ipsilesional M1 activation: r=0.79; iM1-iPMD correlation: r=0.63).

**Figure 5.4.** The interaction between predictor candidates and stroke subtype. A) ipsilesional M1 activation: p=0.057. B) iM1-iPMd connectivity correlation: p=0.004. The change in motor behavior was assessed as the principal component of the change in FM and ARAT scores from baseline to one-month post-therapy. To aid in interpretation, the change in FM score was included on the Y-axis.
Figure 5.5. Greater ipsilesional M1 activation and ipsilesional M1-ipsilesional PMd functional connectivity significantly predicted larger motor gains in patients with lacunar infarcts ($r=0.79$, $p=0.02$; $r=0.81$, $p=0.02$, respectively). The change in motor behavior was assessed as the principal component of the change in FM and ARAT scores from baseline to one-month post-therapy. To aid in interpretation, the change in FM score was included on the Y-axis.

Figure 5.6. Ipsilesional M1 activation and ipsilesional M1-ipsilesional PMd functional connectivity did not significantly predict motor gains in patients with non-lacunar infarcts ($r=0.12$, $p=0.62$; $r=-0.34$, $p=0.13$, respectively). The change in motor behavior was assessed as the principal component of the change in FM and ARAT scores from baseline to one-month post-therapy. To aid in interpretation, the change in FM score was included on the Y-axis.
Discussion

Stroke is a very heterogeneous disease, with wide inter-subject variability seen in measures such as degree of injury, neural function, and response to therapy. These sources of variability complicate prescription of restorative therapies, as the best predictor--or combination of predictors--of response to a post-stroke restorative therapy remains uncertain. Preclinical studies suggest that neural injury, neural function, and clinical status each influence response to a restorative therapy. Results of the current study suggest that neural injury and neural function remain important in human subjects: using multiple forms of assessment to predict gains from a standardized 3-week course of robotic therapy targeting the upper extremity, multivariate modeling found that a measure of brain injury (smaller extent of CST injury) combined with a measure of cortical connectivity (greater iM1-cM1 connectivity) best predicted treatment-induced behavioral gains. This result was also independently confirmed by the Lasso regression method. These variables performed better than traditional predictors of stroke outcome such as age or infarct volume, as well as other measures of demographic/medical history, cognitive/mood, and impairment. Overall, these findings emphasize the importance of a multimodal approach to patient selection and stratification for restorative therapies after stroke.

Prediction of treatment-induced behavioral gains -- all patients

The current study found that a combination of brain injury and cortical connectivity measures was best for predicting treatment gains. These broadly agree with a prior study that also found combining measures of neural injury (CST FA) and neural function (motor evoked potential) to be optimal for predicting upper extremity treatment gains in patients with chronic stroke [249], and agree with preclinical studies that emphasize neural injury and neural function as determinants of training-induced behavioral gains after stroke [54, 284]. This combined
approach explained far more variance in outcome (44%, Table 5.3) than that provided by any single measure (up to 24%, Table 5.2). Clinical status was also hypothesized to be a significant predictor, but none of these measures achieved significance in any of the analyses. The reasons for this result are uncertain but may be related to the study population or may be that the factors related to pre-therapy motor status [300] are not important for therapy gains. The brain injury and the cortical connectivity measures are both neuroimaging measures, a finding that has increased significance considering the triage and distribution of post-stroke rehabilitative care is still primarily based on behavioral assessments [314].

Among the measures of neural injury, extent of CST injury measured with the lesion overlap method best predicted therapy-induced motor gains, consistent with prior studies using this method [150, 285]; indeed, CST injury was the strongest predictor of all measures studied. The current study also confirmed a prior report [150] that neural system injury is a more precise measure than global injury (such as infarct volume) for predicting treatment gains. System-specific measures may be more biologically informative than general measures in the context of brain plasticity. The presence of cortical injury was also an important predictor of treatment gains, being second strongest of all measures, and furthermore was also identified as significant by the Lasso regression method. This finding is consistent with previous studies that found significant differences in treatment response [315] and in neurophysiological integrity [285] depending on whether or not the stroke involved the cerebral cortex. Together, these findings highlight the importance of measuring both white matter and gray matter measures of injury.

Among the measures of neural function, iM1-cM1 functional connectivity best predicted therapy-induced gains, a result confirmed by the Lasso regression method. Specifically, greater baseline iM1-cM1 connectivity predicted higher treatment gains, a finding that agrees with one
[291], but not a second [80] prior study. The divergent results may possibly be related to differences in the population studied or the technique used to measure connectivity. Overall this finding is concordant with cross-sectional studies that have found greater interhemispheric connectivity to be correlated with smaller motor deficits after stroke in humans [316, 317]. A similar finding has been described in an animal stroke model in which increases in interhemispheric connectivity correlated with improved sensorimotor function [318]. The exact manner by which iM1-cM1 connectivity is related to treatment gains in the current cohort is uncertain and could reflect changes in activity within either motor cortex [166, 170, 256], in contralesional hemisphere excitability [319], in ipsilesional hemisphere excitability[320-322], or a more complex pattern of altered interhemispheric inhibition [323-325]. The current results underscore the potential value of connectivity measures for understanding behavioral status after stroke [326], and supports prior reports [327-329] that in some settings connectivity may have advantages over regional assessments of brain activation (Table 5.2).

Prediction of treatment-induced behavioral gains -- stroke subtype

The current results supported the secondary hypothesis that predictive metrics would vary according to stroke subtype (lacunar vs. non-lacunar stroke). Patients with a lacunar stroke, defined here as a small deep infarct, tend to differ in multiple ways from patients with a non-lacunar stroke. This includes risk factor profile [301, 330], lesion volume [310], and clinical prognosis [302, 331] [301-307]. The current study extends these prior observations to predictors of response to a restorative therapy.

