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Correlations Between White Matter Integrity, Structural Connectivity, And Upper and Lower Extremity Motor Function in Individuals With Chronic Stroke

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CORRELATIONS BETWEEN WHITE MATTER INTEGRITY, STRUCTURAL CONNECTIVITY, AND UPPER AND LOWER EXTREMITY MOTOR FUNCTION IN INDIVIDUALS WITH CHRONIC STROKE

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DEDICATION

For my family…

To my partner, who supported me throughout this journey with amazing patience, even as the years kept adding up. You enabled me to pursue this degree while starting a family, something that was not without its challenges for the both of us, and I thank you greatly for this. Through unexpected twists and turns, you lent a guiding hand, a supportive voice, and were someone who I could always count on.

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ABSTRACT

Great variability is seen in the clinical manifestation of and recovery from stroke. Structural abnormalities often extend beyond the infarction site, indirectly affecting nonlesioned areas which can further contribute to motor deficits. Advances in neuroimaging have enabled the examination of white matter integrity and anatomical connectivity within the brain. Evidence is limited, however, regarding the relationship between the structural integrity and connectivity of primary and secondary motor tracts/brain regions and chronic upper and (especially) lower extremity motor impairments post-stroke. Therefore, the current study examined the relationship between upper/lower extremity motor impairments and structural integrity (Aim 1) and connectivity (Aim 2) of motor-relevant brain pathways and regions in individuals with chronic stroke. Forty-three participants completed a comprehensive motor assessment, with MRI scanning performed within two days of behavioral testing. Nonparametric analyses were performed to examine the relationship between structural integrity and connectivity of motor-relevant brain regions and motor function. Regression analyses were performed to assess the amount of variance in upper/lower extremity motor performance explained by ipsilesional corticospinal tract (CST) and red nucleus (RN) integrity, as well as cortical connectivity of the three main brain regions of motor control [primary motor cortex (M1), premotor cortex, and supplementary motor area]. Results indicate that ipsilesional CST and RN structural integrity (as assessed by fractional anisotropy values) are both positively associated with chronic upper/lower extremity

motor function. Ipsilesional CST integrity, however, is a stronger predictor of chronic upper extremity motor function and grip strength post-stroke. Furthermore, cortical integrity and connectivity of ipsilesional M1 is associated with upper extremity motor function of the affected extremity and gait speed, with cortical disconnection of M1 being an independent predictor of chronic motor function. These findings highlight the importance of examining structural changes and cortical disconnection beyond the lesion site post-stroke. Such insight could enhance our understanding of the underlying factors contributing to motor impairments, and improve motor recovery prognosis and help with targeting therapeutic interventions.

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List of Abbreviations

ACA ..... anterior cerebral artery

ADC ..... apparent diffusion coefficient

ADLs ..... activities of daily living

aMF ..... alternate descending motor fibers

BA ..... Brodmann area

BBT ..... Box and Block Test

CMF ..... callosal motor fibers

CRP ..... corticoreticular pathway

CST ..... corticospinal tract

CVA ..... cerebral vascular accident

DTI ..... diffusion tensor imaging

DWI ..... diffusion-weighted imaging

FA ..... fractional anisotropy

fMRI ..... functional magnetic resonance imaging

ICF ..... International Classification of Functioning, Disability, and Health

JHU ..... John Hopkins University

M1 ..... primary motor cortex

MCA ..... middle cerebral artery

MD ..... mean diffusivity

MEPs ..... motor-evoked potentials

MI ..... Motricity Index

MRI ..... magnetic resonance imaging

PLIC ..... posterior limb of the internal capsule

PMC ..... premotor cortex

PPN ..... pedunculopontine nucleus

ROI ..... region of interest

RN ..... red nucleus

RST ..... rubrospinal tract

SMA ..... supplementary motor area

TIA ..... transient ischemic attack

TMS ..... transcranial magnetic stimulation

UE-FM ..... upper extremity – Fugl-Meyer

WD ..... Wallerian degeneration

CHAPTER 1

INTRODUCTION

Stroke is the leading cause of disability in the United States, with approximately 795,000 individuals suffering a stroke each year.[1] Although most survivors recover to some extent,[2, 3] more than 50% are left with residual motor deficits.[4] Weakness or paralysis associated with stroke often results in individuals requiring assistance for activities of daily living (ADLs) and/or gait, with up to approximately half experiencing long-term dependency.[5] Furthermore, the prevalence of stroke is increasing as a result of the aging population, as well as post-stroke life expectancy due to medical advances in early detection and treatment. With approximately 6.4 million Americans who are stroke survivors[6] and many suffering from deficits in motor function, **improving our understanding of neuroplastic changes in the brain post-stroke will offer more insight into predicting level of motor impairment and recovery potential, and assist in targeting therapeutic interventions.**

Recovery of motor function varies considerably following stroke, as individuals with similar lesions on structural scans can exhibit different motor impairments and/or responses to treatments. Motor recovery depends on adaptive processes in both the affected and unaffected hemisphere, although the exact neural mechanisms remain unclear.[7, 8] Results of structural imaging studies suggest that both lesion size[9, 10] and location[11-13] correlate with motor impairment, and lesion-symptom mapping studies

have provided additional information by characterizing the relationship between lesion site and functional deficits,[14-16] yet considerable variance remains unexplained. With advances in neuroimaging techniques, researchers have begun to examine measures of cortical integrity, such as the microstructural properties of white matter and anatomical connectivity, across the whole brain and their role in motor impairment and recovery.

Diffusion tensor imaging (DTI) allows for the examination of the integrity and orientation of white matter in the brain by estimating the magnitude and directionality of water diffusion.[17] Several studies using DTI techniques have demonstrated a correlation between upper extremity motor dysfunction and decreased integrity of white matter tracts in both acute[18-20] and chronic[21-23] stroke. Most of these studies, however, had a small sample size ($n < 20$), used outcome measures that are not as clinically feasible or do not capture different International Classification of Functioning, Disability, and Health (ICF) domains, and/or focused on the major neural pathway for voluntary movement (i.e. corticospinal tract). While the corticospinal tract is important for skilled voluntary movement, other secondary motor pathways may also play a role. Additionally, few studies have examined changes in chronic white matter integrity post-stroke and lower extremity motor function and gait.[24, 25] This highlights the need for more research into the relationship between white matter integrity in primary *and* secondary motor tracts/brain regions and multiple measures of upper *and* lower extremity motor function in a *larger* sample of individuals with chronic stroke. Therefore, the first aim of this study was:

Aim 1a. To investigate the neural basis of upper extremity motor deficits in individuals with chronic stroke by correlating measures of upper extremity motor function (Box and

Block Test, grip strength, and upper limb portion of the Motricity Index for the affected extremity) with DTI-derived indices [fractional anisotropy (FA), mean diffusivity (MD)] of white matter integrity of specific neural tracts (corticospinal and transcallosal tracts) and motor-relevant brain regions (red nucleus, thalamus, substantia nigra, superior cerebellar peduncle).

Hypothesis 1: Poorer performance on upper extremity motor measures will be associated with reduced white matter integrity (as indicated by lower FA and/or higher MD values) in bilateral corticospinal and transcallosal tracts.

Hypothesis 2: Microstructural changes (as indicated by higher FA values) in the ipsilesional red nucleus will be correlated with better performance on upper extremity motor measures.

Aim 1b. To investigate the neural basis of gait and lower extremity motor deficits in individuals with chronic stroke by correlating measures of mobility and lower extremity motor function (gait speed and lower limb portion of the Motricity Index for the affected extremity) with DTI-derived indices (FA and MD) of white matter integrity of specific neural tracts (corticospinal and transcallosal tracts) and motor-relevant brain regions (red nucleus, thalamus, substantia nigra, superior cerebellar peduncle).

Hypothesis 1: Greater walking and lower extremity motor impairment will be associated with reduced white matter integrity (as indicated by lower FA and/or higher MD values) in bilateral corticospinal and transcallosal tracts.

Hypothesis 2: Microstructural changes (as indicated by higher FA values) in the ipsilesional red nucleus will be associated with better walking ability and lower extremity motor performance.

Loss of and/or decreased integrity of white matter tracts after stroke can have both local and remote effects due to the disruption of neural connections between different brain regions that are functionally related. While chronic stroke lesions can be seen easily on structural scans, structural abnormalities often extend beyond the site of tissue necrosis rendering nonlesioned areas dysfunctional,[26, 27] which can further contribute to behavioral deficits. Advances in neuroimaging have enabled the mapping of white matter connections across the entire brain (the brain connectome).[28, 29] This connectivity-mapping technique has only been used to examine the relationship between cortical structural brain connectivity and *language* impairments;[30] this methodology has not yet been used to evaluate the relationship between cortical structural disconnection and *motor* impairments. To address this gap, the second aim of this study was:

Aim 2a. To examine the relationship between chronic upper extremity motor impairments and cortical necrosis and disconnection in individuals with chronic stroke using the connectome-mapping techniques recently developed by Bonilha et al. (2014).[30]

Hypothesis 1: Structural connectivity between cortical/subcortical motor-relevant ROIs will be positively associated with upper extremity motor performance.

Hypothesis 2: Poorer performance on upper extremity motor measures will be associated with residual cortical necrosis and/or disconnection of motor-relevant brain regions (i.e. primary motor cortex, premotor cortex, supplementary motor area).

Aim 2b. To examine the relationship between chronic mobility/lower extremity motor impairments and cortical necrosis and disconnection in individuals with chronic stroke using the connectome-mapping techniques recently developed by Bonilha et al. (2014).[30]

Hypothesis 1: Structural connectivity between cortical/subcortical motor-relevant ROIs will be positively associated with gait and lower extremity motor performance.

Hypothesis 2: Poorer performance on gait and lower extremity motor measures will be associated with residual cortical necrosis and/or disconnection of motor-relevant brain regions (i.e. primary motor cortex, premotor cortex, supplementary motor area).

CHAPTER 2

LITERATURE REVIEW

2.1. PATHOPHYSIOLOGY AND CLINICAL PRESENTATION OF STROKE

Stroke, or a cerebral vascular accident (CVA), is caused by a sudden interruption in cerebral blood flow resulting in damage and/or cell death to neuronal tissues of the brain with neurological deficits persisting for at least 24 hours.[31, 32] There are two main etiological categories of stroke: ischemic and hemorrhagic. An ischemic stroke occurs when a blood clot blocks blood flow to the brain. Two types of ischemic stroke are cerebral thrombosis and cerebral embolism. A cerebral thrombosis occurs when a blood clot (or thrombus) develops in the cerebral artery network, usually secondary to atherosclerosis. A cerebral embolism occurs when a blood clot in the body breaks free (an embolus) and travels through the bloodstream to the brain and becomes lodged in one or more of the small vessels of the brain.[32] Blockage of or low perfusion through the artery leads to a lack of oxygen and glucose, disruption of cellular metabolism, and eventually injury (either reversible or permanent) and/or cell death to neuronal tissue. Ischemic strokes account for 87% of all strokes in the United States.[1]

A hemorrhagic stroke occurs when cerebral blood vessels rupture and cause bleeding in the extravascular regions of the brain, often as a result of head trauma or increased internal pressure. A ruptured aneurysm – a ballooning of a blood vessel caused by a weakened portion of the arterial wall – can also lead to abnormal bleeding in the brain. In hemorrhagic stroke, damage to neuronal tissue is caused by ischemia and

mechanical injury secondary to the accumulation of fluid/blood within the skull creating increased pressure on brain tissue.[32] Two main types of hemorrhagic stroke are intracerebral hemorrhage and subarachnoid hemorrhage. An intracerebral hemorrhage occurs when there is bleeding in the brain itself, while a subarachnoid hemorrhage occurs when blood vessels rupture at the brain's surface just below the arachnoid mater. Intracerebral hemorrhages account for 10% of all strokes, while subarachnoid hemorrhages account for 3%.[1]

Collateral blood flow to the brain helps protect it from various types of vascular compromise.[33] The right and left anterior cerebral arteries (ACAs) supply blood to the anterior 2/3 of the medial aspect of each cerebral hemisphere, including portions of the frontal lobe, parietal lobe, basal ganglia, corpus callosum, and internal capsule. In an ACA stroke, the lower extremity is more involved than the upper extremity due to cortical representation of motor/ somatosensory areas on their respective homunculi. The middle cerebral arteries (MCAs) supply blood to the lateral aspect of the cerebral hemispheres, including portions of the frontal, temporal, and parietal lobes, internal capsule, caudate nucleus, and basal ganglia. The MCA is the most common site of stroke, with increased involvement of the upper extremity compared to the lower extremity due to cortical representation.[32] The posterior cerebral arteries supply blood to the posterior 1/3 of the cortex including the occipital lobe, posterior aspect of the temporal lobe, midbrain, and thalamus. The basilar artery supplies blood to the pons, internal ear, and cerebellum.

The location of an infarct also impacts the behavioral deficits present in an individual post-stroke. In general, a left hemispheric stroke results in right-sided

hemiplegia and/or sensory loss, and can also cause dysfunction in speech and language abilities. A right hemispheric stroke results in left-sided hemiplegia and/or sensory loss, and can also lead to visual neglect of the left-hand side of space. Damage to specific brain regions involved in motor control (to be discussed next) or to the white matter pathways connecting them also impacts the presentation and subsequent recovery of sensorimotor impairment in individuals with stroke.

2.2. NEUROANATOMY OF MOTOR CONTROL

Different parts of the cerebral cortex are functionally specialized, and there are three major cortical areas involved in motor control: (1) primary motor cortex (MI), (2) premotor cortex, and (3) supplementary motor area (SMA)[34] (Figure 2.1). The primary motor cortex corresponds to the precentral gyrus and stimulation of this part of the brain produces isolated movements on the contralateral side of the body; bilateral movements are seen in extraocular muscles and muscles of the face, tongue, jaw, larynx, and pharynx due to bilateral cortical input to cranial nerve motor nuclei. The representation of body regions in the primary motor cortex forms the motor homunculus, with increased cortical area allotted for more skilled movements. The function of the primary motor cortex is to carry out individual and initiate highly skilled movements of various parts of the body. Afferent input to the primary motor cortex includes fibers from the thalamus, premotor cortex, somatosensory cortex, and cerebellum and is involved in coordinating and refining movements. Efferent output from the primary motor cortex contributes to the association, commissural, and corticofugal fiber systems. Approximately one-third of corticospinal tract (CST) fibers originate from this cortical area.[34, 35]

The premotor cortex is located in the frontal lobe anterior to the primary motor cortex. Stimulation of this part of the brain produces movements similar to those elicited by the primary motor cortex (however, with stronger stimulation), as well as gross movements that require increased muscle coordination. The premotor cortex is involved with voluntary movements that depend on visual, auditory, and/or somatosensory input. This area stores motor programs assembled from past experience, and is activated when a new motor program is established or when one is modified based on sensory information. Afferent input to the premotor cortex includes fibers from the somatosensory cortex, thalamus, and basal ganglia. The premotor cortex influences movement via connections with the primary motor cortex or through projections to corticofugal fibers. Approximately one-third of CST fibers originate from the premotor cortex.[34, 35]

The supplementary motor area is situated on the medial surface of the frontal lobe, anterior to the medial extension of the primary motor cortex. Stimulation of this part of the brain also produces contralateral limb movements, but a stronger stimulus is needed compared to the primary motor cortex. The supplementary motor area is important in the temporal/sequential organization of movement and in tasks that require retrieval of motor memory, and becomes more significant in the execution of simple motor tasks if the primary motor cortex is injured. It is connected directly via bi-directional pathways with the ipsilateral primary motor, premotor, and somatosensory cortices and with the contralateral supplementary motor area, and indirectly receives subcortical input mainly from the basal ganglia via corticothalamic pathways. Fibers from the supplemental motor area converge on parts of the caudate nucleus, putamen, and thalamic nuclei. Only about 5% of neurons from this cortical region contribute to the

CST.[34, 35] While the neuronal activity involved in motor control is distributed among various cortical areas, specific motor areas are preferentially recruited based on the type of motor task (e.g., simple vs. complex movements) or due to the presence of disease processes in the brain that may alter normal anatomical and functional brain networks.

Several subcortical regions of the brain are also involved in motor control, including the cerebellum, basal ganglia, and thalamus.[34] The cerebellum is involved in the coordination of movements; it receives input about voluntary movement from the cerebral cortex and compares this information with proprioceptive input it receives from muscles, tendons, and joints. The cerebellum can then make adjustments affecting voluntary movement by indirectly influencing the activity of lower motor neurons. The basal ganglia are a collection of nuclei located near the thalamus that play an important role in postural control and voluntary movement. The largest group of nuclei is called the corpus striatum (comprised of the caudate nucleus, putamen, and globus pallidus); other nuclei include the substantia nigra and subthalamic nucleus. These nuclei have extensive connections with many different regions of the brain. The corpus striatum integrates sensory-motor information from the cerebral cortex, thalamus, and brainstem; efferent information then passes back to motor areas of the cerebral cortex and brainstem influencing the preparation and/or execution of voluntary movements.[34, 35] The thalamus is involved in the integration of sensory information and is closely linked (via axons/reciprocal fibers) to the cerebral cortex. It is an important relay station for sensory-motor neuronal loops involving the cerebellum and basal nuclei, which are important for voluntary movement. Figure 2.2 highlights some of the main connections of the motor cortex.

Nerve fibers from different regions of the brain descend in the white matter to form nerve bundles called descending tracts that project to various subcortical structures. The corticospinal tract (CST) is the major neural pathway for skilled, discrete voluntary movements, especially for fine movements of the hands.[36] The CST (Figure 2.3) is a large collection of axons that originates from neurons in the cerebral cortex; approximately two-thirds of the fibers arise from the precentral gyrus (primary motor and premotor areas) and one-third from the postcentral gyrus (parietal lobe). As the fibers descend from the cortex, they converge in the corona radiata and course through the internal capsule, the cerebral peduncle of the midbrain, the ventral pons and onto the medulla oblongata where they form a swelling of fiber bundles known as the pyramid. At this point, fibers either decussate (cross and descend along the contralateral side of the spinal cord) to form the large, lateral CST (75-90% of fibers), or do not cross over and become part of the anterior or anterolateral CST.[35] This site of fiber crossing is called the pyramidal decussation. CST axons eventually terminate on neurons located in the gray matter of the spinal cord. Damage to the CST rostral to the pyramidal decussation (e.g., cortex, internal capsule) results in contralateral motor deficits, while damage caudal to the decussation (e.g., lesions to the lateral CST) results in ipsilateral motor deficits below the level of the lesion.

While the CST is important for skilled voluntary movement, it is not the only tract serving this function as other neural pathways such as the rubrospinal, reticulospinal, and vestibulospinal tracts can mediate simple voluntary movements.[35] The axons of the rubrospinal tract originate in the red nucleus in the midbrain and then cross at that level before descending through the pons and medulla

oblongata to reach the lateral white column of the spinal cord (Figure 2.4). The fibers then terminate on interneurons in the gray matter of the spinal cord, in close proximity to lateral CST fibers, and facilitate activity of flexor muscles. Since afferent input to the red nucleus includes fibers from the cerebral cortex and cerebellum, it is believed the rubrospinal tract is an indirect pathway by which the cortex and cerebellum can influence motor neurons in the spinal cord.[35, 37] The axons of the reticulospinal tract originate in the reticular formation – a large, diffuse collection of neurons – of the pons (forming the pontine reticulospinal tract consisting of primarily uncrossed fibers) and medulla oblongata (forming the medullary reticulospinal tract consisting of both crossed and uncrossed fibers). The pontine reticulospinal tract descends through the anterior white column, whereas the medullary reticulospinal tract descends though the lateral white column of the spinal cord. Both tracts may facilitate or inhibit the activity of motor neurons, primarily of antigravity muscles of the proximal extremities, influencing voluntary movement, reflexive activity, and postural control.[35] The axons of the vestibulospinal tract originate in the vestibular nuclei of the pons and medulla oblongata, which receive afferent input from the inner ear and cerebellum. This descending pathway influences balance through the facilitation of extensor muscles and inhibition of flexor muscles.[35]

The corpus callosum is the major fiber bundle connecting corresponding motor and sensory regions (i.e. commissural or transcallosal fibers) between the two cerebral hemispheres. The callosal motor fiber tract (CMF) is located in the posterior midbody/isthmus of the corpus callosum.[38] The integrity of the CMF is crucial for interhemispheric inhibition, a process in which activity in the primary motor cortex of

one hemisphere inhibits activity in the homologous region of the opposite hemisphere in order to execute unimanual movements and coordinated bimanual tasks.[39-41] Impairments in interhemispheric inhibition have been associated with motor deficits post-stroke.[40, 42]

Motor weakness is one of the most disabling consequences of stroke, often leading to difficulties in the performance of ADL's, increased energy expenditure, asymmetrical gait patterns, and overall decreased activity levels. Several neuroplastic processes have been suggested for motor recovery post-stroke, such as reorganization of peri-lesional brain areas,[43-45] unmasking of the ipsilateral motor pathway from the nonlesioned cortex to the affected extremities,[46-48] secondary motor area contributions,[49, 50] and recovery of the damaged CST.[51-53] **Modern imaging techniques have allowed for greater examination of the microstructural integrity of white matter motor tracts post-stroke, yet more research needs to be performed to enhance our understanding of the relationship between motor deficits and structural brain connectivity in chronic stroke**.

2.3. IMAGING AND STROKE

2.3.A. *Diffusion Tensor Imaging as an Adjunct to Other Imaging Techniques*

In order to improve prognosis of motor recovery following stroke, clearer identification of the brain regions and structures essential for maintaining motor performance is needed, as well as a better understanding of the damage caused to cortical areas and to local and global neural networks following stroke. Structural scans can provide information concerning lesion size and location, and studies have shown that lesions involving primary and secondary motor cortices and/or corticofugal motor tracts

are associated with decreased upper limb motor recovery.[11, 12] Functional magnetic resonance imaging (fMRI) can provide additional information concerning cortical reorganization following stroke. Changes in brain activity such as unilateral overactivation of primary and association motor areas, perilesional overactivation around the primary motor cortex and premotor areas, and bilateral recruitment of associated motor and nonmotor areas have been associated with motor task performance post-stroke.[54] In patients who demonstrate more favorable recovery, overactivations tend to diminish[55, 56] while persistent recruitment of contralesional motor areas often appears in patients with poorer functional outcomes.[56, 57] **Recent studies using diffusion tensor imaging (DTI) techniques have shown that the *integrity* of corticospinal tracts also plays an important role in the degree of motor impairment and subsequent recovery.[58]** A study by Zhu et al. (2010) showed that degree of lesion-CST overlap, but not lesion size alone, was a significant predictor of motor impairment in individuals with chronic stroke.[59] Another study by Lindenberg et al. (2010) proposed a classification system of motor impairment categories based on CST integrity post-stroke that might be helpful in predicting motor recovery potential.[21] Despite general advances in neuroimaging techniques, a lot remains unexplained in terms of the variability seen in stroke recovery.

2.3.B. *Imaging Techniques for the Corticospinal Tract*

Preservation of the CST is important in the recovery of motor function after stroke.[60-62] Several methods have been used to evaluate the integrity of the CST, including transcranial magnetic stimulation (TMS), fMRI, and DTI. TMS can be used to evaluate the *functional* integrity of the CST by stimulating neurons to try and elicit

motor-evoked potentials (MEPs), and the presence or absence of MEPs has used as a prognostic indicator of motor recovery.[63, 64] Limitations of TMS, however, include poor spatial resolution and the possibility of false negatives.[58] Imaging techniques such as fMRI can be used to assess cortical activity by detecting changes in blood flow in the brain during affected limb movements, demonstrating which sensorimotor areas (ipsilesional and/or contralesional) are active following stroke, thereby providing insight into cortical reorganization.[56, 65-67] Functional MRI is limited, however, in individuals with poor motor function following stroke, and it cannot distinguish the characteristics of the activated motor pathway(s).[58] **DTI, however, can be used to examine the structural integrity of white matter tracts such as the CST.** It can be used to visualize ischemic regions within the CST (and other motor tracts) or to quantify CST integrity, thus offering additional information for predicting motor impairment and recovery following stroke.[60]

2.4. DIFFUSION TENSOR IMAGING

2.4.A. *Tissue Water Diffusion*

When water molecules are unconstrained, they can move at random (i.e., Brownian motion).[68] The microstructure of brain tissue, however, forms physical boundaries (such as cell membranes and white matter tracts) that limit the motion and restrict the overall diffusivity of water molecules.[69] Diffusion is called *isotropic* when molecules can move unconstrained in any direction, and is called *anisotropic* when molecular mobility is not the same in all directions. The movement of water molecules may be restricted depending on the presence and orientation of microstructural obstacles (e.g., axonal fibers), with diffusion being more restricted perpendicular rather than

parallel to such obstacles.[17, 68] **Improvements in imaging have allowed for quantitative measurements of the directionality of water diffusion in brain tissue, providing insight into the orientation and integrity of various motor tracts.**

2.4.B. *Background on Measurement of Water Diffusion*

Magnetic resonance imaging (MRI) is based on the interaction between radio waves and hydrogen nuclei (i.e. protons) in tissues in the presence of a strong magnetic field. Since most hydrogen in the body is in the form of water, MRI distinguishes tissues based on their water content. When placed in a magnetic field, the protons will line up parallel or antiparallel to the main magnetic field and the person temporarily becomes "magnetized." The person is then exposed to a pulse of radiowaves at a particular frequency, which is absorbed by the hydrogen nuclei and causes them to be pushed out of alignment.[70] A spin-echo is generated when a second pulse is used to "refocus" the phase of the protons. The electromagnetic signal given off in the process is received by a radiofrequency coil and transmitted to a computer. Pulse sequences can be applied along different spatial directions (*x, y,* and *z*-axis*),* and the magnitude of the signal at each frequency is proportional to the hydrogen density at certain locations.[70] Different radiofrequency mechanisms can be utilized to decrease "noise" and reduce artifact in the signal.

