Corticoreticular and Reticulospinal Control of Reaching after Stroke: 
Functional, Physiological, and Anatomical Studies

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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2010

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Abstract

The purpose of this study was to develop a new model of focal ischemia with reperfusion in the non-human primate (*M fascicularis*) to explore the contributions of both the corticospinal and reticulospinal systems in recovery of reaching following cortical ischemic injury. First, experiments were conducted to improve understanding of electrophysiological techniques used for studying descending motor systems, and ipsilateral corticospinal control of upper limb movements in the intact animal. Single-pulse stimulation and repetitive stimulation techniques applied at the same pontomedullary reticular formation (PMRF) stimulation site in the same monkey were compared. The type of response (facilitation vs. suppression) was compared between the stimulation methods. The results indicated that for general comparisons of motor outputs, both methods produced comparable responses. This study helped us recognize the possible limitations for our electrophysiological methods for the new cortical injury model. Then, the functional organization of the corticospinal tracts (ipsilateral vs. contralateral) was studied to explore ipsilateral corticospinal outputs to muscles of both upper limbs from cortical stimulation applied at sites in the left cerebral cortex spread across the primary motor cortex, dorsal pre-motor cortex and supplementary motor cortex. Ipsilateral effects were evoked from all
three cortical motor areas. The percentage of ipsilateral effects was higher than the 10 to 20% expected based on anatomy, with the greatest percentage from pre-motor areas. This study provided valuable insights into ipsilateral corticospinal contributions to upper limb motor control, especially proximal limb muscles.

Next, a vasoconstrictive peptide, Endothelin-1 (ET-1) was used to create a focal ischemic injury in the shoulder/elbow representation of left primary motor cortex (M1). Repetitive microstimulation was used to physiologically map motor outputs from right and left cortical motor areas, and upper limb motor outputs from the PMRF. EMG responses were recorded from shoulder girdle, limb extensor and limb flexor muscles of both upper limbs. Functional deficits were assessed using a behavioral reaching task conducted at set time points before and after the ET-1 induced lesion. MRI scans were used for confirmation of lesion location and quantification of lesion volume. In a subject with a mild lesion, reaching was mildly impaired. Changes were evident in the shoulder/elbow representations of both the affected and contralesional M1. No substantial changes were noted in the pattern of PMRF output. In a subject with a severe lesion, reaching was markedly impaired immediately after the lesion. With intensive rehabilitation, gross reaching recovered in a few weeks, and reaching times were slow but comparable to pre-injury levels by 16 weeks post-injury. Surprisingly, the shoulder/elbow representation in the affected M1 remained completely absent after recovery, and there was little change in the
contralesional M1. The novel result was the greater right arm representation from left PMRF sites in this subject. This suggests that there may be increased reliance on PMRF motor outputs associated with upper limb motor recovery after a severe ischemic cortical injury. This opens a new line of investigation to complement cortical plasticity research to understand reticulospinal contributions to functional recovery of reaching after stroke.
Dedication

This document is dedicated to all the stroke survivors who continue to live life with the daily struggles of their physical impairments.
Acknowledgments

First, I would like to thank my advisor, Dr. John Buford for his patience and commitment to my success throughout this project. His expertise and guidance were invaluable. Next, I would like to thank Dr. Lyn Jakeman for dedicating her time and expertise to the development of the anatomical and histological procedures used in the stroke project. Thank you to Drs. Deborah Larsen, Anne Kloos and John Borstad for providing constructive feedback, and for being terrific mentors of the scientific process. I would like to thank Stephanie Moran for her excellent technical support throughout the project, and Lynnette Montgomery for her work on the anatomy procedures and the anatomical reconstructions for the stroke project. I would also like to thank Drs. Kimerly Powell and Anna Bratasz for their time and imaging expertise. Finally, I want to thank my husband, Ron. I couldn’t have done it without your love and support!

This work was partially funded by PODS I and PODS II scholarship awards from the Foundation for Physical Therapy, American Physical Therapy Association, NIH R01 NS037822, and funding from the College of Medicine and the School of Allied Medical Professions at The Ohio State University. Chapter 3 is reprinted with permission from Experimental Brain Research.
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Chapter 1: Introduction

One of the primary functions of the central nervous system is the control of voluntary movement. This is a complex function, requiring coordination between multiple motor systems. Two of these motor systems are the corticospinal and reticulospinal system. The corticospinal system (CST) is the primary descending motor system involved in voluntary motor control, especially fine skilled movements. The predominant role the CST plays in skilled movement has been widely studied in normal and injured states, and its contributions to motor control have been well established.

The reticulospinal system (RST) has been shown to be important in postural control and locomotion\textsuperscript{1-4} and integrated movements of body and limbs.\textsuperscript{5-7} Stimulation of reticulospinal neurons in awake cats produced flexion of the ipsilateral limb, extension of the contralateral limb, and head rotation toward the flexed limb.\textsuperscript{5, 6} This reciprocal pattern was confirmed in the monkey.\textsuperscript{8-10} The RST has also been shown to be involved in control of reaching in non-human primates.\textsuperscript{8, 9}

The RST arises from neurons in the pontomedullary reticular formation (PMRF).\textsuperscript{11, 12} Anatomy and neurophysiology studies\textsuperscript{13-17} have demonstrated that the RST receives input from multiple regions, including ipsilateral and
contralateral cortical motor areas. Diffuse patterns of corticoreticular projections originating from primary motor (M1) and premotor axial and proximal limb movement representation onto both sides of PMRF have been found in the cat.\textsuperscript{13-15, 17, 18} A subpopulation of corticospinal tract neurons from primary motor and premotor axial and proximal limb representation also send collateral branches to the reticular formation in the cat.\textsuperscript{14, 17} Keizer & Kuypers\textsuperscript{15} confirm these findings in the macaque monkey.

The RST has widespread monosynaptic and polysynaptic projections to both sides of the spinal cord, and these extend the entire length of the cord.\textsuperscript{11, 16, 19-21} Anatomical studies indicate that the terminal distributions of reticulospinal axons within the cervical and lumbar enlargements are primarily on interneurons within the intermediate zone and medial regions of the ventral horn of the spinal cord.\textsuperscript{11, 16, 19-22} This termination pattern illustrates that RST projections have an influence over motoneurons that control axial and proximal limb muscles. Although no absolute somatotopic organization has been identified in the PMRF, Kably and Drew\textsuperscript{13} found that the more dorsal regions of PMRF in the cat are the strongest sources of projections to the neck, back, and forelimb muscles. Work in the macaque has shown that PMRF regions just ventral to abducens produce substantial responses for proximal and distal flexor and extensor muscles of both upper limbs.\textsuperscript{8, 9} Both single cell recording and microstimulation studies in monkeys within the PMRF have shown both facilitation and suppression of EMG activity for proximal and distal arm muscles.\textsuperscript{8, 9, 23} Baker’s group has also shown
reticulospinal pathways may influence more distal motoneurons projecting to intrinsic hand muscles.\textsuperscript{24}

Anatomical evidence demonstrates an overlap of CST and RST projections to interneurons and motoneurons in lamina VII of axial and limb girdle muscles,\textsuperscript{11, 21, 25-29} suggesting both motor systems share influence on axial and proximal limb motor control. This was demonstrated by bilateral pyramidalotomy studies in monkeys.\textsuperscript{30, 31} Initially, monkeys were unable to use their extremities, especially their hands independently of whole body movements such as climbing cage bars. After six weeks of recovery, they regained independent limb movements, including gross grasp with the hands. However, fine dexterous hand function never returned. After recovery from pyramidalotomy, interruption of the reticulospinal pathway produced recurrence of axial and proximal limb movement deficits, while the distal hand remained less impaired. This evidence suggests recovery after pyramid lesions could be due to the activity of RST pathways via CST collaterals primarily involved with control of axial and proximal limb muscles.

After brain injury such as stroke, individuals have difficulty performing basic activities of daily living requiring whole body and whole limb motor control such as rolling in bed and putting the affected arm in the sleeve of a shirt. Such motor behaviors preferentially utilizing proximal musculature would presumably require substantial involvement of the RST. Yet, it is unclear what role the RST plays in recovery of arm function following cortical motor stroke. Anatomical findings suggest that corticoreticular and reticulospinal connections may be a feasible
alternative for postural and proximal limb motor control after cortical stroke. If this cortico-reticulo-spinal connection contributes to recovery of functional reaching behaviors, we would expect to observe reorganization of upper limb motor output patterns from PMRF following recovery.

The experiments presented in this dissertation were conducted in an attempt to improve the understanding of electrophysiological techniques for studying descending motor systems, ipsilateral corticospinal control of upper limb movements, and the role of the reticulospinal system in recovery of upper limb function following focal ischemic cortical motor injury in the non-human primate.

The study in chapter 3 has recently been published. This study compared single pulse (stimulus-triggered averaging) and repetitive stimulation (stimulus trains) electrophysiological methods for studying motor outputs of descending systems. The results of this study indicate that differences revealed for stimulus-triggered averaging and stimulus trains for measuring motor outputs of the reticulospinal system should be handled carefully because stimulus trains may underestimate the amount of suppression produced by the PMRF. However, the large agreement between the two methods supports the use of stimulus trains for general description of motor outputs. Stimulus trains produce overt muscle contractions and results can be obtained quickly during data collection, making it ideal for creating gross motor maps of movement representation areas for studying changes after injury. In the second study presented in chapter 4, ipsilateral and contralateral corticospinal upper limb motor outputs were evoked
for Stimulus-triggered averaging in the normal macaque monkey. The results
demonstrate that the motor cortex can recruit muscles of the ipsilateral limb fairly
directly. This could have important implications for understanding mechanisms
of recovery from cortical brain injury. The final study presented in chapter 5 is
the development of a focal ischemic brain injury model in the non-human
primate. Four individual experiments are described in detail providing evidence
for a new approach to studying the reticulospinal contributions to recovery of arm
function after cortical stroke. Chapter 6 is a brief communication written as a
ready for publication manuscript summarizing corticospinal and reticulospinal
contributions to recovery of arm function in one subject after two weeks of
spontaneous recovery and a subject who received intensive rehabilitation
following a focal ischemic brain injury. The data presented in this chapter were
from the two subjects from the preceding chapter where both the corticospinal
and reticulospinal systems were explored following cortical infarct.
Chapter 2: Background and Significance

Stroke is a leading cause of adult disability in the United States.\textsuperscript{32, 33} During the first hours to weeks after cortical injury a cascade of events occurs leading to structural changes within the brain, subsequently leading to loss of voluntary motor control. Early stages of recovery are often attributable to resolution of brain edema and reversal of diaschisis. Recovery after this initial period is likely due to adaptive neural plasticity.

2.1 Adaptive neural plasticity

Neurophysiology studies in human and animal models demonstrate the possibility of widespread structural and functional reorganization within the central nervous system in response to motor skill acquisition, training\textsuperscript{34-40} and brain injury such as stroke.\textsuperscript{41-43} This reorganization or adaptive plasticity can result from a change in function within a particular neural substrate in the central nervous system through alterations in synaptic strength, neuronal excitability, neurogenesis, or neuron death.\textsuperscript{44-46} The mechanisms for long-term functional recovery, however, remain unclear.

The finding that reorganization can occur in the intact brain in association with motor learning indicates that this is a normal part of the brain's function.
Presumably, these normal mechanisms would come into play as part of the process for recovery from injury, as well. In a variety of motor skill acquisition studies in normal subjects, the primary motor cortex (M1) representation of the trained body part has been shown to be modifiable. In rats trained to reach and grab small food pellets, the M1 representation of wrist and digit movements was expanded at the expense of more proximal shoulder and elbow representations. In monkeys trained to retrieve small objects, microstimulation mapping revealed expansion of the digit representation, whereas in monkeys trained to perform forearm pronation/supination movements the forearm movement representations were expanded. Nudo and colleagues showed that these changes were progressive during training and reversed after training was stopped.

Evidence obtained in human subjects also indicates that M1 reorganization can occur both within a single training session as well as over weeks of training. When subjects practiced a novel repetitive finger sequencing task, the amplitude of EMG responses and extent of the cortical representation for finger movements evoked by transcranial magnetic stimulation (TMS) of contralateral M1 increased as the subject’s performance increased over a single day of training. With functional magnetic resonance imaging (fMRI), Karni and colleagues showed that activation of the M1 digit representation was greater during performance of a practiced motor sequence in subjects that practiced the finger movement sequence over several weeks than during
performance of a comparable, but unpracticed sequence. The studies in normal subjects demonstrate the capacity for functional reorganization within the central nervous system that may occur in neural substrates involved in the specific behavior being trained, and further demonstrate that neural plasticity was enhanced when these movements were purposeful and related to the behavior being trained.

2.1.1 Neural substrates for motor recovery

The emphasis of stroke research has primarily been on cortical reorganization. Traditionally, loss of isolated motor control is primarily associated with loss of corticospinal projections, potentially restricting available muscle recruitment patterns. Upper limb functional recovery is largely attributed to reorganization of motor outputs from spared ipsilesional cortical motor structures, including spared M1 territory adjacent to the lesion, pre-motor area (PMA) and supplementary motor area (SMA). Nudo’s group has shown alterations in movement representation area in the peri-lesional M1 tissue in response to ischemic lesion and after intensive rehabilitation in squirrel monkeys. Without training, subjects demonstrate not only loss of hand/digit movement representation within the lesioned M1 region, but also further loss of cortical representation in adjacent, spared tissue. Expansion of spared M1 representation for the trained body part into the adjacent, spared cortical regions was enhanced with training.
While a great deal is known about the physiology and important functional role of the contralateral components of the CST, much less is known about the ipsilateral CST component. The anatomical findings demonstrate that ipsilateral control of movements is possible. Physiological evidence showing a functional ipsilateral connection, however, is sparse. A better understanding of the ipsilateral CST is important for identifying its role in recovery after unilateral injury such as stroke when the only corticospinal input to the hemiplegic side may be the ipsilateral CST projections. Ipsilaterally distributed terminal branches of CST neurons are a potential source from which axonal sprouting could enhance recovery following contralateral CST injury.\textsuperscript{28, 56} In humans, motor cortex damage early during development can often be alleviated by ipsilateral CST projections.\textsuperscript{57} Neuroimaging studies in humans have demonstrated changes in activation patterns of homologous regions of the contralesional hemisphere in response to recovery.\textsuperscript{53, 58} TMS and fMRI evidence in adults indicate that good recovery of distal hand function is associated with recovery of near-normal activation patterns within the lesioned cortex, while incomplete to poor recovery is associated with increased activation in both the ipsilesional and contralesional hemispheres.\textsuperscript{59-62}

2.1.2 Mechanisms underlying upper extremity recovery

The corticospinal system (CST) is often the first system affected by stroke. Since it has been established that motor control requires coordination of multiple motor systems, (ie. rubrospinal, vestibulospinal, reticulospinal) it is likely that
these systems may play a role in motor recovery. However, little is known about the role these other motor systems play in recovery. Up to this point the focus of ischemic stroke research has been on cortical changes and how these changes may be related to upper limb recovery.\textsuperscript{41-43, 63, 64} Both animal and human studies have primarily focused on changes in activation within the cortical motor regions for distal hand function recovery. Upper limb functional recovery, proximal and distal, has been largely attributed to reorganization of motor outputs from spared ipsilesional cortical motor structures.\textsuperscript{42, 43, 55} Besides the CST, other descending pathways conveying motor outputs also undergo reorganization. Small et al\textsuperscript{65} showed correlation of changes in activation in the cerebellar hemisphere opposite the lesioned M1 with recovery. Reorganization in rubrospinal motor outputs to forearm muscles in monkeys also occurs following unilateral pyramidal tract lesions.\textsuperscript{34} The other medial descending motor systems (vestibulospinal, rubrospinal, reticulospinal) work in coordination with the CST to produce upper limb motor outputs. However, to date, no study has explored whether the reticulospinal system undergoes reorganization or contributes to recovery following unilateral cortical ischemic M1 lesion.

The reticulospinal system (RST) influences primarily axial and proximal limb muscles and is well known for its role in postural control and locomotion.\textsuperscript{1, 3, 4, 66} Animal studies involving recording and stimulation methods in the PMRF have also shown that the RST can facilitate or suppress muscles used for control of reaching.\textsuperscript{4, 8, 9, 67}
A unique relationship exists between the cortical motor areas and the PMRF. All three motor areas send projections bilaterally to the PMRF. Hence, based on the anatomical evidence, the PMRF may receive information regarding control of upper extremity function from both cortical hemispheres in the intact and injured state. Given the connectivity of the RST with both hemispheres, and its capacity to influence upper extremity motor control, the RST may be an important pathway for recovery of upper extremity function after stroke.

If the motor cortex is severely damaged from a stroke, motor control of the extremities is still possible but may be constrained to whole limb muscle patterns. Biomechanical analyses in humans demonstrate that movements are constrained to a predominance of specific whole limb flexion or extension muscle patterns (synergies) after stroke. Reaching movements up and across the body, requiring coordination of elbow extensors with shoulder flexors and horizontal abductors or adductors are most notably limited. Comparisons between impaired and non-impaired limb reveal abnormal muscle co-activation patterns, with difficulty moving in directions requiring muscles to co-activate in combinations different from the natural synergies. Animal studies suggest these synergies are consistent with the normal motor output for the PMRF. Drew et al demonstrated that microstimulation of the PMRF in awake cats produced flexion of the ipsilateral limb, extension of the contralateral limb, and head rotation toward the flexed limb. Findings from our lab confirm that these patterns
reflect normal PMRF motor output in the monkey. These findings have long supported the suggestion that increased reliance on the reticulospinal system as an alternative motor pathway may be the reason these abnormal muscle recruitment patterns exist after cortical stroke. An experiment designed to investigate upper limb motor output effects evoked from the PMRF in addition to motor output effects evoked from both cortical hemispheres after cortical stroke would provide further evidence for the roles the ipsilateral corticospinal system and reticulospinal system play in recovery of arm function after stroke.

2.2 Investigative methods for exploring functional reorganization

Existing neuroimaging and TMS techniques are both effective investigative methods for studying reorganization of cortically evoked responses in humans. However, neither technique is capable of examining subcortical structures, such as the PMRF, that may accompany these cortical changes during recovery from stroke. Microstimulation techniques in animal models are essential for investigation of neural changes in brainstem structures such as the PMRF during recovery from stroke. Microstimulation techniques have made it possible to characterize the function of specific motor areas by deriving high resolution functional maps of motor output effects evoked from cortical and subcortical sites in both intact and lesioned areas. Either repetitive stimulation (stimulus trains) delivered at high frequency (~300 Hz) or single pulses (stimulus-triggered averaging) delivered at much lower frequencies (10-20
Hz) can evoke visible movements and/or recordable EMG activity. These techniques have made it possible to investigate brainstem and spinal cord structures previously inaccessible to other methods.