The strongest predictors of treatment-induced behavioral gains among patients in the lacunar subgroup were measures of *cortical function* and intrahemispheric *cortical connectivity*, specifically greater ipsilesional M1 activation and greater iM1-iPMd functional connectivity.
This contrasts with findings among patients in the non-lacunar subgroup where measures of brain injury and interhemispheric cortical connectivity best predicted treatment gains. The importance of cortical function to treatment gains in the lacunar subgroup is consistent with prior results. The degree of ipsilesional sensorimotor cortex activation may play a more important role in recovery from subcortical stroke as compared to recovery from cortical stroke [332]. In addition, a study of 8 patients an average of 20 days after onset of subcortical stroke found that ipsilesional M1 activation, expressed as contrast estimates, predicted motor gains over the subsequent year [333]. The basis for the observed differences between the two stroke subtypes is uncertain but may be related to cortical excitability, as affected arm movements increase motor cortex excitability and intracortical facilitation in patients with subcortical stroke but not in patients with cortical stroke [334], or to lesion-specific differences in patterns of brain plasticity after stroke [332, 334].

Study considerations and limitations

Strengths of the study include the examination of multiple classes of candidate predictor variables in parallel, the attention to stroke subtype, and confirmation of results using independent statistical methods. The heterogeneity of the enrolled population is both a strength and a limitation of the study. There is a balance between studying a narrowly defined population of stroke survivors, where many sources of variance are minimized but results do not generalize, versus a broadly defined population of stroke survivors, among whom heterogeneity limits the ability to identify predictors. A recent report from The NINDS Stroke Progress Review Group recommended enrollment of more homogenous patient populations in studies of stroke recovery and rehabilitation [335]. However, uncertainties remain as to which category of measure serves best for defining homogeneity. The current study prioritized time post-stroke (early after patients
reach a plateau in spontaneous arm motor recovery [4, 308]) and severity of stroke (motor deficits could be neither minor nor devastating). Studies that prioritize enrollment based on different clinical characteristics or that focus on different therapies might reach divergent findings regarding predictors of treatment gains.

Weaknesses of the study include the possibility that genetic analysis results may suffer from Type II error. The current study did not find BDNF or ApoE genotype to be predictive of motor gains. While studies with sample sizes comparable to the current report have found identified significant predictive value for each of these genotypes (e.g., the BDNF val<sup>66</sup>met polymorphism for motor learning [217, 336] and the ApoE4 allele for rate of learning [337] and recall [338]), it is likely that the study was insufficiently powered to detect an association between genotype and degree of gains with therapy. Another weakness is that patients with a lacunar infarct had significantly less CST injury compared to patients with a non-lacunar infarct (Table 5.1), which complicates interpretation of findings related to stroke subtype.

The multivariate predictive model explained only 44% of the variance in therapy-induced behavioral gains. What might account for the remaining variance? A number of candidate measures might be considered, from across the range of biopsychosocial factors important to outcomes after stroke, such as socioeconomic status [339], stress [340], other genes [341], caregiver status [342], neurophysiology [299], and more. These potential cofactors attest to how heterogeneous a condition stroke is [8] and thus the challenges of predicting treatment responses.

The current findings indicate that measures of neural injury and neural function together best predict treatment gains from a standardized course of robotic therapy targeting arm movement in patients who have recently reached a plateau in spontaneous recovery. The results could potentially inform patient selection in other rehabilitation settings after stroke, an important
consideration given decision-making regarding post-stroke rehabilitation therapy is currently driven by clinical assessments [343].
CHAPTER 6

Imaging biomarkers of motor therapy after stroke differ according to stroke severity

Abstract

Stroke heterogeneity is evident not only in initial degree of motor impairment but also in patients' response to restorative therapies. The identification of neural biomarkers of therapy-induced motor gains would shed light on the mechanisms of recovery and how they may differ across the heterogeneous stroke population. MRI-derived neural correlates of motor recovery include regional measures of cortical activation, functional connectivity, and corticospinal tract (CST) integrity. In the current study, serial measures of fMRI motor cortex activation, and functional connectivity were evaluated along with motor gains in patients 3-6 months post-stroke undergoing 3 weeks of upper extremity robotic therapy. The hypothesis was that changes in these measures would correlate significantly with therapy-related motor gains. An exploratory susceptibility-weighted imaging measure of peri-infarct tissue perfusion/angiogenesis was also evaluated. In 13 patients, there was a positive trend for increasing interhemispheric primary motor cortex (M1) connectivity and greater motor gains from therapy (p=0.065). Because recovery is known to vary with degree of motor impairment, a secondary hypothesis was that separate biomarkers may be needed for patient subgroups. For all three measures of functional connectivity, the interaction term between change in connectivity and baseline impairment level was significant: ipsilesional M1-contralesional M1 (p=0.0031), ipsilesional M1-contralesional dorsal premotor cortex (PMd) (p=0.0045), and ipsilesional M1-ipsilesional PMd (p=0.0041).

Specifically, among more impaired patients decreases in functional connectivity correlated with smaller motor gains from therapy whereas in less impaired patients decreases in functional
connectivity correlated with larger motor gains. The current results suggest that cortical function changes at the level of the motor network are strong biomarker candidates of therapy-induced motor gains and, furthermore, that biomarkers of such gains may differ based on patient characteristics like motor impairment severity.

Introduction

Stroke is a leading cause of long-term adult disability in the United States [1], a trend likely to continue given decreases in the stroke mortality rate [344]. Among the most common deficits after stroke are those involving the motor system [345]. In most patients, some degree of spontaneous motor recovery is seen during the three months after stroke onset, but this is often incomplete [3]. In the subsequent chronic stroke phase, motor status can sometimes be further improved with introduction of a restorative therapy that promotes plasticity within surviving neural tissue [7]. However, patient responses to restorative therapies are highly variable [346, 347], the reasons for which are incompletely understood. Improved methods are needed to better understand inter-subject variability in response to restorative therapies after stroke, which could improve individualization of therapy [229, 348]. Identification of biomarkers that provide information about the neural events underlying therapy-related motor gains could have substantial impact in this regard, as post-stroke rehabilitative care is still primarily based on behavioral assessments [314].