Diffusion-weighted imaging (DWI) is a non-invasive way to measure water diffusion within the human brain and is useful in detecting early signs of ischemia.[71, 72] By using additional magnetic field gradients, the spin-echo sequence of MRI can be modified to reflect the mobility of water.[73] The resulting signal is heavily weighted by local differences in water diffusion and less influenced by tissue composition. The

amount of diffusion-weighting is denoted by a "b-value," with larger b-values resulting in increased diffusion-weighting of the magnetic resonance image, i.e. higher sensitivity to the differences between regions of high and low diffusion.[74]

The apparent diffusion coefficient (ADC) describes the amount of random translational motion of protons in a tissue and can be used as a quantitative estimate of diffusion.[75] It can be calculated from DWI by combining several images of the same tissue "slice" into a single image, a process known as "ADC mapping." The ADC represents a mean estimate of water diffusion as measured in mm^2/sec. Larger ADC values represent regions of increased water diffusion, while tissues with lower ADC values correspond to regions of lower diffusion. In tissues where the measured apparent diffusivity is isotropic, it is sufficient to describe water diffusion using the ADC.[17, 76]

Diffusion tensor imaging (DTI) enables the examination of the microstructural integrity and orientation of white matter in the brain *in vivo* by estimating the magnitude and directionality of water diffusion.[17] Water diffuses more freely parallel to normal white matter fibers than perpendicular to them, causing diffusion anisotropy of the white matter. Structural elements such as axonal myelination and density may hinder water diffusion across fiber bundles.[77] In the presence of anisotropy, diffusion needs to be described in each direction with correlations between these directions. DTI takes diffusion measurements in multiple directions and uses tensor decomposition to determine diffusivities parallel and perpendicular to white mater tracts.[78] The diffusion tensor has three eigenvalues ordered as $\lambda_1 \geq \lambda_2 \geq \lambda_3$. Axial diffusion corresponds with diffusion along the principle direction (i.e. λ_1) and reflects fiber orientation since water diffusion is much greater parallel to rather than

perpendicular to white matter tracts. Fiber tract direction, therefore, is determined by the largest eigenvalue. Radial diffusion reflects diffusivity perpendicular to these tracts and corresponds to $[\lambda_2 + \lambda_3] / 2$.[76] These diffusivities are then used to calculate several DTI parameters. Two of the more common indices are mean diffusivity (MD), which represents the overall magnitude of water diffusion, and fractional anisotropy (FA), which reflects the degree of diffusion directionality (Figure 2.5). **The FA value is the most common DTI parameter currently used to assess white matter integrity in individuals with stroke.**[79]

FA values range from 0 (completely isotropic diffusion) to 1 (completely anisotropic diffusion), with higher values indicating greater directionality. White matter tracts typically have an FA > 0.20,[76] and FA maps can be produced to distinguish between different fiber bundles based on diffusion directionality. Loss of microstructural integrity of white matter tracts (e.g., local tissue damage within the primary lesion, anterograde and/or retrograde axonal degeneration) is typically reflected by a reduction in FA value (representing decreased anisotropic diffusion) and/or an increase in MD (representing increased water diffusion in the extracellular space).[76]

2.4.C. *Changes in Water Diffusion After Stroke*

During acute cerebral ischemia, there is a decrease in cerebral blood flow to the affected area and a subsequent influx of extracellular water into the intracellular space resulting in intracellular swelling (cytotoxic edema).[17] Decreases in ADC occur within minutes of the onset of cerebral ischemia, allowing the ischemic region to be visualized as hyperintense in a diffusion-weighted image (DWI).[80] As the infarct progresses into the acute and subacute periods, there is an increase in extracellular water (vasogenic edema).

In the late subacute and chronic periods, continued cellular changes and disintegration can result in cell lysis, axonal degeneration, and decreased interstitial fluid flow.[76] In terms of FA and MD, Yang and colleagues (1999) describe 3 sequential phases of water diffusion changes in the ischemic region post-stroke.[81] In general, FA is mildly elevated while MD is reduced in hyperacute stroke; the acute and subacute periods are characterized by reduced FA and reduced MD; and the late subacute and chronic periods are characterized by reduced FA and elevated MD.

2.4.D. *Wallerian Degeneration*

Wallerian degeneration (WD) is characterized by anterograde degeneration of the distal portion of axons after injury to the cell body and/or proximal nerve and commonly occurs after ischemic stroke. Disintegration of axonal components has been detected as early as within the first 2 weeks post-stroke using DTI.[82] This initial disintegration is followed by progressive myelin degeneration and eventually fibrosis and atrophy of the fiber tracts. Such progressive deterioration may slow down the neurological recovery process. Several studies have examined WD of the pyramidal tract in individuals with stroke and found that reduced FA and/or reduced signal (which were interpreted as a reflection of WD) along the CST was associated with increased motor impairment.[82-84]

2.4.E. *Diffusion Tensor Imaging Tractography*

Different approaches can be used with DTI analyses for the evaluation of white matter integrity post-stroke. Cross-sectional region of interests (ROIs) can be used to delineate fiber tracts that pass through specific ROIs and the FA can be calculated within each ROI, thereby providing a quantitative measure of water diffusion directionality and an assessment of fiber tract integrity within the given ROI volume. Tract-based methods

utilize algorithms to group pixels based on tensor properties to help reconstruct and measure the integrity of specific fiber tracts (Figure 6). Both approaches have demonstrated decreased integrity of the CST post-stroke as indicated by lower FA values,[85, 86] and have revealed an association between interhemispheric asymmetries in FA values and reduced motor recovery[18, 21] and reduced skill improvement post-training[60] in individuals with stroke. The two approaches, however, may evaluate different aspects of white matter tract integrity based on methodological differences. The tract-based approach requires at least part of the tract to be intact for the entire length of its course, and is sensitive to major anatomical deformations. The cross-sectional ROI approach does not typically evaluate the integrity of specific tracts along their entire course, but rather examines a representative section of a tract. Each approach, therefore, may provide complementary information concerning neuroanatomical changes in white matter integrity post-stroke.[87] For the purpose of the proposed study, a cross-sectional ROI approach will be used for Aim 1. For Aim 2, whole brain tractography methods will be used to compute the number of fibers between each pair of cortical/subcortical ROIs for the construction of the brain connectome.[30]

Limitations of FA and DTI tractography need to be discussed. The measured diffusion tensor is an average of several tissue compartments (with different diffusion profiles) within each voxel. Therefore, areas of tissue partial volume (where there is a mixture of white matter/gray matter/cerebrospinal fluid) or of white matter partial volume (where there crossing or diverging fibers) will result in low anisotropy.[78, 88] False tracking can occur in low anisotropic areas and/or in regions with fiber complexity and crossing. Furthermore, many factors influence FA values (e.g., axonal count/density,

degree of myelination, fiber organization) and DTI cannot discern which structural element(s) are contributing to observed changes in FA.[89]

2.5. CORTICAL CONNECTIVITY AND NETWORK DISRUPTION

Motor impairment and recovery can be quite variable among individuals with stroke with similar lesion size and location. Furthermore, patients can often exhibit motor impairments beyond those associated with the lesion size/location on structural scans. Altered structural integrity of white matter occurs not only in the lesioned area but also in brain regions and motor tracts beyond the infarction site. These nonlesioned areas can be indirectly affected by the loss of connections resulting from a stroke and can become dysfunctional,[27] contributing to behavioral deficits. **Advances in neuroimaging have enabled the mapping of white matter connections across the entire brain (the brain connectome)[28, 29] allowing for a more thorough examination of the extent of white matter disconnection after stroke.**

So far, this connectivity-based approach has been used to examine structural disconnection in the lesioned hemisphere (compared to the nonlesioned hemisphere) after ischemic stroke[29] and to examine the relationship between language impairments and structural brain connectivity in individuals with chronic aphasia.[30] Results showed intrahemispheric disconnection extending beyond the necrotic area and fiber reduction in several major white matter tracts underlying the necrotic tissue in eight individuals with chronic stroke, with all participants exhibiting an individual pattern of disconnection.[29] Similarly, structural disconnection was more prevalent in the affected hemisphere (spared by necrotic tissue) than homologous regions in the unaffected hemisphere in a larger sample of individuals with chronic stroke. This finding highlights the idea that the extent

of reduced structural connectivity cannot be surmised based solely on necrotic tissue size/location.[30] Evaluation of the relationship between cortical connectivity and chronic aphasia revealed that structural disconnection of Brodmann area (BA) 45 is independently associated with naming performance (after controlling for BA 45 necrotic tissue volume) in individuals with cortical/subcortical lesions.[30] This finding suggests that preservation of white matter fibers supporting BA 45 is important for accurate naming performance in this patient population. **This connectivity-based approach has not yet been used to examine the relationship between *motor* impairments and impaired cortical connectivity in chronic stroke.** By examining neural connectivity using the brain connectome, the extent of cortical disconnection beyond the lesion site may be more fully revealed, expanding our understanding of motor impairments and recovery after stroke. **The proposed research study will focus, in part, on using this methodology to examine the impact of residual cortical structural disconnection on upper and lower extremity motor function in chronic stroke.**

2.6. DTI AND UPPER EXTREMITY MOTOR IMPAIRMENT/RECOVERY IN ACUTE STROKE

With the development of DTI and tractography, several studies have demonstrated a correlation between motor dysfunction and decreased integrity of white matter tracts in the early stages following stroke. As stated previously, decreases in FA have been demonstrated in acute stroke[81, 86] and have been shown to be correlated with residual motor function of the affected upper extremity.[18, 90] **Since the CST is the major neural pathway for voluntary fine movements of the hand, this motor tract has been a main focus of research.** A study by Radlinska et al. (2010) revealed significant reductions in FA along the entire pyramidal tract within 3 weeks of stroke in 12 patients

who had suffered an infarct affecting the pyramidal tract.[19] Furthermore, decreases in FA ratio (affected/unaffected hemisphere), which indicates higher FA asymmetry, were significantly correlated with poorer upper limb motor function during the acute phase, indicating that the extent of acute pyramidal tract damage as determined by DTI measures is associated with acute motor deficits.

Predicting motor dysfunction and recovery post-stroke can be challenging, as patients with similar lesions on structural scans can often exhibit vastly different motor impairments and/or responses treatment. A few studies have shown that the integrity of the CST during the early stages post-stroke can be used to predict motor outcome and recovery. In a study by Cho et al. (2007), DTI findings obtained from 55 patients within 7-30 days following stroke were classified into 4 groups: Type A, the CST passed around the infarct; Type B, similar to Type A except the CST fibers originated from an area of the brain other than M1; Type C, the infarct interrupted the CST; and Type D, the CST failed to reach the infarct secondary to degeneration.[91] Motor function scores of the affected hand at a 6-month follow-up were significantly different between groups, with the Type A group exhibiting the highest scores (i.e. better motor function) while the lowest scores were seen in the Type D group. These results demonstrate that the integrity of the CST around an acute lesion could be a useful marker for predicting motor outcome of the affected hand at 6 months post-stroke. Similar studies have demonstrated that extent of CST involvement in the infarct is significantly related to upper extremity motor recovery in the acute, subacute and chronic phase,[90, 92, 93] further elucidating the predictive value of DTI and CST integrity for motor outcome in individuals with stroke.

While degree of CST involvement in the infarct is one important prognostic criterion, other parameters can also impact long-term motor outcome. Diffusion anisotropy measures, such as FA symmetry between hemispheres, have also been used as potential markers for the prediction of motor outcome following stroke. An early study by Yang et al. (1999) found a correlation between the diffusion anisotropy ratio (mean values in the lesion ROI/mean values in corresponding contralateral ROI) measured within 12 hours of stroke onset and acute, subacute, and 3-month follow-up clinical scores in 26 patients with cerebral infarct.[81] Subsequent studies also found that FA values were significantly smaller in the lesioned versus contralateral hemisphere, and that FA asymmetry in the early stages of stroke predicted motor outcome of the affected hand at 3 months post-stroke.[18, 90] Overall, individuals with greater FA asymmetry exhibited less improvement in hand motor function across time. More recently, the FA ratio between affected/unaffected CSTs at 30 days post-stroke (compared to $\leq$ 12 hours and 3 days post-stroke) was found to be the only independent predictor of motor outcome at 2 years.[94] These findings suggest that early water diffusion anisotropy changes may help in predicting motor outcome following stroke.

In addition to FA, examining the components that comprise it (i.e., axial and radial diffusivity) may also provide insight into predicting motor dysfunction as each undergoes time-varying changes following stroke.[69] Specifically, axial diffusivity may be a marker of axonal damage while radial diffusivity may reflect the integrity of the myelin sheath.[95, 96] Most studies have used the pooled FA index to quantify white matter integrity following stroke, however a recent study examining white matter integrity in the early stages post-stroke found that acute loss in CST *axial diffusivity* (3-7 days post-

stroke) and subacute loss in CST *FA* (1-2 months post-stroke) were strong predictors of chronic upper limb motor function.[97] These results highlight the prognostic value of both directional diffusivity and FA values in predicting motor outcome. Examination of axial/radial diffusivities in chronic stroke may enhance our understanding of FA alterations and the relationship between structural brain changes and motor impairment/response to treatment in the later stages of stroke.

While the CST is the main motor pathway for voluntary movements of the hand, secondary motor pathways and brain regions also play a role in upper extremity motor function and recovery. Microstructural changes in *commissural pathways* have been found in subacute stroke, with higher FA values in transcallosal fibers of the midbody of the corpus callosum being associated with better motor function at 3 months post-stroke.[20] Yeo & Jang (2010) examined changes in the *red nucleus* (RN) during the early stages post-stroke in 49 individuals with a corona radiata infarct.[37] TMS was also performed, and MEPs were obtained from the abductor pollicis brevis muscle; patients were classified into two groups (MEP+ and MEP-) according to the presence of MEP in the affected hand. **As the red nucleus (located in the rostral midbrain) is the origin of the rubrospinal tract (RST),[34] and the RST and CST are functionally related with their fibers terminating in close proximity in the spinal cord, the RN may have some potential to compensate for injury to the CST following infarct.** The authors found that the mean FA value of the RNs in affected hemispheres was significantly higher than values in the patients' unaffected hemisphere and compared to healthy controls. Mean RN FA values in the MEP- group were significantly higher than the MEP+ group, while clinical scores were greater in the MEP+ group compared to the

MEP- group. A more recent study also found increased FA in the ipsilesional RN at 3 months post-stroke in patients with a pyramidal tract infarction.[20] Additionally, a positive correlation was found between FA value in the RN and recovery of motor function. **These results suggest that neuroplastic changes (as evidenced by increased FA values) occur in the RN of the affected hemisphere during the early stages of stroke, and that these changes may indicate compensation for CST injury and contribute to motor recovery.** The proposed study will examine changes in the RN in individuals with chronic stroke and the relationship to chronic upper/lower extremity motor performance.

2.7. DTI AND UPPER EXTREMITY MOTOR IMPAIRMENT/RECOVERY IN CHRONIC STROKE

Chronic motor impairment after stroke has been associated with lesion size[9, 10] and location,[11, 12] and degree of overlap with the CST.[7, 62] There is great variability, however, in clinical manifestation and recovery from stroke in the chronic phase. More research needs to be performed to clarify structural changes in the brain during the later stages of stroke, such as those related to spontaneous and/or training-induced neuroplastic processes. **With advancements in neuroimaging and quantitative mapping tools, the relationship between lesion size, location, and motor tract integrity can be evaluated throughout the brain.** Such analyses have the potential to further our understanding of structural changes in ipsilesional, commissural, and contralesional white matter and their association with motor outcomes. In addition to the CST, consideration of other descending motor tracts and brain regions might provide a more accurate assessment of the relationship between structural changes in the brain and motor recovery in chronic stroke.

2.7.A. *Corticospinal Tract Integrity and Motor Recovery*

As stated earlier, preservation or recovery of the CST is crucial for good motor function following stroke, especially of fine movements of the hand, as it is the main neural pathway for skilled voluntary movements. CST damage (i.e. lesion overlap with the CST) correlates with residual motor ability, with poorer residual ability observed in patients with greater infarct lesion-CST overlap,[7, 23, 61, 62, 98, 99] **demonstrating that degree of lesion-CST overlap (not lesion size alone) is a major predictor of residual motor function in individuals with chronic stroke.** However, no association has been found between treatment-induced motor improvements following an intensive intervention and either CST integrity or lesion volume in chronic stroke patients with moderate to severe hemiparesis,[23, 99] **indicating that such training-induced gains may be mediated by neuroplastic processes in alternative neural pathways.**

Many researchers have demonstrated that sparing of, or reduced structural damage to the *ipsilesional* CST is associated with better motor outcome following stroke.[21, 62, 100] Microstructural integrity of CST pathways originating from primary *and* secondary cortical motor areas in the affected hemisphere have been shown to be reduced in chronic stroke, with grip strength strongly related to the integrity of fibers originating from the primary motor and (to a lesser extent) dorsal premotor cortices.[101] A study by Jang et al (2014) also showed positive correlations between motor function of the affected upper/lower extremities and ipsilesional CST FA and fiber number ratio, with preservation of CST integrity and absence of Wallerian degeneration important for better motor outcome.[102] Less is known, however, about the contribution and changes in microstructural status of the *contralesional* CST post-stroke. A study by Schaechter et al.

(2009) found that FA of both the ipsilesional and contralesional CST was significantly and positively correlated with motor skill performance of patients' affected hand; those with a poorer level of recovery had lower FA values of bilateral CST compared to controls, while the opposite was true for patients with better motor skill recovery.[85] Accumulating evidence indicates that white matter remodeling occurs in both ipsilesional and contralesional hemispheres, suggesting that structural remodeling of the contralesional motor system also contributes to motor recovery after stroke.[103-105] **The proposed research study will include the examination of white matter integrity in *both* lesioned and nonlesioned hemispheres in an effort to provide a more complete picture of neuroplastic changes in the broader motor system.**

Another study by Lindenberg et al. (2010) found that fiber number and regional FA asymmetry of the posterior limb of the internal capsule (PLIC) were significantly different between patients and healthy controls, with lower values in patients' ipsilesional hemisphere compared to the contralesional hemisphere.[21] Both fiber number and FA asymmetry of the PLIC significantly correlated with upper extremity motor performance when both CST and additional corticofugal tracts (aMF) were grouped together for analyses; correlations were found to be slightly lower when just the CST was analyzed. Furthermore, DTI analyses revealed a pattern of ipsilesional motor tract integrity that the authors used to divide the patients up into three groups: (1) fibers were traceable in both the anterior and posterior pons (CST and aMF), (2) fibers were only traceable in the posterior pons (aMF) but not the anterior pons (CST), and (3) no fibers were traceable in the pons. Significant differences in motor impairment were found between all three groups, with group 3 exhibiting the most impairment while patients in group 2 exhibited

(on average) only moderate impairment. **Overall these results demonstrate that the integrity of multiple motor tracts, not just the CST, play a role in motor impairment and recovery.**

2.7.B. *Transcallosal Fibers and Interhemispheric Changes Post-stroke*

Previous studies have demonstrated an alteration in interhemispheric activity following stroke,[40, 106, 107] and recent neuromodulation studies have revealed increased facilitation of motor recovery via up- or down-regulation of the ipsi- or contralesional motor cortex, respectively.[108, 109] Correspondingly, white matter integrity of transcallosal fibers has been found to be associated with upper extremity motor impairment in chronic stroke. Lower FA and higher radial diffusivity values in transcallosal fibers have been correlated with increased impairment.[22] A recent study by Lindenberg et. al (2012) investigated the relationship between DTI-derived measures of CST, aMF, and transcallosal tracts and motor recovery in 15 individuals with chronic stroke.[110] All patients had lesions which overlapped with the CST, while only 8/15 had lesions which overlapped with transcallosal fibers. The authors found that patients' ipsilesional CST and aMF, as well as transcallosal fibers, exhibited lower FA and higher directional diffusivity values compared to a healthy, age-matched control group. Greater motor gains post-intervention were correlated with higher ipsilesional CST, aMF, and transcallosal FA values and lower aMF and transcallosal directional diffusivities. Furthermore, directional diffusivities of transcallosal tracts had the greatest predictive power of change in Wolf Motor Function Test score. **These results complement earlier studies demonstrating that integrity of both the CST and additional corticofugal fibers plays a role in motor recovery in chronic stroke,[21, 111] and highlights the**

importance of transcallosal fibers and interhemispheric interactions for motor function. Furthermore, the value of examining the relationship between *multiple* DTI-derived measures (FA and directional diffusivities) and motor outcomes is highlighted. As not all patients had lesions overlapping with transcallosal fibers, these results demonstrate broader structural changes in the motor network as described in previous studies in individuals with white matter damage.[85, 112]

Other studies investigating changes in transcallosal fibers following stroke have revealed mixed results. The integrity of callosal motor fibers (CMF) has been shown to be reduced in individuals with subcortical stroke affecting the pyramidal tract (PT) at 6 months post-stroke, but no differences were found between groups (PT stroke, non-PT stroke, TIA) during the acute stage.[113] Another study by Borich et al. (2012) found reduced FA values in the *sensory* region of the corpus callosum, but not the motor region, in 13 individuals with chronic stroke.[114] Overall, these studies reveal disruption (both direct and indirect) of descending motor and transcallosal fibers following stroke. **Based on these results, the structural integrity of different motor tracts (bilateral CST and transcallosal tracts) and structural brain connectivity of motor-relevant cortical ROIs will be assessed in order to gain a better understanding of local and remote residual structural changes post-stroke and the relationship to chronic motor impairments.**

2.7.C. *Fractional Anisotropy Asymmetry and Predicting Motor Impairment*

FA asymmetry between hemispheres has been used as a potential marker for predicting motor function in chronic stroke. Stinear et al. (2007) found that in patients without MEPs in the affected upper extremity, higher FA asymmetry in the internal

capsule predicted lower UE-FM scores with a sharp decrease in score as FA asymmetry exceeded 0.25.[60] This finding may reflect that when damage to the primary motor area is so severe that MEPs cannot be produced, additional loss of PLIC integrity and potential damage to other corticofugal tracts may reduce the capacity for functional reorganization and result in greater impairments in upper limb function. Furthermore, stronger lateralization of cortical activity towards the ipsilesional primary motor area during affected hand use predicted higher UE-FM scores. This finding is consistent with other studies that suggest that while increased contralesional activation often occurs after stroke, functional outcomes remain poor in the presence of persistent recruitment of contralesional motor areas.[61, 115] Following a 30 day program of motor practice, FA asymmetry was found to predict change in UE-FM score across all patients; higher FA asymmetry and motor cortex damage predicted lower UE-FM change scores for individuals without MEPs, suggesting that functional potential is limited in patients with high FA asymmetry (especially above 0.25), which is consistent with previous studies.[18, 82] **These results demonstrate though that functional improvements can occur in the chronic stroke phase, even in individuals who exhibit fair recovery** per UE-FM score. Using neurophysiological (MEPs) and imaging (FA asymmetry) parameters, the authors go on to propose an algorithm for evaluating functional potential of upper extremity recovery to help target rehabilitation techniques in an effort to optimize motor recovery following stroke.[60]

2.7.D. *Cortical Reorganization of Motor Function Post-stroke*

One possible mechanism of motor recovery involves cortical reorganization of the affected motor function and contribution from secondary motor areas. Increased

activation in the supplementary motor area (SMA) and dorsal premotor cortex (dPMC) in the ipsilesional hemisphere have been reported.[116-119] The SMA has direct connections with spinal motor neurons which innervate the hand[120] and the dPMC has connections with the CST;[121] both areas, therefore, can play an augmented functional role in producing simple hand movements post-stroke, and correlations between improved upper extremity motor performance and increased ipsilesional dPMC activation have been found in previous studies.[52, 122, 123] Additionally, a study by Lotze et al. (2012) revealed larger activation increases from paced to maximal velocity fist clenching for patients compared to controls in the primary motor cortex (M1), dPMC, and SMA of the *contralesional* hemisphere.[118] A negative correlation was found between activation in the contralesional dPMC and ipsilesional CST integrity. Patients with a lower proportion of fibers passing through the PLIC exhibited high activation in the dPMC of both hemispheres, highlighting the importance of bihemispheric reorganization in this part of the brain with increasing CST damage after stroke. These studies complement other studies that have found increased activation in contralesional[61, 67, 100] and ipsilesional[61, 100, 124] primary sensorimotor areas following structural damage to the CST post-stroke.