Stimulation for stimulus-triggered averaging (StimulusTA) is delivered while awake behaving animals perform a motor task, and the triggered averaging is used to extract the effects of these pulses from ongoing EMG activity. The movement patterns evoked from StimulusTA in the PMRF are consistent with recruitment of muscles reflected in single neuron extracellular recording studies. Typically, recording data from a single site for StimulusTA takes 5 – 10 minutes, and in the brainstem, several depths must be explored for each electrode track. In a chronic monkey preparation, one or two electrode tracks per day is the limit to what the subject can tolerate, and 50 – 60 penetrations are required to explore the full extent of the PMRF on a 1-mm grid spacing. At least two months are required for a thorough map to be constructed. Even if a very limited subsample of sites was explored, for example 12 tracks per side of the brainstem, at least two weeks would be required for an adequate StimulusTA-based map. Hence, using StimulusTA in a study designed to capture detailed changes in motor output effects evoked from cortical and subcortical sites at set time points during recovery from stroke would be impracticable.

Stimulus trains can evoke detectable movement or EMG activity in anesthetized animals with stimulus intensities comparable to those required in awake subjects. The ability to anesthetize the subject means a much longer
recording session can be tolerated. Because stimulus trains produce visible muscle contractions, results can be obtained quickly during data collection. Hence, the stimulus train method is ideal for creating gross motor maps of movement representations for studying changes after injury, allowing detailed electrophysiological maps in animal models in single recording sessions. While most of the effects from microstimulation result from excitation of neurons, axons or afferent fibers in close proximity to the microelectrode, and other neurons at greater distances may also be excited via trans-synaptic excitation produced from temporal summation. The physiological difference between StimulusTA and stimulus trains is thought to be due to this temporal summation. Stimulus trains are better able to reveal polysynaptic excitation, whereas StimulusTA results tend to reflect mono and disynaptic connections to motoneurons. However, in our recently published comparison of StimulusTA and stimulus train methods we revealed that for PMRF stimulation, both stimulation methods evoked similar response patterns in muscles tested. These results support the use of stimulus trains for a general description of motor output effects (i.e. flexors vs. extensors, ipsilateral vs. contralateral muscles, etc).

2.3 Animal models of focal ischemia

Over the past several years, numerous animal models of stroke have been developed to investigate the consequences of ischemia on brain structure and function. Animal models of ischemic stroke provide invaluable contributions
to our understanding of the neural substrates associated with recovery of function. Given the high incidence of Middle Cerebral Artery (MCA) stroke observed clinically, MCA occlusion (MCAO) is a widely used method for inducing ischemic stroke in rodent models. MCAO methods involve either permanent or temporary blockage of blood flow to the cortex and striatum. Methods include permanent devascularization, artificially generated thrombotic occlusions that can be reversed through natural processes or medical interventions, and transient occlusion with duration sufficient to produce varying levels of ischemia.

The most widely used method for MCAO is the monofilament technique introduced by Koizumi et al. An intraluminal suture is inserted through the External Carotid Artery and advanced until lodged in the junction between the Anterior and Middle Cerebral Arteries. The suture remains in place for a predetermined amount of time, typically 1-2 hours for transient occlusion or permanently. Embolization methods can also be used for inducing MCA occlusion. They involve the injection of microspheres or thrombolytic clots into the Carotid Artery that then lodge in the MCA creating an infarction. These techniques create complex effects spanning the sensorimotor cortex as well as subcortical structures.

Permanent devascularization of the cortical surface either via electrocoagulation of surface blood vessels or pial stripping are also used to create ischemic cortical lesions. Devascularization methods have the
advantage over MCA occlusion methods of producing more focal lesions, but these methods pose some risk of producing unintended trauma during their application and can only be used on surface vessels. Macaque monkeys have a more gyrencephalic cerebral cortex than the squirrel monkey, making the cerebral blood vessels incompletely accessible. As a result, electrocoagulation is not a viable method for creating a controlled focal ischemic injury in this species. As discussed in detail later, the macaque monkey is a more realistic model for investigating the relationship between corticospinal and reticulospinal contributions to recovery from reaching and for this reason, a different method was required.

An alternative method to physically blocking the cerebral vessel is to induce vasoconstriction of the vessel through the stereotaxically guided intracortical injection of endothelin-1 (ET-1), a potent, long-acting vasoconstrictive peptide. ET-1 has been used previously to transiently occlude the MCA in rodent models and the marmoset. This method is less invasive and produces a pattern of ischemic damage similar to traditional MCA occlusion rodent models. Exogenous ET-1 has been shown to be capable of reducing cerebral blood flow to ischemic levels whether applied directly to the MCA surface or via microinjections directly into the brain tissue. Rodent studies have demonstrated that intracortical microinjections of ET-1 can produce focal dose dependent cortical ischemic lesions, and that placement and size can be accurately controlled. Biernaske showed microinjections of ET-
1 reduced cerebral blood flow to 30 to 50% of normal. The duration of decreased cerebral blood flow ranged from 15 to about 90 minutes, with more sustained duration of reduction as the concentration of ET-1 was increased. Others have reported reduction of cerebral blood flow up to 96% of normal with the time taken for blood flow to return to 50% of the original value to take up to four hours after ET-1 injection. Thus, this technique offers the ability to direct focal ischemia within specific brain circuits. By employing focal lesions within the brain, ET-1 enables the investigation of changes in cortical and subcortical representations in adjacent undamaged tissues.

2.4 Methods for measuring brain infarct volume

Since studies have shown that the size of a pathological lesion is an important indicator of stroke outcome, methods have been developed to objectively quantify lesion volumes in experimental and clinical models. There are manual and automated methods for infarct volumetric measurements in MR images found in the literature. The two manual methods commonly used are stereological point counting and manually tracing the region of interest (ROI). Stereological point counting for estimating volume is based on the cavelieri principle. With this method, a set of parallel and equidistant MR images are randomly selected and the region of interest (ROI) estimated on each image by superimposing a grid of points and counting the number of points that fall within the ROI. For manual tracing, a cursor is used to trace the ROI throughout
a defined number of MR sections. The area, determined by pixel counting within
the traced ROI on each section, is summed and then multiplied by the distance
between consecutive sections traced to estimate the volume. Manual tracing
methods are the most common for estimating brain volumes on MR images.²⁹,²⁰ Both point counting and ROI tracing have been shown to be easy to perform and
accurate methods.

2.5 Rationale for macaque stroke model and behavioral reach assessment

There are many differences in motor behaviors between animals used in
previous stroke models. Rats and cats perform gross reaching from a tripod
stance and would not be representative of human reaching behaviors. The
macaque was selected instead of the squirrel monkey for the macaque's larger,
more gyrencephalic cerebral cortex, and the clearer somatotopic distinctions
between proximal and distal limb movement representation, representative of the
human brain. Further, the macaque and human both have a substantial portion
of the corticospinal tract that makes direct, monosynaptic connections from
pyramidal tract neurons in the cortical motor areas directly to motoneurons in the
spinal cord;¹⁰³-¹⁰⁵ these are called corticomotoneuronal cells. For the squirrel
monkey, in contrast corticomotoneuronal cells appear to be non-existent. All
cortical influence over the motor neurons in the squirrel monkey appears to work
through at least one interneuronal link.¹⁰⁵,¹⁰⁶ Hence, the macaque monkey
makes a superior model for the study of stroke that would be translatable to the human.

Behavioral tasks chosen to evaluate motor performance should measure the movement deficits characteristic of the target population. After stroke, reaching with the impaired limb becomes slower, less precise and more segmented at the shoulder and elbow.\textsuperscript{107, 108} The recovery process in stroke patients is characterized by the emergence of stereotypic whole limb flexor and extensor movement patterns\textsuperscript{49, 51} which involve a tight coupling of motion at the shoulder and elbow. Individuals with moderate to severe chronic stroke generate abnormal co-activation of elbow flexors during shoulder flexion with shoulder horizontal abduction or elbow extensors with shoulder horizontal adduction movements.\textsuperscript{50, 52, 68} Therefore, the target locations used to measure reach performance in chapter 5 were chosen because they required coordination of elbow extension concurrently with shoulder flexion and either shoulder abduction or adduction.

Movement analysis can identify changes in the reach behavior following stroke. Kinematic measures of movement time, limb trajectory and inter-joint coordination can be recorded during performance of a motor task and used to measure the subject’s motor performance quantitatively. These kinematic measures have been shown to be strongly correlated to functional measures of upper extremity function, such as the Fugl-Meyer upper extremity score, in individuals after stroke.\textsuperscript{109} Human studies examining reach performance have
demonstrated that whether the paretic arm was supported or unsupported during reach performance, decreased velocity and increased shoulder-elbow segmentation was evident.\(^{110-114}\) It is well established that a speed-accuracy trade off exists during reaching in healthy and stroke subjects. Faster movement times were associated with better shoulder/elbow movement coordination.\(^{107}\) Slower movement times for the affected arm in stroke subjects have been associated with greater corrective movements (abnormal trajectories).\(^{114}\) As an individual's reaching recovery progressed, movement times also improved. Hence, movement time appears to be the one variable most likely to capture changes in reaching behavior during stroke recovery.

2.6 Reorganization of cortex and brainstem after stroke

Theories on recovery suggest lost cortical functions are assumed by undamaged cortical tissue adjacent to the lesioned zone.\(^{55}\) Others have suggested that motor areas in the contralesional cortex or brainstem structures may play a role in recovery.\(^{57, 115}\) Numerous neuroimaging studies have suggested that reorganization occurs in remote cortical areas including homologous motor regions in the contralesional cortex after M1 strokes in humans.\(^{59, 62, 116}\) Functional MRI evidence indicates that the best recovery of function is associated with recovery of near-normal activation patterns within the lesioned cortex, while moderate to poor recovery is associated with increased activation in both the ipsilesional and the contralesional cortex.\(^{61}\) What functional MRI is not
capable of detecting is whether changes occur within the upper limb motor outputs of the PMRF that may accompany these cortical changes during recovery. Our previous work in the intact monkey has demonstrated that the typical output of the PMRF to the upper limbs produces a bilateral reciprocal pattern, with ipsilateral flexor facilitation, contralateral extensor facilitation, and suppression of the antagonists. These movement patterns match the classical whole limb synergy patterns described by Brunnstrom.

It is a common approach for experiments in motor control to study one system at a time. Previous neurophysiological studies on recovery after damage to M1 have mainly focused on reorganization within the intact cortical hemisphere after recovery. Recovery of the corticospinal system is vital for recovery of skilled hand function. Yet, we know that gross motor skills, such as reaching, require the interaction of the lateral and ventromedial systems to coordinate motor control. Therefore, deriving high resolution functional maps of the topographic motor representations of the arm representation of M1 in the ipsilesional and contralesional cortex along with upper limb motor outputs for the PMRF pre- and post-infarct would allow us to more effectively characterize changes in motor output patterns associated with recovery of functional reaching after stroke.
Chapter 3: Measuring the Motor Output of the Pontomedullary Reticular Formation in the Monkey: Do Stimulus-Triggered Averaging and Stimulus Trains Produce Comparable Results in the Upper Limbs?

3.1 Introduction

The pontomedullary reticular formation (PMRF) is the primary source of the reticulospinal system, a major motor system. Reticulospinal neurons have widespread monosynaptic and polysynaptic axonal projections to multiple motor pools on both sides of the spinal cord, with single axons often affecting cervical, thoracic, and lumbar circuits. Reticulospinal neurons terminate primarily in lamina VII and VIII, but also reach motoneurons in lamina IX. Reticulospinal axons usually descend ipsilaterally, but affect contralateral motoneurons through axon collaterals that decussate or via commissural interneurons. Segmental interneurons and motoneurons for axial and proximal limb muscles appear to be the main target of reticulospinal neurons. Reticulospinal outputs may even affect distal motoneurons controlling the hand.

Microstimulation studies in the cat using stimulus trains at rest and during locomotion have shown that the reticulospinal system recruits axial and proximal limb muscles. Previous work in the monkey using stimulus-triggered
averaging (StimulusTA) has also shown the capacity of the reticulospinal system to recruit upper limb muscles during voluntary reaching.\textsuperscript{8, 9} PMRF stimulation evokes a prevalence of ipsilateral limb flexor and contralateral limb extensor muscle excitation, often coupled with reciprocal suppression effects in the antagonist muscles.\textsuperscript{5, 6, 8, 9, 119}

Motor outputs revealed by StimulusTA are thought to primarily reveal monosynaptic and disynaptic connections onto motoneurons.\textsuperscript{75} The results of spike-triggered averaging (SpikeTA) are also thought to be heavily biased towards monosynaptic and disynaptic effects.\textsuperscript{78, 120} A high level of agreement for SpikeTA effects and StimulusTA effects was found in the motor cortex.\textsuperscript{75} This was expected because adjacent cells in motor cortex have similar output fields.\textsuperscript{121} In comparing SpikeTA and StimulusTA effects in arm muscles based on PMRF recordings in the monkey, we found strong agreement in results from these two methods at sites from which SpikeTA effects were found.\textsuperscript{23} This supports the view that StimulusTA reveals the output of reticulospinal neurons through relatively direct pathways, and suggests that local groups of PMRF cells may have similar outputs.

The purpose of this study was to compare the results from StimulusTA and stimulus trains applied at the same PMRF stimulus sites in the monkey to determine whether these two stimulation methods produce similar responses in arm and shoulder muscles. Since StimulusTA effects from the monkey\textsuperscript{9} were similar to the movements evoked with stimulus trains in the cat,\textsuperscript{5, 6, 119} we
hypothesized that effects evoked by stimulus trains and StimulusTA should usually be similar in the monkey. Nevertheless, stimulus trains could engage polysynaptic pathways;\textsuperscript{77} thus, responses evoked with stimulus trains might show characteristics different from those observed with StimulusTA. Preliminary results of these studies have been reported in abstracts.\textsuperscript{122, 123}

3.2 Methods

3.2.1 Subjects and task

Subjects were two male monkeys (\textit{M fascicularis}) trained to perform an instructed-delay, bilateral reaching task controlled by Tempo software (Reflective Computing, Olympia, WA, USA). The details of the task have been described in a previous report.\textsuperscript{9} The task involved a control period, including an instructed-delay interval, when the monkey waited with both hands on start switches at waist level. During the subsequent movement period, he responded to the instruction by reaching with the left or right arm to a target presented on a touch-screen computer monitor. After the trial, he was free to use either hand to retrieve a food reward, and then he started the next trial.

3.2.2 Animal care

Subject care complied with the NIH Guide for the Care and Use of Laboratory Animals, and the institutionally approved animal care protocol for our laboratory. Surgeries were performed under veterinary supervision in aseptic
conditions. As previously reported (Davidson and Buford 2004; 2006), animals were pre-treated with antibiotics and were pre-anesthetized with Ketamine HCL (13 mg/kg im) followed by Isoflurane gas (1-2%). Antibiotics and non-steroidal anti-inflammatory analgesics were administered postoperatively. A stainless-steel recording chamber was mounted to the skull over a craniotomy of the left parietal bone, allowing bilateral access to the PMRF. The EMG electrodes were pairs of multi-stranded, Teflon-coated stainless-steel wires chronically implanted in twelve arm and shoulder muscle pairs per arm, 24 total. The muscles tested were extensor carpi ulnaris (ECU), flexor carpi radialis (FCR), biceps (BIC), brachialis (BRAC), triceps (long head: TRLO; lateral head; TRLA), anterior deltoid (ADLT), middle deltoid (MDLT), posterior deltoid (PDLT), upper trapezius (UTR), middle trapezius (MTR), pectoralis major (PMJ), and latissimus dorsi (LAT). EMG activity was recorded from all implanted muscles during stimulation for both StimulusTA and stimulus train methods using Spike2 software and a Power 1401 data acquisition unit (CED, Cambridge, England). Muscles that showed cross-talk in an off-line analysis or eventual implant failure were removed from the analysis, as described in Davidson and Buford.9

StimulusTA procedures are provided in a previously published report.9 Stimulation was applied following a neural recording without changing the electrode position, and also at 0.5 mm intervals as the electrode was withdrawn from the bottom of the recording track. At each stimulation site, 4,000 biphasic pulses were delivered at a rate of 10 Hz by a digital stimulus controller (Master-8,
connected to an analog stimulus isolator (Model 2200, AM-Systems, Carlsborg, WA, USA). Stimulation for StimulusTA was delivered throughout all phases of the task (pre-trial, instructed-delay, reaching, reward retrieval, etc.). The subjects appeared to be unaware of this background stimulation. The stimulus current used was 30 μA in most cases because previous studies in PMRF output in the macaque indicate this is an effective stimulus intensity for StimulusTA. This current level has also been proven effective for studies in PMRF output in the cat. In 18 cases when single pulses evoked visible muscle twitches at a given site, the current was reduced until twitches were not visible. The lowest current used was 10 μA.

Procedures for compiling StimulusTAs and the acceptance criteria for StimulusTA effects are described in detail in a previous manuscript, only a brief description is presented here. A custom written Spike2 software program was used to compile StimulusTAs and to identify potential EMG responses for analysis. EMG data were averaged over an 80-ms peri-stimulus window, consisting of a 20-ms pre-trigger and a 60-ms post-trigger period. EMG responses that exceeded ± 2 standard deviations of a 15 ms pre-trigger (-20 to -5 ms) baseline EMG mean during the period between 3.5 and 15 ms following the trigger were considered StimulusTA effects. When StimulusTA revealed a sequence of responses in a given muscle, such as suppression followed by facilitation; only the first response was analyzed because this was considered most likely to reflect the direct output of the PMRF.
3.2.4 Stimulus trains

In some cases, stimulus trains were applied following StimulusTA when the StimulusTA was conducted immediately following a neuronal recording. However, the perceptible muscle twitches evoked by stimulus trains distracted the subject and sometimes stopped behavior. To avoid this, stimulus trains were usually applied as the electrode was withdrawn, immediately following StimulusTA from that site. A train of 12 biphasic pulses was applied at 333 Hz (pulse duration 0.2 ms/phase, amplitude = 30 μA). Stimulation was manually triggered during the control period (pre-trial and instructed-delay) while the monkey sat with both hands on start switches awaiting the go cue before reaching. This behavioral state was chosen as the most reproducible because it was impracticable to apply a sufficient number of stimulus trains to create representative averages for all possible phases of the behavior. At some sites, stimulation trains were applied when the subject was not performing the task, but while the arms were at rest on or near the switches. Ten stimulus trains were usually applied at each stimulation site (mean 10.1 ± 1.34 SD, range 5-16).