A biomarker can be defined as a laboratory measurement that reflects the activity of a disease process and can potentially serve as a surrogate marker of treatment effects [349]. The validity of a biomarker depends on a number of considerations, particularly that it must change in parallel with clinical status [350, 351]. A number of non-invasive neuroimaging techniques have been identified that are related to the neural events underlying brain plasticity [90-92, 352, 353] and
are candidate biomarkers of treatment-induced motor gains after stroke [230]. One highly studied technique in this regard is functional MRI (fMRI), which provides various measures of regional activation that, when serially measured during a post-stroke restorative therapy, have been found to decrease within secondary motor areas such as bilateral dorsal premotor cortex (PMd) and contralesional primary motor cortex (M1) [232, 288, 354]. Another set of candidate biomarkers are those derived from studies measuring changes in functional connectivity across brain networks, an approach that at times may be sensitive to alterations in brain function not detected by measures of regional activation [178, 327, 329]. In addition, restorative therapies after stroke have been associated with induction of angiogenesis, which has been captured in preclinical studies using T2*-weighted susceptibility-weighted imaging (SWI) [355-357] but has received limited attention in human studies to date.

The purpose of the current study was to evaluate the comparative utility of these imaging-based biomarker candidates for capturing therapy-related motor gains, based on the extent to which each changed across therapy in parallel with behavioral gains. The first hypothesis was, therefore, that therapy-related motor gains across a 3-week course of standardized motor therapy would correlate significantly with changes in MRI measures of cortical activation and functional connectivity; a correlation with changes in angiogenesis were examined as an exploratory aim. Standardized motor therapy was delivered using a robotic device that has established therapeutic utility [77].

The enormous heterogeneity of the human stroke population suggests that a single biomarker approach may be insufficient. Therefore, the second hypothesis of the current study was that separate biomarkers may be needed for different patient subgroups. For example, although some studies have found that a restorative therapy is associated with decreased fMRI activation within
secondary motor areas [232, 354], as above, other studies have found increased activation [358-360]. Similarly, studies examining changes in connectivity in response to a restorative therapy after stroke have reported both increases [291, 361] and decreases [80, 362] over time. The idea underlying this hypothesis is that, just as the nature of gains from a restorative therapy vary according to level of baseline deficits [236], biomarkers may change in a manner that depends on level of baseline deficits.

**Methods**

Patients enrolled in a clinical trial of robotic therapy (clinicaltrials.gov ID# NCT01244243) underwent a battery of assessments twice, at baseline then again upon completion of treatment. Inclusion and exclusion criteria (Table 4.1) were focused on capturing patients who were close to the time when spontaneous motor recovery is complete [4, 308] and who had motor deficits within a specific range. To confirm that subjects had reached a plateau in arm motor recovery, the arm motor Fugl-Meyer (FM) scale was performed at two baseline exams separated by a week, and patients could only advance if the two scores did not differ by more than 3 points. This study was approved by the UC Irvine Institutional Review Board.

Biomarker testing was obtained in 31 consecutive enrollees (i.e., all study patients beginning with the 10th enrollee). Of these, 4 were excluded because claustrophobia prevented MRI data collection, 7 because of excessive head motion during fMRI scanning at either the pre-therapy or post-therapy scan, 1 was lost to follow-up, and 1 patient moved out of state before post-therapy scanning could be obtained, leaving 18 patients who are the focus of the current report.
Robotic therapy

Patients underwent 12 two-hour treatment sessions of robotic hand therapy over a three-week period. All patients completed the 12 robotic therapy sessions. Therapy consisted of repeated grasp-release ('close' and 'open') movements of the affected hand/wrist, linked to a range of games and exercises, using a pneumatically-actuated robotic device described previously [77].

Image acquisition

An MRI scan was performed at baseline, and again approximately one week post-therapy. MRI images were acquired using a 3.0T Philips Achieva system. Three runs of blood oxygenation level-dependent (BOLD) images were acquired as described in Chapter 4. Anatomical imaging included a high-resolution T1-weighted images using a 3D MPRAGE sequence (TR=8.5 ms, TE=3.9 ms, slices=150, voxel size=1x1x1 mm³) and susceptibility-weighted imaging (TR=35 ms, TE=20ms, slices=128, voxel size=0.72x0.72x1 mm³).

Data analysis

Therapy-induced motor gains: The primary outcome measure of the study was arm motor gains from robotic therapy. This was defined using an impairment-based (Fugl-Meyer: FM) scale and a function-based (Action Research Arm Test: ARAT) assessment that were combined via principal component (PC) analysis [166]; only the first principal component was used given that it accounted for 74% of the variance in these behavioral outcomes. Treatment-induced motor gains were measured as the change from baseline to post-therapy, and were examined in relation to a set of biomarker candidates described below and listed in Table 6.1.
Table 6.1. Biomarker candidates under study.

<table>
<thead>
<tr>
<th>Functional MRI measures of regional activation</th>
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<tbody>
<tr>
<td>Degree of activation (beta contrast estimates) in iM1, cM1, iPMd, and cPMd</td>
<td>Volume of activation in iM1, cM1, iPMd, cPMd</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PPI measures of functional connectivity</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Functional connectivity between iM1 and cM1</td>
<td>Functional connectivity between iM1 and cPMd</td>
</tr>
<tr>
<td>Functional connectivity between iM1 and iPMd</td>
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<table>
<thead>
<tr>
<th>Peri-infarct angiogenesis</th>
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<tbody>
<tr>
<td>T2*-weighted signal in infarct rims of two different diameters</td>
</tr>
</tbody>
</table>

Functional MRI: The fMRI images were analyzed using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). Functional data were processed as previously published [363]. Contrast estimates and activation volume were extract from bilateral M1 and PMd regions.