While several studies have examined changes in motor cortical areas following motor practice post-stroke, fewer have examined changes in the brain more widely. Such insight into more global patterns of functional and structural brain plasticity could help target therapeutic interventions to enhance motor recovery potential. In 2011, Bosnell et al. found baseline differences in brain activation during a visuomotor task between 10 individuals with chronic stroke and healthy controls, with patients exhibiting decreased activation in cortical (ipsilesional primary

sensorimotor, dPMC, and SMA) and subcortical (contralesional cerebellum and thalamus bilaterally) regions involved in motor control.[125] This finding contrasts studies that have found increased functional activation in several brain areas following stroke.[55, 126] These studies, however, used simpler motor tasks than the one used in the aforementioned study. Additionally, there was a positive correlation between white matter integrity (FA) in the PLIC bilaterally and short-term practice gains in visuomotor tracking in patients at baseline. Both patients and controls improved performance following the motor practice period, but they exhibited opposite trends in brain activation patterns. In healthy controls, post-training task performance was associated with decreased activation in several cortical (left inferior frontal gyrus, right superior temporal gyrus and bilateral insula) and subcortical (left thalamus and basal ganglia) regions, which could reflect increased efficiency of motor activity for task performance. Patients, on the other hand, showed either no change or an increase in activation in these same regions, which is consistent with other studies that have found a relationship between increased activation of task-related brain regions and improved motor performance.[117, 127, 128] Furthermore, several regions that showed increased activation after training also exhibited reduced structural connectivity at baseline (left thalamus, basal ganglia, and right superior temporal gyrus). **Overall these results demonstrate that broader structural and functional changes in the brain post-stroke contribute to motor performance, and that repetitive motor practice in the chronic stage of stroke can promote functional recovery in brain regions where white matter integrity/connectivity has either been directly or indirectly reduced.**

Newton et al. (2006) described the trajectories of corticofugal fibers from each major component of the motor system (M1, dorsal premotor area – PMd, ventral premotor area – PMv, and SMA) to the cerebral peduncle in a group of 12 healthy volunteers.[119] The authors then assessed the extent of white matter damage (FA values) in three patients with chronic subcortical stroke and superimposed regions of reduced anisotropy onto the corticofugal fiber trajectories to infer the disconnection between motor areas. They then examined the relationship between this inferred damage/disconnection in the motor system and brain activation patterns as measured with fMRI during a hand grip task. Results revealed varying proportions of damage to corticofugal pathways in this small sample; greater damage to connections, however, was associated with increased ipsilesional motor system activation in secondary motor areas (e.g., PMd) when performing the fMRI task with the affected hand. Furthermore, a recent study by Rüber et al. (2012) found higher FA values in both ipsilesional and contralesional red nuclei in 18 chronic stroke patients compared to healthy controls, with significant positive correlations found between red nuclei FA and level of motor function.[129] These results further indicate that plastic remodeling can occur among secondary motor areas to help drive intact corticofugal connections for restoration of motor function in chronic stroke.

Overall, these studies show that while the integrity of descending neural pathways from the ipsilesional motor system is important for recovery of motor function after stroke, damage to one region may be compensated by increased activation of other regions and contributions from secondary motor areas and tracts (both ipsilesional and contralesional). Structural changes to the broader motor network

have been revealed post-stroke, and the integrity of not only the CST but other corticofugal and transcallosal fibers has been shown to be important in motor recovery.

2.8. DTI AND LOWER EXTREMITY/GAIT IMPAIRMENT IN STROKE

Motor recovery of the affected upper extremity has been highly studied, whereas less is known about the motor recovery mechanisms involved with lower extremity and locomotor function after stroke. While the CST is necessary for fine movements of the hands,[36, 60] locomotion and motor function of the legs is less dependent on the CST.[45, 130, 131] Some studies have suggested that the lateral CST does not play a central role in basic locomotor function in primates or human[45, 79] but rather it is involved in "skilled walking," or the adaption of gait kinematics to environmental demands,[58] and may be more strongly associated with temporal parameters of gait.[130, 132] Other descending neural pathways such as the reticulospinal, rubrospinal, and vestibulospinal tracts could contribute to locomotor function. Research has also demonstrated less lateralization of cortical activity with leg movements compared to hand movements,[133] which is not surprising as lower extremity movements are mainly bilateral (e.g., walking). Additionally, animal studies have revealed the presence of a central pattern generator for locomotion, as cats with completely transected spinal cords have demonstrated the ability to generate hindlimb stepping and speed-appropriate gait cycles.[134, 135] This highlights the complex neuronal circuitry involved in locomotion.

2.8.A. *Damage to the Corticospinal Tract and Locomotor Function*

Degree of lesion-CST overlap has been shown to be more strongly related to upper extremity motor impairment and upper/lower extremity strength than lesion size alone;[62] however, the relationship between extent of CST damage following stroke and

locomotor function remains ambiguous. Greater structural damage to the CST has been associated with decreased knee extensor strength,[136] decreased movement of ankle dorsiflexion, knee internal rotation, and hip flexion[137] and increased walking impairment[24, 137] in chronic stroke. Furthermore, individuals with greater relative ipsilateral connectivity from the nonlesioned motor cortex to the paretic leg may exhibit more lower limb impairment and slower walking speed.[24, 138] Other studies, however, have shown that locomotor ability is still present in some stroke survivors despite complete lateral CST injury in the affected hemisphere,[45, 131] and there is evidence of increased activity in the contralesional sensorimotor cortex during paretic lower limb movements following complete injury to the CST.[131, 138] Studies have also demonstrated that extent of lesion-CST overlap is not strongly correlated with walking performance.[24, 130] **Overall, these findings suggest that the CST is less important in the control of walking, despite the importance of this parameter in predicting upper extremity motor impairment and recovery post-stroke, and that measures of lesion volume/CST overlap alone are not sufficient for indicating the level of locomotor impairment in individuals with chronic stroke.**

2.8.B. *Secondary Descending Motor Tracts and Their Role in Gait*

As stated earlier, non-CST descending motor pathways may play a greater role in gait and recovery of locomotor function after stroke. A recent study by Jang et al. (2013) examined the relationship between the corticoreticular pathway (CRP) – one component of the corticoreticulospinal tract, which is known to be involved in mediating proximal and axial muscles – and walking ability in chronic stroke patients with complete CST injury.[25] Results showed that fiber volume of the CRP in the *unaffected* hemisphere was

higher in patients who could walk independently compared to patients who could not walk and healthy controls, and showed a positive correlation with walking ability and motor function of the affected upper/lower extremities. By contrast, neither FA nor fiber volume of the CST in the unaffected hemisphere showed a correlation with walking ability. **These results highlight the potential role of secondary motor tracts, such as the CRP, in recovery of locomotor function post-stroke.**

2.8.C. *Cortical Reorganization*

The neural control of upper and lower limb movements is not analogous, as spinal interneurons play a role in the central pattern generation of gait while fine hand movements are primarily under cerebral control. Cortical reorganization following stroke, therefore, may be different for lower limb function compared to what has been demonstrated with upper limb function. A study by Luft et al. (2005) revealed differences in brain activation patterns during both paretic and nonparetic knee movements between healthy controls and patients with cortical and brainstem strokes, as well as between patients with cortical and subcortical stroke.[139] Increased activation of the contralateral M1, SMA, and bilateral sensorimotor areas was observed with paretic knee movement yet varied according to lesion location. Less contralateral M1 activation was associated with better walking ability in patients with brainstem stroke, while stronger ipsilateral sensorimotor and bilateral somatosensory activation was associated with better walking ability in patients with subcortical and cortical stroke, respectively. These results are consistent with the hypothesis that compensatory activation of bilateral cortical regions may be needed with increased damage to the cortex itself. Another study by Enzinger et al. (2008) also demonstrated increased cortical activation in the

nonlesioned hemisphere (specifically in the SMA and sensorimotor cortex) during both active and, to a lesser extent, passive ankle dorsiflexion.[140] Cortical activation increased with greater impairment of lower limb function. These results could potentially reflect recruitment of intact motor pathways from the ipsilateral SMA and loss of interhemispheric inhibition of the ipsilateral sensorimotor cortex, which may be maladaptive. **As bilateral movements involve coordinated muscle activation from each cortex, reduced transcallosal inhibition after stroke may contribute to mixed flexor/extensor drive and impaired locomotor function after stroke.**

A recent study by Yeo et al. (2011) highlighted the potential role of the pedunculopontine nucleus (PPN) in locomotor recovery in chronic stroke following damage to the CST.[141] The PPN, which is located in the brainstem, is involved in the control of locomotion[142, 143] and damage to this area has been associated with walking impairments.[144] In individuals who could walk independently, the FA value of the PPN in the lesioned hemisphere was found to be higher than that of the nonlesioned hemisphere (with no significant difference in ADC value) and was positively correlated with degree of walking ability.[141] In individuals who could not walk independently, the ADC value was higher in the lesioned hemisphere (with no significant difference in FA value) and was negatively correlated with walking ability. These results further elucidate the role of multiple brain regions involved in the control of walking and suggest that increased neuronal activity (as evidenced by increased FA without changes in ADC values) in the PPN of the lesioned hemisphere contributes to locomotor ability in individuals with CST injury following stroke.

Overall, little research has been performed examining the relationship between lower extremity motor impairment and the structural integrity of various motor tracts/brain regions in individuals with stroke. Gait has been the main focus of lower extremity research, as walking is often a primary goal of stroke rehabilitation. **The proposed research study will include additional measures of lower extremity motor function, such as active ankle dorsiflexion (tapping) and functional lower extremity strength, in an effort to capture a more comprehensive assessment of white matter structural connectivity and lower extremity dysfunction in chronic stroke.**

2.9. DTI AND LOWER EXTREMITY/GAIT IMPAIRMENT IN OTHER PATIENT POPULATIONS

A few studies have examined the association between DTI measures of white matter integrity and gait in patient populations other than stroke. **Imaging studies of gait in the elderly have demonstrated that white matter degeneration in the corpus callosum, specifically the genu, is associated with gait impairment.**[145, 146] This relationship was independent of other factors that affect gait and balance (e.g. age, stroke).[145] The genu of the corpus callosum contains fibers connecting the prefrontal cortices, which are involved in the cognitive control of motor performance.[147, 148] Given that walking is a bimanual activity and requires coordination between various components of the nervous system, integration of right and left frontal lobe executive and cognitive functioning is important for successful gait.[149] White matter lesions in bilateral frontal and periventricular areas involving thalamic radiations and corticofugal motor tracts, as well as other adjacent association fibers (e.g., short cortical association fibers), have also been associated with poorer gait in the elderly population.[146] Disruption of one or more of these tracts may negatively affect the motor networks involved with gait.

Loss of white matter integrity is also associated with gait impairment in older individuals with cerebral small vessel disease (SVD). White matter degeneration (as indicated by a lower FA and higher MD) across multiple brain regions, especially within normal-appearing white matter as well as in white matter lesions, was found to be associated with poorer gait performance in large samples of individuals with SVD.[150, 151] White matter degeneration of the internal capsule and genu of the corpus callosum showed the strongest relationship to gait performance.[150] **Compared to young healthy adults, elderly individuals use more brain regions for motor control and are thought to rely on increased bilateral activation of the frontal cortices during motor performance,[152] underscoring the significance of commissural fibers in this age group. Overall, these studies highlight the importance of the microstructural integrity of fiber tracts in normal-appearing white matter (on structural scans) and their relationship to gait.**

Similar findings have been found in other patient populations. A recent study of individuals with vascular parkinsonism found that a disruption in the microstructural integrity of bilateral fiber tracts that pass from the frontal lobe through the anterior limb of the internal capsule, as well as tracts in the genu of the corpus callosum, was associated with gait impairments in this patient sample.[153] Another study examining white matter integrity in individuals with idiopathic normal pressure hydrocephalus found a negative correlation between gait disturbance and FA values in the left SMA and anterior limb of the internal capsule.[154] These studies further support the theory that a disconnection or disruption between cortical/subcortical structures is related to gait impairment.

A review of the literature has highlighted the value of examining white matter integrity post-stroke to gain a better understanding of structural brain damage (both local and remote) and neuroplastic changes after stroke and the relationship to motor dysfunction. Most studies to date have had a small sample size ($n < 20$), used outcome measures that are not as clinically feasible or do not capture different ICF domains, and/or primarily examined the integrity of the CST. Few studies have investigated changes in chronic white matter integrity and lower extremity motor function and gait. Additionally, new neuroimaging methodologies that enable mapping of white matter connections across the entire brain have yet to be applied to examine the relationship between cortical structural disconnection and motor impairments. Results from the proposed study will offer more insight into the extent of structural brain damage and neuroplastic changes that can occur in individuals with chronic stroke and how these changes relate to chronic upper and lower extremity motor performance. Such information may eventually be used to assist in targeting therapeutic interventions in the chronic stages of stroke recovery.

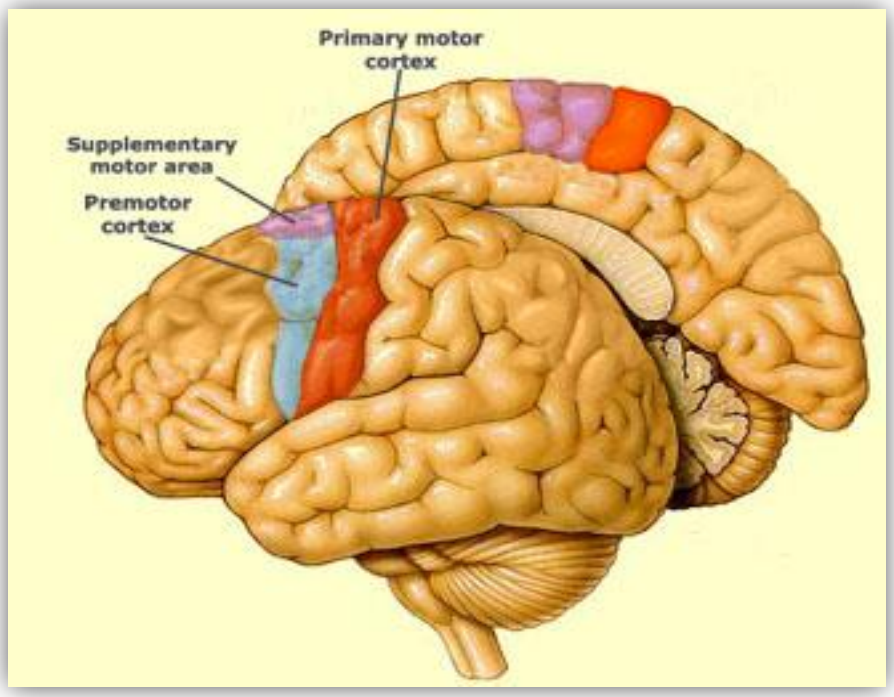

Figure 2.1. Cortical areas of motor control
http://thebrain.mcgill.ca/flash/i/i_06/i_06_cr/i_06_cr_mou/i_06_cr_mou.html

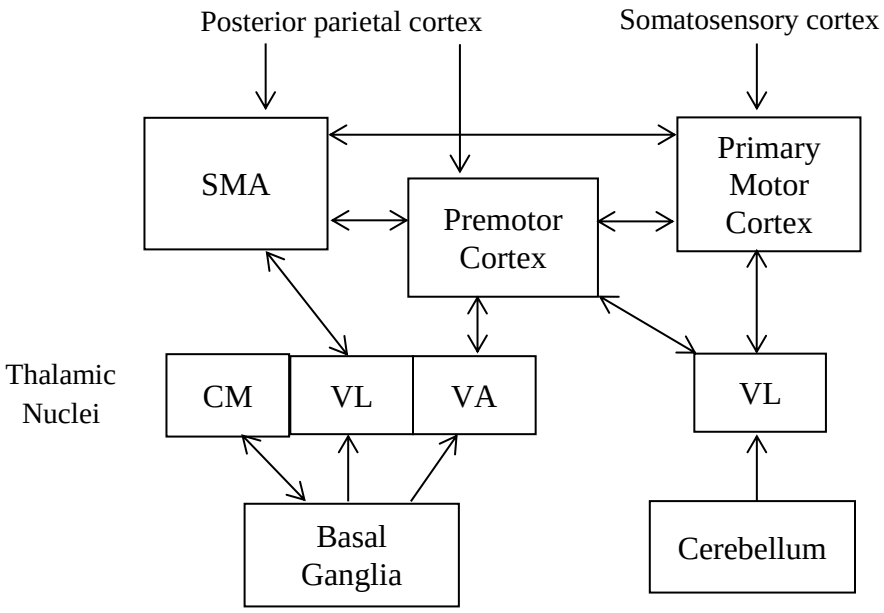

Figure 2.2. Afferent and efferent pathways of the motor cortex. Abbreviations: SMA=supplementary motor area; CM=centromedian nucleus; VL=ventral lateral nucleus; VA=ventral anterior nucleus.
http://neuroscience.uth.tmc.edu/s3/chapter03.html

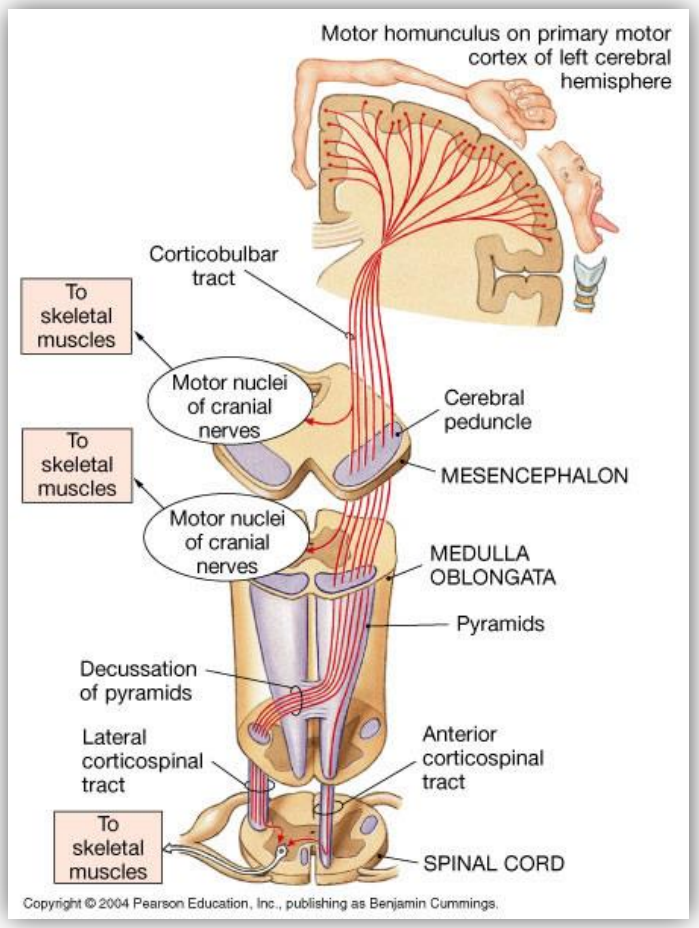

Figure 2.3. Corticospinal tract
http://www.as.miami.edu/chemistry/2008-1-MDC/2085/Chap-15_New/chap_15.htm

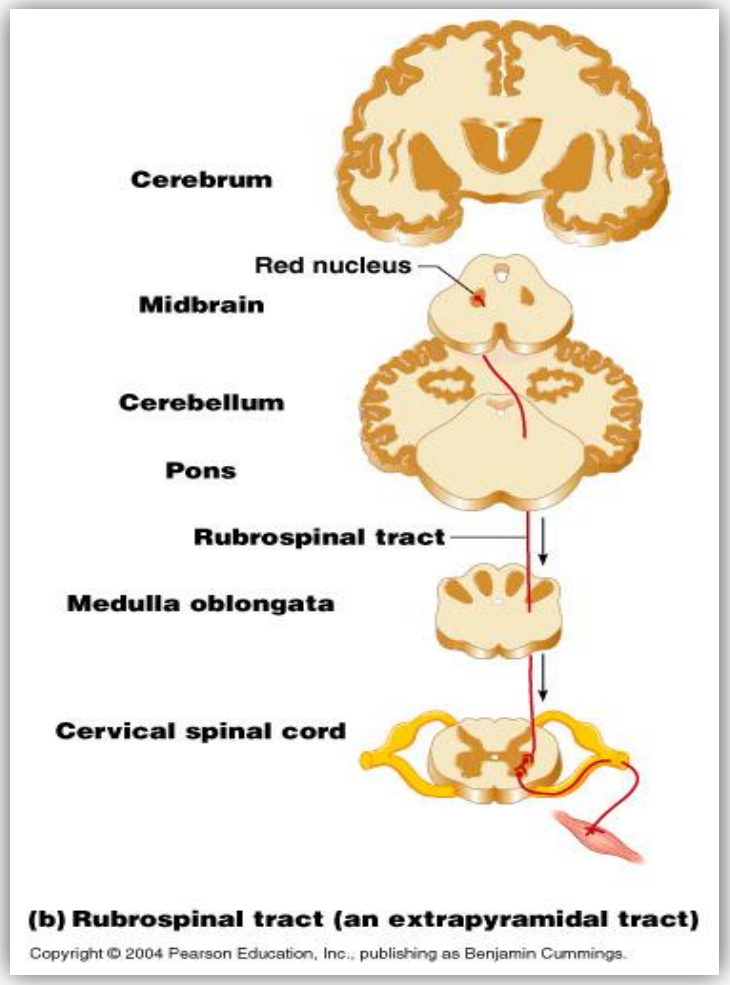

Figure 2.4. Rubrospinal tract
http://www.profelis.org/webpages-cn/lectures/neuroanatomy_3.html

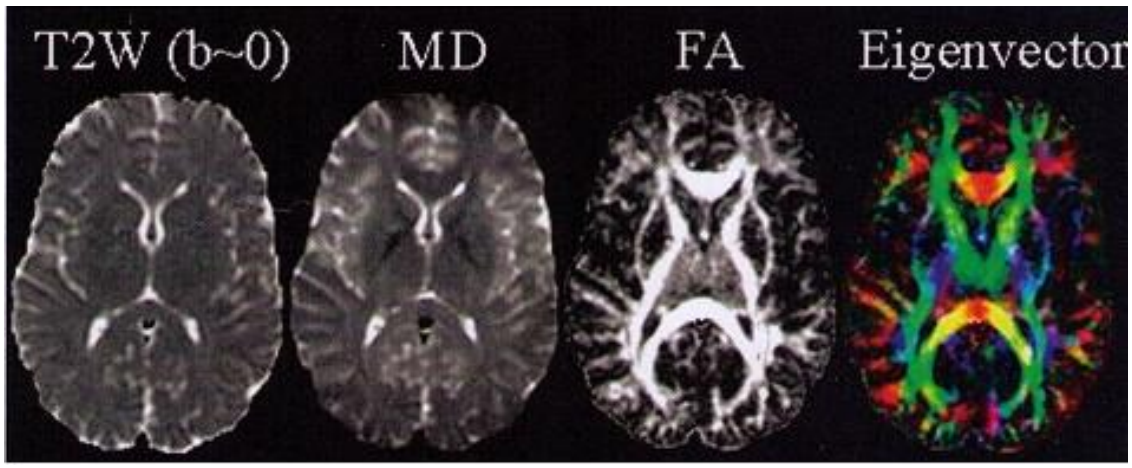

Figure 2.5. DTI measures
http://bme240.eng.uci.edu/students/08s/jlisinsk/DTI.html

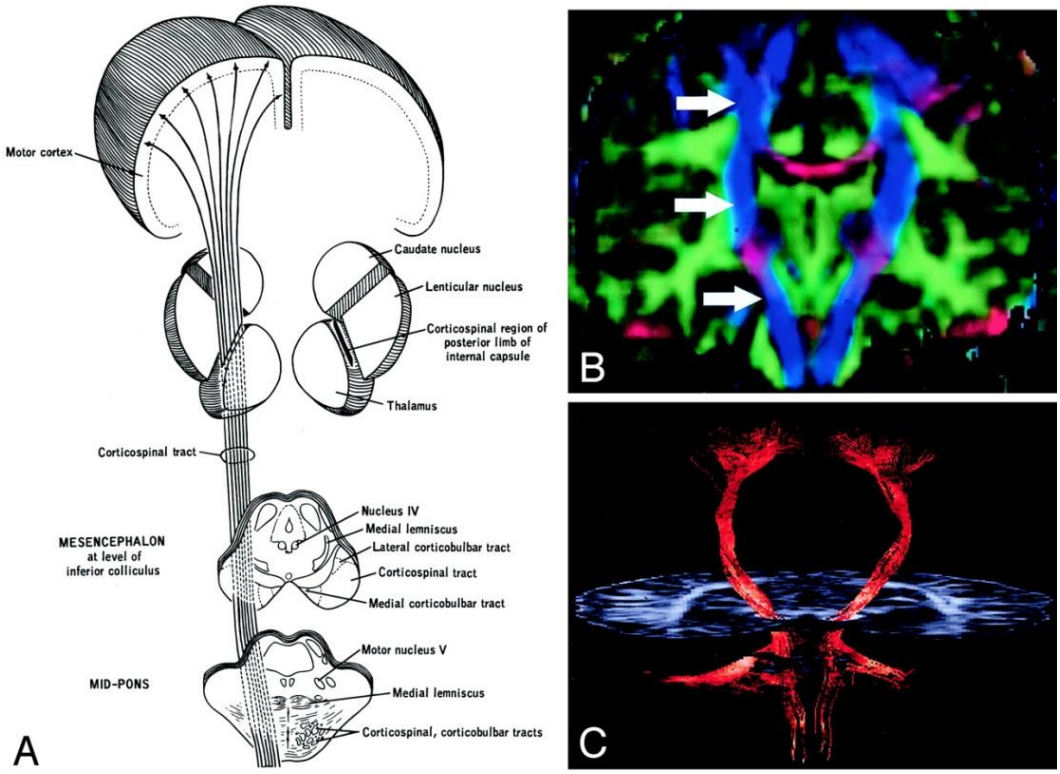

Figure 2.6. Corticospinal tract and diffusion tensor imaging
http://www.ajnr.org/content/25/3/356.figures-only

CHAPTER 3

METHODS

3.1. RESEARCH DESIGN

A cross-sectional design was used to address both aims of the study.