EMG responses to stimulus trains were averaged and then smoothed using an 80 Hz low pass zero-lag Butterworth filter (Fig. 3.1, black traces) to capture the main features of the responses. A 36-ms period beginning 47 ms before stimulus train onset (ending before the train actually began) was used to calculate the mean baseline level of this smoothed EMG activity. Having the baseline period end before the train began prevented any effects of stimulus
artifact from entering the baseline. Potential responses to stimulus trains were automatically detected if the smoothed EMG departed from the baseline mean by +2 SD for facilitation or -1.65 SD for suppression. The -1.65 SD threshold was required to detect suppression because there was little background EMG to begin with, making suppression relatively hard to detect. Onset and offset times determined from the smoothed data were manually adjusted to match the time when the raw EMG data (Fig. 3.1, grey traces) crossed the mean and SD thresholds calculated from the unsmoothed averages (e.g., Fig. 3.2). Only responses with onsets that began during the stimulus train or within three milliseconds after the last stimulus pulse were accepted for further analysis. There was often a sequence of responses in a given muscle in the stimulus train data. To match the approach used to analyze the StimulusTA data, only the first response was analyzed in these cases. A response duration of at least 10-ms was required to avoid spurious responses from entering the dataset for responses from stimulus trains, since a relatively small number of trains were averaged in these awake subjects. With a 33-ms stimulus train duration, it was reasonable to expect at least a 10-ms response.
Figure 3.1 Example of potential and accepted responses for stimulus trains. The two vertical lines for each panel represent the 33-ms stimulation period. The gray traces show the raw averages and the black traces show the smoothed responses. The solid horizontal lines indicate the mean EMG levels for the baseline period; dashed horizontal lines above and below the mean represent +2 SD and -1.65 SD thresholds used for response detection. Gray boxes outline potential responses, black boxes outline accepted responses.
Figure 3.2. Example of responses for StimulusTA and stimulus trains from a PMRF site. Facilitation is indicated by a filled bar below the trace, suppression by an open bar. The solid horizontal lines indicate the mean EMG levels for the baseline period; dashed horizontal lines above and below the mean represent +2 SD and -1.65 SD thresholds used for response detection.
With these objective criteria, visual inspection of the data was still required in order to eliminate responses that were clearly noise. In Fig. 3.1, for example, the automatic response detection indicated a possible facilitation in contralateral LAT. Visual inspection, however, showed this response was due to stimulus artifact superimposed on noise, so this was omitted. The automatic response detection also indicated a facilitation response in contralateral MTR and contralateral UTR; these facilitation events were accepted.

3.2.5 Data analysis

Because of the differences in stimulation methods, which would be expected to result in latency and amplitude differences, and due to the differences in detection criteria required for the two methods, detailed comparisons of response amplitude and onset latencies were not conducted. The sign of the response detected, facilitation or suppression, was considered the most reliable finding, and this was compared in detail for results from the two stimulation methods. The StimulusTA dataset from previously published StimulusTA results, referred to here as the main StimulusTA dataset, was one source of data for comparison. However, because that dataset was constructed from stimuli applied during reaching as well as during rest, it was not the most comparable data. Hence, the results from these studies were reanalyzed using only stimuli applied while both hands were resting on the start switches to create the averages. This control period before reaching provided a behavioral task epoch in which the monkey’s behavior was relatively consistent across trials and
most comparable to the conditions under which stimulus trains were applied. Stimulus triggered averages constructed from stimulation during this period are referred to as the control-period StimulusTA dataset.

In our previously published report\(^9\) a minimum of 500 triggers were required for a StimulusTA response to be included in the analysis. Application of this criterion for control-period StimulusTA data was too stringent because with only 4000 stimuli to begin with and much of the time spent reaching, there were often not enough triggers to test. McKiernan et al.\(^{125}\) found that spike triggered averages of EMG for cortical recordings based on a minimum of 200 triggers were not substantially different from those with 1000 or more. Davidson et al.\(^{23}\) presented spike triggered averages of EMG for cortical recordings from primary motor cortex that contain visible spike triggered effects with only 100 triggers. Based on these findings, we required at least 200 triggers for a StimulusTA response during the control period to be included in the analysis for comparison with stimulus trains. To avoid introduction of spurious results from this lower number of triggers, StimulusTA responses found in this data subset were used for comparison only if a corresponding response was found in the main StimulusTA data.

SPSS 17.0 and Microsoft Office Excel 2003 were used for all data analyses. Descriptive statistics were used to describe characteristics of evoked responses. Chi-squared tests were used to investigate significant differences in proportions of stimulus-evoked effects (facilitation vs. suppression responses,
extensor vs. flexor responses, etc.). The Wilcoxon signed rank test was used to test for differences in stimulation effectiveness between StimulusTA and stimulus trains. A criterion level of P < 0.05 was considered statistically significant in each of these statistical tests.

3.3 Results

3.3.1 Comparison of main StimulusTA and control-period StimulusTA datasets

As described in METHODS, our previous results from StimulusTA\textsuperscript{9} were obtained from single pulse stimulation applied during all phases of performance of a bilateral reaching task. The StimulusTA data presented here are based on a reanalysis including StimulusTA data compiled only from the control period between trials, while the hands rested on start switches. This is the only behavioral state during which stimulus trains were applied. Before comparing these StimulusTA results with responses to stimulus trains, we first performed a comparison of the control-period StimulusTA dataset to the main (previously published) StimulusTA dataset to determine whether the responses obtained during the control period data were similar. Of the 1611 responses from the previously published, main StimulusTA dataset, 1257 potential responses were found for stimuli applied during the control period; 864 of these were also represented in the main StimulusTA dataset. The remaining 393 control period responses that had no corresponding event in the main dataset were not
significantly different from 864 comparable control-period responses in terms of distribution of suppression vs. facilitation or ipsilateral vs. contralateral effects. Likewise, the 747 unmatchable responses in the main dataset were not significantly different from the 864 that could be compared.

The 864 comparable control-period StimulusTA responses were elicited from 293 of the 535 PMRF sites (55%). In the main dataset, the full complement of 1611 responses came from 435 sites (81%). Hence fewer PMRF sites are represented in the control-period dataset. However, when the 864 responses were compared for the control-period vs. main StimulusTA datasets, agreement was 97%, with agreement defined as the same type of response (facilitation or suppression) in a given muscle from a given stimulation site. For the 3% that differed, half were facilitation in main dataset and suppression in the control-period data, and the other half were the opposite. As would be expected with 97% agreement, the proportions of facilitation and suppression by muscle and laterality in these 864 responses were not significantly different for the control-period and main StimulusTA datasets. For the remainder of this report, only these 864 control-period StimulusTA responses are used for comparison with responses to stimulus trains.

### 3.3.2 Overall comparison of StimulusTA and Stimulus train evoked effects

Stimulus trains were applied at 473 PMRF sites; 444 of these sites (94%) were effective for activating one or more muscles. A total of 2582 EMG responses were included in the database from stimulus trains. Compared to
StimulusTA, stimulus trains were more effective for evoking EMG responses (Wilcoxon Signed Rank Test, p < 0.0001). In sum, nearly twice as many sites were effective for stimulus trains as for StimulusTA, and about three times as many responses were found for stimulus trains as for StimulusTA.

Figure 3.2 presents a comparison of StimulusTA and stimulus train responses evoked from one of the most effective sites stimulated. In this example, seven muscles showed StimulusTA effects, eleven muscles showed responses to stimulus trains, and both methods produced bilateral responses. Of the six StimulusTA responses in Fig. 3.2 for which there was a corresponding stimulus train response, all of the responses had matching signs (CPMJ, IUTR, CUTR, CPDLT, CTRLO, ITRLA), and the analyses below will indicate this was a common finding. Likewise, this example shows that stimulus trains were more effective – there are five stimulus train responses (ILAT, IMTR, IADLT, IFCR, CFCR) for which the same muscle had no response detected in the StimulusTA record. This was expected as stimulus trains are more likely to excite neurons via polysynaptic pathways. Conversely, there was one StimulusTA response (CLAT) for which the same muscle did not show a response to stimulus trains. This can be expected when the signal-to-noise ratio is better for StimulusTA.

The acceptance criteria resulted in some plausible responses not being accepted in both StimulusTA and stimulus train records. For example, in the StimulusTA record (Fig. 3.2), because the traces for IFCR and CFCR did not have corresponding responses in the main StimulusTA dataset they were not
accepted. In the stimulus train record (Fig. 3.2), because the trace for CMTR never went below threshold, this was not accepted as a suppression response. One could also question why a facilitation response was not found in IBIC even though there was one in IADLT. Again, the response detection in the smoothed data (see methods, Fig. 3.1), did not identify this BIC response as a viable candidate. Hence, some apparent facilitation and suppression responses were hard to detect for both stimulation methods. Within the limits of experimental error, the acceptance criteria were designed to ensure that those responses which were ultimately accepted into the dataset for comparison were valid.

3.3.3 Effectiveness of stimulation by muscle.

Figure 3.3 compares the effectiveness of stimulation for each muscle for stimulus trains vs. StimulusTA. Effectiveness was calculated as the number of sites from which a stimulus-evoked response could be observed in a muscle divided by the total number of sites where EMG was recorded for that muscle during a stimulation attempt. A response was counted as one or more muscles being facilitated or suppressed from a given site. For StimulusTA, effectiveness varied among muscles from 0% to 35%, with the highest degree of effectiveness found in limb girdle muscles (15%), moderate effectiveness found in the limb extensors (10%), and the lowest effectiveness found in the limb flexors (2%) ($\chi^2 = 324.2, p < 0.0001$). For stimulus trains, effectiveness varied from 18% to 42%, and there was barely a significant difference by muscle group. Overall stimulus train effectiveness was 31% for the limb girdle muscles, 29% for limb extensors,
and 27% for limb flexors ($\chi^2 = 8.4 \ p = 0.014$). The tendency for more limb girdle and fewer flexor muscles to respond, with intermediate responsiveness for extensors, was significantly more disparate for StimulusTA than for stimulus trains ($\chi^2 = 142.1, \ p < 0.0001$).

For each effective stimulation site, the number of muscles responding for stimulus trains ranged from one to fourteen, with a median of six and a mean of $6 \pm 3$ (SD). On average, half as many muscles responded per site for StimulusTA, with a range of one to eleven muscles per site, a median of two and a mean of $3 \pm 2$ (SD).

![Figure 3.3](image)

Figure 3.3. Comparison of the effectiveness of stimulation for StimulusTA vs. stimulus trains. The effectiveness of stimulation was determined as a percentage for each muscle by counting the number of sites at which a stimulus-evoked response was observed and dividing this number by the total number of sites where EMG was recorded for that muscle during a stimulation attempt.

### 3.3.4 Effects from StimulusTA vs. stimulus trains

For stimulus trains, ipsilateral and contralateral responses were equally prevalent, with 51% of the effects observed in ipsilateral muscles and 49% in
contralateral muscles. Bilateral responses were evoked by stimulus trains from 83% of all effective sites; 9% of sites evoked only ipsilateral responses and 8% of sites evoked only contralateral responses. Most StimulusTA sites (57%) also evoked bilateral outputs, 18% of sites evoked only ipsilateral responses and 25% of sites evoked only contralateral responses. Hence, there was a greater tendency for bilateral outputs to be revealed from stimulus trains, but neither method favored ipsilateral or contralateral effects.

Facilitation was the prevailing response to stimulus trains for both upper limbs, accounting for 71% of ipsilateral responses and 73% of contralateral responses. Suppression was more common (61%) than facilitation (39%) in the results for StimulusTA. This was true for both upper limbs, with suppression accounting for 67% of ipsilateral responses and 54% of contralateral responses.

In summary, stimulus trains and StimulusTA had a similar capacity to affect ipsilateral and contralateral muscles, with bilateral responses being most common, especially for stimulus trains. Facilitation was more common in response to stimulus trains, but suppression was more common in the results for StimulusTA ($\chi^2 = 294.3$, $p < 0.0001$).

3.3.5 Comparison of effects by muscle

Figure 3.4 provides a schematic diagram of the proportion of stimulus-evoked facilitation and suppression responses in upper limb muscles for each method. The top two diagrams (Fig. 3.4a) illustrate ipsilateral and contralateral muscles color coded for all 864 control-period StimulusTA and all 2582 stimulus
train responses. The colors represent the overall percentage of facilitation effects evoked for each muscle, with red indicating a preponderance of facilitation effects, blue indicating a preponderance of suppression effects, and green representing equal proportions of facilitation and suppression. The white color indicates that no StimulusTA responses in ipsilateral ADLT, and contralateral ADLT and BRAC were available for comparison. In agreement with the results from the main StimulusTA dataset (Davidson and Buford 2006), the overall pattern for the control-period StimulusTA dataset was a double reciprocal pattern between the upper limbs, with ipsilateral flexors and contralateral extensors facilitated and ipsilateral extensors and contralateral flexors suppressed.

For ipsilateral flexors and contralateral extensors, StimulusTA and stimulus trains both produced a preponderance of facilitation. As shown in Table 3.1, there was more facilitation from stimulus trains than for StimulusTA for many of these muscles. However, in two ipsilateral extensors, UTR and PDLT, where suppression was the typical response for StimulusTA, facilitation was the prevalent response for stimulus trains (Fig 3.4a, Table 3.1). In two contralateral flexors (FCR, and MTR), stimulus trains also resulted in a prevalence of facilitation even though suppression was typically the response for StimulusTA (Fig 3.4a, Table 3.1). In sum, stimulus trains favored facilitation of ipsilateral flexors and contralateral extensors even more strongly than StimulusTA. Conversely, the tendency for stimulus trains to suppress ipsilateral extensors and
contralateral flexors was less pronounced, and in some cases stimulus trains were most likely to facilitate muscles that were typically suppressed for StimulusTA. This prevalence of facilitation from stimulus trains was found throughout the muscles studied, regardless of the muscle’s location (proximal vs. distal) or function (flexor vs. extensor).
Figure 3.4. This schematic representation color codes muscles according to the proportion of facilitation versus suppression for each stimulation method. As shown in the key, shades of red indicate muscles that were mostly facilitated, shades of blue indicate muscles that were mostly suppressed, and colors in between represent a mixture of responses; white no response. Responses are referred to as ipsilateral or contralateral to the stimulation site. The top two diagrams A illustrate the response patterns for all control-period StimulusTA and stimulus train results. The bottom two B illustrate the response patterns for all control-period StimulusTA and stimulus train results constrained to sites where both methods evoked responses for a given muscle. In order to portray the triceps muscle, the proportion of responses for TRLA and TRLO were averaged. Muscle key: a ECR, b TRI, c PDLT, d LATS, e UTR, f MDLT, g FCR, h BRACH, i BIC, j ADLT, k PMJ.
Table 3.1 Frequency of responses for Stimulus TA and stimulus trains

<table>
<thead>
<tr>
<th>Muscle</th>
<th>FAC</th>
<th>SPR</th>
<th>% FAC</th>
<th>FAC</th>
<th>SPR</th>
<th>% FAC</th>
</tr>
</thead>
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<tr>
<td>CECU</td>
<td>21</td>
<td>4</td>
<td>85 (88)</td>
<td>148</td>
<td>8</td>
<td>95 (94)</td>
</tr>
<tr>
<td>CFRC</td>
<td>3</td>
<td>20</td>
<td>13 (50)</td>
<td>91</td>
<td>25</td>
<td>78 (50)</td>
</tr>
<tr>
<td>CBIC</td>
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<td>3</td>
<td>0 (0)</td>
<td>38</td>
<td>1</td>
<td>51 (50)</td>
</tr>
<tr>
<td>CBRAC</td>
<td>0</td>
<td>0</td>
<td>- (-)</td>
<td>64</td>
<td>0</td>
<td>100 (-)</td>
</tr>
<tr>
<td>CTRLA</td>
<td>43</td>
<td>3</td>
<td>93 (95)</td>
<td>92</td>
<td>8</td>
<td>92 (95)</td>
</tr>
<tr>
<td>CTRLB</td>
<td>47</td>
<td>14</td>
<td>77 (73)</td>
<td>84</td>
<td>45</td>
<td>65 (77)</td>
</tr>
<tr>
<td>CADLT</td>
<td>0</td>
<td>0</td>
<td>- (-)</td>
<td>22</td>
<td>0</td>
<td>63 (-)</td>
</tr>
<tr>
<td>CPMJ</td>
<td>3</td>
<td>93</td>
<td>3 (2)</td>
<td>8</td>
<td>6</td>
<td>85 (42)</td>
</tr>
<tr>
<td>CPDLT</td>
<td>3</td>
<td>1</td>
<td>75 (57)</td>
<td>62</td>
<td>0</td>
<td>100 (100)</td>
</tr>
<tr>
<td>CLAT</td>
<td>4</td>
<td>61</td>
<td>7 (4)</td>
<td>63</td>
<td>14</td>
<td>48 (54)</td>
</tr>
<tr>
<td>CMTR</td>
<td>2</td>
<td>9</td>
<td>18 (100)</td>
<td>106</td>
<td>17</td>
<td>86 (100)</td>
</tr>
<tr>
<td>CUTR</td>
<td>72</td>
<td>25</td>
<td>74 (72)</td>
<td>136</td>
<td>43</td>
<td>76 (67)</td>
</tr>
<tr>
<td>IECU</td>
<td>5</td>
<td>32</td>
<td>14 (5)</td>
<td>53</td>
<td>39</td>
<td>58 (21)</td>
</tr>
<tr>
<td>IFCR</td>
<td>13</td>
<td>16</td>
<td>45 (33)</td>
<td>152</td>
<td>8</td>
<td>95 (92)</td>
</tr>
<tr>
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<td>11</td>
<td>90 (100)</td>
</tr>
<tr>
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<td>94</td>
<td>0</td>
<td>100 (100)</td>
</tr>
<tr>
<td>ITRLA</td>
<td>4</td>
<td>62</td>
<td>6 (5)</td>
<td>50</td>
<td>60</td>
<td>45 (29)</td>
</tr>
<tr>
<td>ITRB</td>
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<td>59</td>
<td>13 (27)</td>
<td>37</td>
<td>81</td>
<td>31 (59)</td>
</tr>
<tr>
<td>IADLT</td>
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<td>0</td>
<td>- (-)</td>
<td>46</td>
<td>2</td>
<td>96 (-)</td>
</tr>
<tr>
<td>IPMJ</td>
<td>56</td>
<td>8</td>
<td>88 (97)</td>
<td>79</td>
<td>18</td>
<td>81 (92)</td>
</tr>
<tr>
<td>IPDLT</td>
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<td>12</td>
<td>14 (17)</td>
<td>48</td>
<td>1</td>
<td>98 (100)</td>
</tr>
<tr>
<td>ILAT</td>
<td>19</td>
<td>47</td>
<td>29 (21)</td>
<td>66</td>
<td>70</td>
<td>48 (34)</td>
</tr>
<tr>
<td>IMTR</td>
<td>3</td>
<td>2</td>
<td>60 (100)</td>
<td>118</td>
<td>31</td>
<td>79 (100)</td>
</tr>
<tr>
<td>IUTR</td>
<td>19</td>
<td>56</td>
<td>25 (42)</td>
<td>98</td>
<td>58</td>
<td>63 (45)</td>
</tr>
</tbody>
</table>

Facilitation and suppression evoked responses during the control phase of the task in each muscle for both methods are expressed as counts. The numbers in parentheses are control data constrained to sites where both methods evoked a response in a given muscle. The prevalence of facilitation responses evoked in each muscle is expressed as a percentage. Each percentage of facilitation is calculated by dividing the number of facilitation responses by the total number of evoked responses for that muscle. I ipsilateral, C contralateral
3.3.6 Site-by-site comparison of effects from stimulus trains and StimulusTA

3.3.6.1 Directly Comparable Responses

To follow up on the finding that stimulus trains were more likely to produce facilitation, a more specific comparison was conducted with the analysis constrained to directly comparable responses where both methods evoked a response in a given muscle from the same site, the best chance for agreement. Of 356 directly comparable responses from 164 sites, facilitation accounted for 58% of the responses for stimulus trains, a higher proportion than the 45% found for StimulusTA ($\chi^2 = 11.9$, $p = 0.001$).