Psychophysiological interaction: A psychophysiological interaction (PPI) analysis was used to assess the relationship between therapy-induced motor gains and changes in intra- and interhemispheric motor network functional connectivity. Psychophysiological interaction analysis is a tool that can evaluate the influence of psychological variables (e.g., different contexts, here pre- vs. post-treatment MRI session) on physiological variables (i.e., BOLD response) [364, 365], and that reflects underlying neuronal changes [366]. For each patient, time series data were extracted from the fMRI images within the four ROIs defined above. The PPI design matrix contained the following 3 regressors: 1) the psychological variable (i.e. pre- vs. post-treatment session); 2) the physiological variable (i.e., ROI BOLD time series data); and 3) the interaction term of these two variables (i.e., the PPI term). Changes in motor network
Functional connectivity were evaluated as the changes in PPI regression slopes between brain regions based on the psychological variable. Specifically, to evaluate change in functional connectivity between two brain regions, the time series data from one ROI was plotted against the time series of a second ROI, for both the pre- and the post-treatment fMRI sessions. Next, the slopes of the regression lines for the two fMRI sessions were extracted [363, 367] and the difference in slope across treatment (post-treatment minus pre-treatment; Figure 6.1) was calculated. An increase in the PPI slope from pre- to post-therapy indicates that activity between the two brain regions has become more correlated, and thus increased functional connectivity; and thus a decrease in the PPI slope over time indicates decreased functional connectivity. In this way the connectivity was measured for iM1 with cM1, iPMd, and cPMd. As a negative control, functional connectivity was also evaluated in non-motor regions, specifically between bilateral primary visual cortex (V1).

![Image](image.jpg)

**Figure 6.1.** Example of change in psychophysiological interaction (PPI) slope as a measure of functional connectivity. Each data point represents a single brain volume.
**Peri-infarct angiogenesis:** Serial T2*-weighted SWI scans were used to estimate angiogenesis across the period of treatment. Two infarct rims were generated in native SWI space, similar to methods used in a prior report [368]. First, the infarct was outlined on the baseline T1-weighted images. Using FSL (www.fmrib.ox.ac.uk/fsl), each lesion mask was dilated twice, by 1mm. Rim 1 was defined as the first dilatation minus the infarct, while Rim 2 was defined as the second dilatation minus the infarct plus Rim 1. For post-treatment, the patients’ lesion masks were transformed into post-treatment SWI space, respectively, then the two post-treatment Rims were created. The FAST module in FSL was used to generate pre- and post-treatment CSF masks that were then subtracted from each infarct Rim, to insure that Rims did not extend beyond the brain. The mean intensity within each rim was calculated, at both time points, to calculate the pre-treatment to post-treatment difference in SWI intensity.

**Statistics**

To address the first study hypothesis, linear regression was used to compare the change in each biomarker measure from pre-therapy to post-therapy with therapy-induced motor gains over the same time period. To address the second study hypothesis, patients were divided into two subgroups based on a median split of baseline impairment, using pre-therapy FM scores. In order to determine whether the relationship between biomarker and treatment gains varied according to baseline impairment, an interaction term ("measurement" X "baseline impairment subgroup") was added to each biomarker measurement in the model to predict treatment-induced motor gains. For any model in which the interaction term was significant, post-hoc testing was done separately for each baseline impairment subgroup. Analysis of fMRI and PPI data excluded any patient in whom substantial (>50%) damage was present in any of the ROIs [369]. Parametric statistical methods were used for measures that were normally distributed or could be
transformed to a normal distribution otherwise non-parametric methods were used. All analyses were two-tailed with alpha=0.05 and used JMP-8 software (SAS Institute, Inc., Cary, NC).

**Results**

**Patients**

The 18 patients were 61±10 years of age (mean±SD) and 4.1±1 months post-stroke, all but one were right-handed, and all but two were ischemic. Overall, patients showed wide variation in baseline clinical status, such as impairment level (FM scores ranging from 20-60, see Table 6.2). Treatment-induced motor gains from baseline to end of therapy varied greatly across patients (2.9±2.7 points for FM, p=0.0003; 2.5±2.6 points for ARAT, p=0.0008).

**Table 6.2.** Patient characteristics.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61 ± 10</td>
</tr>
<tr>
<td>Gender</td>
<td>3 F / 15 M</td>
</tr>
<tr>
<td>Time post-stroke (months)</td>
<td>4.1 ± 0.97</td>
</tr>
<tr>
<td>Side of stroke</td>
<td>10 L / 8 R</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 Y / 15 N</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>9 Y / 9 N</td>
</tr>
<tr>
<td>Hypertension</td>
<td>11 Y / 7 N</td>
</tr>
<tr>
<td>Infarct volume (cc)</td>
<td>27 ± 45 (0.5-166)</td>
</tr>
<tr>
<td>Mean baseline Fugl-Meyer (normal = 66)</td>
<td>39 [20 - 60]</td>
</tr>
<tr>
<td>NIH Stroke Scale (normal = 0)</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Nottingham Sensory Scale (normal = 17)</td>
<td>13 ± 4.5</td>
</tr>
<tr>
<td>Mini Mental State Exam (normal = 30)</td>
<td>28 ± 2.6</td>
</tr>
<tr>
<td>Geriatric Depression Scale (normal = 0)</td>
<td>3.2 ± 2.1</td>
</tr>
</tbody>
</table>

*For the 18 individuals, values presented are mean±SD (range) or median [IQR].

**Change in biomarker assessments across therapy**

Across all 18 patients, no significant changes were found across therapy in any of the biomarker assessments.
Biomarker performance -- all patients

Biomarker performance was evaluated by examining the extent to which changes in these assessments over time paralleled treatment-induced motor gains. When all patients were examined, a relationship with behavioral gains was suggested for only one of the biomarker candidates, with increased iM1-cM1 connectivity associated with larger motor gains ($r=0.53, p=0.065$). The plots for the variables that did not survive as potential biomarkers of therapy-induced behavioral gains can be found in Appendix 3.

Biomarker performance in relation to level of baseline deficits

The relationship that each biomarker had with therapy-induced motor gains was examined as a function of baseline motor impairment. For all three measures of functional connectivity, the interaction term between change in connectivity and baseline impairment was significant (Figure 6.2): iM1-cM1 ($p=0.0031$), iM1-cPMd ($p=0.0045$), and iM1-iPMd ($p=0.0041$); change in the degree of activation (beta contrast estimates) within cPMd was also significant ($p=0.02$). These relationships were further examined by examining the relationship in each specific baseline impairment subgroup. The two impairment groups did not differ with regard to baseline values for any biomarker assessment or the extent of treatment-induced motor gains.