3.2. POWER ANALYSES

For Aim 1, a sample size of 50 will provide 90% power to determine the relationship between grip strength asymmetry and FA in the ipsilesional CST ($\alpha = 0.05$) using bivariate parametric correlation (one-tailed test). This estimate assumes a null correlation ρ H0 = 0 with an alternative hypothesis correlation ρ H1 = 0.4 (a medium to low correlation value). This ρ H1 correlation value was chosen for a more conservative estimate as published articles in this area have reported a range of correlation values (from approximately 0.3-0.9) between measures of motor function and white matter integrity.[23, 97, 110, 114] Additional correlations will be powered off of this model.

For Aim 2, a sample size of 48 will provide 90% power to determine the relationship between grip strength asymmetry and cortical necrosis/disconnection in M1 ($\alpha = 0.05$) using multiple linear regression. This a conservative estimate and assumes an effect size $f^2 = 0.287$ (calculated by using partial $R^2 = 0.223$) with 4 predictors (age, time post-stroke, percentage of necrotic lesion damage to M1, percentage fiber number of M1) entered into the full model. This partial R^2 estimate was based on a review of the literature which reported significant partial R^2 values ranging from 0.223 to 0.613.[7, 110]

Additional multiple linear regressions will be powered off of this model. Sample size calculations were performed using G*Power 3.1.9.2 software.

3.3. PARTICIPANTS

Fifty-three participants (ages 20-85) with chronic stroke who had either participated in previous studies at the University of South Carolina, expressed interest in or were currently participating in ongoing research studies, or had participated in aphasia groups at the University of South Carolina Speech and Hearing Center were recruited. Participants were contacted via phone, and prior to entry all individuals underwent safety screening for MRI and provided written informed consent as approved by the institutional review board at the University of South Carolina.

As this study was part of a larger study investigating lesion-impairment mapping of speech and language processing, spatial processing, and motor execution in individuals with chronic stroke, all participants were monolingual speakers of English pre-stroke. Additionally, the following inclusion criteria had to be met: 1) occurrence of a single ischemic or hemorrhagic stroke at least 6 months prior to study inclusion, 2) able to follow simple instructions, and 3) able to walk 8 meters with or without an assistive device (advancing their lower extremities independently). Potential participants with a contraindication for MRI examination (claustrophobia, pregnancy, metal implants, etc.) or clinically reported history of dementia, alcohol abuse, psychiatric disorder, traumatic brain injury, or extensive visual acuity or visual-spatial problems were excluded from participating in the study.

3.4. MOTOR ASSESSMENT

Tables 3.1 and 3.2 list the behavioral measures used to assess upper and lower extremity motor function and gait. Test administration did not follow a specific order as testing was coordinated with MRI scanning and data collection performed by other researchers involved in the larger study. Participants were given rest breaks as needed, and used any assistive devices or orthotics that they commonly used in community ambulation during the gait assessment. Primary behavioral measures are bolded in the aforementioned tables. These measures were chosen as they capture different aspects of motor function (body functions/structure vs. activity ICF domain) and are commonly used measures. Refer to Appendix B for the complete testing packet.

3.5. MRI ACQUISITION

All participants underwent scanning using a 3T Siemens Trio system with a 12 element head coil at the McCausland Center for Brain Imaging (Columbia, SC). High-resolution T_1-MRI scans [repetition time (TR) = 2250 ms, echo time (TE) = 4.15 ms, field of view (FOV) = 256 mm and voxel size = 1.0 x 1.0 x 1.0 mm] and T_2-MRI scans [TR = 3200 ms, TE = 212 ms, FOV = 256 mm and voxel size = 1.0 x 1.0 x 1.0 mm] were acquired for determination of lesion size and location. DTI was performed with a single shot gradient echo planar imaging (EPI) sequence using the following parameters: TR = 4987 ms, TE = 79.2 ms, flip angle (α) = 90°, FOV = 207 mm, voxel size = 2.3 x 2.3 x 2.3 mm, slice thickness = 2.3 mm, noncollinear diffusion directions = 40 with a *b*-value of 1000 s/mm^2, number of slices = 50.

3.6. IMAGE PREPROCESSING FOR AIM 1

MRI images were converted to NIfTI format using the dcm2nii tool from the MRIcron software package.[155] Stroke lesions were manually outlined (by Bonilha) on the T1 image. Using tools from the FMRIB Software Library (http://www.fmrib.ox.ac.uk/fsl),[156] DTI data was corrected for head motion and eddy current distortion prior to brain extraction, and was then analyzed by fitting a diffusion tensor model at each voxel to generate FA and MD images. FA/MD maps were then aligned into a common (standard) space using the nonlinear registration tool FNIRT.[157, 158]

3.7. IMAGE PREPROCESSING FOR AIM 2

After conversion to NIfTI format, T1-weighted images were normalized into standard MNI space (utilizing a cost-function mask of the brain lesion)[159] using unified segmentation-normalization routines as part of the Clinical Toolbox for the software Statistical Parametric Mapping (SPM) 8.[160] This step also provided probabilistic gray and white matter maps in standard space.[161] The invert transformation was then applied to a John Hopkins University (JHU) template and to the gray and white matter probabilistic maps in order to transform these maps/template onto native T1 space. The probabilistic gray matter map (now in T1 space) was then segmented into a map of cortical/subcortical JHU ROIs (excluding the lesion area).

In order to improve registration between T1 and DTI spaces, the T2 image was linearly coregistered to the T1 image to create a T2-weighted image matched to T1 space. This "matched" image was then coregistered to the B0 image (DTI space) using FMRIB's Linear Image Registration Tool (FLIRT). The same transformation matrix was

applied to the map of segmented cortical ROIs and the probabilistic white matter map (which are in T1 space) to transform these maps onto DTI space.

Probabilistic DTI tractography was performed using FDT's probtrackx[162] (with 5000 streamline samples) to determine the number of white matter streamlines connecting each JHU ROI. For each possible pair of cortical/subcortical ROIs ***i*** and ***j***, the number of iterative streamlines connecting the pair was computed for the creation of a connectivity matrix ***A***, where each ***Aij*** entry represented the weighted link between ROIs (adjusted based on ROI size and distance streamlines travelled).[30] The weighted sum of all connections to the 3 main cortical areas involved in motor function (M1, premotor cortex, and SMA) was then computed to assess overall connectivity of these cortical regions. The percentage of fibers compared to the homologous region in the unaffected hemisphere was calculated to assess fiber reduction in these motor areas in the affected hemisphere, and to normalize the final connectivity measure based on each subject's contralateral hemisphere and imaging properties. The percentage of necrotic lesion damage to each cortical ROI was also calculated by overlaying the manually outlined lesion onto the segmented cortical map in native T1 space. These steps were performed through in-house scripts written in MATLAB.[30]

3.8. STATISTICAL ANALYSES

Descriptive statistics were obtained for both demographic characteristics and behavioral measures. Normality of data was established using the Shapiro-Wilk test of normality along with visual inspection of plots. Analyses were performed using SPSS (Statistical Package for the Social Sciences) version 20.0 (Chicago, IL).

3.8.A. *Aim 1*

Differences in motor scores between the affected versus unaffected extremities were evaluated using two-tailed paired *t*-tests (or Mann-Whitney U tests if the data was not normally distributed). ROI analyses were conducted using John Hopkins University maps overlaid on the voxelwise skeletons of FA and MD. Nonparametric correlations were performed to examine the relationship between FA/MD of *a priori* motor tracts (bilateral CST and body of the corpus callosum) and brain regions (bilateral red nuclei, substantia nigra, thalamus, and superior cerebellar peduncle) and upper/lower extremity motor function. Behavioral data was adjusted for variability described by the overall lesion volume, ensuring that statistical analyses were performed on ROI's with a lesion load of <5% to minimize the influence of necrotic tissue on FA/MD values.

For Aim 1a, correlations were computed to examine the relationship between indices of white matter integrity (*FA and MD*) of specific neural tracts/brain regions (*ipsilesional/contralesional corticospinal and transcallosal tracts*; *red nucleus; thalamus; substantia nigra; and superior cerebellar peduncle*) and upper extremity motor performance (primary measures: *BBT, grip strength, upper limb portion of MI for the affected extremity*), for a total of 36 correlations each for FA/MD assessments. For Aim 1b, correlations were computed to examine the relationship between indices of white matter integrity (*FA and MD*) of specific neural tracts/brain regions (*ipsilesional/contralesional corticospinal and transcallosal tracts*; *red nucleus; thalamus; substantia nigra; and superior cerebellar peduncle*) and lower extremity motor performance (primary measures: *gait speed and lower limb portion of MI for the affected extremity*), for a total of 24 correlations each for FA/MD assessments.

Hierarchical multiple linear regression analyses were then performed to assess the amount of variance in upper/lower extremity motor performance explained by the integrity of the ipsilesional CST and RN. Separate multiple regressions were performed for each behavioral measure. Ipsilesional CST FA was entered first into the model, as the CST is the major neural pathway for skilled, discrete voluntary movements.[36] Ipsilesional RN FA was the next predictor entered into the model, as studies have shown neuroplastic changes in the red nucleus following CST injury,[20, 129] as well as a positive correlation between RN FA value and recovery of motor function post-stroke.[129] Age and/or time since stroke were controlled for as covariates if significantly correlated with motor performance. Significance level was set at $P<0.05$, corrected based on the number of behavioral measures assessed.

3.8.B. *Aim 2*

Differences in motor scores between the affected versus unaffected extremities were evaluated using two-tailed paired *t*-tests (or Mann-Whitney U tests if the data was not normally distributed). Standard multiple linear regression analyses were performed to examine the relationship between cortical necrosis and disconnection and upper/lower extremity motor performance. The analyses focused on primary behavioral measures: BBT, grip strength, and upper limb portion of the MI for the affected extremity (Aim 2a); gait speed and lower limb portion of the MI for the affected extremity (Aim 2b).

Hemispheric differences in anatomical connectivity between a subsection of the motor network were assessed using nonparametric statistics. The analyses focused on connectivity between the three main cortical areas of motor control (M1, PMC, and SMA) and several important subcortical regions involved in motor control (cerebral

peduncle, red nucleus, thalamus, basal ganglia, and cerebellum). Studies have shown that selective disruption of corticofugal fibers from multiple motor regions can impact functional reorganization and motor recovery following stroke,[119] and neuroplastic changes in the red nucleus (RN) of the affected hemisphere have been found post-stroke,[37, 127] which may indicate a compensatory role of this brain region in motor recovery. Nonparametric correlation analyses were then performed to examine the relationship between structural connectivity amid these *a priori* ROIs (including inter-hemispheric connectivity between homologous regions) and motor function. Significance level was set at $P<0.05$, corrected based on the number of ROI connectivity calculations.

Separate multiple linear regressions were performed for each upper extremity and lower extremity measure, and included the clinical score as the dependent variable with the following independent variables: 1) percentage of necrotic lesion damage to each motor area, and 2) percentage fiber number of each motor area. Either of the descriptive statistics (age, time since stroke) significantly correlated with motor performance were controlled for by entering them into the regression model as covariates, as age and time post-stroke have been shown to have an effect on motor outcome.[163, 164] Each brain motor area (M1, PMC, and SMA) was analyzed separately, for a total of 9 multiple linear regressions for Aim 2a and 6 multiple regressions for Aim 2b. Significance level was set at $P<0.05$, corrected based on the number of behavioral measures assessed.

Table 3.1. Upper Extremity Motor Test Battery

UPPER EXTREMITY MEASURES			
Measure	**Assessing**	**Calculation**	**Reliability/Validity**
Box and Block Test[165,166]	Gross manual dexterity of the hand	# blocks moved in one minute	Valid measure with high test-retest reliability in multiple populations, including stroke
Grip Strength[167, 168]	Force production in the hand	Assessed using handheld dynamometer (kg), averaged over 3 trials	Valid and reliable in stroke population, with excellent intra-rater and test-retest reliability
* **Motricity Index**[169, 170]	Measure of limb impairment; assesses motor function, strength	Upper and lower limb sections each have a maximum score of 100	High inter-rater reliability in stroke, with strong concurrent validity with other established measures
Index Finger Tapping Test[171, 172]	Motor function of the hand, specifically finger movement speed	# taps performed in 10 seconds with index finger (averaged over 2 trials); asymmetry in finger tapping calculated as ($IFT_{unaff} - IFT_{aff}$ / $IFT_{unaff} + IFT_{aff}$)	Good inter-rater and test-retest reliability demonstrated in acute stroke
Modified Brunnstrom Classification[173, 174]	Motor function of the paretic hand	Graded on a scale from 1-6, with higher values indicating better motor function	Valid and reliable in stroke population
* Stroke Impact Scale[175, 176]	Self-reported perceived recovery related to upper/lower extremity function and mobility	Items scored on a 5-point ordinal scale, total scores calculated for separate domains [strength (max=20), hand function (max=25), mobility (max=45)]; stroke recovery scale 0-100	Reliable and valid instrument for measurement of participation post-stroke

* Also used as a lower extremity behavioral measure

Table 3.2. Lower Extremity Motor Test Battery

LOWER EXTREMITY MEASURES			
Measure	**Assessing**	**Calculation**	**Reliability/Validity**
GAITRite[177, 178]	Spatiotemporal parameters of gait	Self-selected gait speed (m/s) averaged over 3 trials	Valid and reliable clinical measure in chronic stroke; identifies gait asymmetries and deviations
30 Seconds Sit to Stand Test[179]	Lower limb strength and balance	# full stands performed in 30 seconds	Valid and reliable in older adults; similar test (5 Times Sit to Stand) valid and reliable in chronic stroke population
Foot Tapping Test[180]	Motor function of the foot/ankle, specifically ankle movement speed	# foot taps performed in 10 seconds (averaged over 2 trials); asymmetry in foot tapping calculated as $(FT_{unaff} - FT_{aff} / FT_{unaff} + FT_{aff})$	Validity and reliability have not been established for stroke

CHAPTER 4

IPSILESIONAL CORTICOSPINAL TRACT AND RED NUCLEUS STRUCTURAL INTEGRITY IS ASSOCIATED WITH UPPER AND LOWER EXTREMITY MOTOR FUNCTION IN CHRONIC STROKE

4.1. ABSTRACT

Background: While the integrity of the ipsilesional corticospinal tract (CST) is important for recovery of motor function after stroke, limited research has examined the relationship between changes in white matter integrity in primary *and* secondary motor tracts/brain regions and upper *and* lower extremity motor function in chronic stroke.

Objective: To investigate the neural basis of upper/lower extremity motor deficits in individuals with chronic stroke by correlating measures of motor function with diffusion tensor imaging (DTI)-derived indices of white matter integrity [fractional anisotropy (FA) and mean diffusivity (MD)] in primary and secondary motor tracts/brain regions.

Design: Cross-sectional

Methods: Forty-three participants post-stroke [age: 59.7 (11.2) years; time since stroke: 64.4 (58.8) months] underwent motor assessments and MRI scanning. Nonparametric correlation analyses were performed to examine the relationship between FA/MD of *a priori* motor tracts/brain regions and motor function. Hierarchical multiple regression analyses were performed to assess the amount of variance in upper/lower extremity motor performance explained by the integrity of the ipsilesional CST and red nucleus (RN).

Results: FA of the ipsilesional CST and RN was significantly correlated with motor function across all upper extremity measures, with correlation coefficients ranging from 0.36-0.55. FA of the ipsilesional CST was significantly correlated with motor function of the affected lower extremity (Leg MI_{Aff}, r=0.44), and FA of the ipsilesional RN was correlated with both Leg MI_{Aff} score (r=0.49) and gait speed (r=0.50). No significant ipsilesional MD-behavior correlations were found. Ipsilesional CST FA significantly explained 37.3% of the variance in grip strength of the affected hand (F(1, 38)=22.00, P<0.001) and 31.5% of the variance in Arm MI_{Aff} score (F(1, 23)=10.11, P=0.004). Adding ipsilesional RN FA to the models did not significantly explain an additional amount of variance in upper extremity motor performance.

Limitations: These results should be interpreted in the context of the limitations of DTI and FA.

Conclusions: The microstructural integrity of the ipsilesional (but not contralesional) CST is correlated with both upper/lower extremity motor function. The CST appears to be less important, however, for gait speed than to the control of upper extremity dexterity and strength. Additionally, microstructural integrity of the ipsilesional RN was fair-moderately associated with upper and lower extremity motor function, suggesting that this region may contribute to motor recovery.

4.2. INTRODUCTION

Motor weakness is one of the most disabling consequences of stroke, often leading to difficulties in the performance of ADL's, increased energy expenditure, asymmetrical gait patterns, and overall decreased activity levels.[1, 2, 4] Recovery of motor function varies considerably following stroke, as patients with similar lesions on

structural scans can exhibit different motor impairments and/or responses to treatments. Motor recovery depends on adaptive processes in both the affected and unaffected hemisphere, although the exact neural mechanisms remain unclear.[7, 8] Results of structural imaging studies have suggested that both lesion size[9, 10] and location[11-13] correlate with motor impairment, and lesion-symptom mapping studies have provided additional information by characterizing the relationship between lesion site and functional deficits,[14-16] yet considerable variance remains unexplained. With advances in neuroimaging techniques, recent research has begun to examine measures of cortical integrity, such as the microstructural properties of white matter and anatomical connectivity, across the whole brain and their role in motor impairment and recovery.

Diffusion tensor imaging (DTI) allows for the examination of the integrity and orientation of white matter in the brain by estimating the magnitude and directionality of water diffusion.[17] Water diffuses more freely parallel to normal white matter fibers than perpendicular to them, causing diffusion anisotropy of the white matter. Two of the more common DTI parameters used to assess white matter integrity are mean diffusivity (MD), which represents the overall magnitude of water diffusion, and fractional anisotropy (FA), which reflects the degree of diffusion directionality.[76, 78] Loss of microstructural integrity of white matter tracts (e.g., local tissue damage within the primary lesion, anterograde and/or retrograde axonal degeneration) is typically reflected by an increase in MD (representing increased water diffusion in the extracellular space) and/or a reduction in FA (representing decreased anisotropic diffusion).[76] Axonal properties such as density, myelination, diameter, and orientation contribute to overall FA values.[77, 181]

Several studies using DTI techniques have demonstrated a correlation between upper extremity motor dysfunction and decreased integrity of white matter tracts in both acute[18-20] and chronic[21-23] stroke. Many researchers have demonstrated that reduced structural damage to the ipsilesional corticospinal tract (CST) is associated with better motor outcome following stroke.[21, 62, 100] While the CST is the main motor pathway for voluntary movements of the hand, secondary motor pathways and brain regions also play a role in upper extremity motor function and recovery. Microstructural changes in transcallosal fibers have been found in subacute[21] and chronic stroke[23, 108] with higher FA values associated with better motor function. Additionally, neuroplastic changes have also been shown to occur in the red nucleus (RN) of the affected[37, 127] and unaffected hemisphere,[127] which may indicate compensation for CST injury and contribute to motor recovery. The red nucleus (located in the rostral midbrain) is the origin of the rubrospinal tract (RST).[34] The RST and CST are functionally related with their fibers terminating in close proximity in the spinal cord, suggesting the RN may have some potential to compensate for injury to the CST following stroke. Studies have suggested that anisotropy within deep nuclear structures (such as the RN) may be related to the axon bundles traveling within them,[182] and that increased FA values in such nuclei could indicate remodeling and neuroplastic changes following stroke.[37, 129]

Motor recovery of the affected upper extremity has been highly studied, whereas less is known about the motor recovery mechanisms involved with lower extremity and locomotor function after stroke. While the CST is necessary for fine movements of the hands,[36, 60] locomotion and motor function of the legs is less dependent on the CST.[45, 130, 131] Degree of lesion-CST overlap has been shown to be more strongly related to upper

extremity motor impairment and upper/lower extremity strength than lesion size alone;[7, 23, 62] however, the relationship between extent of CST damage following stroke and locomotor function remains ambiguous. Greater structural damage to the CST has been associated with decreased knee extensor strength,[136] decreased movement of ankle dorsiflexion, knee internal rotation, and hip flexion[137] and increased walking impairment[24, 137] in chronic stroke. Other studies, however, have shown that locomotor ability is still present in some stroke survivors despite complete lateral CST injury in the lesioned hemisphere.[45, 131] In other patient populations, imaging studies of gait in the elderly have demonstrated that white matter degeneration in the corpus callosum is associated with gait impairment.[145, 146] Loss of white matter integrity is also associated with gait impairment in older individuals with cerebral small vessel disease, with white matter degeneration of the internal capsule and corpus callosum showing the strongest relationship to gait performance.[150]

Together, these finding suggest that while the integrity of descending neural pathways from the ipsilesional motor system is important for recovery of motor function after stroke, damage to one region may be compensated for by increased activation of other regions and contributions from secondary motor areas and tracts. Most studies to date, however, have had small sample size ($n < 20$), used outcome measures that are not as clinically feasible or do not capture different International Classification of Functioning, Disability, and Health (ICF) domains, and/or focused on the major neural pathway for voluntary movement (i.e. CST). Additionally, few studies have examined changes in chronic white matter integrity post-stroke and lower extremity motor function and gait.[24, 25] Therefore, the aim of the present study was to investigate the neural basis

of both upper and lower extremity motor deficits in a larger sample of individuals with chronic stroke by correlating measures of motor function with DTI-derived indices of white matter integrity in primary and secondary motor tracts/brain regions. We hypothesized that 1) poorer performance on upper and lower extremity motor measures would be correlated with reduced white matter integrity (as indicated by lower FA and/or higher MD values) in the ipsilesional CST and corpus callosum, and 2) microstructural changes (as indicated by higher FA values) in brain regions associated with motor control (e.g., red nucleus) would be positively associated with better performance on upper and lower extremity motor measures.

4.3. METHODS

4.3.A. *Participants*

Behavioral assessments were performed on 52 participants. Nine participants were excluded from analyses due to incomplete MRI data. A summary of demographic, clinical, and imaging data for the remaining 43 participants is presented in Table 4.1 (see Appendix C for detailed sample characteristics). The mean age of participants (16 female, 27 male) was 59.7 ± 11.2 years, with a mean time post-stroke of 64.4 ± 58.8 months. All participants gave written informed consent to participate in this study that was approved by the Institutional Review Board at the University of South Carolina. As this study was part of a larger study investigating lesion-impairment mapping of speech and language processing, spatial processing, and motor execution in individuals with chronic stroke, inclusion criteria consisted of 1) monolingual speaker of English (pre-stroke), 2) occurrence of a single left hemispheric ischemic or hemorrhagic stroke at least 6 months prior to study inclusion, 3) able to follow simple instructions, and 4) able to

walk 8 meters with or without an assistive device (advancing their lower extremities independently). Exclusion criteria included contraindication for MRI examination (claustrophobia, pregnancy, metal implants, etc.) or clinically reported history of dementia, alcohol abuse, psychiatric disorder, traumatic brain injury, or extensive visual acuity or visual-spatial problems.

4.3.B. *Motor Assessment*

All participants underwent a comprehensive behavioral assessment of upper and lower extremity motor function and gait. Testing was performed by a physical therapist and included the Box and Block Test (BBT),[165] grip strength,[167] Motricity Index (MI),[170] and gait speed[177] (Table 4.2). For the gait speed assessment, participants were allowed to use any assistive device or orthotic that they commonly use with community ambulation.

4.3.C. *MRI Acquisition*

All participants underwent scanning using a 3T Siemens Trio system with a 12 element head coil at the McCausland Center for Brain Imaging (Columbia, SC) within two days of behavioral testing. High-resolution T_1-MRI scans [repetition time (TR) = 2250 ms, echo time (TE) = 4.15 ms, field of view (FOV) = 256 mm and voxel size = 1.0 x 1.0 x 1.0 mm] and T_2-MRI scans [TR = 3200 ms, TE = 212 ms, FOV = 256 mm and voxel size = 1.0 x 1.0 x 1.0 mm] were acquired for determination of lesion size and location. DTI was performed with a single shot gradient echo planar imaging (EPI) sequence using the following parameters: TR = 4987 ms, TE = 79.2 ms, flip angle (α) = 90°, FOV = 207 mm, voxel size = 2.3 x 2.3 x 2.3 mm, slice thickness = 2.3 mm, noncollinear diffusion directions = 40 with a *b*-value of 1000 s/mm^2, number of slices = 50.