The bottom two diagrams in Fig. 3.4b illustrate ipsilateral and contralateral muscles color coded for these corresponding StimulusTA and stimulus train responses. The white color indicates that no responses in ipsilateral ADLT and contralateral ADLT and BRAC were available for direct comparison between methods. For StimulusTA, the bilateral reciprocal response pattern was still evident in this subset of the data. Stimulus trains also usually facilitated ipsilateral flexors and contralateral extensors, consistent with the typical response for StimulusTA. However, stimulus trains reduced the tendency for reciprocal suppression, especially for ipsilateral extensors.
3.3.6.2 Matching StimulusTA and stimulus train responses.

Overall, 80% of the 356 comparable responses matched, with a match being defined as the same type of response (facilitation or suppression) evoked in a given muscle for a given stimulus site for both methods. Matches were equally common for facilitation and suppression, and for ipsilateral and contralateral effects. Figure 3.5a is representative of matching StimulusTA and stimulus train responses from a single stimulation site. StimulusTA and stimulus trains both evoked facilitation of CECU and CTRLA. These were typical StimulusTA responses for these muscles. Examples of responses that matched for StimulusTA and stimulus trains were also presented in Fig. 3.2.

3.3.6.3 Mismatched StimulusTA and stimulus train responses.

At sites with comparable stimulus train and StimulusTA responses, 20% (72/356) of the responses were mismatches, with a mismatch being defined as a case where facilitation was evoked by one method and suppression was evoked by the other. Most (82%) mismatches were cases where facilitation was evoked by the stimulus trains but suppression was evident in the record for StimulusTA ($\chi^2 = 29.4, p < 0.0001$). Mismatches were also more common ipsilaterally (63%) than contralaterally (38%) ($\chi^2 = 4.5, p = 0.03$), and more common in extensors (81%) than flexors (19%) ($\chi^2 = 26.9, p < 0.0001$). The most common type of mismatch (40%) was when ipsilateral extensors were suppressed by StimulusTA, but this typical effect was replaced by facilitation for stimulus trains. Many of the mismatches (35%) were also cases where the response for StimulusTA was
suppression in a muscle that was typically facilitated, and the stimulus train produced the more typical facilitation response; this was most common in contralateral extensors.

Figure 3.5b illustrates an example of a mismatch in responses from StimulusTA and stimulus train for a single stimulation site. In this example, suppression responses were evoked in IFCR and ILAT by StimulusTA, stimulus trains produced facilitation for these two muscles. The suppression response in IFCR was atypical from StimulusTA, but the suppression response in ILAT was typical.
Figure 3.5. Selected examples of matching and mismatching effects for StimulusTA and stimulus trains. a Illustrates matching responses, and b illustrates mismatches. Formatted like Fig. 3.2.

3.3.7 Additional control analyses for mismatches

As noted earlier in the results, 3% of effects from the control-period StimulusTA were opposite in sign, altered from the effect from the corresponding site in the main StimulusTA dataset. We checked to see whether these altered effects showed up with a disproportionately high incidence in the mismatches between StimulusTA and stimulus train responses described above, but this was not the case. Only three of these altered responses entered into the data. Two of these were atypical facilitation from StimulusTA replaced by suppression from
stimulus trains and one was atypical suppression from StimulusTA replaced by facilitation from stimulus trains. Therefore, responses in the control-period StimulusTA dataset that were altered from the response in the main StimulusTA data could not account for the finding that suppression from StimulusTA was often replaced by facilitation from stimulus trains.

Next, we examined the extent to which measurement error could account for different responses observed between methods. To test for this possibility, the control-period StimulusTA dataset the data file was split in two and the StimulusTAs compiled from these two datasets were compared. Each response found in the split data had to come from at least 100 triggers, half the overall acceptance criteria of at least 200 triggers for a StimulusTA response. In this comparison, 496 StimulusTA responses were found. There was 96% agreement in response type (facilitation or suppression) for each response found in the split StimulusTA datasets.

Referring back to the actual comparison of StimulusTA and stimulus train responses, for the 72 mismatches found, only one came from a site that produced different responses for the two halves of the StimulusTA data. Hence, sites from which StimulusTA responses could be described as unstable were not the source of differences between the StimulusTA and stimulus train responses. There was a significantly higher proportion of disagreement in the comparison between StimulusTA and stimulus train responses than there was between the two halves of the StimulusTA dataset ($\chi^2 = 19.11, p < 0.001$). Consequently, the
20% difference found between the stimulus train and StimulusTA results exceeds the expected measurement error.

### 3.3.8 Unmatched responses

For 86% (n=2222) of the stimulus train responses, there was no corresponding StimulusTA response in that muscle for that site; these were called unmatched responses, and were present in one or more muscles from 243 sites. A smaller proportion (56%) of the StimulusTA responses (n=464) were unmatched; these were evoked from 78 sites. For the unmatched subset of stimulus train responses, facilitation was even more prevalent (74%) than in the matched subset (57%). In these unmatched train responses, 52% of the effects were ipsilateral, 48% were contralateral, and the bilateral reciprocal pattern of ipsilateral flexor and contralateral extensor facilitation was evident in the data. For the unmatched StimulusTA responses there was 65% suppression, more than for matched StimulusTA responses (55%). Again, 52% of the effects were ipsilateral and 48% were contralateral, and the bilateral reciprocal pattern was evident.

In sum, data that entered into the site by site comparison for directly comparable responses appear the most similar overall as subsets of the data from the two stimulation methods. In fact, the StimulusTA responses available for direct comparison with stimulus train results had more, not less facilitation, than could be found in the overall control-period dataset. Hence, a lack of
facilitation in the StimulusTA results cannot account for the excess facilitation from stimulus trains.

3.3.9 Anatomical organization of effective PMRF stimulus-evoked responses

The locations of sites evoking responses for StimulusTA were published in Davidson and Buford. As stated in METHODS, stimulus trains were applied at most StimulusTA sites. Sites evoking facilitation and suppression responses for given muscles with stimulus trains were interspersed. There was no special concentration of stimulus train sites producing the most effects, nor were there special concentrations of sites producing ipsilateral, contralateral, or bilateral effects. There were not certain locations from which facilitation versus suppression responses were elicited. PMRF sites evoking matched and mismatched StimulusTA and stimulus train responses were also evenly dispersed throughout the PMRF.

3.4 DISCUSSION

3.4.1 Double reciprocal pattern of PMRF output

In the aggregate, results from StimulusTA and stimulus trains revealed a common pattern of bilateral motor output to the upper limbs: ipsilateral flexor and contralateral extensor muscle facilitation, with suppression (or at least less facilitation) of the reciprocal muscles. One explanation for this consistent finding
could be that most reticulospinal neurons distribute their outputs in a way that contributes to this overall pattern. Matsuyama et al.\textsuperscript{21} traced individual reticulospinal axons and found that many terminated ipsilaterally, but some terminated bilaterally. These axons terminated in laminae VII and VIII, where they could contact interneurons or the dendrites of motoneurons. Classical studies indicate that there are monosynaptic pathways from the PMRF to motoneurons in the cat\textsuperscript{11, 12, 16, 126} and in the monkey.\textsuperscript{127} Jankowska’s physiological data indicate that bilateral PMRF effects are mediated by pathways comprised of monosynaptic synapses on the motoneurons or disynaptic connections onto excitatory, inhibitory and commissural interneurons.\textsuperscript{20, 117, 128} Thus, there is ample evidence for mono and disynaptic pathways from the PMRF to motoneurons that are bilaterally distributed. However, most of these studies are in the lumbar cord of the cat and there is a need to define this in the cervical cord of the macaque.

A pattern combining extension of one limb with flexion of the other has its roots in the control of locomotion. Activation of the PMRF directly, or indirectly from mesencephalic locomotor region stimulation, is sufficient for initiation of locomotion in reduced preparations.\textsuperscript{129-131} Hence, the tendency for PMRF output to produce this double reciprocal pattern is well established.\textsuperscript{6, 119, 132} Data from our previous work and from the cat also shows that PMRF neurons are strongly modulated during voluntary reaching.\textsuperscript{4, 6, 67, 132-134} As Drew et al.\textsuperscript{130} noted, these
findings indicate the pathways used for initiation and regulation of locomotion by the PMRF may overlap with pathways engaged by the PMRF during reaching.

The double reciprocal pattern observed with PMRF stimulation is also consistent with the upper limb movements associated with the asymmetric tonic neck reflex (ATNR), which is thought to reflect brainstem output after cortical injury.71 Humans often present with movement patterns that mimic the ATNR after a cortical stroke. Dewald et al.50 demonstrated a predominance of stroke-related muscle synergies in the hemiparetic upper limb during reaching that are consistent with the ATNR and the historical descriptions of stroke synergies from Brunnstrom.49 Subjects in Dewald’s50 study typically flexed the elbow when elevating the shoulder, and had the greatest difficulty recruiting elbow extensors coupled with shoulder flexors. Our results from both stimulation methods are consistent with Dewald’s argument that these stroke synergies may be explained by increased reliance on the reticulospinal system for voluntary control of reaching.

3.4.2 Differences in results for stimulus trains vs. StimulusTA

Although both methods produced results that largely agreed and reflected the double reciprocal pattern, there were two important differences. First, there was excess facilitation from stimulus trains. Second, stimulus trains were more effective for recruiting limb muscles, whereas StimulusTA results were more heavily represented in the limb girdle muscles.
Despite the excess facilitation from stimulus trains, only 20% of responses were different in the directly comparable individual responses. However, in the larger datasets, the difference was more pronounced. Facilitation accounted for 72% of the responses to stimulus trains, but only 39% of the responses to StimulusTA. For an investigator forced to choose between one method or the other, this could present somewhat different conclusions from a study. One factor that probably contributed to this difference in results for the two methods was the design of our study. Suppression of EMG when movement is not underway is relatively hard to detect. The improvement of signal-to-noise with the relatively large number of triggers in StimulusTA would be expected to make that the more sensitive technique for subtle responses, since a relatively small number of stimulus trains were applied. Perhaps if stimulus trains were applied during reaching movements, more suppression could have been detected. For practical reasons explained in the methods, however, this was not feasible in the present study. Even considering this limitation, however, the analysis of the directly comparable responses along with the control analyses described at the end of the results show that there was still a clear tendency for stimulus trains to evoke facilitation where the response for StimulusTA was suppression. This was especially true for extensor muscles. Our conclusion is that at least some of this over-representation of facilitation from stimulus trains represents a true physiological difference between the pathways for muscle recruitment for the two methods.
Studies in the cat where stimulus trains were used also report a preponderance of facilitation.\(^5\) Previously, we attributed the difference between the findings from the cat and our findings with StimulusTA\(^9\) to the lack of EMG activity in the resting state for the cat making suppression harder to detect. In the present analysis, we controlled for that and still found facilitation in response to stimulus trains more often than facilitative StimulusTA effects. The present findings suggest that responses to stimulus trains are similar in the cat and monkey. Further, we suspect a StimulusTA study in the cat would yield results similar to those found in the monkey, where suppression was more common than facilitation. Indeed, when SpikeTA has been conducted from PMRF neurons in the cat,\(^6,7\) suppression was more common than facilitation, and SpikeTA and StimulusTA results tend to agree.\(^23, 75, 120\)

The second difference was the relatively equal distribution of effects among proximal and more distally located muscles for responses to stimulus trains. For StimulusTA, there was clearly a preponderance of effects in the limb girdle muscles, moderate responsiveness in limb extensors, and very few responses in limb flexors. But with stimulus trains, all three muscle groups were quite responsive and there was only a small difference among groups. Again, this is consistent with what was described in the cat with stimulus trains, where flexor responses in limb muscles were common.\(^5\) Recent data from Baker’s lab indicates that stimulation of reticulospinal and other axons in the medial longitudinal fasciculus (MLF) of the rhesus monkey can evoke monosynaptic
EPSPs in forearm motoneurons and even in intrinsic hand motoneurons. The tendency for stimulus trains to have more effects distally suggests that these connections may be mostly polysynaptic.

The unmatched results in the StimulusTA and stimulus train datasets may be explained by differing sensitivities and specificities of the two methods. StimulusTA by its nature is able to extract very small effects as long as they are consistently present. Stimulus trains, in comparison would be expected to reveal a larger number of effects overall by engaging polysynaptic pathways, but might not succeed at revealing real but very weak effects. A stimulus train that succeeded as well as possible at eliciting a very weak effect would still potentially be hard to recognize because there are very few stimulus trains to average and the weak effect might be hard to discern from the background EMG activation that was present in these awake subjects.

3.4.3 Implications for reticulospinal output pathways

The physiological difference between stimulus trains vs. StimulusTA is thought to be that through temporal summation, stimulus trains are better able to reveal polysynaptic pathways, whereas StimulusTA results tend to reflect monosynaptic and disynaptic connections to motoneurons. Accepting for the moment that stimulus train and StimulusTA results were different in the ways described above, this suggests that a slightly different set of neural pathways was being studied for the two methods. Similarities could be attributed to the most direct pathways activated well by both methods, while differences could be
attributed to more polysynaptic routes better revealed by stimulus trains. The tendency to facilitate ipsilateral flexors and inhibit ipsilateral extensors may be explained by monosynaptic reticulospinal projections to motoneurons in combination with disynaptic connections through excitatory and inhibitory interneurons. The tendency to facilitate contralateral extensors while simultaneously inhibiting contralateral flexors may be explained by known populations of excitatory and inhibitory commissural interneurons.\textsuperscript{20, 117}

This double reciprocal pattern is present with both StimulusTA and stimulus trains. Results from previous SpikeTA and StimulusTA studies in the monkey have also revealed the typical, double reciprocal pattern,\textsuperscript{8, 9, 23} which further indicates that the double reciprocal pattern with a preponderance of suppression revealed by StimulusTA is more likely to reflect relatively direct pathways. However, facilitation of ipsilateral extensors and contralateral flexors also exists and may become more prevalent with stimulus trains. The finding that suppression responses from StimulusTA could be replaced by facilitation from stimulus trains suggests that there is an alternative route, where the more direct pathway promotes suppression and a more polysynaptic pathway allows for an alternative response of facilitation, especially in extensors.

This is not the first evidence that reticulospinal motor outputs may take alternative pathways with opposite effects for a given motor pool. In the cat, reciprocal activation patterns between flexors and extensors in the forelimbs can vary depending on the behavioral state (rest or movement) of the animal. Drew
and Rossignol\textsuperscript{5} reported that stimulus trains applied at rest facilitated ipsilateral forelimb flexors and contralateral forelimb extensors, but rarely suppressed EMG activity in any limb muscles. Stimulation applied during the swing phase of locomotion for the ipsilateral forelimb also facilitated ipsilateral flexors and contralateral extensors, but stimulation applied during swing for the contralateral forelimb facilitated contralateral flexors, opposite to the pattern observed at rest and during ipsilateral swing.\textsuperscript{118, 132, 136} Drew and colleagues have interpreted this result to suggest that reticulospinal outputs can facilitate or suppress flexors and extensors bilaterally, but the pathways capable of conducting responses to stimulation will depend on behaviorally appropriate gating of spinal circuits through which the reticulospinal effects are conveyed.\textsuperscript{4, 67, 137}

Drew et al.\textsuperscript{130} suggested that the direct and alternative pathways explaining the nature of reticulospinal outputs exist at the spinal level. We concur that there is ample evidence that well established spinal circuits associated with locomotion can support this double reciprocal pattern and that circuits for coactivation of flexors and extensors can also be demonstrated at the spinal level, so this proposal has the advantage of parsimony and plausibility. However, there is another possibility worth considering: polysynaptic pathways revealed by stimulus trains may also involve circuitry intrinsic to the PMRF. In primary motor cortex, intracortical microstimulation activates cells in the immediate vicinity of corticomotoneuronal cells indirectly\textsuperscript{135, 138} and activates corticospinal cells trans-synaptically.\textsuperscript{76, 138} Stimulus trains applied to the MLF
activate some reticulospinal neurons directly and some trans-synaptically via antidromic activation of reticulospinal collaterals.\textsuperscript{20, 139} Therefore, it seems likely that stimulus trains delivered in the PMRF would recruit polysynaptic pathways within the reticular formation. In contrast to findings in motor cortex of adjacent cells showing similar motor outputs,\textsuperscript{121} our findings showed no anatomical organization within PMRF producing facilitation and suppression effects. Hence, engagement of polysynaptic circuits to spread the activation within the PMRF might have mixed effects, whereas polysynaptic engagement within a local region of the motor cortex might not produce as much difference.

Another interesting polysynaptic route with known projections to the more distal muscles in cat and in monkey is the C3-4 propriospinal system.\textsuperscript{140, 141} C3-4 propriospinal neurons are also a strong target of reticulospinal projections in the cat, but may be a weaker target in the monkey.\textsuperscript{142, 143} This system has been implicated even in control of skilled digit movements in monkeys via direct cortical projections to the propriospinal neurons\textsuperscript{141} and with corticoreticular influences on reticulospinal cells that project to the propriospinal neurons.\textsuperscript{144, 145}

### 3.5 Further implications

Finally, on a methodological note, a common neurophysiological technique in cerebral cortex is to use stimulus trains to map motor outputs, recording movements evoked without EMG analysis. Can simple observation of movement or even EMG recording with stimulus trains in the PMRF suffice, or is
the extra time associated with StimulusTA required for an accurate representation of motor outputs from this part of the brain? The large agreement between StimulusTA and stimulus trains in these results support the use of stimulus trains for a general description of motor outputs. However, stimulus trains may under estimate the amount of suppression produced by the PMRF. If a comparison was planned as the result of a treatment that could change the effects of the more direct PMRF output pathways or to specifically focus on changes in suppression, then the StimulusTA method might be required to reveal those changes. If the intent was to study effects that were mediated by polysynaptic routes, stimulus trains would be a better approach. Future investigations should choose methods carefully with these differences in mind.
Chapter 4: Ipsilateral Corticospinal Outputs to the Upper Limbs of the Monkey Revealed with Stimulus-Triggered Averaging

4.1 Introduction

The corticospinal system (CST) is the primary descending motor pathway mediating skilled voluntary motor control, especially of fine, dexterous hand movements. It is well established that the CST is predominantly a crossed motor pathway, however approximately 10-20% of CST fibers reach the spinal cord undecussated.\textsuperscript{146-148} Both contralateral (crossed) and ipsilateral (uncrossed) CST projections originate in the cerebral cortex and terminate predominantly onto interneurons in the intermediate zone and lamina IX motoneurons.\textsuperscript{27-29, 146, 147, 149, 150} The capacity to execute highly fractionated distal extremity movements is provided by the presence of direct cortico-motoneuronal connections onto motoneurons within lamina IX.\textsuperscript{30, 105, 151, 152}

The anatomic substrate and function of ipsilateral CST projections to upper limb motoneurons remains debated. Anatomical evidence of primary and premotor cortical areas having ipsilateral terminations within lamina IX where upper limb motoneurons are located,\textsuperscript{105, 147} is indicative that ipsilateral CST projections from the motor cortex may make fairly direct connections with upper limb motoneurons. These ipsilateral CST pathways are thought to originate
primarily from areas of the motor cortex that mediate motor control over trunk and proximal limb girdle muscles. The possibility of ipsilateral CST control as one route of recovery of arm function after cortical injury such as stroke has been studied in humans via trans-cranial magnetic stimulation and inferred from functional MRI. However, there is only a small amount of physiologic evidence of CST connections to ipsilateral upper limb motoneurons in healthy subjects, perhaps because few studies exploring corticospinal motor outputs have recorded from muscles of the ipsilateral limb.