In the more impaired group (baseline FM $\leq 36$), larger therapy-induced motor gains were associated with greater increases in functional connectivity between iM1 and cM1 ($r=0.65$, $p=0.058$), and with greater increases in degree of activation within cPMd ($r=0.74$, $p=0.02$). Excluding the three patients with substantial damage to an ROI revealed that treatment-induced motor gains were significantly related to increased connectivity between iM1-cM1 ($r=0.98$, $p=0.0007$; Figure 6.3A), iM1-cPMd ($r=0.82$, $p=0.046$; Figure 6.3B), and iM1-iPMd ($r=0.94$, $p=0.005$).
In the less impaired group (baseline FM >36), larger therapy-induced motor gains were associated with significantly greater decreases in functional connectivity between iM1 and cPMd \((r=-0.70, p=0.04)\). Excluding two patients with substantial damage to an ROI revealed that treatment-induced motor gains were significantly related to decreased connectivity between iM1-iPMd \((r=-0.83, p=0.02; \text{Figure 6.4A})\), with a trend for decreased iM1-cPMd connectivity \((r=-0.68, p=0.09; \text{Figure 6.4B})\).

The plots for the interactions that were not significant can be found in Appendix 3.

**Figure 6.2.** The significant interaction between changes in functional connectivity and baseline impairment. A) iM1-cM1: \(p=0.0031\). B) iM1-cPMd: \(p=0.0045\). C) iM1-iPMd: \(p=0.0041\). Behavioral gains were assessed as the principal component of the change in FM and ARAT scores from baseline to post-therapy. To aid interpretation, the change in FM score is included on the Y-axis.
**Figure 6.3.** In *more impaired* patients decreased functional connectivity correlates with smaller motor gains from therapy. A) Ipsilesional primary motor (M1)-contralesional connectivity ($r=0.98$, $p=0.0007$). B) Ipsilesional M1-contralesional dorsal premotor (PMd) cortex connectivity ($r=0.82$, $p=0.046$). Behavioral gains were assessed as the principal component of the change in FM and ARAT scores from baseline to post-therapy. To aid interpretation, the change in FM score is included on the Y-axis.

**Figure 6.4.** In *less impaired* patients, decreased functional connectivity correlates with larger motor gains from therapy. A) Ipsilesional primary motor (M1)-ipsilesional dorsal premotor (PMd) cortex connectivity ($r=-0.83$, $p=0.02$). B) Ipsilesional M1-contralesional PMd cortex connectivity ($r=-0.68$, $p=0.09$). Behavioral gains were assessed as the principal component of the change in FM and ARAT scores from baseline to post-therapy. To aid interpretation, the change in FM score is included on the Y-axis.
Discussion

Stroke is a very heterogeneous disease in humans, complicating prescription of restorative therapies. Biomarkers may be useful to inform treatment decisions. Numerous candidate measures have been identified, but to date results across studies have been variable. The current study compared the performance of a number of imaging-based measures suggested as useful in prior investigations, and examined how results differed in patient subgroups as defined by level of baseline deficits. The main findings were that the strongest biomarkers were identified when focusing on specific patient subgroups rather than the entire population, that measures of functional connectivity performed best, and that there were major differences in biomarker performance across the two subgroups.

Across the entire population of enrollees, no biomarker changed over time in a manner that correlated significantly with treatment-induced motor gains, although the data suggested potential utility for change in iM1-cM1 connectivity. Mixed results have been seen in prior studies examining changes in connectivity as a biomarker of restorative therapy effects after stroke, with increased [291, 361] and decreased [80, 362] connectivity over time correlating with treatment gains. These differences may be due to differences between studies in factors such as the method used to measure connectivity or the choice of therapeutic intervention.

Further insight was obtained by examining biomarker performance in specific patient subgroups. This approach was motivated by the frequent finding that features of spontaneous and treatment-induced recovery vary tremendously according to level of baseline deficits [3, 193, 236]. Consistent with this, the current study found that results differed substantially according to level of baseline deficits (Figures 6.3 and 6.4). These findings emerged in relation to functional connectivity biomarkers, confirming the utility of such measures for understanding behavioral
recovery after stroke [329]. Furthermore, changes in regional measures of cortical activation did not relate to changes in arm motor status neither in all patients nor according to baseline impairment. This suggests that a network-level approach provides greater granularity into changes in the motor system arising from therapy than can be gleaned from evaluating changes in isolated regions within the network. Also, changes in iV1-cV1 connectivity did not correlate with changes in motor status, corroborating a finding by van Meer et al [318], which together provide evidence that functional connectivity changes are not ubiquitous throughout the brain with stroke recovery but, rather, they are specific to the system and resultant behavior of interest.

Among more impaired patients, larger treatment-induced motor gains were significantly associated with increases in functional connectivity between iM1 and all three cortical regions examined, particularly between iM1 and cM1. Although an increased role of contralesional brain regions after stroke has been associated with greater motor impairment, evidence suggests this is nonetheless an important substrate for treatment gains in more impaired subjects. Activity in multiple bilateral motor regions grows largest in those patients experiencing the smallest amount of spontaneous motor recovery after stroke [174]. A larger facilitatory effect of cPMd on iM1 is seen in patients with greater impairment after stroke [172]. Similarly, interfering with the function of cPMd interferes with behavioral state to a greater extent among patients with greater impairment [171] than in less impaired patients [370]. The behavioral relevance of these observations in more impaired patients may be similar to greater recruitment of secondary motor areas observed in healthy subjects during performance of increasingly complex tasks [371-374]. Together, these findings suggest a model for patients with more severe deficits post-stroke whereby the contralesional hemisphere plays an important role in supporting motor behavior after stroke, and this role increases in support of therapy-induced motor gains.
In less impaired patients, larger treatment-induced motor gains were associated with decreased motor system functional connectivity, particularly between iM1-iPMd. Previous activation studies have shown that, while recruitment of secondary motor areas can support motor recovery after stroke, restitution of normal circuitry is likely a more successful strategy [174, 375, 376]. Therefore, a model is suggested for patients with less severe deficits post-stroke such that decreased reliance on activity within bilateral motor regions is useful for motor recovery after stroke, and these relationships become more pronounced when a restorative therapy induces motor gains.