4.3.D. *Image Preprocessing*

MRI images were converted to NIfTI format using the dcm2nii tool from the MRIcron software package.[155] Stroke lesions were manually outlined by one of the authors (Bonilha) on the T2 image, which was then coregistered to the T1 image. The T1-weighted image was then normalized into standard MNI space utilizing unified segmentation-normalization routines of the clinical toolbox for SPM8,[160, 161] which also applied a lesion-masked cost function.[159] The normalized lesion mask was then binarized. Using tools from the FMRIB Software Library (http://www.fmrib.ox.ac.uk/fsl),[156] DTI data was corrected for head motion and eddy current distortion prior to brain extraction, and was then analyzed by fitting a diffusion tensor model at each voxel to generate FA and MD images. FA/MD maps were aligned into a common (standard) space using FMRIB's Nonlinear Image Registration Tool (FNIRT).[157, 158]

4.3.E. *Statistical Analyses*

Differences in motor scores between the affected and unaffected extremities were evaluated using two-tailed paired *t*-tests (or Wilcoxon matched-pairs signed rank tests if the data was not normally distributed). ROI analyses were performed using the John Hopkins University (JHU) template overlaid on the voxelwise skeletons of FA and MD. Mean FA and MD values were calculated in each ROI within the JHU atlas. Statistical analyses were performed on ROI's with a lesion load of <5% to minimize the influence of necrotic tissue on FA/MD values.

Due to the non-normal distribution of our behavioral data, nonparametric correlation analyses were performed to examine the relationship between FA/MD of *a priori* motor tracts (bilateral CST and body of the corpus callosum) and brain regions

(bilateral red nuclei, substantia nigra, thalamus, and superior cerebellar peduncle) and upper/lower extremity motor function. We focused our analyses on these motor tracts/brain regions as the CST is the major descending motor pathway, and previous studies have highlighted an association between FA changes in the corpus callosum/red nucleus and motor performance following stroke.[21, 22, 110, 129] Additionally, we wanted to investigate microstructural changes in specific subcortical regions involved in motor control and their relationship with upper/lower extremity motor performance in chronic stroke. Correlations were interpreted as poor (<0.25), fair (0.25 to <0.5), moderate (0.5 to 0.75), and strong (>0.75).[183]

Regions of interest with a significant bivariate correlation were entered into a regression model to assess the amount of variance in upper/lower extremity motor performance explained by the integrity of each ROI. In order to normalize data for regression analyses, participants who scored 0 on BBT were removed as the BBT has a low floor effect.[165, 166] Similarly, participants who scored 99 on the MI for the affected upper/lower extremity were removed due to the high ceiling effect.[169, 170] A reflect and square root transformation was also used with Arm MI_{Aff} data to obtain a normal distribution. Separate multiple regressions were performed for each behavioral measure. Age and/or time since stroke were controlled for as covariates if significantly correlated with motor performance. Significance level was set at $P<0.05$, corrected based on the number of behavioral measures assessed (corrected $P\leq 0.01$).

4.4. RESULTS

4.4.A. *Behavioral Measures*

Motor performance of the affected extremities was significantly reduced compared to the unaffected extremity. The median BBT score for the affected extremity was 34.5 [interquartile range (IQR), 7.25-46.5]; median BBT score for the unaffected extremity was 51.0 (IQR, 44.0-56.25) ($P<0.001$, Wilcoxon matched-pairs signed rank test). Average grip strength for the affected hand was 23.46 ± 15.72 kg and for the unaffected hand was 34.64 ± 10.51 kg ($P<0.001$, paired *t*-test). The median Motricity Index score for the affected upper extremity was 88 (IQR, 56.5-100) and for unaffected extremity was 100 (IQR, 100-100) ($P<0.001$, Wilcoxon matched-pairs signed rank test). The median Motricity Index score for the affected lower extremity was 79 (IQR, 59-100) and for the unaffected extremity was 100 (IQR, 100-100) ($P<0.001$, Wilcoxon matched-pairs signed rank test). Average gait speed was 0.94 ± 0.31 m/s. These scores/values indicate that participants, on average, exhibited a mild to moderate degree of motor impairment.

For the subgroup of participants with normalized data for the regression analyses, average BBT score for the affected extremity was 37.8 ± 17.4 (n=34), average Arm MI score for the affected extremity was 59.8 ± 28.1 (n=25), and average Leg MI score for the affected extremity was 64.9 ± 18.4 (n=28).

4.4.B. *Necrotic Lesion Location*

All participants exhibited a cortical/subcortical lesion in the left hemisphere, broadly distributed within the territory of the middle cerebral artery. For one participant, the lesion was too small to adequately outline; all other lesions were clearly visible on T2

weighted images. Locations of maximal lesion overlap were the extra-nuclear and subgyral areas, as demonstrated in Figure 4.1.

4.4.C. *Lesion Size and Motor Impairment*

Total lesion volume was not significantly correlated (Spearman's rho) with motor function of the affected extremities as measured by the BBT (r=-0.17, P=0.29), grip strength (r=0.10, P=0.54), Arm MI (r=-0.15, P=0.37), Leg MI (r=-0.06, P=0.71), and gait speed (r=0.15, P=0.35).

4.4.D. *FA/MD of A Priori ROI's and Motor Function*

FA values were significantly reduced in the ipsilesional hemisphere compared to the contralesional hemisphere in *a priori* ROIs (P<0.01, Wilcoxon matched-pairs signed rank tests), except for in the body of the corpus callosum (P=0.20) and superior cerebellar peduncle (P=0.06) (Figure 4.2). Correlations between upper/lower extremity motor function and mean FA values of *a priori* ROIs are presented in Table 4.3. FA of the ipsilesional CST and RN was significantly correlated with motor function across all upper extremity measures, with correlation coefficients ranging from 0.36-0.55. FA of the ipsilesional CST was significantly correlated with motor function of the affected lower extremity (Leg MI_{Aff}, r=0.44), and FA of the ipsilesional RN was correlated with both Leg MI_{Aff} score (r=0.49) and gait speed (r=50). Additionally, FA of the ipsilesional substantia nigra was significantly correlated with Leg MI_{Aff} score (r=0.37). No significant correlations were found between upper/lower extremity motor function and mean FA values of contralesional ROIs.

A significant MD-behavior correlation was observed between the contralesional superior cerebellar peduncle and grip strength of the affected hand (r=-0.37, P=0.008,

n=42) and Leg MI (r=-0.40, P=0.005, n=42). Otherwise, no significant MD correlations were found across behavioral measures.

4.4.E. *Multiple Regression Analyses*

Only the two ROIs with the strongest bivariate correlation with motor function (i.e. CST and RN) were entered into the regression models due to our sample size. Ipsilesional CST FA was entered first into each model, as the CST is the major neural pathway for skilled, discrete voluntary movements.[36] Ipsilesional RN FA was the next predictor entered into the models, as studies have shown neuroplastic changes in the red nucleus following CST injury[20, 129] as well as a positive correlation between RN FA value and recovery of motor function post-stroke.[129] A significant, positive correlation was found between ipsilesional CST and RN FA (Spearman's r=0.37, P=0.02); however, since the correlation between these independent variables was fairly low, both variables were retained for analyses. Results of the regression analyses are summarized in Table 4.4, with the bivariate relationship between grip strength/Leg MI_{Aff} and ipsilesional CST/RN FA illustrated in Figure 4.3. Ipsilesional CST FA significantly explained 37.3% of the variance in grip strength of the affected hand (F(1, 38)=22.00, P<0.001) and 31.5% of the variance in Arm MI_{Aff} score (F(1, 23)=10.11, P=0.004). Adding ipsilesional RN FA to the models, however, did not significantly explain an additional amount of variance in upper extremity motor performance as assessed by these measures. No other regression analyses revealed significant models (at corrected $P \leq 0.01$), but several models approached significance (at $P \leq 0.05$).

4.5. DISCUSSION

The aim of the current study was to investigate the association between the integrity of different motor tracts/brain regions and upper and lower extremity motor function in individuals with chronic stroke. While chronic motor impairment after stroke has been associated with lesion size,[9, 10] others have found no statistically significant relationship.[99] In our study, total lesion volume did not correlate with any of the upper or lower extremity motor measures. In terms of FA, our findings showed that ipsilesional CST FA was associated with both upper and lower extremity motor performance, providing further evidence that sparring of, or reduced structural damage to, the ipsilesional CST is critical for chronic motor performance and recovery.[21, 62, 100] Less is known, however, about the contribution and changes in microstructural status of the contralesional CST post-stroke. A study by Schaechter et al.[85] found that FA of both the ipsilesional and contralesional CST was significantly and positively correlated with motor skill performance of patients' affected hand; those with a poorer level of recovery had lower FA values of bilateral CST compared to controls, while the opposite was true for patients with better motor skill recovery. Accumulating evidence indicates that white matter remodeling occurs in both ipsilesional and contralesional hemispheres, suggesting that structural remodeling of the contralesional motor system also contributes to motor recovery after stroke.[103-105] Our results, however, did not find a significant correlation between contralesional CST FA and motor performance of the affected upper/lower extremities. This difference could be attributed to different sample characteristics (e.g., higher degree of motor impairment in previous studies) and/or to differences in measures

of motor function (e.g., Schaechter et al.[85] used the Purdue Pegboard Test and maximum speed of index finger tapping as measures of hand motor function).

In addition to the ipsilesional CST, FA of the ipsilesional RN was also associated with both upper and lower extremity motor function. Anisotropy within the RN has been postulated to be associated with its afferent and efferent fibers,[182] such as the rubrospinal tract (RST). Animal studies have shown that the RST has connections with contralateral spinal motor neurons,[184] and have suggested a compensatory role of the RST in motor recovery following CST injury in non-human primates.[185, 186] Although the RST is more anatomically prominent in animals compared with humans,[187] the RST may undergo neuroplastic changes post-stroke that promotes increased functional contribution to motor recovery. Only a few studies, however, have examined neuroplastic changes in the RN following CST injury in humans after stroke. Yeo and Jang[37] examined changes in the RN 8-21 days post-stroke and found higher mean FA in the ipsilesional RN compared to the contralesional RN and healthy controls. A more recent study by Takenobu et al.[20] also found increased FA in the ipsilesional RN compared to the contralesional RN at 3 months post-stroke in patients with a pyramidal tract infarction. Additionally, a positive correlation was found between FA value in the RN and recovery of motor function. In regards to chronic stroke, Rüber et al.[129] found higher FA values in both ipsilesional and contralesional RN in 18 chronic stroke patients compared to healthy controls, with significant positive correlations found between red nuclei FA and level of motor function. These results suggest that remodeling and neuroplastic changes occur in the RN during the early stages of stroke (and possibly later) and are still evident in the chronic stage of stroke, and that these changes may indicate compensation for CST injury and contribute

to motor recovery. Our results show that variability in upper and lower extremity motor function is associated with variability in ipsilesional RN FA in chronic stroke, although we cannot directly infer a compensatory role of the RST in motor recovery. The RN receives extensive input from the cerebral cortex and has been shown to be involved with sensorimotor integration,[188] executive control,[189] and motor planning/execution;[190, 191] however, more clarity is still needed regarding the function of the human RN and its role in motor recovery post-stroke.

Of the other *a priori* ROIs involved in motor control, only ipsilesional SN FA showed a significant positive correlation with motor performance, with correlations observed with both BBT_{Aff} and Leg MI_{Aff}. The substantia nigra is involved in motor control and movement and is part of the basal ganglia. It receives input from other basal nuclei, the cerebral cortex, and midbrain reticular formation; efferent information then passes back to the cerebral cortex, basal ganglia, red nucleus, thalamus, and amygdala.[34] Our results suggest that the SN may also be involved in the recovery of both upper and lower extremity motor function in chronic stroke.

In contrast to previous studies,[20, 22, 110, 192] we did not find any significant correlations between the microstructural integrity of the corpus callosum and motor performance. This difference could be attributed to different patient sample characteristics (higher versus lower functioning), different motor assessments (e.g., prior studies commonly used the Fugl-Meyer assessment), and/or differences in how the corpus callosum was delineated or whether lesioned voxels were included in the analyses. We examined only the body of the corpus callosum, which contains the commissural

fibers connecting bilateral motor cortices, using the JHU ROI atlas and did not include lesioned voxels in our analyses.

Regression analyses revealed that ipsilesional CST FA was a predictor of grip strength of the affected hand and Arm MI_{Aff} score. These results compliment other studies that have shown that the integrity of the ipsilesional CST[7, 110] or CST/PLIC FA asymmetry[21, 60] is related to upper extremity motor performance. Adding ipsilesional RN FA to the models, however, did not significantly explain more variance in motor performance beyond that predicted by ipsilesional CST FA. In terms of lower extremity motor performance, neither ipsilesional CST FA alone or in combination with ipsilesional RN FA were significant predictors. This finding may be a reflection of the complex neural circuitry involved with locomotion. The neural control of upper and lower limb movements is not analogous, as spinal interneurons play a role in the central pattern generation of gait[134, 135] while fine hand movements are primarily under cerebral control. Studies have shown that locomotor ability is still present in some stroke survivors despite complete lateral CST injury in the affected hemisphere.[45, 131] Other descending neural pathways such as the reticulospinal, rubrospinal, and vestibulospinal tracts could contribute to locomotor function and recovery after stroke. As stated previously, significant nonparametric correlations were found between ipsilesional RN FA and lower extremity motor performance in our study.

This study is one of the first studies to incorporate a large sample size that is left hemisphere damage biased rather than right hemisphere when examining the relationship between motor impairment and structural brain integrity in chronic stroke, and may explain some of the differences with previous studies. As there are hemispheric

differences in motor structure and activation related to motor attention,[193] action selection,[194] and task complexity,[195] examining changes in structural integrity following left hemispheric stroke and the relationship to motor function is also imperative. Increasing our understanding of the relationship between structural integrity of motor pathways/brain regions and motor impairment post-stroke may provide insight into mechanisms that support brain plasticity and motor recovery. Such information could improve motor recovery prognosis and access to therapeutic resources for stroke survivors, as well as assist in evaluating rehabilitation potential and targeting therapeutic interventions. For example, researchers and clinicians could design treatment approaches that utilize different motor pathways/brain regions depending on the structural integrity post-stroke. Adding the RN to studies that predict baseline status or response to treatment may provide more insight to help guide rehabilitative strategies, as well as further examination of the potential role of basal ganglia structural integrity on motor function. Future studies that examine training-induced neuroplastic changes in the integrity of structural and functional networks are also warranted.

Our findings need to be interpreted in the context of our study design and the limitations of DTI and FA. The measured diffusion tensor is an average of several tissue compartments (with different diffusion profiles) within each voxel. Therefore, areas of tissue partial volume (where there is a mixture of white matter/gray matter/cerebrospinal fluid) or of white matter partial volume (where there crossing or diverging fibers) will result in low anisotropy.[78, 88] Furthermore, many factors influence FA values (e.g., axonal count/density, degree of myelination, fiber organization) and DTI cannot discern which structural element(s) are contributing to observed changes in FA.[89] Additionally,

our ROIs were defined by the atlas we used; for example, we assessed only the distal portion of the CST. Other studies have used alternative analysis techniques that incorporate different ROI parameters or evaluate different subsections of white matter tracts.[21, 60, 85]

4.6. CONCLUSION

Our findings demonstrate that the microstructural integrity of the ipsilesional (but not contralesional) CST is correlated with both upper and lower extremity motor function across different ICF domains. The CST appears to be less important, however, for gait speed than to the control of upper extremity dexterity and strength. Additionally, microstructural integrity of the ipsilesional RN was fair-moderately associated with upper and lower extremity motor function, suggesting that this region may contribute to motor recovery. Ipsilesional RN FA, however, did not significantly explain an additional amount of variance in affected grip strength or Arm MI beyond that explained by ipsilesional CST FA. Future work is needed to continue to elucidate the relationships between the integrity of secondary motor tracts/brain regions and motor performance in the chronic phase of stroke, as well as their relationship with functional reorganization. Such insight could help with targeting rehabilitation techniques in an effort to optimize motor recovery following stroke. Further clarification of the neuroplastic processes that underlay changes in structural integrity is also warranted, as well as a careful comparison between right and left stroke to determine if there are differences in neuroplastic changes.

Table 4.1. Summary of Demographic and Behavioral Data

Age, yr	59.7 (11.2); 31-80
Female*	16 (37.2)
Time Since Stroke, mo	64.4 (58.5); 10-284
Box & Block Test$_{Aff}$	30.6 (21.7); 0-64
Grip$_{Aff}$, kg	23.46 (15.72); 0-56.67
Arm Motricity Index$_{Aff}$	74.5 (29.2); 0-99
Leg Motricity Index$_{Aff}$	76.2 (22.1); 28-99
Gait Speed, m/s	0.94 (0.31); 0.21-1.46

Values are presented as mean (SD); range
* indicates n (percentage)
Abbreviations: yr = years; mo = months; $_{Aff}$ = affected extremity; kg = kilograms; m/s = meters per second.

Table 4.2. Upper and Lower Extremity Behavioral Measures

BEHAVIORAL MEASURES			
Measure	**Assessing**	**Calculation**	**Reliability/Validity**
Box and Block Test[165,166]	Gross manual dexterity of the hand	# blocks moved in one minute	Valid measure with high test-retest reliability in multiple populations, including stroke
Grip Strength[167, 168]	Force production in the hand	Assessed using handheld dynamometer (kg), averaged over 3 trials	Valid and reliable in stroke population, with excellent intra-rater and test-retest reliability
Motricity Index[169, 170]	Measure of limb impairment; assesses motor function, strength	Upper and lower limb sections each have a maximum score of 100	High inter-rater reliability in stroke, with strong concurrent validity with other established measures
Walking Speed[177, 178]	Temporal parameter of gait	Self-selected walking speed (m/s) averaged over 3 trials using the GAITRite electronic walkway	Valid and reliable clinical measure in chronic stroke; identifies gait asymmetries and deviations

Table 4.3. Nonparametric Correlations Between Motor Function and Mean FA Values of *A Priori* ROIs

	Behavioral Measures				
FA of *a priori* ROIs	BBT_{Aff} (n=42)†	$Grip_{Aff}$ (n=42)†	Arm MI_{Aff} (n=40)†	Leg MI_{Aff} (n=42)†	Gait Speed (n=40)†
Ipsilesional					
CST	0.55** *(n=41)*	0.38* *(n=41)*	0.52** *(n=39)*	0.44* *(n=41)*	0.35 *(n=39)*
Body of CC	0.05 *(n=34)*	-0.25 *(n=34)*	0.12 *(n=33)*	-0.03 *(n=34)*	-0.27 *(n=33)*
Red nucleus	0.45**	0.36*	0.45*	0.49**	0.50**
Thalamus	0.33 *(n=33)*	0.11 *(n=33)*	0.39 *(n=32)*	0.14 *(n=34)*	-0.12 *(n=32)*
Substantia nigra	0.36* *(n=41)*	0.19 *(n=41)*	0.36 *(n=39)*	0.37* *(n=41)*	0.17 *(n=39)*
SCP	0.10	0.17	0.19	0.26	0.20
Contralesional					
CST	0.13	0.23	0.15	0.11	0.11
Body of CC	0.11	-0.05	0.10	0.01	-0.04
Red nucleus	0.01	-0.08	0.06	0.08	0.22
Thalamus	0.02	0.07	0.02	-0.02	-0.19
Substantia nigra	-0.25	-0.24	-0.26	-0.25	-0.15
SCP	0.09	0.26	0.18	0.30	0.36

Values in the table are Spearman's coefficients (*r*). Abbreviations: ROIs = regions of interest; BBT_{Aff} = Box and Block Test (affected extremity); $Grip_{Aff}$ = grip strength (affected extremity); Arm MI_{Aff} = Arm Motricity Index score (affected extremity); Leg MI_{Aff} = Leg Motricity Index score (affected extremity); CST = corticospinal tract; CC = corpus callosum; SCP = superior cerebellar peduncle

† overall *n* value (exceptions noted in parentheses)

** $P \leq 0.001$; * $P \leq 0.01$

Table 4.4. Multiple Regression Analyses of Tract/Region-Specific FA and Upper and Lower Extremity Motor Performance

Behavioral Measure	Predictors	R2	F Statistic	P-value for F	β_{TSS}	β_{CST}	β_{RN}
BBT_{Aff} *(n=32)*							
Model 1	TSS, FA CST_{ipsi}	0.264	5.21	0.012	-0.258	0.335	
Model 2	TSS, FA CST_{ipsi}, FA RN_{ipsi}	0.270	3.45	0.030	-0.230	0.344	0.076
$Grip_{Aff}$ *(n=39)*							
Model 1	FA CST_{ipsi}	0.373	22.03	0.000†		0.611**	
Model 2	FA CST_{ipsi}, FA RN_{ipsi}	0.374	10.74	0.000†		0.597**	0.027
Arm MI_{Aff} *(n=24)*[a]							
Model 1	FA CST_{ipsi}	0.315	10.13	0.004†		-0.561**	
Model 2	FA CST_{ipsi}, FA RN_{ipsi}	0.337	5.34	0.013		-0.472*	-0.173
Leg MI_{Aff} *(n=27)*							
Model 1	FA CST_{ipsi}	0.186	5.72	0.025		0.432*	
Model 2	FA CST_{ipsi}, FA RN_{ipsi}	0.241	3.82	0.036		0.282	0.278
Gait speed *(n=37)*							
Model 1	TSS, FA CST_{ipsi}	0.162	3.28	0.050	-0.200	0.270	
Model 2	TSS, FA CST_{ipsi}, FA RN_{ipsi}	0.247	3.61	0.023	-0.160	0.175	0.315

Abbreviations: BBT_{Aff} = Box and Block Test (affected extremity); $Grip_{Aff}$ = grip strength (affected extremity); Arm MI_{Aff} = Arm Motricity Index score (affected extremity); Leg MI_{Aff} = Leg Motricity Index score (affected extremity); TSS = time since stroke; FA = fractional anisotropy; CST = corticospinal tract; RN = red nucleus.

† = significant at corrected $P \leq 0.01$

** $P \leq 0.01$; * $P \leq 0.05$

[a] Arm MI_{Aff} behavioral scores reflect and square root transformed in order to normalize data.

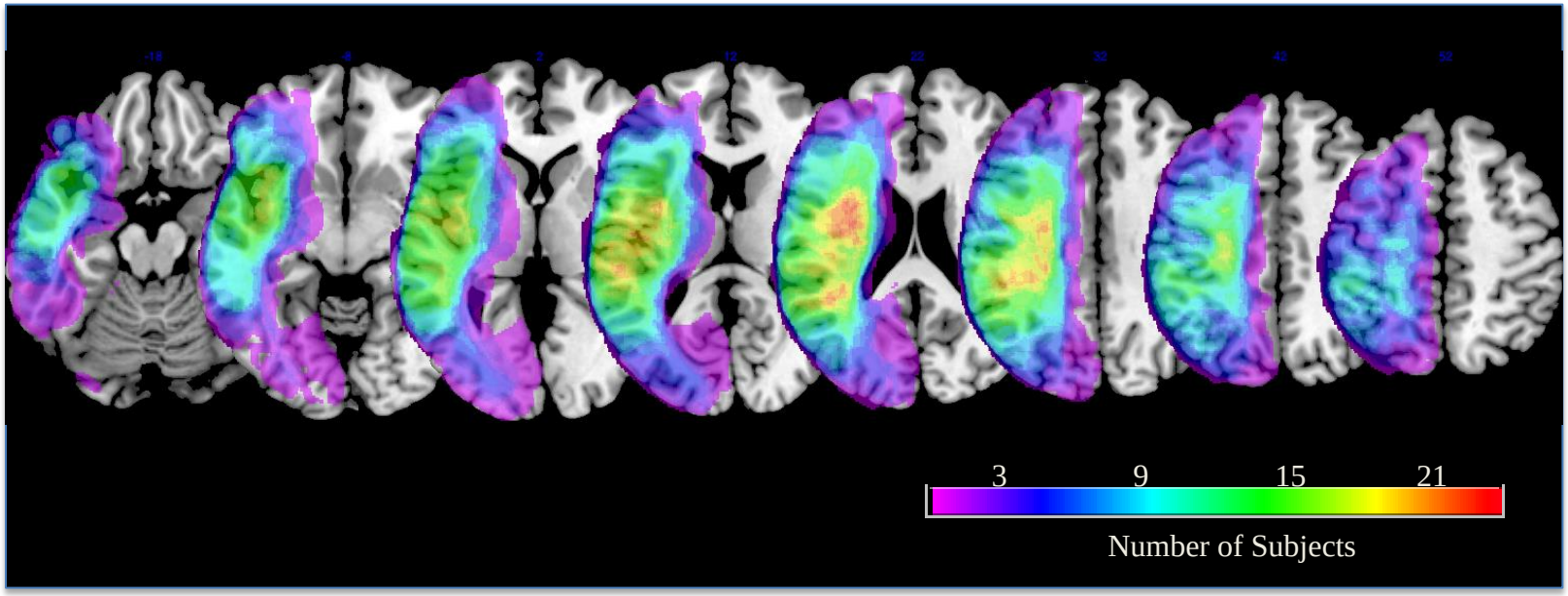

Figure 4.1. Sum lesion mask (axial view). Lesions were drawn on the native T2 image and then coregistered to the T1 image, which was then normalized into standard MNI space. The number of lesions overlapped is color coded from pink (n=1) to red (n=24).