As part of a larger study of combined (paired) corticospinal and reticulospinal outputs to both upper limbs, ipsilateral and contralateral muscles were both studied during cortical stimulation. The purpose of the present study was to investigate ipsilateral and contralateral corticospinal outputs from three cortical motor areas in the macaque monkey (M fascicularis) to muscles of both upper limbs with single pulse stimulation (Stimulus-triggered averaging) to test the hypothesis that direct ipsilateral corticospinal outputs could be evoked from primary motor cortex and the premotor areas.

4.2 Methods

4.2.1 Subjects and task

Subjects were three male monkeys (M fascicularis) trained to perform an instructed-delay, bilateral reaching task controlled by Tempo software (Reflective computing, Olympia, WA, USA). The details of the task have been described in
a previous report based on a different set of subjects. The task involved a control period, including an instructed-delay interval, when the monkey waited with both hands on start switches at waist level. During the subsequent movement period, he responded to the instruction by reaching with the left or right arm to a target presented on a touch-screen computer monitor. After the trial, he was free to use either hand to retrieve a food reward, and then he started the next trial. Hence, this task provided a broad base of EMG activity in both upper limbs upon which to base EMG averages for stimulus-triggered averaging (StimulusTA).

4.2.2 Animal Care

Subject care complied with the NIH Guide for the Care and Use of Laboratory Animals, and the institutionally approved animal care protocol for our laboratory. Surgeries were performed under veterinary supervision under aseptic conditions. As previously reported, animals were pre-treated with antibiotics and were pre-anesthetized with Ketamine HCL (12 mg/kg im) followed by isoflurane gas (1-2%). Antibiotics and non-steroidal anti-inflammatory analgesics were administered postoperatively. A recording chamber (25mm x 25mm square internal grid) was mounted to the skull over a craniotomy of the left parietal bone and secured with dental acrylic. The center of the recording chamber was aimed towards stereotaxic coordinates AP 15, ML 12, allowing access to all three cortical motor areas. A 19 mm circular recording chamber was also mounted over the right parietal bone providing access for deep-brain recording and
stimulation in the pontomedullary reticular formation, but data from the brainstem is not reported here. Chronic indwelling EMG electrodes (fine-wire) recorded activity from the trunk and shoulder girdle, limb extensor and limb flexor muscles of both arms; 24 muscles were implanted per subject. The muscles implanted were flexor carpi ulnaris (FCU), extensor carpi radialis (ECR), biceps brachii (BIC), triceps (TRI), middle deltoid (MDLT), latissimus dorsi (LAT), lumbar paraspinals (LUMB), supraspinatus (SUPRA), upper trapezius (UTR), pectoralis major (PMJ), sternocleidomastoid (SCM), and cervical paraspinals (CERV).

4.2.3 Stimulation procedure

Stimulation for StimulusTA was applied to cortical motor sites in the left cerebral cortex spread across the primary motor cortex (M1), dorsal pre-motor cortex (PMd) and supplementary motor cortex (SMA). Glass coated platinum-iridium electrodes (impedance 500 kΩ) were positioned with an alpha omega system (Alphalab, Alpharetta, GA) to record cortical neurons with activity related to reaching. Then the system was set up for stimulation at these reach-related recording sites. These cortical stimuli came in the midst of a series of pulses applied for a paired pulse paradigm which included randomly ordered sets of reticular formation stimulation alone, cortical stimulation alone, and cortical and reticular formation stimulation paired at various latencies. Data for the current study were from instances of cortical stimulation alone. The experimental paradigm was controlled with custom-written software using a CED Power 1401 System and Spike 2 version 6 software (CED Cambridge, England). The voltage

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outputs from the CED system were converted to constant-current stimulus pulses with an AM-Systems model 2200 stimulus isolator (AM-Systems, Carlsborg, WA, USA). At each stimulation site, 2000 biphasic pulses (0.2 ms per phase) were delivered at a rate of 10 Hz.

4.2.4 Response detection and analysis

The EMG signals were amplified with gains ranging from 200-1000 and filtered with a bandpass of 20Hz–5 KHz prior to recording with custom-built EMG amplifiers (Washington National Primate Research Center, Seattle, WA). An 8-pole, 10 KHz low pass filter was also applied to limit high-frequency noise. Procedures for compiling StimulusTAs are described in detail in a previous manuscript; only a brief description is presented here. A custom written Spike2 software program was used to compile StimulusTAs and to identify potential EMG responses for analysis. Rectified EMG data were averaged over an 80-ms peri-stimulus window, consisting of a 20-ms pre-trigger and a 60-ms post-trigger period. The averaged EMG responses that differed from the 15 ms pre-trigger (-20 to -5 ms) baseline level mean by more than 2 standard deviations for at least 3 ms were considered candidates for analysis. A response duration of at least 3 ms was required to avoid spurious responses from entering the dataset. The peak of each effect was defined as the highest point in the post-stimulus effect between the onset and offset latencies. Onset and offset latencies were determined by the points of intersection between the EMG averages and the ±2 standard deviation threshold. The amplitude of the post-
stimulus effect was quantified by the standard deviation score of the peak (SDPeak). Responses were only accepted with a peak at least 4 standard deviations from the baseline with onsets between 2 and 30 ms after the stimulus.\textsuperscript{160} Stimuli were applied throughout all phases of the task, and the assessment of effects was based on StimulusTAs of at least 200 trigger events.\textsuperscript{23, 125}

As one part of the detection decision, the presence of corresponding responses in the record for paired stimulation at the same site was considered. If a marginal response evident for cortical stimulation alone was also evident in response to cortical stimulation in the paired paradigm, then the response was retained. For instances where StimulusTA revealed a sequence of responses in a given muscle such as suppression followed by facilitation; only the first response was analyzed because this was considered most likely to reflect the direct output of the cortical motor area. With these objective criteria, visual inspection of every candidate response was still required in order to eliminate spurious responses that were due to stimulus artifact or noise.

\textbf{4.2.5 Test for EMG implant integrity}

Procedures for EMG implant failure and cross talk analyses followed a previously published report.\textsuperscript{9} Cross talk was identified if a muscle’s averaged EMG-triggered responses exceeded the amplitude of the triggering motor unit potential by at least 15% during the period of a motor unit action potential that served as the trigger.\textsuperscript{161} When cross talk was revealed, data from the EMG
implant with the higher quality EMG signal was retained. EMG implant failure was identified as the time point where the EMG quality from the given channel deteriorated and/or the size of the artifact grew, or the channel completely failed. Data from these implants were removed from analysis from the determined time point of failure. Table 4.1 represents muscles removed due to cross talk or implant failure.

Table 4.1. Test for muscle integrity

<table>
<thead>
<tr>
<th>MUSCLE</th>
<th>Subject H</th>
<th>Subject N</th>
<th>Subject O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Implant integrity</td>
<td>Implant Integrity</td>
<td>Implant Integrity</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>R</td>
<td>L</td>
</tr>
<tr>
<td>CERV</td>
<td>+</td>
<td>C</td>
<td>f</td>
</tr>
<tr>
<td>LUMB</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SCM</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>UTR</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LAT</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PMJ</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SUPRA</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MDLT</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TRI</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BIC</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ECR</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FCU</td>
<td>f</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Muscle integrity key: + muscle passed all tests; X muscle completely removed from analysis due to bad implant; f implant failed before end of study and responses were removed from time point of failure; C muscle eliminated due to cross talk

4.2.6 Histological procedure

After completion of the study the subject was deeply anesthetized with a lethal dose of sodium pentobarbital and then perfused transcardially with phosphate-buffered saline followed by phosphate buffered para-formaldehyde. This was followed by a perfusion with 10% sucrose in phosphate buffer, followed by 30%
sucrose in phosphate buffer. The brain was removed and soaked in 30% sucrose for cryoprotection and then frozen using isopentane at -20 degrees. Coronal sections were cut at 50 µm on a freezing microtome and every tenth section (every ½ mm) was mounted and stained with cresyl violet (CV).

4.2.7 Reconstruction of stimulation sites

Anatomical sections with evident gliosis from electrode penetrations were selected as representative for each coronal plane on the 1-mm based grid in the recording chamber (see Fig. 4.1A for representative recording grid). Selected electrolytic marking lesions made at a few locations were identified under a light microscope in order to determine the locations of specific electrode tracks in the brain, allowing identification of the relative positions of the remainder of the tracks. Depths were determined based on the distance from the site where the first neural activity was heard in the background, taken as the approximate surface of the cortex. Adjustments in depth were made as indicated to account for potential dimpling of the brain and changes in cortical thickness associated with histological processing as the approximate stimulation sites were plotted on digital images of each section in accordance with the notes taken during recording about depths where cells v. fibers were found.

Based on this reconstruction, any responses evoked from deep white matter, cingulate cortex or sites that appeared to have crossed midline were excluded from the analysis. For SMA and M1, we considered stimulation depths ≤ 3mm to represent superficial cortical layers, and represented those sites on a
surface map at their respective stereotactic coordinate locations. Sites at depths >3mm were considered to represent deep cortical layers, and are represented in an unfolded view (Fig. 4.1C).

4.2.8 Data analysis

SPSS 17.0 and Microsoft Office Excel 2007 were used for all data analyses. Descriptive statistics were used to describe characteristics of evoked responses. The Friedman test was used to test for differences in stimulation effectiveness between the cortical motor areas. Chi-squared tests were used to investigate significant differences in proportions of stimulus-evoked effects (ipsilateral vs. contralateral responses, proximal vs. distal, etc.). A criterion level of P < 0.05 was considered statistically significant in each of these statistical tests. The Bonferroni family wise correction was used to prevent Type I errors for comparisons of differences in stimulation effectiveness and proportions of stimulus-evoked effects.

4.3 Results

4.3.1 General characteristics of post-stimulus effects

Stimuli were applied at 263 cortical motor sites from 249 tracks in the left cerebral cortex spread across the primary motor cortex (M1, 108 sites), dorsal premotor cortex (PMd, 84 sites and supplementary motor cortex (SMA, 71 sites). By the final acceptance criteria, 626 post-stimulus effects (PSTE) were evoked
from 173 sites, 67% of all cortical motor sites tested. On average $3.6 \pm 2.2$ (SD) of the muscles analyzed responded per effective stimulus site.

### 4.3.2 Ipsilateral versus contralateral post-stimulus effects

Of the 626 PSTEs, facilitation was the most common type of PSTE from all cortical motor areas (81%), especially for ipsilateral effects (see Table 4.2). This trend is reflected in Fig. 4.2, where 5 of the 6 muscles responding at a single site in a single subject demonstrate facilitation and 2 demonstrated suppression. Even though there was an EMG response for contralateral CERV muscle for stimulation at this site, this response was eliminated from analysis due to cross talk for this subject.
Figure 4.1. (A) Surface map representing aggregate stereotactic coordinate records of cortical motor stimulation sites for accepted responses for the three monkeys. Green, Supplementary motor cortex (SMA); Blue, dorsal Pre-motor cortex (PMd); Red, primary motor cortex (M1). Sites double-labeled blue/red represent sites that were PMD sites in one subject and M1 in another subject based on electrophysiological data and anatomical reconstruction of stimulation sites. (B) Frontal section of SMA to illustrate reconstruction of unfolded view of SMA used for the unfolded view in C. D=cingulate sulcus (CGS); C-D represents medial wall of SMA. Black filled dots represent stimulation sites. (C) Unfolded view of SMA accepted stimulation sites. Colored circles represent accepted sites for each subject. (S1= first subject; S2, second subject; S3, third subject). Dashed horizontal lines represent the superficial (b-c) and deep (c-d) layers of SMA.
Figure 4.2. Representative record from stimulus triggered averaging for a primary motor cortex site for a single subject. Facilitation is indicated by a filled bar below the trace, suppression by an open bar. The solid horizontal lines indicate the mean EMG levels for the baseline period; dashed horizontal lines above and below the mean represent ±2 SD thresholds used for response detection. Ipsilateral, left arm muscles; Contralateral, right arm muscles. Although a response was evoked from stimulation for contralateral CERV at this site, this response was eliminated from analysis due to cross talk.
Table 4.2. Post-stimulus effects by muscle

<table>
<thead>
<tr>
<th>MUSCLE</th>
<th>Location</th>
<th>IPSILATERAL RESPONSES</th>
<th>CONTRALATERAL RESPONSES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FAC</td>
<td>SPR</td>
</tr>
<tr>
<td>CERV</td>
<td>S/T</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>LUMB</td>
<td>S/T</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>SCM</td>
<td>S/T</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>UTR</td>
<td>S/T</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>LAT</td>
<td>S/T</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>PMJ</td>
<td>S/T</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>SUPRA</td>
<td>S/T</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>MDLT</td>
<td>S/T</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>TRI</td>
<td>L</td>
<td>26</td>
<td>5</td>
</tr>
<tr>
<td>BIC</td>
<td>L</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>ECR</td>
<td>L</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>FCU</td>
<td>L</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

S/T Shoulder/Trunk; L Limb; FAC facilitation; SPR suppression

4.3.3 Effectiveness of stimulation by cortical motor area

Fig.4.3 compares the effectiveness of stimulation for each muscle by laterality (ipsilateral versus contralateral) for each cortical motor area. Effectiveness was determined as a percentage for each muscle by counting the number of sites from which a stimulus-evoked response could be observed in a given muscle divided by the total number of sites where EMG was recorded for that muscle during a stimulation attempt. A response was counted as one or more muscles being facilitated or suppressed from a given site. Overall, M1 was more effective than both premotor areas ($\chi^2 = 6.96, P = 0.031$). For M1, effectiveness varied among muscles from 3 to 35%, with the highest degree of
effectiveness found in contralateral muscles, especially in the shoulder and trunk muscles. Effectiveness was similar for PMd and SMA, ranging from 1 to 20% among muscles.

4.3.4 Differences in ipsilateral and contralateral effects by cortical motor area

As expected, contralateral responses were more prevalent overall (63%). To explore a possible difference in ipsilateral effects across cortical motor areas, chi-square analyses were performed weighted by the overall distribution of ipsilateral (37%) and contralateral (63%) effects for the observed number of StimulusTA effects in each area, and in proportion to the number of StimulusTA sites per area (Table 4.3). All three cortical motor areas produced ipsilateral effects. Overall, the premotor areas evoked more ipsilateral responses than M1, with similar proportions produced from PMd (46%) and SMA (43%). M1 produced 30% ipsilateral and 70% contralateral responses; representative of the overall pattern for the dataset.
Figure 4.3. Comparison of the effectiveness of stimulation between cortical motor areas. The effectiveness was determined as a percentage for each muscle by counting the number of sites at which a stimulus-evoked response was observed and dividing this by the total number of sites where EMG was recorded for that muscle during a stimulation attempt. M1 primary motor area; SMA supplementary motor area; PMD dorsal premotor area. **Black bars** = contralateral muscle responses; **Grey bars** = ipsilateral muscle responses.
Table 4.3. Chi Square analysis of ipsilateral effects by cortical motor area

<table>
<thead>
<tr>
<th>Cortical Area</th>
<th>StimulusTA Sites</th>
<th>Observed</th>
<th>Expected per Areaa</th>
<th>Expected across Areab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IPSI</td>
<td>CONTRA</td>
<td>IPSI</td>
</tr>
<tr>
<td>M1</td>
<td>105</td>
<td>99</td>
<td>234</td>
<td>123.21</td>
</tr>
<tr>
<td>PMd</td>
<td>83</td>
<td>77</td>
<td>92</td>
<td>62.53</td>
</tr>
<tr>
<td>SMA</td>
<td>71</td>
<td>53</td>
<td>71</td>
<td>45.88</td>
</tr>
</tbody>
</table>

a based on the overall distribution of ipsilateral and contralateral effects for the observed number in each area. b weighted as in “a” and in proportion to the number of StimulusTA sites per area. IPSI = Ipsilateral muscle responses; CONTRA = Contralateral muscle responses. (M1) primary motor cortex; (PMd) dorsal premotor area; (SMA) supplementary motor area

Next, we explored differences in ipsilateral effects by muscle location (proximal versus distal) weighted in proportion to the number of muscles per category. Ipsilateral effects were equally prominent in shoulder/trunk and limb muscles ($\chi^2 = 0.48$, P= 0.49) (see Table 4.4).

Table 4.4. Chi square analysis of ipsilateral effects by muscle location

<table>
<thead>
<tr>
<th>LOCATION</th>
<th>Count (n)</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IPSI</td>
<td>CONTRA</td>
</tr>
<tr>
<td>Shoulder/Trunk</td>
<td>8</td>
<td>149</td>
<td>269</td>
</tr>
<tr>
<td>Limb</td>
<td>4</td>
<td>80</td>
<td>128</td>
</tr>
</tbody>
</table>

Count = number of muscles represented in each category. IPSI = Ipsilateral muscle responses; CONTRA = Contralateral muscle responses. Expected numbers are based on the number of muscles per category.

4.4 Discussion

The results of the current study support previous anatomical evidence that primary and premotor cortical neurons related to ipsilateral upper limb movements exist in the macaque monkey. As expected, a greater proportion of
ipsilateral effects were produced from the premotor areas compared with M1.\textsuperscript{147}

The percentage of ipsilateral CST effects (36\%) was higher than the general distribution of between 10 – 20\% of ipsilateral effects expected based on previous anatomical data in the monkey.\textsuperscript{27, 29, 146-148, 156} However, the current study targeted motor cortex sites for proximal output areas, and the most distal muscles recorded from were extrinsic hand muscles (wrist flexors and extensors). Other studies reporting higher proportion of contralateral effects targeted more intrinsic hand and finger muscles, which based on anatomy have the largest density of CST terminals onto lamina IX motoneurons.\textsuperscript{147, 150-152}

Perhaps we may have seen a greater proportion of ipsilateral versus contralateral effects among the cortical motor areas in the current study had we included intrinsic hand and finger muscles.

It is well established that CST neurons can act through both direct and indirect excitatory and inhibitory pathways influencing both contralateral and ipsilateral spinal motoneurons.\textsuperscript{28, 139, 162} Projections from CST neurons to ipsilateral spinal motoneurons can be mediated by brainstem neurons or excitatory, inhibitory or commissural interneurons within the spinal cord.\textsuperscript{139, 162} Based on this anatomical evidence, it has been suggested that ipsilateral CST motor outputs to upper limb muscles are likely due to activation of indirect cortico-bulbar connections or crossed connections at the spinal cord level. However, StimulusTA is thought to act mainly by relatively direct mono and disynaptic pathways,\textsuperscript{75} and often resembles post-spike effects produced by
single neurons recorded at the same motor cortex site.\textsuperscript{23, 75, 135} Thus, in the current study is it likely that StimulusTA revealed the output of ipsilateral CST effects through relatively direct projections to spinal motoneurons.