The current findings have implications for therapies aiming to modulate the interhemispheric balance of excitation and inhibition after stroke. Brain stimulation using techniques such as transcranial direct current stimulation or repetitive transcranial magnetic stimulation is under study to improve motor function after stroke. One strategy advocated is to decrease excitability within cM1 [377], based on the model that stroke results in increased inhibition of iM1 from cM1 [378]. However, the current results suggest that this approach may be more beneficial in patients with milder baseline deficits and less useful in those with the greatest deficits. This view is concordant with prior findings whereby dampening excitability in cM1 improved arm motor control in patients with milder impairment and worsened control in those with more severe post-stroke impairment [379].

Strengths of the current study include direct comparison of multiple biomarker candidates, including those with demonstrated value as well as the experimental SWI-based measure. Biomarkers were studied across a treatment regimen that used a robotic device to deliver therapy in a highly standardized manner. A relatively heterogeneous stroke population was intentionally enrolled, addressing the concern that studies examining effects restorative
therapy effects on functional imaging measures have preferentially enrolled patients with mild impairment or small infarcts [232], and enabling analysis of biomarker performance in relation to level of baseline impairment. Weaknesses include sample size, with serial data unavailable in a number of patients due to factors such as claustrophobia or head motion artifact during fMRI scanning. The biomarker candidates were limited to imaging-based measures, mainly because biomarker candidates in other categories such as blood tests of molecules related to cerebral plasticity are less well developed. Further studies are needed to better understand the performance of functional connectivity measures as biomarkers of treatment-induced motor gains. For example, a biomarker should not only lie in the causal pathway of the disease process but also mediate the treatment's effect on the outcome measure [351] -- that is, is a therapy improving hand function by promoting neural plasticity, as seems to be the case herein, or by decreasing hand edema and tone? Animal and human studies of motor learning, an important component to stroke recovery [380], provide evidence of rapid changes in network connectivity [190, 381-383] suggesting that functional connectivity is in the pathway of motor learning effects on behavior. Other regions within the motor network might contribute to motor recovery [178, 384, 385] but the current study was interested in evaluating the primary and secondary motor areas.

The human stroke population is heterogeneous. A number of promising restorative therapies are under study. Their efficacy may be best appreciated by connecting the right patients with the right therapies [229]. As noted by Bradnam et al [379], this task "is not a 'one size fits all' approach." Therefore, the same treatment may yield different, and in some cases undesirable, results depending on factors like stroke severity. A crutch that improves function among more impaired patients hinders those who are less impaired. The current findings may be
of guidance to defining biomarkers for restorative therapies after stroke.
CHAPTER 7

Summary and Conclusions

Stroke is a heterogeneous disease not only in its initial presentation but in the likelihood of recovery and response to restorative therapies. With 50-75% of survivors experiencing long-term motor deficits, new ways are needed to improve motor function. Rehabilitation is the current gold standard of therapy but, unfortunately, patient response to therapy is highly variable and the reasons are not well understood. The advent of structural and functional neuroimaging has enabled researchers to identify neural correlates of motor impairment and recovery that could help guide treatment and explain patient heterogeneity. However, previous studies have a) enrolled small numbers of patients, b) enrolled homogeneous patient cohorts, and/or c) examined only one or a few measures of neural injury and function at a time. These approaches are limited in their scope and prevent discernment of the factors most significant to recovery and most explanatory of treatment response heterogeneity. Therefore, the salience of the current studies lies in the enrollment of a heterogeneous sample of patients with chronic stroke and utilization of a multimodal approach to better understand motor recovery and interindividual differences. Specifically, this dissertation research set out to identify predictors and biomarkers of therapy-induced motor gains.

The aim of the first study (Chapter 4) characterized the patient sample and identified the factors most strongly related to pre-therapy motor impairment and disability. Among variables belonging to 6 categories (demographics/medical history, cognitive/mood, genetics, neurophysiology, brain injury, and cortical function), measures of brain injury and neurophysiology were related to motor impairment. Specifically, reduced CST integrity (by DTI) and reduced corticospinal integrity (absence of TMS MEP) independently correlated with greater
motor impairment. This corroborates prior cross-sectional studies' findings in humans that motor system injury provides insight into post-stroke motor status. A novel finding was that the correlates of post-stroke disability differed from the correlates of motor impairment such that lower CST integrity and poorer cognitive status (lower MMSE score) independently correlated with greater disability. Furthermore, in mildly impaired patients (the most frequently studied stroke subgroup), greater contralesional motor cortex activation was associated with greater motor impairment. In sum, the correlates of stroke outcome vary according to the behavioral endpoint or the patient group studied. These points have great relevance for aspects of clinical trial design such as choice of entry criteria or stratifying variable [229]. Finally, the current findings support a body of data suggesting that a measure of injury to a specific neural system might be useful for distinguishing patient subgroups on a biological basis.

The second study (Chapter 5) examined which baseline measures explained the greatest proportion of the variance in improvements with therapy and, therefore, were strong predictors of motor gains. Variables belonging to the same prior 6 categories, with the addition of a cortical connectivity category, were examined for their predictive potential. Across all patients, significant predictors of treatment gains were identified in the brain injury, cortical function, and cortical connectivity categories such that less CST injury (lesion load), greater ipsilesional M1 activation, and greater interhemispheric M1-M1 connectivity each predicted larger treatment gains. Together in a multivariate model, CST injury and M1-M1 connectivity explained 44% of the variance in treatment gains. Interestingly, results varied according to whether patients had a lacunar or non-lacunar stroke. For patients with lacunar stroke, gains were best predicted by a measure of intrahemispheric functional connectivity (ipsilesional M1-ipsilesional PMd). However, in patients with non-lacunar stroke greater motor gains were still predicted by less
CST injury and greater interhemispheric functional connectivity (ipsilesional M1-ipsilesional M1). Considering post-stroke rehabilitation decision-making is currently driven by clinical assessments [343], the finding that measures of neural injury and neural function together best predict treatment gains from a course of robotic therapy, above and beyond what was predicted by baseline impairment, highlights the important utility of these measures to inform patient selection and optimize gains from restorative stroke therapies.