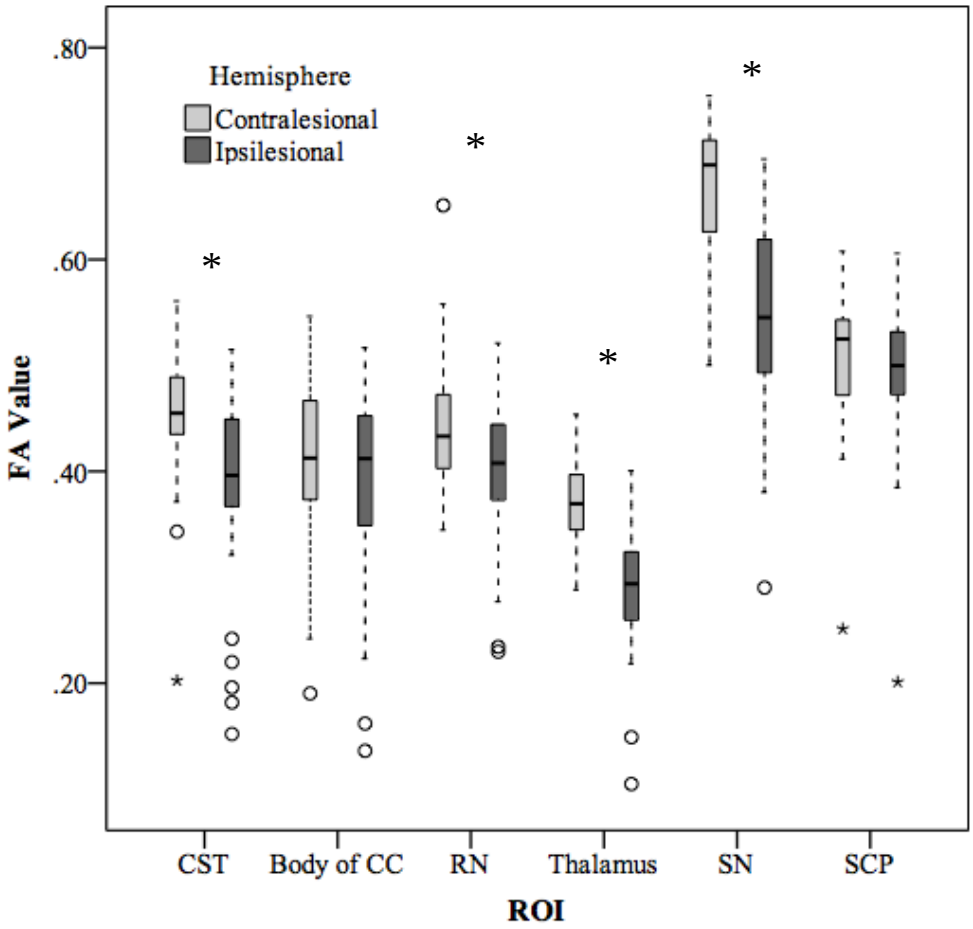

Figure 4.2. Differences in contralesional and ipsilesional white matter integrity in *a priori* ROIs. Reduced FA values were observed in the ipsilesional hemisphere. The median FA value is represented by the solid black line. The circles and stars represent outliers. The range lines indicate the limits of the first and third quartile of the IQR.
* indicates significant difference between ipsilesional and contralesional FA values

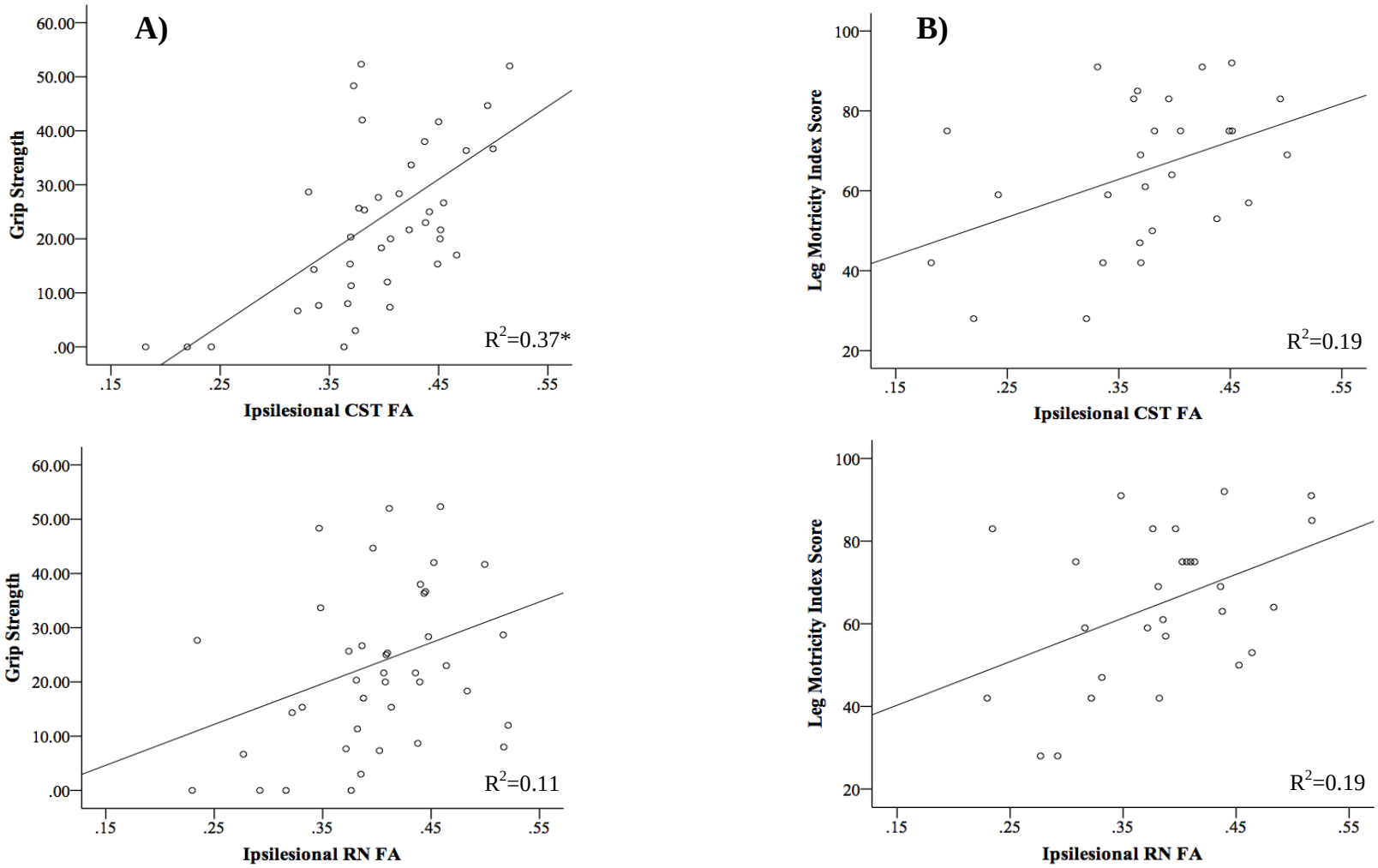

Figure 4.3. Associations between ipsilesional CST and RN FA and A) grip strength of the affected hand, and B) leg Motricity Index scores. A significant relationship was observed between CST FA and grip strength. * $P \leq 0.01$.

CHAPTER 5

CORTICAL DISCONNECTION OF THE IPSILESIONAL PRIMARY MOTOR CORTEX IS ASSOCIATED WITH GAIT SPEED AND UPPER EXTREMITY MOTOR IMPAIRMENT IN CHRONIC STROKE

5.1. ABSTRACT

Background: Recent advances in neuroimaging have enabled the mapping of white matter connections across the entire brain, allowing for a more thorough examination of the extent of white matter disconnection after stroke. This connectivity-based approach has not yet been used to examine the relationship between *motor* impairments and impaired cortical connectivity in chronic stroke.

Objective: To examine the relationship between upper/lower extremity motor impairment and structural brain connectivity in individuals with chronic stroke using the connectome-mapping techniques recently developed by Bonilha et al. (2014).[30]

Design: Cross-sectional

Methods: Forty-three participants post-stroke [age: 59.7 (11.2) years; time since stroke: 64.4 (58.8) months] underwent motor assessments and MRI scanning. Nonparametric correlation analyses were performed to examine the relationship between structural connectivity amid a subsection of the motor network and upper/lower extremity motor function. Standard multiple linear regression analyses were then performed to examine the relationship between cortical necrosis and disconnection of the three main cortical

areas of motor control [primary motor cortex (M1), premotor cortex (PMC), and supplementary motor area (SMA)] and motor function.

Results: Anatomical connectivity between ipsilesional M1/SMA and the 1) cerebral peduncle, 2) thalamus, and 3) red nucleus were significantly correlated ($P \leq 0.003$) with upper and lower extremity motor performance, with gait speed exhibiting the weakest correlations. Inter-hemispheric connectivity between M1-M1 was also significantly correlated with gross manual dexterity of the affected upper extremity (BBT_{Aff}, $r=0.46$, $P=0.001$). Regression models composed of M1 lesion load and M1 disconnection explained a significant amount of variance in BBT_{Aff} score ($R^2=0.41$), $Grip_{Aff}$ strength ($R^2=0.36$), Arm MI_{Aff} score ($R^2=0.41$), and gait speed ($R^2=0.35$). M1 disconnection was an independent predictor of motor performance while M1 lesion load was not.

Limitations: These results should be interpreted in the context of the limitations of DTI tractography.

Conclusions: Cortical disconnection, especially of ipsilesional M1, could significantly contribute to the variability seen in locomotor and upper extremity motor function and recovery in chronic stroke.

5.2. INTRODUCTION

In order to improve prognosis of motor recovery and enhance development and targeting of therapeutic interventions post-stroke, an increased understanding of the damage caused to both local and global neural networks following stroke is needed. With advancements in neuroimaging and quantitative mapping tools, the relationship between lesion size, location, and motor tract integrity can be evaluated throughout the brain. Diffusion tensor imaging (DTI) enables the examination of the microstructural

integrity and orientation of white matter in the brain *in vivo* by estimating the magnitude and directionality of water diffusion.[17, 76] Several studies using DTI techniques have demonstrated a correlation between upper extremity motor dysfunction and decreased integrity of white matter tracts in both acute[18-20] and chronic[21-23] stroke.

Motor impairment and recovery after stroke can be variable among individuals with similar lesion size/location, as the full extent of white matter injury may not be revealed by traditional structural MRI scans.[26, 196] Wallerian degeneration (WD) is characterized by anterograde degeneration of the distal portion of axons after injury to the cell body and/or proximal nerve and commonly occurs after ischemic stroke. WD has been detected as early as within the first 2 weeks post-stroke using DTI[82] and can eventually lead to fibrosis and atrophy of the fiber tracts. Several studies have examined WD of the corticospinal tract (CST) in individuals with stroke and found that reduced fractional anisotropy (FA) and/or reduced signal (which were interpreted as a reflection of WD) along the CST was associated with increased motor impairment.[82-84]

In addition to altered white matter integrity in the lesioned area, brain regions and motor tracts beyond the infarction site may also exhibit structural abnormalities. These nonlesioned areas can be indirectly affected by the loss of connections resulting from a stroke and can become dysfunctional,[27] contributing to behavioral deficits. Recent advances in neuroimaging have enabled the mapping of white matter connections across the entire brain (the brain connectome)[28, 29] allowing for a more thorough examination of the extent of white matter disconnection after stroke.

To date, this connectivity-based approach has been used to examine overall structural disconnection in the lesioned hemisphere (compared to the nonlesioned

hemisphere) after ischemic stroke[29] and to examine the relationship between language impairments and structural brain connectivity in individuals with chronic aphasia.[30] Results showed intrahemispheric disconnection extending beyond the necrotic area and fiber reduction in several major white matter tracts underlying the necrotic tissue in individuals with chronic stroke, highlighting that the extent of reduced structural connectivity cannot be surmised based solely on necrotic tissue size/location.[29, 30] Evaluation of the relationship between cortical connectivity and chronic aphasia revealed that structural disconnection of Brodmann area (BA) 45 is independently associated with naming performance (after controlling for BA 45 necrotic tissue volume) in individuals with cortical/subcortical lesions.[30] This connectivity-based approach, however, has not yet been used to examine the relationship between *motor* impairments and impaired cortical connectivity in chronic stroke. By examining neural connectivity using the brain connectome, the extent of cortical disconnection beyond the lesion site may be more fully revealed, expanding our understanding of motor impairments and recovery after stroke.

The aim of the present study was to examine the relationship between upper and lower extremity motor impairment and structural brain connectivity in individuals with chronic stroke using the connectome-mapping techniques recently developed by Bonilha et al. (2014).[30] We hypothesized that poorer performance on gait, upper and lower extremity motor measures would be associated with residual cortical necrosis and/or overall disconnection of ipsilesional motor-relevant brain regions [e.g., primary motor cortex (M1), premotor cortex (PMC), supplementary motor area (SMA)].

5.3.METHODS

5.3.A. *Participants*

Behavioral assessments were performed on 52 participants. Nine participants were excluded from analyses due to incomplete MRI data. A summary of demographic, clinical, and imaging data for the remaining 43 participants is presented in Table 5.1 (see Appendix C for detailed sample characteristics). The mean age of participants (16 female, 27 male) was 59.7 ± 11.2 years, with a mean time post-stroke of 64.4 ± 58.8 months. All participants gave written informed consent to participate in this study that was approved by the Institutional Review Board at the University of South Carolina. As this study was part of a larger study investigating lesion-impairment mapping of speech and language processing, spatial processing, and motor execution in individuals with chronic stroke, inclusion criteria consisted of 1) monolingual speaker of English (pre-stroke), 2) occurrence of a single left hemispheric ischemic or hemorrhagic stroke at least 6 months prior to study inclusion, 3) able to follow simple instructions, and 4) able to walk 8 meters with or without an assistive device (advancing their lower extremities independently). Exclusion criteria included contraindication for MRI examination (claustrophobia, pregnancy, metal implants, etc.) or clinically reported history of dementia, alcohol abuse, psychiatric disorder, traumatic brain injury, or extensive visual acuity or visual-spatial problems.

5.3.B. *Motor Assessment*

All participants underwent a comprehensive behavioral assessment of upper and lower extremity motor function and gait. Testing was performed by a physical therapist and included the Box and Block Test (BBT), grip strength, Motricity Index (MI), and gait

speed (Table 5.2). Assistive devices and/or orthotics commonly used with community ambulation were allowed for the gait speed assessment.

5.3.C. *MRI Acquisition*

All participants underwent scanning using a 3T Siemens Trio system with a 12 element head coil at the McCausland Center for Brain Imaging (Columbia, SC) within two days of behavioral testing. High-resolution T_1-MRI scans [repetition time (TR) = 2250 ms, echo time (TE) = 4.15 ms, field of view (FOV) = 256 mm and voxel size = 1.0 x 1.0 x 1.0 mm] and T_2-MRI scans [TR = 3200 ms, TE = 212 ms, FOV = 256 mm and voxel size = 1.0 x 1.0 x 1.0 mm] were acquired for determination of lesion size and location. DTI was performed with a single shot gradient echo planar imaging (EPI) sequence using the following parameters: TR = 4987 ms, TE = 79.2 ms, flip angle (α) = 90°, FOV = 207 mm, voxel size = 2.3 x 2.3 x 2.3 mm, slice thickness = 2.3 mm, noncollinear diffusion directions = 40 with a *b*-value of 1000 s/mm^2, number of slices = 50.

5.3.D. *Image Preprocessing*

MRI images were converted to NIfTI format using the dcm2nii tool from the MRIcron software package.[155] Stroke lesions were manually outlined by a neurologist (Bonilha) on the T2 image, which was then coregistered to the T1 image. T1-weighted images were normalized into standard MNI space (utilizing a cost-function mask of the brain lesion)[159] using unified segmentation-normalization routines as part of the Clinical Toolbox for the software Statistical Parametric Mapping (SPM) 8.[160] This step also provided probabilistic gray and white matter maps in standard space.[161] The invert transformation was then applied to a John Hopkins University (JHU) template and to the

gray and white matter probabilistic maps in order to transform these maps/template onto native T1 space. The probabilistic gray matter map (now in T1 space) was then segmented into a map of cortical/subcortical JHU ROIs (excluding lesioned voxels).

In order to improve registration between T1 and DTI spaces, the T2 image was linearly coregistered to the T1 image to create a T2-weighted image matched to T1 space. This "matched" image was then coregistered to the B0 image (DTI space) using FMRIB's Linear Image Registration Tool (FLIRT).[197] The same transformation matrix was applied to the map of segmented cortical ROIs and the probabilistic white matter map (which were in T1 space) to transform these maps into DTI space.

Probabilistic DTI tractography was performed using FDT's probtrackx[162] (with 5000 streamline samples) to determine the number of white matter streamlines connecting each JHU ROI. For each possible pair of cortical/subcortical ROIs ***i*** and ***j***, the number of iterative streamlines connecting the pair was computed for the creation of a connectivity matrix ***A***, where each ***Aij*** entry represented the weighted link between ROIs (adjusted based on ROI size and distance streamlines travelled).[30]

5.3.E. *Statistical Analyses*

Differences in motor scores between the affected and unaffected extremities were evaluated using two-tailed paired *t*-tests (or Wilcoxon matched-pairs signed rank tests if the data was not normally distributed). Hemispheric differences in anatomical connectivity between a subsection of the motor network (Figure 5.1) were also assessed using nonparametric statistics. The analysis focused on connectivity between the three main cortical areas of motor control [M1 (corresponds to JHU precentral gyrus ROI), PMC (corresponds to JHU middle frontal gyrus – posterior segment ROI), and SMA

(corresponds to JHU superior frontal gyrus – posterior segment ROI)] and several important subcortical regions involved in motor control (cerebral peduncle, red nucleus, thalamus, basal ganglia, and cerebellum). Studies have shown that selective disruption of corticofugal fibers from multiple motor regions can impact functional reorganization and motor recovery following stroke,[119] and neuroplastic changes in the red nucleus (RN) of the affected hemisphere have been found post-stroke,[37, 127] which may indicate a compensatory role of this brain region in motor recovery.

Due to the non-normal distribution of our behavioral data, nonparametric correlation analyses were performed to examine the relationship between structural connectivity amid these *a priori* ROIs (including inter-hemispheric connectivity between homologous regions) and motor function. Correlations were interpreted as poor (<0.25), fair (0.25 to <0.5), moderate (0.5 to 0.75), and strong (>0.75).[183] Significance level was set at $P<0.05$, corrected based on the number of ROI connectivity calculations (corrected $P\leq0.003$).

The weighted sum of all connections to the three main cortical areas involved in motor function (M1, PMC, SMA) was computed to assess overall connectivity of these cortical regions. The percentage of fibers compared to the homologous region in the unaffected hemisphere was calculated to assess fiber reduction in these motor areas in the affected hemisphere, and to normalize the final connectivity measure based on each subject's contralateral hemisphere and imaging properties. The percentage of necrotic lesion damage to each motor cortical ROI was also calculated by overlaying the manually outlined lesion onto the segmented cortical map in native T1 space. These steps were performed through in-house scripts written in MATLAB.[30]

Standard multiple linear regression analyses were then performed to examine the relationship between cortical necrosis and disconnection of M1, PMC, and SMA and upper/lower extremity motor performance. In order to normalize data for regression analyses, participants who scored 0 on BBT were removed as the BBT has a low floor effect.[165, 166] Similarly, participants who scored 99 on the MI for the affected upper/lower extremity were removed due to the high ceiling effect.[169, 170] A reflect and square root transformation was also used with Arm MI_{Aff} data to obtain a normal distribution. Separate multiple linear regressions were performed for each behavioral measure, and included the clinical score as the dependent variable with the following independent variables: 1) percentage of necrotic lesion damage to each motor area, and 2) percentage fiber number of each motor area. Each cortical motor area was analyzed separately. Significance level was set at $P<0.05$, corrected based on the number of behavioral measures assessed (corrected $P\leq0.01$).

5.4. RESULTS

5.4.A. *Behavioral Measures*

Motor impairment of the affected upper extremity was evidenced by significantly reduced gross manual dexterity of the hand [median BBT_{Aff} score = 34.5 (interquartile range (IQR), 7.25-46.5), median BBT score for the unaffected extremity = 51.0 (IQR, 44.0-56.25), $P<0.001$, Wilcoxon matched-pairs signed rank test], decreased grip strength ($Grip_{Aff}$ = 23.46 ± 15.72 kg, grip strength unaffected hand = 34.64 ± 10.51 kg, $P<0.001$, paired *t*-test), and impaired motor function [median Arm MI_{Aff} score = 88 (IQR, 56.5-100), median Arm MI score for the unaffected extremity = 100 (IQR, 100-100), $P<0.001$, Wilcoxon matched-pair signed-rank test]. The affected lower extremity also exhibited

significant residual motor impairment [median Leg MI_{Aff} score = 79 (IQR, 59-100), median Leg MI score for the unaffected extremity = 100 (IQR, 100-100), $P<0.001$, Wilcoxon matched-pair signed-rank test]. Average gait speed was 0.94 ± 0.31 m/s.

For the subgroup of participants with normalized data for the regression analyses, average BBT score for the affected extremity was 37.8 ± 17.4 (n=34), average Arm MI score for the affected extremity was 59.8 ± 28.1 (n=25), and average Leg MI score for the affected extremity was 64.9 ± 18.4 (n=28).

5.4.B. *Necrotic Lesion Location*

All participants exhibited a cortical/subcortical lesion in the left hemisphere, broadly distributed within the territory of the middle cerebral artery. For one participant, the lesion was too small to adequately outline; all other lesions were clearly visible on T2 weighted images. Locations of maximal lesion overlap were the extra-nuclear and subgyral areas, as demonstrated in Figure 5.2.

5.4.C. *Lesion Size and Motor Impairment*

Total lesion volume was not significantly correlated (Spearman's rho) with motor function of the affected extremity on BBT (r=-0.17, P=0.29), grip strength (r=0.10, P=0.54), Arm MI (r=-0.15, P=0.37), Leg MI (r=-0.06, P=0.71), or with gait speed (r=0.15, P=0.35).

5.4.D. *Cortical/Subcortical Connectivity and Motor Impairment*

Anatomical reciprocal connectivity within the lesioned hemisphere between the following *a priori* ROIs was decreased overall compared with the homologous connectivity in the nonlesioned hemisphere: M1 <-> thalamus (P=0.002); PMC <-> thalamus (P=0.001); SMA <-> to thalamus (P<0.001); M1 <-> cerebral peduncle

(P=0.002); SMA <-> cerebral peduncle (P=0.001); M1 <-> PMC (P<0.001); and caudate nucleus <-> thalamus (P<0.001). Connectivity between the remaining ROIs was not significantly different between hemispheres (P>0.003).

Correlations between motor function and structural connectivity of *a priori* ROIs (lesioned hemisphere and inter-hemispheric) are presented in Table 5.3. Anatomical connectivity between ROIs corresponding to two of the main cortical areas of motor control (M1 and SMA) and the 1) cerebral peduncle, and 2) thalamus were significantly correlated ($P \leq 0.003$) with motor performance across all behavioral measures, with gait speed exhibiting the weakest correlations. Structural connectivity between M1/SMA and the RN was significantly correlated with motor performance across all upper extremity motor measures and one lower extremity measure (Leg MI_{Aff}). In terms of basal ganglia connectivity, only anatomical connectivity between the putamen and thalamus was significantly correlated with motor performance (gait speed, r=0.43, P=0.003). Analyses of connectivity between the three main cortical areas of motor control revealed a significant correlation between M1 <-> SMA connectivity and grip strength (r=0.46, P=0.001) and BBT_{Aff} (r=0.42, P=0.003). Inter-hemispheric connectivity between M1-M1 was also significantly correlated with gross manual dexterity of the affected upper extremity (BBT_{Aff}, r=0.46, P=0.001).

5.4.E. *Multiple Regression Analyses*

Results of the regression analyses are summarized in Table 5.4. For upper extremity motor performance, a model composed of M1 lesion load and M1 disconnection explained a significant amount of variance in BBT_{Aff} score [R^2=0.41, $F(2,30)$=10.48, P<0.001; β_{LL}=-0.145, P=0.32.; $\beta_{disconnection}$=0.59, P<0.001], $Grip_{Aff}$

strength [R^2=0.36, F(2,38)=10.82, P<0.001; β_{LL}=0.035, P=0.81; $\beta_{disconnection}$=0.616, P<0.001], and Arm MI_{Aff} score [R^2=0.41, F(2,21)=7.41, P=0.004; β_{LL}=0.11, P=0.54; $\beta_{disconnection}$=-0.601, P=0.003]. Whereas M1 disconnection was an independent predictor of upper extremity motor performance across all three measures, M1 lesion load was not independently associated with upper extremity motor performance. We also observed a significant relationship between BBT_{Aff} score and a model composed of SMA lesion load and SMA disconnection [R^2=0.29, F(2,28)=5.61, P=0.009; β_{LL}=-0.35, P=0.056; $\beta_{disconnection}$=0.285, P=0.115]; however, neither SMA lesion load or disconnection were independent predictors of BBT_{Aff} score. Upper extremity motor performance was not associated with a model composed of PMC lesion load and disconnection.