4.5 Conclusions

The present investigation provides further electrophysiological evidence that primary and premotor cortical areas can recruit proximal and distal muscles in the ipsilateral limb fairly directly. What we learn about the functional anatomy of the corticospinal tracts can help us to understand their contributions to motor control of the upper limbs, especially after injury such as stroke.
5.1 Introduction

Stroke is a leading cause of permanent long-term disability, and the loss of upper extremity motor function is one of the most devastating consequences. The prognosis for recovery of arm function remains poor. Over half of stroke survivors have chronic motor impairments and very few ever regain full use of the arm. We do not fully understand the basis for these chronic upper limb impairments following stroke or the neural substrates underlying recovery. In order to develop more effective therapeutic interventions to address these deficits, we require better understanding of the neural substrates that underlie normal upper limb motor function and recovery following stroke.

After stroke, altered reaching is evident in the arm contralateral to the lesion, along with subtle deficits in movement control in the ipsilateral arm. Biomechanical analyses in humans demonstrate that movements of the contralesional limb are constrained to a predominance of specific whole limb flexion or extension muscle patterns (synergies) after stroke. Reaching movements up and across the body, requiring coordination of elbow extensors with shoulder flexors and horizontal abductors or adductors, are most notably
Comparisons between contralateral and ipsilateral limb reveal abnormal muscle co-activation patterns, with difficulty moving in directions requiring muscles co-activate in combinations different from the natural synergies. The patterns of recruitment observed in these synergies are consistent with the patterns associated with pontomedullary reticular formation (PMRF) motor outputs in animal studies. Drew et al demonstrated that microstimulation of PMRF in awake cats produced flexion of the ipsilateral limb, extension of the contralateral limb, and head rotation toward the flexed limb. Findings from our lab confirm that these patterns reflect typical PMRF motor output in the monkey. These findings are consistent with the hypothesis that increased reliance on the reticulospinal system as an alternative motor pathway is the reason these synergistic patterns are seen after cortical stroke.

Anatomical studies have shown that a unique relationship exists between the cortical motor areas and the PMRF. In addition to sending direct projections to spinal motoneurons, all three cortical motor areas send projections bilaterally to the PMRF. By receiving bilateral projections from the cortical motor areas, the PMRF may receive information regarding control of the upper extremity function from both cortical hemispheres. Both the corticospinal (CST) and reticulospinal (RST) system have been shown to contribute to bilateral cortical control of spinal motoneurons. Since both systems are known to terminate on interneurons in the intermediate zone related to motoneurons of axial and proximal limb muscles, in the absence of CST control, RST
pathways may be capable of controlling independent limb movements in addition to integrated whole body/whole limb movements. This was demonstrated in monkeys where bilateral pyramidal tract lesions initially resulted in severe paresis of the extremities followed by recovery of gross extremity function, suggesting CST collateral branches to brainstem structures exist.\textsuperscript{30, 31} Brainstem pathways, such as the RST may be largely responsible for gross extremity motor control that gradually reemerged after pyramidal tract lesions. These results suggest recovery after CST lesions may be due to activity of RST pathways.

Both animal and human studies have primarily focused on changes in activation within cortical motor regions for distal hand function recovery. Besides the CST, other descending pathways conveying motor outputs also undergo reorganization. Small et al\textsuperscript{65} showed correlation of changes in activation in the cerebellar hemisphere opposite the lesioned M1 with recovery. Reorganization in rubrospinal motor outputs to forearm muscles in monkeys also occurs following a unilateral lesion.\textsuperscript{34} The evidence from these studies and the anatomical and electrophysiological evidence of cortico-reticulo-spinal influences on axial and proximal limb muscles suggest neural plasticity within motor outputs from the PMRF as a contributor to recovery is a reasonable expectation. However, despite long-standing clinical theories that the RST is a source of upper limb recovery,\textsuperscript{50, 57} there are no studies investigating reorganization of upper limb motor outputs from the PMRF following focal motor cortex lesions.
Animal models of ischemic stroke provide invaluable contributions to our understanding of neural substrates associated with recovery of function. The macaque monkey and humans both have a substantial portion of the CST that makes monosynaptic connections from pyramidal tract neurons in the cortical motor areas to spinal motorneurons.\textsuperscript{103-105} Hence, this makes the macaque an excellent model for the study of stroke that would be translatable to the human. However, because of the macaque’s more gyrencephalic cortex, the cerebral blood vessels are incompletely accessible, making experimental methods such as electrocoagulation\textsuperscript{43} not feasible for creating a controlled focal ischemic injury in this species. An alternative model used extensively in rodent models\textsuperscript{91, 94} and the marmoset\textsuperscript{81} is to induce an ischemic injury by either topical application or stereotaxically guided microinjections of a potent vasoconstrictive peptide, Endothelin-1 (ET-1) directly into the brain tissue.\textsuperscript{90, 94-96} Because the purpose of this study was to investigate the contributions of the RST to recovery of arm function, we wanted a model that allowed selective injury of the proximal shoulder/elbow motor area while leaving distal arm/hand motor function intact to the extent possible. Intracortical ET-1 injections appeared to be a viable method for creating a controlled focal ischemic injury.\textsuperscript{94-96} However, to our knowledge, there are no published reports of ET-1 in the macaque monkey.

Therefore, the purpose of the current study was two-fold: 1) to investigate the effectiveness of ET-1 in inducing a focal ischemic infarct in the macaque motor cortex, and 2) to explore whether recovery of arm function following a focal
ischemic cortical lesion in the shoulder/elbow representation of M1 is associated with reorganization of upper limb motor outputs from the ipsilesional or contralesional M1 or PMRF or both. Unlike existing stroke studies in the monkey focusing on recovery of distal hand function, this project studied proximal upper limb control. Our question was, what role does the reticulospinal system play in recovery of arm function following cortical ischemic infarct? The typical motor output pattern for the PMRF is a reciprocal pattern of ipsilateral flexor and contralateral extensor facilitation with ipsilateral extensor and contralateral flexor suppression.\textsuperscript{5, 6, 8-10} The question becomes, do we see changes in this response pattern after recovery? Would we see an increase in variety of movement combinations elicited by PMRF stimulation outside the typical response pattern? For example, after recovery, could stimulation elicit facilitation of ipsilateral shoulder flexors with elbow extensors? Our hypothesis was that recovery of reaching abilities elicited in the contralateral limb deviating from the stereotypical whole limb flexion or extension synergies may be correlated with adaptive plasticity of motor output patterns from both sides of the PMRF.

This paper is a compilation of four individual case studies. The paper begins with the experimental methods common to all four subjects, and then refinements to these methods are described within each individual subject’s method section. Results are divided into anatomical, physiological and behavioral findings for each subject.
5.2 General methods

Fig. 5.1 Behavioral Reach Task. Overhead view of the monkey seated in primate chair with non-reaching arm restrained (indicated by yellow strap) to the table top (white outlined area). Non-reaching arm was positioned at her side in approximately neutral shoulder flexion/abduction and 90° elbow flexion. Black square centered in front of the monkey on the table top represents start switch. The grey box is a schematic diagram of the front of the reaching task apparatus. The apparatus was attached to the table so that the front of the box made a 90° angle with the table. The center of the display was centered on the subject’s reaching shoulder. The four white squares represent positioning of the four food well doors. The numbering on the doors illustrates the Cartesian coordinates used for ordering reaching trials during assessments and training. 1 and 2, high targets; 3 and 4, low targets. These numbers are for illustration purposes only. No visible numbers were used during the actual task. Red and green circles represent LED lights centered on the display at eye level.

5.2.1 Subject and task

Subjects were four female monkeys (M fascicularis) trained to perform a unilateral reaching task with either arm. The motor task required monkeys reach to retrieve food rewards, with the direction of reach positioned to facilitate co-activation of elbow extension with shoulder flexion and horizontal
abduction/adduction. These combinations were chosen to make specific challenges on muscle synergies known to be affected by human stroke. The task consisted of four targets; two high (eye level) and two low targets (waist level) (Fig. 5.1). The subject sat in a primate chair with both arms resting on a table top, facing the reach apparatus. The subject's non-reaching arm was restrained at her side, with the shoulder positioned in neutral flexion/abduction, elbow in 90 to 95 degrees flexion, with the forearm resting on the table (see Fig. 5.1). The center of the display was centered on the shoulder of the reaching arm. Target positions required subjects reach either across the body (crossing midline) to targets in line with the opposite shoulder, or to targets five inches lateral to the body. The centers of targets were ten inches apart horizontally and six inches apart vertically, and their locations are numbered in quadrants corresponding to standard Cartesian coordinates, as indicated in Fig. 5.1. The food wells were instrumented with photoelectric sensors (IDEC Corporation) that detected entry and exit of the hand from each well. The doors to each food well were instrumented with simple momentary switches (Crouzet Corporation) that detected when the door was opened and closed.

To start a trial, the subject rested her hand on the table top in front of her body. A green ‘ready’ light in the center of the display, at eye level, was then illuminated indicating the beginning of a new trial. The subject then depressed the start switch centered in front of her body, requiring no dexterity and no more than 25 grams of force to actuate. When the start switch was depressed, a red
‘go’ light just above the green light was illuminated, indicating a requirement to reach when the food well door opened. The subject was required to hold the start switch closed until the food well door opened. The hold time on the start switch was random between one to two seconds. Once the red light was illuminated, the researcher manually opened the selected food well door. After three seconds, if the subject had not begun to reach for the food, the ready light was extinguished and the food well door was manually shut, terminating the trial. If the subject began the reach within three seconds, she was allowed to continue as long as she was attending to the food and persisting in her effort. However, if the subject gave up or became distracted, the door was shut and the trial was terminated. Then the task was reset for the next trial.

For each behavioral reach assessment, subjects performed two to three sets of 24 reaches with each arm. The subject was required to reach to each target six times within each set of 24 reaches. Thus, subjects performed 25% (12/48) of reaches to each target with each arm. Target order was randomized during each set of 24 reaches in attempt to prevent order effect. The subject alternated arms after each set of 24 reaches. The order for arm tested was randomized for each testing session to prevent order effect. The monkey’s unimpaired limb was used as the animal’s own control.
5.2.2 Assessment of motor performance on the behavioral reach task

The subject was trained on the behavioral reach task for several months. Once 85% success on the task was achieved, baseline performance was measured and surgery was carried out. Then, reaching performance was tested at set time points after ischemia (see Fig.5.2).

![Timeline for Experimental Design](image)

Figure 5.2. Timeline for Experimental Design

The time required for the hand to leave the start switch, enter the well, and exit, as well as success or failure in retrieving and eating the food, was noted for every trial. Custom-written software was used to locate task-related events (onset of trial, movement initiation, entry and exit of food well) within each trial. Each trial was recorded on videotape and later analyzed frame-by-frame to assess times of task-related events and quality of reach performance such as number of entry attempts before success or failed entry attempts. From these
times, mean reaction times, movement times (hand to target), dwell times (time
to retrieve reward from well) and transport times (hand to mouth) were calculated
for each target direction.

Reach performance was divided into two phases, a movement phase and
a transport phase. The movement phase of the reach was defined as the time
the hand left the start switch until the hand entered the well. The transport phase
was defined as the time the hand exited the well with the food until the monkey
brought the hand to mouth. Dwell time was used to characterize the time
required to retrieve the reward from the well. This time started with initial hand
entry into the well, included manipulation of food in the well, and concluded when
the hand was withdrawn from the well for the last time for the given trial.

5.2.3 Animal care

Subject care complied with the NIH Guide for the Care and Use of
Laboratory Animals and the approved animal care protocol for our lab. Surgeries
were performed under veterinary supervision in aseptic conditions. Animals were
pre-treated with antibiotics (Florfenicol) and were pre-anesthetized with Ketamine
HCL (10 mg/kg im). Atropine (0.03 mg.kg s.q.) was given pre-operatively to
decrease secretions and facilitate intubation. Following tracheal intubation, the
animal was anesthetized with isoflurane gas (1-2%) and placed in a stereotaxic
frame. During the microstimulation portion of the procedure, the isoflurane was
discontinued and Ketamine and Dexmedetomidine (alpha-2 agonist) were
administered IV with boluses supplied as needed based on anesthetic plane. A
local anesthetic (bupivicaine) was injected into the tissue around the incision every 3-5 hours. Mannitol (1g/kg IV) was administered every 8 hours to reduce intracerebral pressure and prevent the likelihood of cerebral edema. Fluid levels, glucose levels and electrolyte balance were closely monitored throughout the procedure. Core temperature was monitored using a rectal probe and a convective air warming system maintained body temperature to within 99 to 101°C for the entire procedure (typically 16-18 hr). An indwelling catheter was inserted into a hindlimb vein for delivery of intravenous fluids. ECG, pulse oximetry and capnography were used to monitor physiological status. Venous blood gases were drawn every 2 to 6 hours.

A removable bone plug was fabricated using dental acrylic and secured by suturing temporalis muscle and skin closed to allow for ease of reaccess for subsequent surgeries which require repeating the microstimulation portion of the study. Antibiotics and non-steroidal anti-inflammatory analgesics were administered postoperatively. Subjects required physical assistance with natural cage activities such as sitting balance, positioning the affected limb(s), hygiene and feeding during the initial hours to first day following surgery. During the two week spontaneous recovery period subjects required frequent monitoring throughout the day and assistance was provided with feeding and hygiene activities as needed if the monkey was unable to perform these activities independently. Subjects received water ad libitum in their cages. Monkey chow and fruits and vegetables formed the balance of their diet.
5.2.4 Electrophysiological mapping procedure

Microstimulation techniques described by Cheney and Fetz\textsuperscript{75} were used to map motor outputs of right and left cortical motor shoulder/elbow representation areas for all four monkeys. Microstimulation techniques previously described by Davidson and Buford\textsuperscript{9} were used to map upper limb motor outputs from PMRF bilaterally in two monkeys at set time points after infarct (subject A03354 and A01183). Outputs to both cortical and PMRF stimulation were verified by visible movement and muscle contractions. Cortical and PMRF motor outputs were mapped at set time points across the study for each subject.

Cortical stimuli were delivered through a glass-insulated platinum iridium microelectrode [(impedance 1MΩ)(Frederick Haer, Bowdoinham, ME)]. The electrode was introduced on a grid pattern (1 mm interpenetration distances) using central sulcus as the caudal border and spur of arcuate sulcus as the rostral border. At each penetration site, the electrode was held vertical relative to the stereotaxic frame and then lowered to depths ranging from 2.5 to 10.5 mm, targeting layer V (the location of the corticospinal neurons). The territorial extent of the area 4 shoulder/elbow representation was explored as completely as possible using the following criteria. Penetrations were made at each coordinate of a 1 mm grid pattern until the shoulder/elbow representation was circumscribed by 2 mm of sites evoking other body part movements such as lower extremity, trunk or facial movements or no visible responses.
The subject was maintained in a prone position in the stereotaxic frame with the limbs consistently supported. Outputs to stimulation were verified by visible movement, muscle contractions and EMG responses. At each site, the visible movement or muscle contraction evoked by stimulation at near-threshold current levels was determined by the following procedure. Current was gradually decreased from 80 μA by applying a stepwise current change of 10 μA until the movement disappeared, and then gradually increased again in 5 μA steps to determine the lowest current level required for evoking the movement by at least three consecutive train bursts. This current was defined as the threshold current. The response evoked at threshold current level was called the threshold or primary response. Each track was explored in 0.5 mm increments up to depths of 10.5 mm at the more lateral cortical sites, and if a response was not evoked at 80 μA, stimulation was halted and the site was designated as non-responsive.

PMRF stimuli were delivered through a tungsten epoxy and polyimide insulated electrode (Frederick Haer, Bowdoinham, ME). The electrode was conditioned with gold plating on the tip to produce recording impedance of 100 to 200 KΩ. The electrode was inserted through a thin-walled stainless 23-guage steel guide cannula. The electrode was positioned using stereotactic coordinates and lowered into the brainstem using a manual hydraulic microdrive. Stimulation sites were in the right and left PMRF. The dorsal boundary of the stimulating region was the abducens nucleus and eye-related sites; the ventral boundary was the inferior olive and pyramidal tract; the lateral boundary was the spinal
trigeminal nucleus. Sites estimated to be on the midline or less than 0.5 mm lateral to midline were excluded from subsequent analyses. Arm-related sites were identified by visibly evoked movements in response to stimulation. Stimulation was applied at 1.0 mm intervals for subject A01183 and 0.5 mm intervals for subject A03354 as the electrode was withdrawn from the bottom of the tracts.

5.2.5 Stimulation procedure

For each site, a train of 36 biphasic pulses was applied at 333 Hz (pulse duration 0.2 ms/phase). Ten stimulus trains were applied at each stimulation site at threshold, 1.5 times threshold and then a fixed current of 30 μA, as controlled by a constant current stimulus isolator. At each site, the electromyographic (EMG) activity was recorded from all implanted muscles during all three stimulation current conditions using Spike2 software.

EMG was recorded from percutaneous fine-wire electrodes. Percutaneous EMG electrodes were pairs of multi-stranded, Teflon-coated stainless-steel wires implanted in eight arm and shoulder muscle pairs per arm; sixteen total. The muscles tested were extensor carpi radialis (ECR), flexor carpi ulnaris (FCU), biceps (BIC), triceps (TRI), latissimus dorsi (LAT), pectoralis major (PMJ), middle deltoid (MDLT), and upper trapezius (UTR).
5.2.6 Cortical infarct procedure

After the cortical electrophysiological mapping procedure was completed, a focal ischemic infarct was created in the shoulder/elbow representation area of left primary motor cortex (M1). Microinjections of Endothelin-1[(ET-1; 400pmol/μl in sterile H₂O); human, porcine endothelin-1(American Peptide, USA)], a potent vasoconstrictive peptide, were used to induce a focal ischemic injury of the cortical tissue. Using a 10-μl Hamilton Syringe, ET-1 was injected into left M1. The needle was lowered and after 30 seconds ET-1 was injected at a rate of 1.0 μl/2 min with a 30 sec pause between each 0.25 μl and a 1 min delay before needle withdrawal. The location and appropriate depths of injection sites were guided by stereotactic coordinates and based on microstimulation mapping results, targeting the shoulder/elbow representation and avoiding wrist and hand representations to the extent possible. The dosage and number of ET-1 injections were chosen based on previous studies in rats showing that small quantities produced localized ischemic lesions through all six cortical layers while sparing the underlying white matter.

All four monkeys received a focal ischemic cortical infarct targeting the shoulder/elbow representation of left M1. The first monkey (A01183) received 10 injections of ET-1 targeting sites distributed throughout the shoulder/elbow representation. We found this procedure to be less effective than planned due to substantial recovery of behavioral performance. Thus, the other three monkeys (A01219, A03354 and A01074) received two rounds of ET-1 injections to every
site that evoked a shoulder or elbow response to stimulation. Expanding the protocol to two rounds of ET-1 injections at every shoulder/elbow site increased the time to complete this part of the experiment from approximately 30 to 40 minutes for the first subject to nearly two hours for the other three subjects.

5.2.7 Magnetic resonance imaging acquisition and analysis

All scans were acquired using the Biospec 94/30 (Bruker BioSpin, Germany) with a horizontal 30 cm bore magnet operating at 400 MHz (9.4T). The monkey was intubated and anesthetized with 1.5 to 2% isoflurane gas throughout the scans. She was scanned in the supine position using a volume coil 1 H, rabbit body (154 mm inner diameter). Scans were acquired before the first electrophysiological mapping surgery (baseline), after nine to ten days post-infarct (spontaneous recovery) and after completion of rehabilitation (for A03354 only). T1 and T2-weighted magnetic resonance (MR) images were acquired from gradient-echo and spin-echo multi-slice imaging sequences. Coronal, axial, and sagittal scans through the cortex and brainstem were acquired in contiguous (1 mm slice thickness) slices spanning the brain.