The third study (Chapter 6) moved beyond identifying predictors of therapy-induced motor gains to identifying potential biomarkers of therapy-induced motor gains. Changes in measures of cortical function (i.e., cortical activation and functional connectivity) and an exploratory measure of peri-infarct perfusion, were evaluated in parallel with motor gains with robotic therapy. Although a trend existed for increasing interhemispheric M1-M1 connectivity correlating with larger gains, the more salient finding was that there was a significant interaction between the changes in functional connectivity and the patients' level of motor impairment. Less impaired patients exhibited decreases in intrahemispheric (ipsilesional M1-ipsilesional PMd) and interhemispheric (ipsilesional M1-contralateral PMd) functional connectivity with larger gains from therapy. Conversely, in more impaired patients, decreases in interhemispheric (ipsilesional M1-contralateral M1; ipsilesional M1-contralateral PMd) and intrahemispheric (ipsilesional M1-ipsilesional PMd) functional connectivity correlated with smaller motor gains. In both groups baseline connectivity between ipsilesional M1 and contralateral PMd significantly correlated with motor impairment in the same manner as the subsequent change with therapy. The data suggest that measures of functional connectivity -- that is, network level probes of motor cortex function -- are more closely linked with treatment gains than are regional measures of cortical activation. In sum, functional connectivity measures are likely strong biomarker
candidates of therapy-induced motor gains and could help define biomarker differences in the heterogeneous stroke population.

**Implications and future directions**

The studies comprising this dissertation contribute corroborative, as well as novel, data towards the need to incorporate neuroimaging measures of neural injury and function into therapeutic decision-making after stroke. Together the data provide strong evidence for the need to move beyond bedside clinical measures as determinants of the likelihood of recovery. Moreover, because different brain-behavior relationships were observed according to impairment level or stroke subtype in all 3 studies, patient heterogeneity should become a focus of research efforts. Considering the failure of translating new stroke therapeutics, identifying factors responsible for the observed treatment variability will not only improve our general understanding of stroke heterogeneity but also improve our ability to individualize therapies, reduce disability, and allocate costly resources appropriately. The current data have important scientific implications for studies aiming to understand the neural factors important for restorative therapy-induced recovery and patient heterogeneity of recovery.

Future neuroimaging studies of restorative therapies would benefit from enrolling a larger number of patients. While the enrollment of 41 patients with chronic stroke was an improvement upon samples sizes of earlier studies, clearly larger numbers are needed to have sufficient power for generating robust patient subgroups. We did find significant edifying brain-behavior relationships in subgroups of 6-8 patients but their generalization and interpretation are tentative until further confirmation is received. Also, the number of patients may have been partly responsible for our inability to find a significant genetic influence on outcome [15, 224].
Because task-related head motion confounds resulted in the loss of usable fMRI data, reducing the sample size available for fMRI-based analyses ( Chapters 4 and 5), an adjunct resting-state fMRI scan should be included in task-based studies of patients with a wide range of deficits [329]. The scientific questions asked (and neural demands) with each type of fMRI scan are different and, therefore, mutually informative.

The current study examined changes in cortical function (regional activation and functional connectivity) and CST integrity (by DTI) in parallel with therapy-induced motor gains but other biomarker candidates may exist as well. Studies of healthy controls have observed changes in cortical thickness occurring with motor training [386, 387]. However, the studies were not concordant in whether the structural changes correlated with behavioral changes. One study of constraint-induced movement therapy in chronic stroke patients did find increases in sensorimotor cortex volume that correlated with motor improvements [388]. Efforts are currently underway to determine if changes in cortical thickness occurred in parallel with the current therapy and whether changes correlate with gains. Additionally, the method for analyzing serial SWI images was an important albeit rudimentary first attempt to explore changes in peri-infarct perfusion. A more sophisticated method has been explored and may provide greater granularity into such changes.

Within the central nervous system, the motor network consists of regions beyond the primary motor and dorsal premotor cortices. The coordinated descending and ascending information required for voluntary movement also involves the basal ganglia, thalamus, midbrain, and cerebellar cortex. Future work should explore how activation and connectivity of these regions [384, 389] predict treatment gains and/or change in parallel with treatment gains.
Conclusions

In summary, neuroimaging-derived measures of motor system injury and function, connectivity specifically, are the strongest determinants of motor status after stroke and the likelihood of recovery with motor rehabilitative therapy. No clinical measure or global measure of injury exhibited such utility. The data also highlight the diversity of brain-behavior relationships after stroke and, therefore, the hurdle patient heterogeneity poses to the understanding and translation of restorative therapies [8, 65]. These studies will hopefully galvanize support for incorporating neuroimaging, and examining patient heterogeneity, in large restorative therapy clinical trials.
REFERENCES


with fMRI and TMS


After chronic
fMRI and
326.
325.
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neuroscience
Grefkes, C., et al., Modulating cortical connectivity in stroke patients by rTMS assessed with
42. Greifkes, C., et al., Modulating cortical connectivity in stroke patients by rTMS assessed with fMRI and


**APPENDIX 1**

**Negative data for Chapter 4**

**Note** that parametric plots are presented but where appropriate the non-parametric statistics are provided as in the Table 4.3. The statistics are the bivariate correlations of variables with greater motor impairment (lower Fugl-Meyer score).

**Demographics/medical history**

![Graph 1](image1)

- \( r = 0.005, p = 0.98 \)
- \( r = 0.25, p = 0.14 \)

![Graph 2](image2)

- \( r = 0.08, p = 0.66 \)
- \( r = 0.15, p = 0.41 \)
Cognitive/mood

Genetics

r = -0.16, p = 0.34

r = 0.11, p = 0.51

r = -0.08, p = 0.57

r = 0.09, p = 0.55
Brain injury

$r = 0.30, p = 0.08$

$r = 0.26, p = 0.14$

$r = 0.18, p = 0.30$

$r = 0.19, p = 0.25$

$r = 0.27, p = 0.11$
Cortical function

$r = -0.01, p = 0.94$

$r = -0.03, p = 0.87$

$r = 0.30, p = 0.12$

$r = 0.04, p = 0.82$

$r = -0.10, p = 0.60$

$r = -0.23, p = 0.22$
$r = 0.30, p = 0.12$

$r = -0.02, p = 0.93$

$r = -0.18, p = 0.39$

$r = -0.30, p = 0.19$
APPENDIX 2

Negative data for Chapter 5

**Note that parametric plots are presented but where appropriate the non-parametric statistics are provided as in the Table 5.2.