In terms of lower extremity motor performance, a significant relationship was found between gait speed and a model composed of M1 lesion load and M1 disconnection [R^2=0.35, F(2,36)=9.64, P<0.001; β_{LL}=0.225, P=0.13; $\beta_{disconnection}$=0.63, P<0.001]. Again, M1 disconnection was an independent predictor of gait speed while M1 lesion load was not. Lesion load and disconnection in PMC or SMA was not associated with lower extremity motor performance.

5.5. DISCUSSION

The aim of the current study was to investigate the relationship between upper and lower extremity motor impairment and structural brain connectivity among a subsection of the motor network in individuals with chronic stroke. Anatomical reciprocal connectivity within the lesioned hemisphere was significantly reduced between M1/PMC/SMA and the thalamus, as well as between MI/SMA and the cerebral peduncle, compared with the nonlesioned hemisphere. There was no significant hemispheric

difference, however, in connectivity between MI/PMC/SMA and the red nucleus, as well as between SMA and M1/PMC. This may reflect adaptive changes in structural connectivity between these three cortical regions and the red nucleus, as well as between SMA and M1/PMC, in the lesioned hemisphere following stroke. The red nucleus is the origin of the rubrospinal tract; afferent input to the red nucleus includes fibers from the cerebral cortex and cerebellum, and it is believed the rubrospinal tract is an indirect pathway by which the cortex and cerebellum can influence motor neurons in the spinal cord.[35, 37] Previous studies in both non-human primates[184, 185] and humans[20, 129] suggest that remodeling and neuroplastic changes occur in the RN during the early stages of stroke (and possibly later) and are still evident in the chronic stage of stroke, and that these changes may indicate compensation for CST injury and contribute to motor recovery. Our results show that variability in upper and lower extremity motor function is associated with variability in structural connectivity among ipsilesional M1/SMA and the RN, suggesting that connectivity between these cortical regions and midbrain nucleus may play a role in motor recovery in chronic stroke.

Connectivity between ipsilesional M1/SMA and the cerebral peduncle significantly correlated with motor function across all behavioral measures (with gait speed exhibiting the weakest correlation), while PMC <-> cerebral peduncle connectivity only correlated with BBT_{Aff} and Arm MI_{Aff}. Research has shown that selective disruption of corticofugal fibers from multiple motor regions can impact functional reorganization and motor recovery following stroke.[119] Microstructural integrity of CST pathways originating from primary *and* secondary cortical motor areas in the affected hemisphere have been shown to be reduced in chronic stroke, with grip strength strongly

related to the integrity of fibers originating from the primary motor and (to a lesser extent) dorsal premotor cortices.[101] A study by Jang et al (2014) also showed positive correlations between motor function of the affected upper/lower extremities and ipsilesional CST FA and fiber number ratio, with preservation of CST integrity and absence of Wallerian degeneration important for better motor outcome.[102] Additionally, connectivity between ipsilesional M1/SMA and the thalamus was significantly correlated with motor function across all behavioral measures. This finding is not surprising, as the thalamus is involved in the integration of sensory information and is closely linked to the cerebral cortex. It is a main relay station for sensory-motor neuronal loops involving the cerebellum and basal nuclei, which are important for voluntary movement.[34]

Investigation of the influence of basal nuclei connectivity on motor performance revealed only a significant correlation between gait speed and structural connectivity between the ipsilesional putamen and thalamus, indicating a potential role of the basal ganglia in lower extremity motor recovery following stroke. These nuclei have extensive connections with many different regions of the brain and are known to play an important role in postural control and voluntary movement.[34] Research has demonstrated increased putamen activation during imagined standing and walking in healthy adults,[198] and damage to the putamen has been associated with temporal gait asymmetry[199] and decreased walking speed[200] in individuals with chronic stroke.

Interhemispheric structural connectivity between M1s was also significantly and positively correlated with BBT_{Aff} score, suggesting that communication between primary motor cortices is important for upper extremity movement and dexterity of the hand post-stroke. Structural connectivity between M1s is crucial for interhemispheric inhibition, a

process in which activity in the M1 of one hemisphere inhibits activity in the homologous region of the opposite hemisphere in order to execute unimanual movements and coordinated bimanual tasks.[39-41] Previous studies have found reduced white matter integrity of transcallosal fibers to be associated with upper extremity motor impairment in chronic stroke.[22, 110] Functional neuroimaging studies have also demonstrated that decreased functional connectivity[106, 192] and impaired interhemispheric inhibition[40, 42] between motor cortices is associated with upper extremity motor impairment post-stroke. Additionally, our results could suggest a role for contralesional M1 involvement in upper extremity motor recovery, which is supported by previous studies investigating functional connectivity and cortical reorganization following stroke.[100, 118]

Our findings reveal that cortical disconnection occurs alongside cortical necrosis, and that cortical disconnection is independently associated with upper/lower extremity motor performance. We observed that grip strength and motor function of the affected upper extremity is associated with preservation of cortical integrity and connectivity of ipsilesional M1, while gross manual dexterity of the affected hand is associated with preservation of both ipsilesional M1 and SMA cortical integrity and connectivity. Furthermore, preserved cortical connectivity of M1 is an independent predictor of upper extremity motor performance across all measures, after controlling for lesion load. As the CST is the major neural pathway for skilled, discrete voluntary movements (especially for fine movements of the hands),[36] preservation of white matter fibers supporting ipsilesional M1 is important as approximately one-third of the CST fibers arise from M1.[35] The SMA is connected directly via bi-directional pathways with the ipsilateral primary motor, premotor, and somatosensory cortices and indirectly receives

subcortical input mainly from the basal ganglia via corticothalamic pathways.[34] It has direct connections with spinal motor neurons which innervate the hand,[120] and therefore can play an augmented functional role in producing simple hand movements post-stroke. The SMA is important in the temporal/sequential organization of movement and becomes more significant in the execution of simple motor tasks if the primary motor cortex is injured.[34, 201] In general, chronic motor impairment after stroke has been associated with lesion size[9, 10] and location,[11, 12] and degree of overlap with the CST.[7, 62] There is great variability, however, in clinical manifestation and recovery from stroke in the chronic phase. Our results suggest that cortical disconnection of these motor regions, especially of ipsilesional M1, could significantly contribute to the variability seen in upper extremity motor function/recovery, as the extent of brain damage may be underestimated by examination of the necrotic tissue alone if more salient, global effects on the motor network are not taken into account.

Gait speed was also associated with preservation of cortical integrity and connectivity of ipsilesional M1, with cortical connectivity of this brain region being an independent predictor. Motor recovery of the affected upper extremity has been highly studied, whereas less is known about the motor recovery mechanisms involved with lower extremity and locomotor function after stroke. While the CST is necessary for fine movements of the hands,[36, 60] locomotion and motor function of the legs is less dependent on the CST.[45, 130, 131] Some studies have suggested that the lateral CST does not play a central role in basic locomotor function in primates or human[45, 79] but rather it is involved in "skilled walking," or the adaption of gait kinematics to environmental demands,[58] and may be more strongly associated with temporal parameters of gait.[130, 132] Other

descending neural pathways such as the corticoreticulospinal, rubrospinal, and vestibulospinal tracts could contribute to locomotor function. Furthermore, the neural control of upper and lower limb movements is not analogous, as spinal interneurons play a role in the central pattern generation of gait[134, 135] while fine hand movements are primarily under cerebral control. Cortical reorganization following stroke, therefore, may be different for lower limb function compared to what has been demonstrated with upper limb function. Our results suggest that while we might not know the precise pathways that play a role in locomotor recovery, preservation of white matter fibers supporting ipsilesional M1 is important for locomotor function in individuals with chronic stroke.

Overall, these results compliment the findings of Bonilha et al.[30] that examined language impairments and cortical disconnection after stroke, and further highlight the discrepancy of brain regions that appear intact on structural scans but actually exhibit reduced structural integrity that may contribute to motor impairments and affect recovery post-stroke. Improving our insight and understanding of the broader structural and functional changes that occur in these "nonlesioned" brain regions can help improve motor recovery prognosis and target therapeutic interventions in a more tailored fashion for stroke survivors, thereby improving the clinical management of chronic mobility impairments in this patient population. More research needs to be performed to clarify the mechanisms that underlie changes in structural integrity and connectivity post-stroke, as well as examine training-induced changes in structural connectivity of motor networks in both subacute and chronic stroke.

This study is one of the first studies to incorporate a large sample size with left hemisphere necrotic damage when examining the relationship between motor function

and structural brain connectivity in chronic stroke. As there are hemispheric differences in motor structure and activation related to motor attention,[193] action selection,[194] and task complexity,[195] examining changes in motor network integrity and connectivity following left hemispheric stroke and the relationship to motor function is also imperative. The results of our study, however, should be interpreted in the context of the limitations of DTI tractography (e.g., false tracking in low anisotropic areas and/or in regions with fiber complexity and crossing). Additionally, as this study was part of a larger study investigating lesion-impairment mapping of speech and language processing in individuals with chronic stroke, the majority of participants had lesions involving language regions of the brain resulting in a large number of subjects with minimal motor impairment. Future studies involving lesions primarily distributed in sensorimotor brain regions may provide a broader range of motor impairments and allow for the examination of structural disconnection related to more direct necrotic lesion damage to the motor network.

5.6. CONCLUSION

Our findings demonstrate that ipsilesional structural connectivity between several brain regions involved in the motor network (particularly between M1/SMA and the cerebral peduncle, red nucleus, and thalamus) is associated with both upper and lower extremity motor performance in individuals with chronic stroke. Furthermore, upper extremity motor function of the affected extremity and gait speed is dependent upon the preservation of cortical integrity and connectivity of ipsilesional M1 (and to a lesser degree ipsilesional SMA with BBT), with cortical disconnection of M1 being an independent predictor of motor function. Our findings highlight the importance of

examining structural changes and cortical disconnection in the broader motor network post-stroke. Such insight could enhance our understanding of the underlying factors contributing to motor impairments post-stroke. Future research examining structural integrity and connectivity following a treatment intervention may provide insight into more global patterns of structural brain plasticity that could help clinicians and researchers better target therapeutic interventions to enhance motor recovery potential.

Table 5.1. Summary of Demographic, Behavioral, and Imaging Data

Age, yr	59.7 (11.2); 31-80
*Female	16 (37.2)
Time Since Stroke, mo	64.4 (58.5); 10-284
^M1 lesion load, %	21.3 (23.3); 0-91.0
^PMC lesion load, %	15.3 (25.5); 0-98.4
^SMA lesion load, %	6.4 (14.8); 0-59.4
Box & Block Test$_{Aff}$	30.6 (21.7); 0-64
Grip$_{Aff}$, kg	23.46 (15.72); 0-56.67
Arm Motricity Index$_{Aff}$	74.5 (29.2); 0-99
Leg Motricity Index$_{Aff}$	76.2 (22.1); 28-99
Gait Speed, m/s	0.94 (0.31); 0.21-1.46

Values are presented as mean (SD); range
* indicates n (percentage)
^ ipsilesional hemisphere
Abbreviations: yr = years; mo = months; M1 = primary motor cortex; PMC = premotor cortex; SMA = supplementary motor area; $_{Aff}$ = affected extremity; kg = kilograms; m/s = meters per second.

Table 5.2. Upper and Lower Extremity Behavioral Measures

BEHAVIORAL MEASURES			
Measure	**Assessing**	**Calculation**	**Reliability/Validity**
Box and Block Test[165,166]	Gross manual dexterity of the hand	# blocks moved in one minute	Valid measure with high test-retest reliability in multiple populations, including stroke
Grip Strength[167, 168]	Force production in the hand	Assessed using handheld dynamometer (kg), averaged over 3 trials	Valid and reliable in stroke population, with excellent intra-rater and test-retest reliability
Motricity Index[169, 170]	Measure of limb impairment; assesses motor function, strength	Upper and lower limb sections each have a maximum score of 100	High inter-rater reliability in stroke, with strong concurrent validity with other established measures
Walking Speed[177, 178]	Temporal parameter of gait	Self-selected gait speed (m/s) averaged over 3 trials using the GAITRite electronic walkway	Valid and reliable clinical measure in chronic stroke; identifies gait asymmetries and deviations

Table 5.3. Nonparametric Correlations Between Motor Function and Structural Connectivity of *A Priori* ROIs

	Behavioral Measures				
Structural Connectivity of *a priori* ROIs	BBT_{Aff} (n=42)	$Grip_{Aff}$ (n=42)	Arm MI_{Aff} (n=40)	Leg MI_{Aff} (n=42)	Gait Speed (n=40)
Ipsilesional Hemisphere					
M1 <-> PMC	0.35	0.24	0.28	0.17	0.10
M1 <-> SMA	0.42*	0.46*	0.41	0.38	0.25
PMC <-> SMA	0.31	0.22	0.22	0.14	-0.05
M1 <-> CP	0.74*	0.67*	0.67*	0.63*	0.55*
PMC <-> CP	0.45*	0.30	0.45*	0.41	0.34
SMA <-> CP	0.66*	0.56*	0.63*	0.63*	0.54*
M1 <-> RN	0.53*	0.49*	0.49*	0.43*	0.24
PMC <-> RN	0.35	0.28	0.35	0.34	0.23
SMA <-> RN	0.52*	0.42*	0.46*	0.45*	0.38
M1 <-> Thalamus	0.65*	0.56*	0.61*	0.66*	0.54*
PMC <-> Thalamus	0.39	0.21	0.37	0.37	0.23
SMA <-> Thalamus	0.65*	0.57*	0.63*	0.64*	0.46*
Basal Ganglia & Cerebellum					
Caudate <-> Thalamus	0.05	-0.07	0.10	0.10	-0.09
Putamen <-> Thalamus	0.37	0.36	0.27	0.40	0.43*
GP <-> Thalamus	0.23	0.29	0.25	0.33	0.36
Cerebellum <-> Thalamus	0.50*	0.51*	0.43*	0.48*	0.39
Interhemispheric Connectivity					
M1 <-> M1	0.46*	0.30	0.31	0.30	0.14
PMC <-> PMC	0.24	0.01	0.16	0.08	0.06
SMA <-> SMA	0.35	0.14	0.22	0.21	0.23

Values in the table are Spearman's coefficients (*r*). Abbreviations: ROIs = regions of interest; BBT_{Aff} = Box and Block Test (affected extremity); $Grip_{Aff}$ = grip strength (affected extremity); Arm MI_{Aff} = Arm Motricity Index score (affected extremity); Leg MI_{Aff} = Leg Motricity Index score (affected extremity); M1 = primary motor cortex; PMC = premotor cortex; SMA = supplementary motor area; CP = cerebral peduncle; RN = red nucleus; GP = globus pallidus.
* significant at corrected $p \leq 0.003$

Table 5.4. Multiple Regression Analyses of Cortical Necrosis and Disconnection and Upper/Lower Extremity Motor Performance

Behavioral Measure	Predictors	R2	F Statistic	P-value for F	β_{LL}	$\beta_{disconnection}$
BBT_{Aff} *(n=33)*						
M1	LL_{M1}, % fiber number $_{M1}$	0.41	10.48	0.000†	-0.145	0.590**
PMC[a]	LL_{PMC}, % fiber number $_{PMC}$	0.01	0.89	0.915	-0.053	0.037
SMA[b]	LL_{SMA}, % fiber number $_{SMA}$	0.29	5.61	0.009†	-0.350	0.285
$Grip_{Aff}$ *(n=41)*						
M1	LL_{M1}, % fiber number $_{M1}$	0.36	10.82	0.000†	0.035	0.616**
PMC[c]	LL_{PMC}, % fiber number $_{PMC}$	0.02	0.38	0.687	0.027	0.161
SMA	LL_{SMA}, % fiber number $_{MA}$	0.19	4.38	0.019	-0.059	0.404*
Arm MI_{Aff} *(n=24)*^						
M1	LL_{M1}, % fiber number $_{M1}$	0.41	7.41	0.004†	0.110	-0.601**
PMC[a]	LL_{PMC}, % fiber number $_{PMC}$	0.28	3.86	0.038	0.688*	0.410
SMA	LL_{SMA}, % fiber number $_{SMA}$	0.34	5.39	0.013	0.402*	-0.305
Leg MI_{Aff} *(n=27)*						
M1	LL_{M1}, % fiber number $_{M1}$	0.18	2.55	0.099	0.118	0.460*
PMC	LL_{PMC}, % fiber number $_{PMC}$	0.01	0.12	0.890	-0.051	0.059
SMA	LL_{SMA}, % fiber number $_{SMA}$	0.07	0.91	0.417	-0.166	0.149
Gait speed *(n=39)*						
M1	LL_{M1}, % fiber number $_{M1}$	0.35	9.64	0.000†	0.225	0.630**
PMC	LL_{PMC}, % fiber number $_{PMC}$	0.02	0.36	0.699	0.070	0.165
SMA[b]	LL_{SMA}, % fiber number $_{SMA}$	0.19	4.02	0.027	-0.336	0.170

Abbreviations: BBT_{Aff} = Box and Block Test (affected extremity); $Grip_{Aff}$ = grip strength (affected extremity); Arm MI_{Aff} = Arm Motricity Index score (affected extremity); Leg MI_{Aff} = Leg Motricity Index score (affected extremity); M1 = primary motor cortex; PMC = premotor cortex; SMA = supplementary motor area; LL = lesion load.

^ Arm MI_{Aff} behavioral scores reflect and square root transformed in order to normalize behavioral data

† = significant at corrected $P \leq 0.01$; ** $P \leq 0.01$; * $P \leq 0.05$

[a] 1 outlier removed; [b] 2 outliers removed; [c] 4 outliers removed

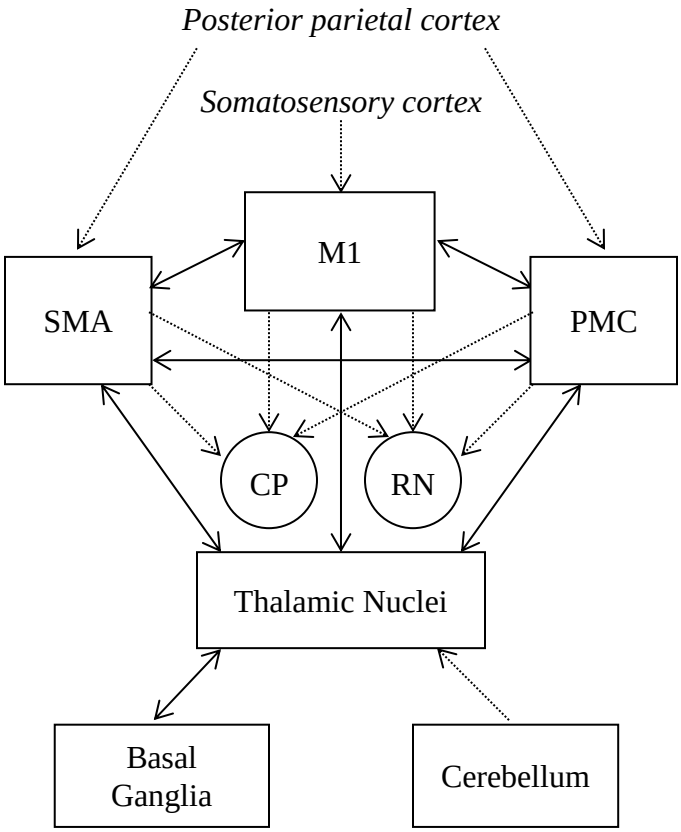

Figure 5.1. Subsection of the motor network.

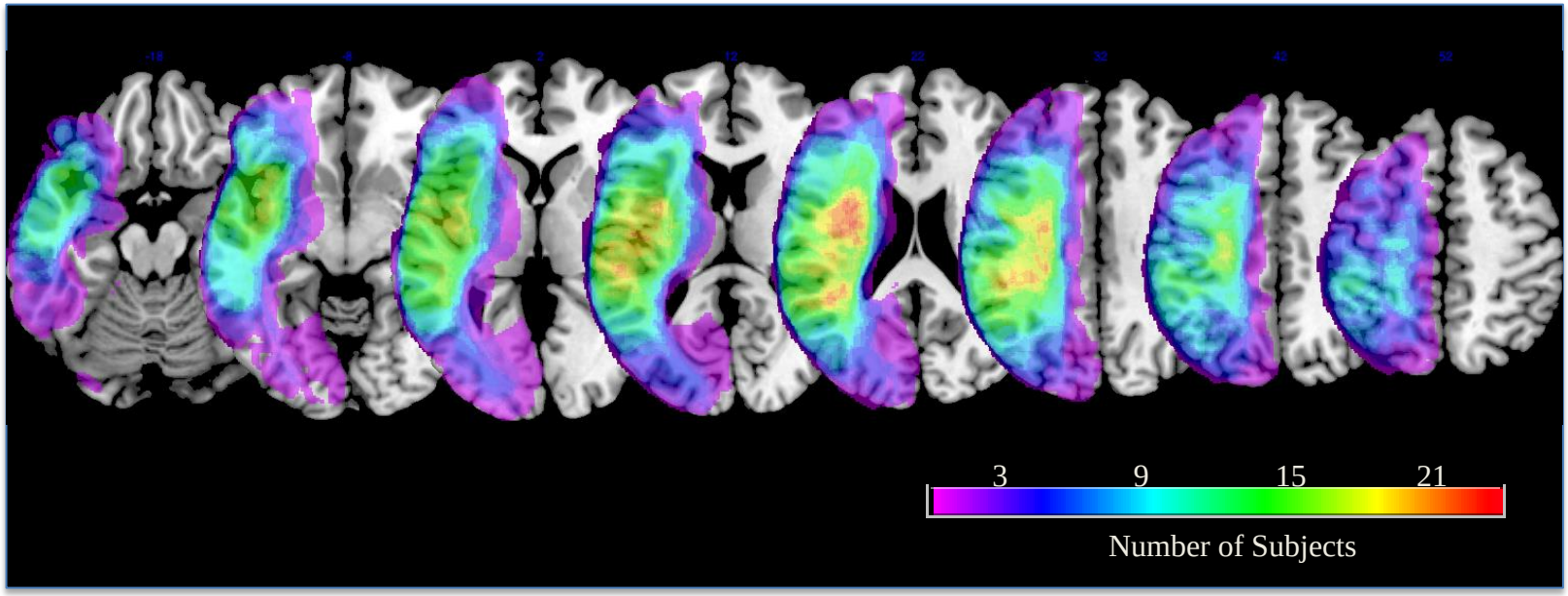

Figure 5.2. Sum lesion mask (axial view). Lesions were drawn on the native T2 image and then coregistered to the T1 image, which was then normalized into standard MNI space. The number of lesions overlapped is color coded from pink (n=1) to red (n=24).

CHAPTER 6

DISCUSSION

Stroke is the leading cause of disability in the United States.[1] Recovery of motor function varies considerably following stroke, as individuals with similar lesions on structural scans can often exhibit vastly different motor impairments and/or responses to treatments. Structural abnormalities often extend beyond the site of tissue necrosis, rendering nonlesioned areas dysfunctional[26, 27] which can further contribute to behavioral deficits. With approximately 6.4 million Americans who are stroke survivors[6] and many suffering from deficits in motor function, improving our understanding of structural changes in the brain post-stroke would help improve motor recovery prognosis and assist in targeting therapeutic interventions. Advances in neuroimaging have enabled the examination of the integrity and orientation of white matter in the brain[17, 21] and the mapping of white matter connections across the entire brain (the brain connectome).[28, 29] Limited research has examined the relationship between upper *and* lower extremity motor performance and the structural integrity of both primary *and* secondary motor tracts/brain regions in individuals with chronic stroke. Furthermore, the connectivity-based approach developed by Bonilha et al.[30] has not yet been used to examine the relationship between *motor* impairments and impaired cortical connectivity in chronic stroke. Therefore, the aim of the current study was to examine the relationship between

upper/lower extremity motor impairments and structural integrity/connectivity of motor-relevant regions of interest in individuals with chronic stroke.

6.1. IPSILESIONAL STRUCTURAL INTERGRITY AND MOTOR FUNCTION

Our results showed that ipsilesional CST FA was associated with both upper and lower extremity motor performance, providing further evidence that sparring of, or reduced structural damage to the ipsilesional CST is critical for chronic motor function and recovery.[21, 100, 101] The CST appears to be less important, however, for gait speed than to the control of upper extremity dexterity and strength. Less is known about the contribution and changes in microstructural status of the *contralesional* CST post-stroke. A few studies have suggested that structural remodeling of the contralesional motor system also contributes to motor recovery after stroke.[103-105] Our findings did not reveal a significant correlation between contralesional CST FA and motor performance, suggesting that microstructural integrity of the ipsilateral CST is not strongly associated with motor function and recovery of the affected extremity post-stroke. On the other hand, this difference could be attributed to different sample characteristics (e.g., higher degree of motor impairment in previous studies) and/or to differences in measures of motor function compared to previous studies.

In addition to the ipsilesional CST, microstructural integrity of the ipsilesional RN was fair-moderately associated with both upper/lower extremity motor performance. The red nucleus is the origin of the rubrospinal tract (RST),[34] which has connections with contralateral spinal motor neurons, and studies in both non-human primates[185] and humans[20, 129] have suggested a compensatory role of the RST in motor recovery following CST injury. Our results show that while we cannot directly infer a compensatory role of the RST in motor recovery, variability in upper and lower extremity motor function is

associated with variability in ipsilesional RN FA in chronic stroke, providing further evidence of potential RN involvement to motor recovery in humans. Ipsilesional RN FA, however, did not significantly explain an additional amount of variance in affected grip strength or Arm MI beyond that explained by ipsilesional CST FA. Therefore, while integrity of the ipsilesional CST and RN are both associated with upper extremity motor performance, ipsilesional CST alone is a stronger predictor of upper extremity motor function post-stroke.