5.2.8 Estimation of lesion location and volume

MR images were used for confirmation of ET-1 injection site locations, lesion location and calculation of lesion volume. MR image analysis was performed using NIH Image-J v1.43 software (Bethesda, MD, USA). Coregistration of MRI scans and neurophysiological maps were used for ET-1
injection site reconstruction. Pre-infarct T1-weighted MR images acquired in the coronal plane were selected based on actual stereotactic coordinates of injection sites, using the Szabo and Cowan atlas\textsuperscript{171} as a reference. The rostral edge of the precentral dimple was used as the relative reference point to co-register the MRI images with the stereotactic coordinates. Then, each ET-1 injection site was marked on the MR images according to the stereotactic coordinate records (see Fig. 5.3 for example).

The lesion was reconstructed from MR images of individual 1-mm contiguous sections that comprised the region of interest (ROI). An alignment of the sections allowed reconstruction of the location and extent of the lesion on a coronal view of the brain. The abnormal bright area on the image (ROI) defined as signal hyper-intensity was manually traced slice by slice in T2-weighted images, and lesion volume was calculated by multiplying the total area of traced ROI by slice thickness. The ROI was manually traced on three separate occasions and the individual volume measurements were used to calculate a mean lesion volume (± standard deviation). A RARE (rapid acquisition with relaxation enhancement) sequence was used for acquisition of T2-weighted coronal images used for lesion measurements because of their greater sensitivity for grey matter,\textsuperscript{172} thus providing higher resolution for manually tracing the ROI.

5.2.9 Reproducibility of measurements

Manual ROI tracing methods are established for lesion volume measurements.\textsuperscript{102} To test for intra-rater variability with the manual tracing
method for the current study, the same researcher (WJH) outlined the ROIs on the same MRI set on three separate occasions and calculated the coefficient of variation (CV). The CV ranged from 2 to 6%, thus, indicating strong repeatability with manually ROI tracing for measuring lesion volume.

5.2.10 Analysis procedure for change in cortical movement representation

First, the total number of sites explored was calculated for left and right motor cortex. Next, the total number of sites explored was subdivided into movement areas represented. Sites were coded as shoulder, elbow, wrist, hand/fingers, forearm (supination/pronation), leg/tail (hip/knee/ankle/foot/toes), or trunk and face (included anterior neck) representations. A site was coded as a dual response site if movement was evoked at two separate joints (ie. shoulder and wrist, elbow and wrist). A site was coded as complex if movement was evoked at more than two joints, movement was evoked in the upper limb ipsilateral to stimulation, or movement was evoked bilaterally.

The arm area representation included all sites at which electrical stimulation elicited movements of digits, hand, wrist, forearm, elbow or shoulder. The proportion of arm-related sites for right and left motor cortex was calculated from the aggregate of single, dual and complex arm-related sites and presented as a percentage. From this value, the proportion of shoulder/elbow and wrist/hand/forearm dedicated sites was determined. Dual and complex movement sites were described separately. Each animal’s pre-infarct cortical motor maps were used as its own control. This approach enabled us to explore
longitudinal changes in arm-related movement representations after infarct, and was similar to approaches used by other cortical mapping studies characterizing motor output organization of specified representation areas.\textsuperscript{36, 42, 43, 55, 74}

5.2.11 Data analysis

SPSS 17.0 and Microsoft Office Excel 2007 were used for all data analyses. Descriptive statistics and repeated measures ANOVA were used to explore differences in arm and target direction on behavioral reach performance. Chi-squared tests were used to investigate significant differences in proportions of cortical and PMRF arm movement representation areas (ipsilesional vs. contralesional responses, shoulder/elbow vs. wrist/hand/forearm, etc). The Friedman Test was used to investigate significant differences in threshold currents for movement representation areas within right and left motor cortex post-infarct. A criterion level of $P < 0.05$ was considered statistically significant in each of these statistical tests The Bonferroni family wise correction was used to adjust the $P$ value for the threshold current analyses and changes in cortical motor output map comparisons.
5.3 Experiment one (subject A01183): ischemic injury with PMRF and cortical mapping at baseline and two weeks post

5.3.1 Methods

5.3.1.1 Electrophysiological mapping procedure

Under sterile conditions, two limited craniotomies (approximately 3.5 cm diameter each) were made over the left and right frontoparietal cortex and the dura mater was removed allowing access to M1 and PMRF. The animal was administered Ketamine HCL intravenously at the rate of 14mg/kg/hr, and after about 20 minutes were allowed for infusion to take effect, the isoflurane was withdrawn. In addition, Dexmedetomidine was administered at the rate of 0.15 ml every two hours IV. The rate of Ketamine HCL and Dexmedetomidine infusion was occasionally adjusted to maintain a relatively stable anesthetic state. Repetitive microstimulation mapping was only conducted during periods of stable anesthesia. It was halted during periods of light anesthesia marked by excessive muscle tone in the forelimb muscles, or during periods of deep anesthesia marked by unusually high thresholds or non-responsive sites in the midst of M1 that should have evoked responses. When in doubt about the depth of anesthesia, threshold at a previously responsive site was re-tested, and if necessary, anesthesia was reduced until the previously obtained threshold was reproduced to within 5 µA.
Microstimulation techniques described above were used to map motor outputs of right and left cortical motor shoulder/elbow representation areas and upper limb motor outputs from PMRF bilaterally. Right and left cortical motor outputs were mapped at two time points across the study; pre-infarct and two weeks post-infarct. Right and left PMRF upper limb motor outputs were also mapped pre-infarct and two weeks post-infarct.

5.3.1.2 Detection and analysis criteria for PMRF responses

A custom written Spike2 software program was used to compile results from stimulus trains. EMG data were averaged for each stimulation site on a muscle by muscle basis. An 89 ms period beginning 99 ms before stimulus train onset was used to calculate the mean baseline level of rectified EMG activity. Having a baseline period end before the stimulus train began prevented any effects of stimulus artifact from entering baseline. The 105 ms stimulus train was divided into two equal duration periods of 36 ms each for response detection, beginning 33 ms after stimulus train onset. If the onset of responses occurred during the first period (the middle 36 ms), we theorized that these were most likely the result of relatively direct pathways, and responses with onset latencies during the last 36 ms period, were theorized to be most likely the result of polysynaptic pathways. Based on our experience, early responses are evident in the first period and all responses, early and late, are evident in the second period. For the present analysis, we did not distinguish between early and late responses.
Using a 2 standard deviation excursion from baseline as the acceptance criteria\(^8,75\) for suppression in the anesthetized monkey for this study was found to be too stringent. With little background EMG to begin with, these responses were relatively hard to detect, especially suppression, causing responses to be rejected that based on visual inspection should have been accepted. Therefore, new objective criteria for EMG response acceptance were determined and the validity of these criteria was evaluated for their ability to test whether a stimulus-evoked EMG response was really present or not, especially during suppression.

Upon inspection, during responses that appeared to be suppression, not only did the mean level of the EMG decrease, but the variability in the EMG baseline decreased as well; it became quiet. To measure this, in addition to the stimulus train EMG dataset based on mean EMG levels, we created a dataset of the averaged standard deviations of the EMG levels, which we referred to as the standard deviation plots. This provided further comparison of variability between baseline and potential responses detected for analysis. EMG response acceptance was based on three criteria: 1) peak excursion of response from baseline mean, described as a Z-score; 2) percent change of the mean response from baseline; and 3) percent change of the mean standard deviation of the response from the mean standard deviation of the baseline. A Z-score of \(\geq 1.96\), percent change of the response mean from baseline mean of \(\geq 30\%\) and percent change of the standard deviation plot of \(\geq 30\%\) were required to detect facilitation. Different criteria were required to detect suppression because there
was little background EMG to begin with, making suppression relatively hard to detect. A Z-score of $\leq -0.75$ and percent change of the response mean of $\leq -30\%$ and percent change of the standard deviation of $\leq -30\%$ were required to detect suppression. A visual inspection of all the responses was compared to the objective criteria, taking the visual inspection as the gold standard. Visual inspection was based on past experience with response detection. For questionable cases where there were discrepancies between visual inspection and objective criteria for response detection, the response was manually marked using a minimum $\pm 2$ SD threshold as the cut-off for clarification. The objective criteria was used to form the dataset. The sensitivity of the acceptance criteria was good at 65 to 81%, and the specificity of the acceptance criteria was strong at 87%. Visual inspection was still required to omit spurious responses for which the measurements were contaminated by stimulus artifact.

5.3.1.3 Cortical infarct procedure

From the descriptions of the lesion volumes in rat studies,\textsuperscript{94-96, 170} we theorized the spread of ET-1 to be approximately 1.5 to 2 mm per injection. Therefore, for the present study the ET-1 injection sites were staggered about 2 mm apart within the shoulder/elbow representation area for optimal coverage. Ten 1 $\mu$l injections were made within the shoulder/elbow representation (see Fig 5.3 and Fig. 5.5a for location of injection sites).
5.3.1.4 Assessment of motor performance on the behavioral reach task

The monkey was trained on the behavioral reach task for several months. Once 85% success on the task was achieved, baseline performance was measured. Then, reaching performance was tested on days three, seven and thirteen after ischemia.

5.3.1.5 Magnetic resonance imaging acquisition and analysis

Scans were acquired before the first electrophysiological mapping surgery (baseline) and then nine days post-infarct. T1-weighted fast low-angle shot (FLASH) images and T2-weighted rapid acquisition relaxation enhancement (RARE) images were obtained from gradient-echo and spin-echo multi-slice imaging sequences. Multiple coronal, axial, and sagittal brain slices through the cortex and brainstem were acquired in contiguous slices (1 and 2 mm thick) spanning the brain.

5.3.1.6 Histological procedure

Two days after the final electrophysiological mapping study the subject was deeply anesthetized with a lethal dose of sodium pentobarbital and then perfused transcardially with phosphate-buffered saline followed by phosphate buffered para-formaldehyde. This was followed by a perfusion with 10% sucrose in phosphate buffer, followed by 30% sucrose in phosphate buffer. The brain was then removed and soaked in a 30% sucrose solution for cryoprotection.
5.3.2 Results

5.3.2.1 Anatomical Results: Ischemic infarct size and location

10 1µl ET-1 injections were made in the shoulder/elbow representation of left M1, covering approximately 45% of the movement area. As stated in METHODS, the distribution and depth of ET-1 injection sites was identified based on electrophysiological data. ET-1 injection depths ranged from 2.5 to 4 mm. The placement and distribution of ET-1 injection sites in the left primary motor cortex (M1) is shown in Fig. 5.3 and 5.5. Based on our reconstruction, injections were made within the superficial and deep layers of the motor cortex.

At 9 days post-infarct, volumetric analysis was carried out by manually tracing the outer edge of the hyper intense lesioned tissue in the T2-weighted MR images in the left motor cortex using the Image-J software. Eight image slices were affected, with a volume mean of 281.87± 6.4 (SD) mm³ that involved the pre-central gyrus and extended through all layers of grey matter and into the superficial white matter. No abnormal signal intensity changes were noted in subcortical structures (see Fig. 5.4).
Figure 5.3. Coregistration of MRI sections and neurophysiological map was used to reconstruct ET-1 injection site locations (indicated by white markings) in left motor cortex with respect to stereotactic coordinate records. **A.** T1-weighted MRI scan (2 mm slice thickness contiguous slices) acquired in the coronal plane (TR=500.4, TE=4.854, flip angle = 35º) was used for reconstruction. Images are ordered from rostral (left) to caudal (right). Since images were 2 mm slice thickness, each scan represents 2 mm of the injection grid. The laterality of each site was referenced from midline of the rostral surface of each scan. Dashed line represents arbitrary demarcation between motor and sensory cortex. CS, central sulcus; LS, lateral sulcus; STS, superior temporal sulcus; CGS, cingulate sulcus; M1, primary motor cortex; S1, primary sensor cortex; SMA, supplementary motor cortex. Scale bar = 4 mm. **B.** Schematic representation of target area for ET-1 injection sites in left motor cortex outlined by dark grey box. PRCS, pre-central sulcus (dimple); PCS, post-central sulcus; CS, central sulcus; LS, lateral sulcus; TS, temporal sulcus; AS, arcuate sulcus.
Figure 5.4. Represents the ET-1 induced lesioned tissue in the left motor cortex. T2-weighted MRI scans (1 mm slice thickness contiguous slices; 0.43 x 0.43 mm pixel spacing) acquired in the coronal plane were used to manually trace the region of interest. Region of interest (ROI) was defined as the area of abnormal signal intensity change (outlined in yellow). Coronal images through the lesion are arranged by rows from rostral (top left) to caudal (bottom right). Abnormal signal intensity changes were manually traced on each individual section using Image-J software. The estimated lesion volume was then calculated by multiplying the total ROI area (mm²) by slice thickness.
5.3.2.2 Physiological results

We first tested to determine whether a comparable number of sites were explored in the left and right cortex to ensure that any difference in responsiveness was not due to experimental bias. Overall, the total number of sites explored in the left versus right motor cortex, and the number of sites explored within each motor cortex were similar between pre- and post-infarct mapping procedures ($\chi^2=3.27$, $P=0.07$).

5.3.2.2.1 General organization of M1 arm area before and after ischemic infarct

On a surface map of left and right M1 (Fig 5.5a), each electrode penetration is represented at its corresponding position by a color indicating the body territory activated at threshold current. Arm area(s) was defined as a cortical region where microstimulation at threshold elicited movements of the shoulder, elbow, forearm (supination, pronation), wrist and fingers. The post-lesion maps, established after two weeks of spontaneous recovery, are shown in Fig 5.5b. For both pre- and post-lesion maps, the shoulder/elbow representation was bounded medially by trunk and lower limb movements, rostrally by face movements and non-responsive sites, laterally by wrist/hand and forearm movements and arcuate sulcus (AS), and caudally by the central sulcus (CS). In figure 5.5a, sites where ET-1 was injected are indicated with a syringe symbol ( ).
Fig. 5.5 Surface map of microstimulation evoked motor outputs from right and left motor cortex a pre-infarct, b post-spontaneous recovery period. Each electrode penetration is represented at its corresponding position by a color indicating the body territory activated at threshold current. Shoulder/elbow representation area was circumscribed by 2-mm of other movements or non-responsive sites. Rostral and caudal boundaries indicated by arcuate sulcus (AS) and central sulcus (CS); Middle Cerebral Artery and branches indicated by red lines. Dual response sites are represented by both body territory colors. Sites that evoked bilateral arm responses are outlined in black; star symbol indicate complex and whole arm responses. ET-1 injection sites are indicated by the syringe symbol.

*continued*
5.3.2.2.2 Ipsilesional cortical changes

The majority of the 71 sites explored in the ipsilesional (left) motor cortex pre-infarct (63%) evoked visible arm-related movement responses to stimulation. This was a consequence of the study design, which focused on mapping the arm representation of the motor cortex. Of the 45 arm-related sites, 44% evoked shoulder/elbow movements, 47% evoked wrist/hand/forearm movements, and 4% evoked complex movements of hip and shoulder or whole arm movements. After the two week spontaneous recovery period, there was relatively little
change in the overall proportion of arm-related responses, with 50% of the 60 sites explored evoking arm-related responses to stimulation. Surprisingly, most sites effective before the ET-1 induced lesion remained microexcitable after the lesion; there was no zone of non-responsive sites where the ET-1 was injected. As expected, the number of arm-related sites eliciting shoulder/elbow movements post-infarct was reduced (8 of 30 arm-related sites) (Fig. 5.5b). This result is supported by previous work in adult squirrel monkeys that received focal ischemic lesions in M1 hand area, which showed that without training, movements formerly represented in the infarcted zone did not reappear in adjacent cortex. In the current study, after spontaneous recovery, wrist/hand/forearm movement representations expanded into the shoulder/elbow territory (see Fig. 5.5b).

5.3.2.2.3 Contralesional cortical changes

Similar to the lesioned cortex, the majority of the 55 sites explored in the contralesional (right) motor cortex pre-infarct (67%) evoked visible arm-related movement responses to stimulation. Of these 37 arm-related sites, the proportion of sites evoking shoulder/ elbow movements (41%) and wrist/hand/forearm movements (54%) was similar ($\chi^2=0.71$, $P=0.398$). Again, there was relatively little change in the overall number of sites explored that elicited arm-related movements after spontaneous recovery (51% of 71 sites). However, both the proportion of sites eliciting shoulder/elbow movements (8 of 36 sites) and wrist/hand/forearm movements (9 of 36 sites) post-infarct were
reduced. Surprisingly, the novel change was a shift in sites that pre-infarct evoked single joint shoulder/elbow or wrist/hand/forearm movements of the contralateral upper limb to 36% of sites post-infarct that elicited slow, complex bilateral shoulder and bilateral elbow movements, and one site that evoked ipsilateral arm responses (see Fig. 5.5b).

5.3.2.2.4 Comparison of ipsilesional and contralesional cortical changes

When changes in arm representation area for the ipsilesional and contralesional pre-infarct cortical maps to post-infarct maps were compared, we found the greatest reduction in the number of sites that evoked arm-related responses in the ipsilesional cortex after recovery ($\chi^2=4.65$, $P=0.031$). This was expected since the study aimed to create a focal lesion in the arm-representation of left primary motor cortex. Next, changes of specified movement categories within the arm representation areas (shoulder/elbow and wrist/hand/forearm) for the ipsilesional and contralesional motor cortex pre- and post-infarct were compared using the Chi-square test weighted by the number of arm-related sites tested for each motor cortex (see Fig. 5.6). The largest difference was found in the proportion of wrist/hand/forearm (distal arm) responses post-infarct ($\chi^2=12.76$, $P=0.005$). After spontaneous recovery, not only was there expansion of the distal arm representation for the ipsilesional cortex and reduction of the distal arm representation for the contralesional cortex, but there was also a change in responsiveness of sites in the contralesional cortex that prior to the
infarct had evoked single joint responses, but after the recovery evoked complex and bilateral arm responses (see Fig.5.5b).

### Change in Arm Representation Areas

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5.6. Represents change in proportion of sites that evoked shoulder/elbow and wrist/hand/forearm related responses for ipsilesional (left) and contralesional (right) cortex pre- and post-infarct. Results are presented as a percentage of the total number of arm-related sites for each motor cortex. Blue bars = shoulder/elbow sites; Red bars = wrist/hand/forearm sites

### 5.3.2.2.5 Arm representation threshold current comparison

Next, we explored whether changes in threshold current levels (µA) were required to evoke the specified responses within the arm representation areas for pre- and post-spontaneous recovery maps for the ipsilesional and contralesional motor cortex (see Table 5.1). To test for this possibility, the mean threshold current levels for all shoulder/elbow and wrist/hand/forearm responses at each
time point were compared using the non-parametric Friedman Test. The
threshold stimulation current levels required to elicit shoulder/elbow or
wrist/hand/forearm movements remained comparable pre- and post-infarct for
the ipsilesional and contralesional motor cortex ($\chi_0^2 = 0.429, P = 0.934$
shoulder/elbow; $\chi_0^2 = 4.74, P = 0.192$ wrist/hand/forearm) (Table 5.1).