Demographics/medical history

![Graph 1](image1)

$r = 0.07, p = 0.72$

![Graph 2](image2)

$r = 0.06, p = 0.76$

![Graph 3](image3)

$r = 0.03, p = 0.80$

![Graph 4](image4)

$r = -0.04, p = 0.71$
Cognition/mood

Impairment
$r = 0.04, p = 0.82$

$\text{Genetics}$

$r = 0.25, p = 0.20$

$r = 0.19, p = 0.35$

$r = 0.19, p = 0.43$

$\text{Brain injury}$

$r = -0.15, p = 0.54$

$r = -0.18, p = 0.36$
$r = -0.28, p = 0.07$

$\textbf{Cortical function}$

$r = -0.36, p = 0.06$

$r = 0.18, p = 0.34$

$r = 0.36, p = 0.05$

$r = 0.28, p = 0.14$
$r = -0.01, p = 0.95$

$r = 0.16, p = 0.40$

$r = 0.02, p = 0.92$

$r = -0.01, p = 0.94$

$r = -0.10, p = 0.60$
Cortical connectivity

$r = 0.11, p = 0.58$

$r = 0.13, p = 0.50$
APPENDIX 3

Negative data for Chapter 6

**Note that parametric plots are presented but where appropriate the non-parametric statistics are provided.

Changes in regional activation - activation magnitude

![Graphs showing changes in regional activation with correlation coefficients.]

$r = 0.20, p = 0.50$

$r = 0.16, p = 0.61$

$r = -0.05, p = 0.86$

$r = 0.37, p = 0.22$
Changes in regional activation - activation volume

- $r = -0.13, p = 0.67$
- $r = -0.12, p = 0.70$
- $r = 0.03, p = 0.91$
- $r = 0.02, p = 0.95$
Changes in cortical connectivity

$r = 0.53, p = 0.065$

$r = 0.29, p = 0.34$

$r = 0.08, p = 0.79$

$r = 0.13, p = 0.68$
Interaction between biomarker candidate and baseline impairment (FM>36?) versus motor gains

Changes in regional activation - activation magnitude

Interaction term:  $p = 0.15$

Interaction term:  $p = 0.11$

Interaction term:  $p = 0.83$
Changes in regional activation - activation volume

Interaction term:  \( p = 0.80 \)  

Interaction term:  \( p = 0.64 \)  

Interaction term:  \( p = 0.30 \)  

Interaction term:  \( p = 0.74 \)  

Changes in cortical connectivity

Interaction term:  \( p = 0.19 \)
**APPENDIX 4**

95% Confidence intervals and sample size calculations for Chapter 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>95% Confidence Interval</th>
<th>Sample size for 80% power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.3628&lt;B&lt;0.3728</td>
<td>313952</td>
</tr>
<tr>
<td>Time post-stroke (months)</td>
<td>-4.37136&lt;B&lt;4.87136</td>
<td>123</td>
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<tr>
<td>Gender (M/F)</td>
<td></td>
<td>1224</td>
</tr>
<tr>
<td>Diabetes mellitus? (y/n)</td>
<td></td>
<td>346</td>
</tr>
<tr>
<td>Hypertension? (y/n)</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Hypercholesterolemia? (y/n)</td>
<td></td>
<td>304</td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>-2.3445&lt;B&lt;1.66446</td>
<td>65</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>-1.88790&lt;B&lt;2.10790</td>
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<tr>
<td>BDNF val&lt;sup&gt;66&lt;/sup&gt; met polymorphism present? (y/n)</td>
<td></td>
<td>1224</td>
</tr>
<tr>
<td>ApoE4 allele present? (y/n)</td>
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<td>967</td>
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<tr>
<td>Infarct volume (cc)</td>
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<tr>
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<td>Precentral gyrus injury (cc)</td>
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<td>Total cortical injury (cc)</td>
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<tr>
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<tr>
<td>Percent injury to CST</td>
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<tr>
<td>Ipsilesional M1 activation - contrast estimate</td>
<td>-3.03252&lt;B&lt;3.01252</td>
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<tr>
<td>Ipsilesional PMd activation - contrast estimate</td>
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<td>Contralesional M1 activation - contrast estimate</td>
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<td>Activation laterality M1</td>
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<tr>
<td>Activation laterality PMd</td>
<td>-7.48405 &lt; B &lt; 6.88405</td>
<td>85</td>
</tr>
</tbody>
</table>

* Values presented are for the 36 patients in Chapter 4. 95% CI are only presented for continuous/ordinal variables.
### APPENDIX 5

95% Confidence intervals and sample size calculations for Chapter 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>95% Confidence Interval</th>
<th>Sample size for 80% power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
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<td>Time post-stroke (months)</td>
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<td>Gender (M/F)</td>
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<tr>
<td>Hypertension? (y/n)</td>
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<td>Hypercholesterolemia? (y/n)?</td>
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<td>NIH Stroke Scale</td>
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<td>Fugl-Meyer Scale</td>
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<tr>
<td>Action Research Arm Test</td>
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<tr>
<td>Precentral gyrus injury (cc)</td>
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<td>190</td>
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<tr>
<td>Cortical PMd injury?</td>
<td></td>
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<tr>
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<tr>
<td>Cortical injury?</td>
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<td>35</td>
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<tr>
<td>Total cortical injury (cc)</td>
<td>0.17098&lt;B&lt;0.0171</td>
<td>266</td>
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<tr>
<td>CST integrity (DTI FA)</td>
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<td>229</td>
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<tr>
<td>Percent injury to CST</td>
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<td>31</td>
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<td>Estimate</td>
<td>95% CI</td>
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<td>-3.557&lt;B&lt;3.817</td>
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* Values presented are for the 29 patients in Chapter 5. 95% CI are only presented for continuous/ordinal variables.