In contrast to previous studies,[20, 22, 110, 192] we did not find any significant correlations between the microstructural integrity of the corpus callosum and motor performance. This difference could be attributed to different patient sample characteristics (higher versus lower functioning), different motor assessments (e.g., prior studies commonly used the Fugl-Meyer assessment), and/or differences in how the corpus callosum was delineated or whether lesioned voxels were included in the analyses.

While ipsilesional CST FA was a significant predictor of upper extremity motor performance, neither ipsilesional CST FA alone or in combination with ipsilesional RN FA were significant predictors of lower extremity motor performance. This finding may be a reflection of the complex neural circuitry involved with locomotion. The neural control of upper and lower limb movements is not analogous, as spinal interneurons play a role in the central pattern generation of gait[134, 135] while fine hand movements are primarily under cerebral control. Studies have shown that locomotor ability is still present in some stroke survivors despite complete lateral CST injury in the affected hemisphere.[45, 131] Secondary descending neural pathways could play a greater role in locomotor function and recovery after stroke, and nonparametric correlations were

strongest between ipsilesional RN FA and lower extremity motor performance in our study.

6.2. CORTICAL CONNECTIVITY AND MOTOR FUNCTION

Our results showed reduced anatomical reciprocal connectivity within the lesioned hemisphere between several motor-relevant regions of interest (e.g., between M1/SMA and the thalamus/cerebral peduncle). There was no significant hemispheric difference, however, in connectivity between M1/PMC/SMA and the RN, as well as between SMA and M1/PMC. This may reflect adaptive changes in structural connectivity between these regions in the lesioned hemisphere following stroke. Similarly, our results showed significant correlations between upper and lower extremity motor performance and structural connectivity among ipsilesional M1/SMA and the RN, suggesting that connectivity between these cortical regions and midbrain nucleus may play a role in motor recovery in chronic stroke.

Connectivity between ipsilesional M1/SMA and the cerebral peduncle significantly correlated with motor function across all behavioral measures (with gait speed exhibiting the weakest correlation). This finding is in line with previous studies that have shown selective disruption of corticofugal fibers from multiple motor regions can impact functional reorganization and motor recovery following stroke.[101, 119] Additionally, interhemispheric structural connectivity between primary motor cortices was significantly and positively correlated with BBT_{Aff} score, suggesting that communication between M1s is important for upper extremity movement and dexterity of the hand post-stroke. These results could also suggest a role for contralesional M1 involvement to upper extremity motor recovery, which is supported by previous studies investigating functional connectivity and cortical reorganization following stroke.[100, 118]

Upon examining the relationship between cortical disconnection and motor performance in chronic stroke, our results indicate that cortical disconnection occurs alongside cortical necrosis and is independently associated with upper/lower extremity motor performance. We observed that gait speed, grip strength, and motor function of the affected upper extremity is associated with preservation of cortical integrity and connectivity of ipsilesional M1, while gross manual dexterity of the affected hand is associated with preservation of both ipsilesional M1 and SMA cortical integrity and connectivity. Furthermore, preserved cortical connectivity of M1 is an independent predictor of gait speed and upper extremity motor performance, after controlling for lesion load. Overall, these findings suggest that cortical disconnection of these motor regions, especially of ipsilesional M1, could significantly contribute to the variability seen in locomotor and upper extremity motor function/recovery. The extent of brain damage after stroke, therefore, may be underestimated by examination of the necrotic tissue alone if more salient, global effects on the motor network are not taken into account.

6.3. LIMITATIONS AND FUTURE STUDIES

The results of this study should be interpreted in the context of the limitations of FA and DTI tractography. The measured diffusion tensor is an average of several tissue compartments (with different diffusion profiles) within each voxel. Therefore, areas of tissue partial volume (where there is a mixture of white matter/gray matter/cerebrospinal fluid) or of white matter partial volume (where there crossing or diverging fibers) will result in low anisotropy.[78, 88] False tracking can occur in low anisotropic areas and/or in regions with fiber complexity and crossing. Furthermore, many factors influence FA

values (e.g., axonal count/density, degree of myelination, fiber organization) and DTI cannot discern which structural element(s) are contributing to observed changes in FA.[89]

Additionally, as this study was part of a larger study investigating lesion-impairment mapping of speech and language processing in individuals with chronic stroke, the majority of participants had lesions involving language regions of the brain, resulting in a large number of subjects with minimal motor impairment. Future studies involving lesions primarily distributed in sensorimotor brain regions may provide a broader range of motor impairments and allow for the examination of structural disconnection related to more direct necrotic lesion damage to the motor network. Lastly, our ROIs were defined by the atlas we used. Other studies have used alternative analysis techniques that incorporate different ROI parameters or evaluate different subsections of white matter tracts.[21, 60, 85]

Future work is needed to continue to elucidate the relationships between the integrity of secondary motor tracts/brain regions, cortical/subcortical connectivity and motor performance in the chronic phase of stroke, as well as their relationship with functional reorganization. Further clarification of the neuroplastic processes that underlie changes in structural integrity and connectivity is also warranted.

6.4. CLINICAL IMPLICATIONS

The findings of this project highlight the importance of examining structural changes and cortical disconnection in the broader motor network post-stroke. Brain regions that appear intact on structural scans may actually exhibit reduced structural integrity/connectivity that may contribute to the severity of motor impairments and affect recovery post-stroke. Additionally, secondary motor tracts/brain regions have been shown to be associated with both upper/lower extremity motor function in chronic stroke.

Such insight into the more salient, global effects of stroke could enhance our understanding of the mechanisms that support brain plasticity and motor recovery. This information could help improve motor recovery prognosis, assist with identifying patients with the potential to recover, and help target therapeutic interventions based on an individual's structural brain scaffolding, thereby improving the clinical management of chronic mobility impairments in this patient population. Measures of CST and RN integrity and cortical connectivity of motor-relevant brain regions could be used to help guide clinicians in their choice of treatment interventions (e.g., unilateral or bilateral motor practice augmented by sensorimotor stimulation of appropriate brain regions). Future research examining structural integrity/connectivity among primary and secondary sensorimotor brain regions following a treatment intervention may provide insight into training-induced neuroplastic processes that could help clinicians and researchers better target therapeutic interventions to enhance motor recovery potential.

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APPENDIX A – TESTING PACKET

BOX AND BLOCK TEST

Subject ID:_____ Date:_____ Affected Side: R / L

BBT	Unaffected		Affected (Right / Left)	
	#	Comments	#	Comments
Number of Blocks				

Additional Comments:_____

Starting Position: Subject sits at table in "front-close" position. Hand being tested should be on the side of the box with base of MCP lined up with and near edge of box. Box should be placed ~ 1 inch from edge in line with table edge.

Instructions for Patient: "I want to see how quickly you can pick up one block at a time with your (right or left) hand, carry it to the other side of the box and drop it. Make sure your fingertips cross the partition [demonstrate]. Do not throw the block over the partition. Also make sure you only pick up ONE block at a time. Before you start, you will have a chance to practice for 15 seconds. Do you have any questions?"

Upon completion of practice period, continue with the following instructions:

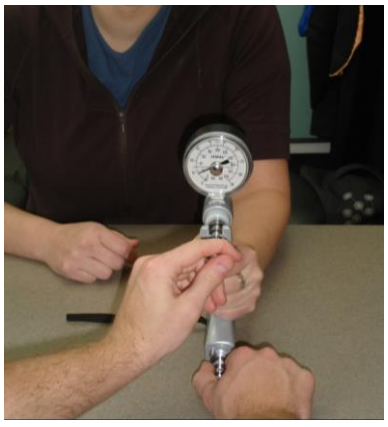
"This will be the actual test. You will have one minute to move as many blocks as you can."

Always start with unaffected hand.
Allow 15 sec practice trial for each hand.

Grip Strength

SET UP	TASK	VERBAL INSTRUCTIONS
Starting Position: • Chair Position (Front-Close). • Hand not to be tested on thigh. • Hips against back of the chair. • Upper extremity to be tested placed on table, olecranon process at front edge of table, forearm in neutral position, elbow flexed, shoulder slightly flexed and in 0° abduction. • The hand-held dynamometer is set on the second setting position. • Grip strength dynamometer placed in hand that is resting on the table. The tester or an assistant should stabilize the dynamometer for the patient from the front of the patient. • Not Filmed.	Task Description: Patient attempts to grip the dynamometer with greatest grip strength possible. The test should be conducted 3x with a 30 sec rest between trials. Timing Procedure: Not applicable. Measure: The mean of grip strength exerted (kg) on 3 trials.	Verbal Instructions: • "Squeeze the handle down as far as you can for at least 3 seconds and then let go when I say "Release." I will ask you to do this 3 times with a 30 sec rest between attempts." (repeat instructions) • "Do you have any questions?" • "Ready, set, go."

Chair Position (Front-Close): Chair is facing the table and centered. The front edge of the back legs of the chair are approximately 36-cm from the front table edge.

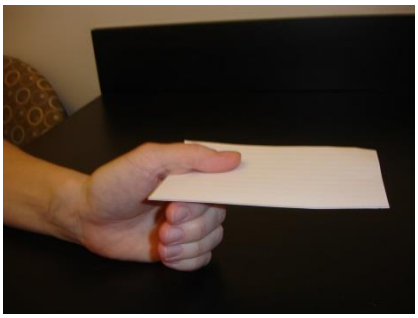


Unaffected Hand	Affected Hand
Trial 1: _____	Trial 1: _____
Trial 2: _____	Trial 2: _____
Trial 3: _____	Trial 3: _____

Evaluation and grading of motor function of the paretic hand according to modified Brunnstrom classification

Stage	Description
1	unable to move fingers voluntarily
2	able to move fingers voluntarily
3	able to close hand voluntarily; unable to open hand
4	able to grasp a card between thumb & radial side of index finger; able to extend fingers slightly
5	able to pick up & hold a glass; able to extend fingers fully
6	able to catch & throw a ball in a near-normal fashion; able to button & unbutton a shirt

Example of Stage 4 activity:



Instructions for Patient (along with demonstration of movement by tester):

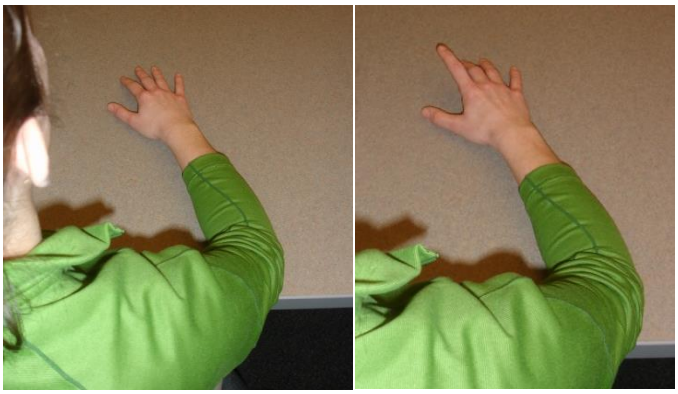
(2) "Can you move your fingers?"
(3) "Can you close your hand…and then open it?"
(4) "Can you grasp this card between your thumb and finger? Can you extend or straighten your fingers slightly?"
(5) "Can you pick up and hold this glass? Can you extend or straighten your fingers all the way?"
(6) "Can you throw/catch this ball? Can you button/unbutton this shirt?"

Scoring:
Circle the highest stage in which patient can perform movement. If unable to perform a certain movement, do not proceed to next stage.

Index Finger Tapping Test

General Information:

- Perform first with non-paretic hand, followed by paretic hand.
- The participant is allowed a 10 second practice trial for each hand prior to testing.
- Participants are to be seated comfortably at a table with their forearm resting on the table in a pronated position and slight shoulder internal rotation.
- Additionally, participants are instructed to try and keep their other fingers down when tapping, and to try and rest the heel of their hand on the table when performing the task. If needed, a research assistant can stabilize the participant's distal forearm.



Instructions for Participant:
"I want to see how quickly you can tap your index finger [demonstrate]. Before you start you will have a chance to practice. Once testing begins, you will have 10 seconds to perform as many taps as you can. Do you have any questions?"

Scoring:
Count the total number of taps in **10 seconds**.

	Unaffected Hand	**Affected Hand**
Index finger tapping	Trial 1: _____	Trial 1: _____
	Trial 2: _____	Trial 2: _____
Alternate index/middle finger tapping	Trial 1: _____	Trial 1: _____
	Trial 2: _____	Trial 2: _____
Alternate R/L index finger tapping	Trial 1: _____	
	Trial 2: _____	

Motricity Index

ARM (conducted in sitting position)	**Right Side**	**Left Side**
(1) Pinch grip (2.5 cm cube between thumb and forefinger)	_____	_____
(2) Elbow flexion (from 90°, so that hand touches shoulder)	_____	_____
(3) Shoulder abduction (move flexed elbow off of chest)	_____	_____
LEG (conducted in sitting position)		
(4) Ankle dorsiflexion (from plantar flexed position)	_____	_____
(5) Knee extension (from 90°, foot unsupported)	_____	_____
(6) Hip flexion (from 90°, move knee towards the chin)	_____	_____

**** Always assess non-paretic side first.***

<u>Test 1 (Pinch grip)</u>
***0** = No movement*
***11** = Beginnings of prehension*
***19** = Grips cube but unable to hold against gravity*
***22** = Grips cube, held against gravity, but not against weak pull*
***26** = Grips cube against pull but weaker than other side*
***33** = Normal pinch grip*

<u>Tests 2-6</u>
***0** = No movement*
***9** = Palpable contraction in muscle but no movement*
***14** = Visible movement, but not full range/not against gravity*
***19** = Full range against gravity, not against resistance*
***25** = Full movement against resistance but weaker than other side*
***33** = Normal power*

ARM SCORE: R _____ L _____

LEG SCORE: R _____ L _____

SIDE SCORE: R _____ L _____
[Arm + Leg] / 2

Stroke Impact Scale

Instructions for Participant:
"The purpose of this questionnaire is to evaluate how stroke has impacted your health and life. We want to know from **YOUR POINT OF VIEW** how stroke has affected you. We will ask you questions about impairments and disabilities caused by your stroke, as well as how stroke has affected your quality of life. Finally, we will ask you to rate how much you think you have recovered from your stroke."

These questions are about the physical problems which may have occurred as a result of your stroke.

In the past WEEK, how would you rate the strength of your…	A lot of strength	Quite a bit of strength	Some strength	A little strength	No strength at all
a. Arm that was most affected by your stroke?	5	4	3	2	1
b. Grip of your hand that was most affected by your stroke?	5	4	3	2	1
c. Leg that was most affected by your stroke?	5	4	3	2	1
d. Foot/ankle that was most affected by your stroke?	5	4	3	2	1

The following questions are about your ability to use your hand that was MOST AFFECTED by your stroke

In the past 2 weeks, how difficult was it to use your hand that was most affected by your stroke to…	Not difficult at all	A little difficult	Somewhat difficult	Very difficult	Could not do at all
a. Carry heavy objects (e.g., bag of groceries)?	5	4	3	2	1
b. Turn a doorknob?	5	4	3	2	1
c. Open a can or jar?	5	4	3	2	1
d. Tie a shoe lace?	5	4	3	2	1
e. Pick up a dime?	5	4	3	2	1

The following questions are about your ability to be mobile at home and in the community.

In the past 2 weeks, how difficult was it to…	Not difficult at all	A little difficult	Somewhat difficult	Very difficult	Could not do at all
a. Stay sitting without losing your balance?	5	4	3	2	1
b. Stay standing without losing your balance?	5	4	3	2	1
c. Walk without losing your balance?	5	4	3	2	1
d. Move from a bed to a chair?	5	4	3	2	1
e. Walk one block?	5	4	3	2	1
f. Walk fast?	5	4	3	2	1
g. Climb one flight of stairs?	5	4	3	2	1
h. Climb several flights of stairs?	5	4	3	2	1
i. Get in and out of a car?	5	4	3	2	1

No Problem

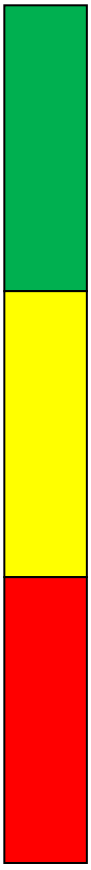
Problem

5 4 3 2 1

*Will be used to help participant understand/express rating on SIS scale if needed secondary to aphasia.

Stroke Recovery

"On a scale of 0 to 100, with 100 representing full recovery and 0 representing no recovery, how much have you recovered from your stroke?"



_____ 100 Full Recovery

_____ 90

_____ 80

_____ 70

_____ 60

_____ 50

_____ 40

_____ 30

_____ 20

_____ 10

_____ 0 No Recovery

GAITRite

Participants will perform 3 trials on the GAITRite, starting and ending ~5 feet before and after the mat to allow for acceleration and deceleration.

Instructions:

We will perform three trials and I will instruct you when to begin. **"When you walk across the mat please walk at your normal pace. You may walk across the mat."**

*Denote how many trials with brace and/or AD used.

Assistive Device type: _____

Brace: _____ L R

Trials with AD and/or Brace:_____

Trials without AD and/or Brace:_____

30 Seconds Sit to Stand Test

Directions for Evaluator:

1. Have the subject sit in a straight backed chair (seat height approximately 17"). The chair is placed against the wall to prevent it from moving during test. A stopwatch is also required.
2. Tell the participant to sit in the middle of the chair with back straight and feet on floor with arms crossed over their chest. If patient has to use an upper-extremity or both to stand please notate.
3. Instruct the patient to rise to a full stand and return back to a fully seated position after the signal "go" is given. They are to complete as many full stands as possible within a 30-second time limit.
4. The evaluator should demonstrate the test for the patient and allow a practice trial of 1 to 2 repetitions to ensure correct form. One 30-second trial is performed and recorded. The examiner needs to stand facing the side of the patient.

Directions for Participant:

1. **"Sit in the middle of the chair with back straight and feet on floor with arms crossed over your chest. You can use one or both of your hands if needed. Rise to a full stand and return to a fully seated position after I say 'go.' Complete as many full stands possible until I say 'stop.' "**

Scoring: The score is the total number of full stands executed correctly within 30 seconds. **If the patient is more than half way up at the end of 30 seconds it is counted as a full stand.**

Use of hands required: ☐ **NO** ☐ **YES** ☐ RIGHT ☐ LEFT ☐ BOTH

Number of repetitions completed in 30 seconds: _____

1. Rikli RE, Jones CJ. Senior Fitness Test Manual. Champaign, IL: Human Kinetics. 2001.
2. Jones CJ, Rikli RE, Beam W. A30-s chair stand test as a measure of lower body strength in community-residing older adults. Res Q Exerc Sport. 1999;70:113-119.

Foot Tapping Test

General Information:

- Perform first with non-paretic foot, followed by paretic foot.
- The participant is allowed a 10 second practice trial for each foot prior to testing.
- Participants are to be seated comfortably with their feet resting on the floor.
- Additionally, participants are instructed to keep their heel down when tapping. If needed, a research assistant can stabilize the participant's hip/knee to minimize compensation.

Instructions for Participant:

"I want to see how quickly you can tap your foot [demonstrate]. Before you start you will have a chance to practice. Once testing begins, you will have 10 seconds to perform as many taps as you can. Do you have any questions?"

Scoring:

Count the total number of taps in 10 seconds.

Unaffected Foot	**Affected Foot**
Trial 1: _____	Trial 1: _____
Trial 2: _____	Trial 2: _____

Alternate L/R foot

Trial 1: _____

Trial 2: _____

APPENDIX B – PILOT STUDY

INVESTIGATOR TRAINING IN DTI ANALYSES

B.1. BACKGROUND/PURPOSE

While the primary investigator has previous experience with administration of the majority of proposed outcome measures and training in MRI safety and obtaining MRI images, the investigator has no previous experience with neuroimaging analyses. With the recent development of the nii_stat software, most of the brain analyses will be automated for this proposed study. However, to improve the investigator's baseline understanding of DTI analyses and the various components involved in such analyses, a pilot study was designed with the following objectives: 1) familiarize the investigator with how to use/navigate FSL and MRIcron imaging software, 2) train the investigator on how to manually delineate lesions on T2-weighted images, how to coregister images between native and standard space, and how to obtain FA/MD values in native and standard space for a specified ROI in both normal and lesioned brains, and 3) determine the reliability of the investigator's manually-delineated lesions and FA/MD values compared to an experienced neuroimaging researcher (JR).

B.2. METHODS

The primary investigator met with JR multiple times and progressively practiced various components of DTI analyses. Reliability analyses for lesion drawing were

performed on 4 individuals with chronic stroke, while reliability analyses for FA/MD values were obtained on 3 healthy older adults and 3 individuals with chronic stroke.

B.3. RESULTS

The primary investigator was able to successfully negotiate FSL and MRIcron imaging software and improve problem-solving skills (especially with lesioned brains) during the DTI analysis process, demonstrating that the first objective of the pilot study was met. Reliability analyses between investigator and JR lesions and FA/MD values are forthcoming as JR's values are in the process of being calculated. These results will provide information concerning the second and third objectives of the pilot study. As a result of the pilot study, the investigator now has a baseline foundation of DTI analyses and a greater understanding of the various components involved in such analyses (which applies to the nii_stat program as well). Familiarity with FSL and MRIcron will help with subsequent analyses if needed with the proposed study.

B.4. CONCLUSIONS

The pilot study has allowed the primary investigator to develop a baseline knowledge and skill set related to DTI analyses necessary to complete the proposed study. Compared to normal brains, analyses on lesioned brains were more complex leading to increased problem-solving skills. Collaboration and feedback from JR throughout the pilot study process helped to clarify the specific aims of the proposed study.

APPENDIX C – DETAILED SAMPLE CHARACTERISTICS

Subject	Age (yr)	Gender	Time Since Stroke (mo)	Lesion Volume (mm^3)	BBT_{Aff}	$Grip_{Aff}$ (kg)	Arm MI_{Aff}	Leg MI_{Aff}	Gait speed (m/s)
1	57	M	67	156492	40	52.33	91	99	1.24
4	55	M	47	141372	26	27.67	83	83	0.67
5	57	M	129	117207	5	20.33	70	69	0.34
6	65	M	113	159516	36	44.00	83	75	0.90
8	77	M	284	290223	0	0.00	0	59	0.62
9	60	F	185	80028	2	14.33	50	42	0.21
10	80	F	83	108	50	12.00	99	99	1.21
11	60	M	42	7290	9	8.00	33	85	1.37
14	56	F	80	177471	45	21.67	99	99	0.95
15	62	M	65	69660	42	28.67	92	91	0.90
17	65	M	18	88155	56	36.33	91	99	1.07
19	70	F	93	2835	22	20.00	85	92	1.01
20	43	M	27	151578	0	6.67	23	28	0.85
21	66	F	28	1863	46	25.33	99	75	0.56
22	66	M	53	378	24	11.33	55	42	0.65
23	37	F	86	80973	33	25.00	99	99	0.71
24	42	F	20	31239	18	23.00	61	53	1.33
25	62	M	54	93852	0	0.00	--	28	--
26	52	M	36	220509	46	41.67	99	99	1.46
27	76	M	10	76572	42	36.67	99	99	1.43
28	70	M	173	338310	0	0.00	0	42	--
29	62	F	20	1809	29	18.33	70	64	0.55
31	55	M	27	8208	0	15.33	33	47	0.76
32	60	M	11	242028	33	15.33	76	75	1.30
34	64	F	22	54	59	28.33	99	99	1.12
35	78	M	68	648	0	8.67	50	63	0.57
36	62	M	60	71361	44	42.00	99	50	--
37	61	M	24	2511	51	33.67	99	91	0.98
39	50	M	51	71982	0	7.67	39	59	0.77

40	73	M	22	93312	54	52.00	99	99	1.38
41	67	M	57	55836	8	7.33	--	75	0.94
42	31	F	27	14877	--	--	--	69	0.52
43	50	M	13	177174	44	21.67	91	75	1.02
44	48	M	20	30078	63	56.67	99	99	1.31
45	53	F	103	164619	32	25.67	70	--	1.09
46	69	M	202	235683	48	48.33	99	99	1.15
47	63	F	27	--	46	17.00	70	57	0.77
48	69	F	12	47385	58	20.00	99	99	1.02
49	63	M	93	207333	45	44.67	91	83	1.11
50	39	F	87	62532	9	3.00	61	61	0.53
52	52	F	13	783	64	26.67	99	99	1.06
53	72	M	12	28242	57	38.00	99	99	1.13
54	49	F	101	69903	0	0.00	28	83	0.91
Avg	59.7	16F/27M	64.4		30.6	23.46	74.5	76.2	0.94
SD	11.2		58.8		21.7	15.72	29.2	22.1	0.31

Abbreviations: yr = years; mo = months; BBT_{Aff} = Box and Block Test (affected extremity); $Grip_{Aff}$ = grip strength (affected extremity); Arm MI_{Aff} = Motricity Index score for the affected upper extremity; Leg MI_{Aff} = Motricity Index score for the affected lower extremity; Avg = average; SD = standard deviation.