Table 5.1 Arm representation threshold current levels

<table>
<thead>
<tr>
<th></th>
<th>Left Cortex</th>
<th>Right Cortex</th>
<th>Left Cortex</th>
<th>Right Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>Pre</td>
<td>38.16 ± 16.6</td>
<td>37.0 ± 16.45</td>
<td>36.96 ± 21.59</td>
<td>43.47 ± 24.25</td>
</tr>
<tr>
<td>Post</td>
<td>32.5 ± 8.02</td>
<td>32.5 ± 8.02</td>
<td>28.25 ± 13.98</td>
<td>29.25 ± 12.9</td>
</tr>
</tbody>
</table>

Mean threshold current levels (µA) ± SD pre versus post-infarct for ipsilesional (left) and
contralesional (right) cortex subcategories of arm representation area. Proximal =
shoulder/elbow responses; Distal = wrist/hand/forearm responses

5.3.2.2.5 Effectiveness of PMRF stimulation

Stimulation was applied at 38 sites (15 left, 23 right) from 12 electrode
penetrations (6 left, 6 right). The locations of stimulus sites were anatomically
reconstructed and plotted by locus and side of brainstem using stereotaxic
coordinates and Szabo and Cowan atlas$^{171}$ as reference. After anatomical
reconstruction, 16 sites were found to be outside the predetermined boundaries
of the PMRF and were excluded. This left 22 stimulation sites for the study. By
the final acceptance criteria, 85 responses were evoked from a balanced number
of effective sites from left (9/20 sites) and right (11/20 sites) sides of the
brainstem ($\chi^2=0.2, P=0.655$). As previously reported in awake behaving
animals,\textsuperscript{9,10} repetitive stimulation in this acute recovered animal had a strong tendency towards bilateral outputs (65%); 10% of sites evoked only ipsilateral responses; 25% of sites evoked only contralateral responses.

5.3.2.2.5 Response pattern of PMRF following recovery

Following two weeks of spontaneous recovery, overall there were similar proportions of right (39/85) and left (46/85) arm responses ($\chi^2=0.576$, $P=0.45$). Yet, more responses were evoked from right (57/85) versus left (28/85) PMRF stimulation ($\chi^2=9.89$, $P=0.0017$). For each effective stimulation site in right PMRF, the number of muscles responding per site ranged from 0 to 10, with a median of 3 and a mean of $3 \pm 3$ (SD). Half as many muscles responded per site for left PMRF, with a range of 0 to 5 muscles per site, a median of 0 and a mean of $1 \pm 2$ (SD).

Based on anatomical findings from previous studies in intact animals,\textsuperscript{173-177} we expected the proportion of ipsilateral to contralateral responses for a single PMRF side stimulation pattern to be a 60:40 split. In this acute recovered animal, ipsilateral responses were common from left and right PMRF stimulation. In fact, the results exceeded this expectation for left PMRF stimulation. In fact, the results exceeded this expectation for left PMRF stimulation.
stimulation, with 79% ipsilateral and 21% contralateral responses ($\chi^2 = 4.02$, $P = 0.045$). For right PMRF, although there was a slightly smaller difference in proportion of right and left arm responses ($\chi^2 = 0.105$, $P = 0.745$), the expected proportion for a single PMRF side stimulation was upheld with 58% ipsilateral and 42% contralateral responses.

5.3.2.2.5 Agreement with typical PMRF response pattern

We then compared the sign of response (facilitation versus suppression) for flexor and extensor muscles responding for a given site ipsilateral and contralateral to side of PMRF stimulation with the typical response pattern based on our previous findings in awake behaving monkeys. Agreement was defined as the percentage of responses matching the expected sign of response. Overall, 54% of the 85 evoked responses matched the expected motor output pattern of ipsilateral flexor and contralateral extensor facilitation with ipsilateral extensor and contralateral flexor suppression. Overall, agreement was stronger for right (61%) versus left PMRF responses (39%). Next, we compared the percentage of agreement within the left and right brainstem (see Table 5.2). Agreement for ipsilateral and contralateral responses was similar for right PMRF stimulation ($\chi^2 = 1.29$, $P = 0.26$). For left PMRF, agreement for ipsilateral responses was double that of contralateral responses ($\chi^2 = 4.86$, $P = 0.028$). Thus, suggesting left PMRF response pattern changes for the right arm in the acute recovered animal. Taking this comparison a step further, ipsilateral and contralateral response agreement was further divided by muscle function (flexor
versus extensor) for each side of PMRF stimulation. Agreement for ipsilateral flexor muscle responses was similar for right and left PMRF stimulation. Agreement for contralateral extensor muscle responses for right PMRF was almost double that for left PMRF stimulation. Interestingly, ipsilateral extensor and contralateral flexor muscle responses from both the right and left PMRF did not match the expected response pattern. This mismatch in the response pattern for ipsilateral extensors and contralateral flexors may be explained by the lack of ability to detect suppression in the anesthetized animal. If some of this over-representation of facilitation for these muscle groups is a true physiological change, then there may be subtle changes in the response pattern after recovery.
Table 5.2 Agreement with expected PMRF response pattern

<table>
<thead>
<tr>
<th>PMRF</th>
<th>Left</th>
<th>Right</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ipsilateral</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flexor</td>
<td>12</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td>Extensor</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Contralateral</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flexor</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Extensor</td>
<td>6</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18</td>
<td>28</td>
<td>46</td>
</tr>
</tbody>
</table>

Table represents the aggregate number of responses for the flexor and extensor muscles for the ipsilateral and contralateral arm from PMRF sites that matched the expected sign of response (facilitation versus suppression) for typical awake behaving animals. Ipsilateral, same side as brainstem stimulation; Contralateral, opposite to side of brainstem stimulation.

5.3.2.3 Behavioral results

5.2.3.3.1 Clinical observations

Initially after recovering from anesthesia, the subject presented with posturing of whole limb flexion of the shoulder and elbow of the upper limb contralateral to the lesion (right arm). Within the first 24 hours, she showed signs of subtle weakness of the affected upper limb (right arm). The subject was able to slowly climb about the cage using both upper limbs, but displayed difficulty releasing her right grasp on the cage bars. She kept the right elbow tucked close to the body when reaching out directly in front of her body within arm’s length, and would not reach away from her torso in any direction for food. Subtle trunk weakness was also evident with reaching behaviors, requiring her to brace herself on an extended arm to prevent loss of balance. By day two post-infarct, the subject had full use of her right hand, was able to reach away from her body...
with the right arm and was able to reach for food with either arm. However, if
given the choice, she would retrieve food with the left arm. Three days post-
infarct, the subject was climbing about the cage using all four limbs. She had
difficulty climbing up and positioning into the primate chair for the behavior reach
task.

5.2.3.3.2 Behavioral reach task performance

At baseline (pre-infarct), movement times were similar for the right and left
arm (see Fig. 5.8). Immediately post-infarct, reach performance, as measured by
the behavioral task (see Fig. 5.1), was diminished bilaterally (F=17.76,
P<0.0001). During the first reach assessment post-infarct (day 3), she required
physical assistance with pressing the start switch with the non-impaired arm (left)
before resuming full task performance independently. She became increasingly
distracted during right arm trials. Therefore, she was only able to complete one
set of 24 reaches with each arm during this assessment period. This confusion
may have been a side effect of the stronger pain medication (buprenorphine) on
day 3, because by day 7, when only ibuprofen was administered, she was able to
physically perform all components of the task with either arm without difficulty.

Although no significant interaction was found for arm or target location, left
arm (non-impaired) reach performance was slowest at 7 days post-infarct, and
returned to near baseline reach performance levels by the end of the two week
spontaneous recovery period. Immediately after infarct (3 days post), right arm
(impaired) movement times had nearly doubled baseline performance. Right arm
performance gradually improved over the spontaneous recovery period, but never fully returned to pre-infarct performance levels. As Fig. 5.9 illustrates, target direction appeared to have a slightly greater impact on right arm reach performance during recovery, with movement times remaining slower to high and low targets away from the body.

**Movement Time**

![Movement Time Graph](image)

Figure 5.8 Represents mean time to successfully reach into wells for the left (blue bars) and right (red bars) arm at each assessment period expressed in milliseconds. (error bars = standard error)

Even though immediately after infarct the subject had difficulty focusing on the task, and would occasionally overshoot well openings with right arm reaches, physical impairments for retrieving food from wells were minimal as indicated by the negligible change in mean dwell times for left and right arms from the pre-infarct assessment regardless of target location (F=1.92, P=0.167; Figure 5.10).
**Figure 5.9.** A illustrates direction of target locations with task centered on the right shoulder. Lateral up = high targets away from the body; Lateral down = low targets away from the body; Medial up = high targets across the body (crossing midline); Medial down = low targets across the body. Blue bars = left arm reaches; Red bars = right arm reaches. B Represents mean time for successful reach performance by arm and target direction expressed in milliseconds (error bars = standard error). Pre = baseline; 3D = 3 days post-infarct; 7D = 7 days post-infarct; 13D = 13 days post-infarct.
Figure 5.10 Represents the mean time required to retrieve food rewards from wells for the left (blue bars) and right (red bars) arm at each assessment period expressed in milliseconds (error bars = standard error).

After infarct, the subject was able to transport food directly from the well to mouth with the left and right arm without dropping rewards. Similar to movement time data, she was slowest transporting food to mouth immediately post-infarct (F= 4.61, P= 0.012). Left arm (non-impaired) trials were slightly slower, especially transporting food from high targets out and away from the body, but quickly recovered to pre-infarct levels within the spontaneous recovery period (see Fig. 5.11).
5.3.3 Lessons learned from this subject

ET-1 was effective at inducing a focal ischemic cortical lesion in the macaque monkey. Based on the reconstruction of injection sites, estimation of lesion size and behavioral outcomes, our findings are in agreement with previous ET-1 studies.\(^91, 94, 96, 178\) The spread of ET-1 was more extensive caudally than medial-lateral. Previous studies indicate that lesion size and behavioral outcomes appear to be dose dependent.\(^94-96\) Therefore, it is plausible that only injecting a
percentage of the shoulder/elbow representation area in the macaque monkey may have created very subtle proximal upper limb motor deficits that the subject was able to recover from fairly quickly.

Although rat models have suggested estrogen may have vascular and neuroprotective effects by counteracting the effects of vasoconstriction and improving blood flow during and after the ischemic insult,\textsuperscript{179} the use of female monkeys did not prevent ET-1 from demonstrating an effect in the current study. However, the question remains, would we have produced greater functional deficits using the same dose of ET-1 in male monkeys, or were the subtle functional deficits the result of not injecting enough sites or not inducing long enough duration of ischemia?

Limitations in repeated electrophysiological mapping studies such as this include controlling for the variability with movements evoked by repetitive microstimulation due to the effects of different anesthetic agents and anesthesia levels, and measurement error due to stimulating slightly different sites across multiple mapping studies\textsuperscript{180, 181} First, we attempted to control for some of this variability by having a second experimenter independently determine the evoked movements. The movement categories defined by the two experimenters rarely differed. Next, although difficult to maintain a completely stable anesthetic plane, we were able to lessen the fluctuations by using intravenous infusion of a diluted solution of Ketamine and Dexmedetomidine. Penetrations for stimulation specifically avoided surface blood vessels to minimize local ischemia caused by
rupture or trauma. Even though the exact site may not have been stimulated during subsequent mapping studies, given the columnar organization of the cortex, it is likely that the repeated electrophysiological maps produced across the time span of the study are representative of motor outputs from the specified cortical movement representation area(s).\textsuperscript{121}

Neurophysiological and behavioral results in the current study are in agreement with previous animal studies in squirrel monkeys and rats that underwent focal ischemic infarcts to hand and digit representation areas followed by spontaneous recovery period. The monkey demonstrated the ability to perform natural cage behaviors but exhibited motor deficits on the skilled behavior task. The electrophysiological map showed reduction of the representation of the impaired body part within the lesioned area and further reduction in the adjacent non-lesioned cortical region without training.\textsuperscript{43, 55, 182} It was not unexpected, though not completely anticipated that we would see the extent of distal hand area expansion in the ipsilesional cortex due to the design of the study. Enrichment activities in her cage did not encourage bilateral arm use, but didn’t prevent it, either. Therefore, behaviorally, the subject was free to use her impaired limb for all natural cage behaviors.

In the current study, there was a novel change in the contralesional cortex, with the increase in sites that post-injury elicited complex bilateral arm responses. The expansion of complex sites in the contralesional cortex suggests some contralesional contribution to recovery in the relatively mild lesion after two
weeks of spontaneous recovery. To date, there is little electrophysiological evidence for this change after recovery, perhaps because few studies exploring recovery of arm function after cortical stroke have recorded the changes in motor outputs for the non-lesioned cortex.

The original plan was to conduct a longitudinal study which allowed us to investigate changes in PMRF motor outputs pre and post recovery from an ischemic cortical injury. However, the MRI data after the baseline PMRF mapping session for this subject provided evidence that the approach for accessing the brainstem and running a small subset of electrode tracks on both sides of the PMRF pre-injury was going to be traumatic to the brain tissue. Thus, based on these findings, we concluded that PMRF mapping would be conducted post recovery and compared with the typical output patterns in intact behaving animals from previously published results from our lab.

5.3.4 Plans implemented for next subject

ET-1 was effective at producing a focal controlled ischemic lesion in the M1 shoulder/elbow representation of the macaque monkey. A longer duration of ischemia seemed necessary to produce the extent of functional deficits we targeted for this study. Based on the behavioral, electrophysiological and imaging data, we concluded that the spread of intracortical ET-1 injections was minimal and greater concentration/duration of ischemia was required to create greater functional deficits in the proximal upper limb.
5.4 Experiment two (subject A01219): pre and post ischemia cortical mapping, with increased dosage of ET-1

5.4.1 Methods

5.4.1.1 Electrophysiological Mapping Procedure
The electrophysiological mapping procedures were as described above except the PMRF was not explored in this subject. The primary purpose was to increase the severity of the ischemic injury.

5.4.1.2 Cortical Infarct Procedure
The ET-1 injection protocol was as described above. For this subject, every site that evoked a visible shoulder/elbow response to stimulation was injected with two rounds of ET-1. A total of 32 injections were made in the arm representation area of the left M1. The depth of ET-1 injections ranged from 3 to 5.5 mm. To complete this many injections using our established protocol of waiting 30 seconds after needle insertion and slowly injecting each 1 µl over 2 minutes, required approximately 90 minutes. See Fig. 5.12 and 5.14 for location of injection sites.

5.4.1.3 Magnetic Resonance Imaging Acquisition and Analysis
The MRI protocol was changed to 1-mm slice thickness for all images pre and post to correspond with the stereotaxic grid for anatomical reconstruction and electrophysiological data analysis. In addition to T1-weighted images and
T2-weighted RARE images, T2-weighted fluid attenuated inversion recovery (FLAIR) images were obtained post-infarct for better illustration of infarcted tissue for reconstruction.

5.4.1.4 Assessment of motor performance on the behavioral reach task

After several months of training on the behavioral reach task, one pre-infarct (baseline) reaching performance assessment was taken. Then, reaching performance was tested on days four, seven, and twelve after ischemia. A final behavioral reach assessment was added after the second electrophysiological mapping surgery to explore any potential changes in reach performance due to the additional surgical procedure.

5.4.2 RESULTS

5.4.2.1 Anatomical Results

5.4.2.1.1 Ischemic infarct size and location

As stated in METHODS, 32 1µl ET-1 injections were made in the shoulder/elbow representation of the left motor cortex; 2 rounds of injections to each of 16 sites. As for the previous subject, coregistration of MRI scans and the neurophysiological map was used to reconstruct ET-1 injection site locations (Fig. 5.12). Based on this reconstruction, injections made greater than 4 mm at the more rostral sites may have been along the grey-white matter border vs. possibly just below, penetrating the superficial white matter.
As for the previous subject, volumetric analysis was carried out by manually tracing the outer edge of the hyper intense lesioned tissue in the MR images using the Image-J software. Eight image slices were affected (see Fig. 5.13). The ET-1 induced ischemic lesion resulted in a total volume of 286.24 ± 17.03 (SD) mm³ of signal intensity changes within the left motor cortex involving grey and white matter. The lesion spanned the region of the pre-central gyrus corresponding with the coordinates of cortical ET-1 injection sites. The subcortical structures remained intact.
Figure 5.12. Coregistration of MRI sections and neurophysiological map was used to reconstruct ET-1 injection sites in left motor cortex (indicated by white markings) with respect to stereotactic coordinates recorded.  
**A.** T1-weighted MRI scan (1 mm slice thickness contiguous slices) acquired in the coronal plane (TR = 589.05 ms, TE = 4.854 ms, flip angle = 35º, pixel spacing .43x.43 mm). Images are ordered in columns from rostral (top left) to caudal (bottom right). The laterality for each injection site was referenced from midline on each representative section. Dashed line represents arbitrary demarcation between motor and sensory cortex. SPCD, superior pre-central dimple; CS, central sulcus; LS, lateral sulcus; STS, superior temporal sulcus; CGS, cingulated sulcus; M1, primary motor cortex; S1, primary sensory cortex; SMA, supplementary motor cortex. Scale bar = 4 mm.  
**B.** Schematic representation of target area for ET-1 injection sites in left motor cortex outlined by dark grey box. PRCS, pre-central sulcus (dimple); PCS, post-central sulcus; TS, temporal sulcus; AS, arcuate sulcus.
Figure 5.13. Represents the ET-1 induced lesioned tissue in left motor cortex. T2-weighted MRI scans (1mm slice thickness contiguous slices, 0.43 mm x 0.43 mm pixel spacing) acquired in coronal plane were used to manually trace the region of interest on each individual section using Image-J software. Region of interest (ROI) was defined as the area of abnormal signal intensity change (outlined in yellow). Coronal images through the lesion are arranged by rows from rostral (top left) to caudal (bottom right). The estimate lesion volume was then calculated by multiplying the total ROI area (mm²) by slice thickness. The unexpected complication of brain herniation is visible in right cortex.
5.4.2.2 Physiological Results

Again, the mapping studies were balanced. There was no significant difference in the total number of sites explored in the left versus right motor cortex, and the number of sites explored within each motor cortex were similar for pre- and post-infarct mapping procedures ($\chi^2=0.016$, $P=0.90$).

5.4.2.2.1 Ipsilesional cortex changes

At baseline, 42% of the 73 sites explored in the ipsilesional (left) motor cortex evoked visible arm-related movements to stimulation. Within the arm representation area, similar proportions of sites evoked shoulder/elbow responses (55%) and wrist/hand/forearm responses (45%) ($\chi^2=0.29$, $P=0.59$). Although a comparable number of arm-related sites were explored pre and post-infarct, there were significant changes in the electrophysiological map after two weeks of spontaneous recovery. Most sites within the expected shoulder/elbow representation corresponding to ET-1 injection sites were non-responsive after recovery. Only one site evoked a response to stimulation; elbow flexion. The shoulder/elbow representation did not expand into adjacent movement representations. Wrist/hand/forearm movement representation expanded from 45% (14/31) of arm-related sites pre-infarct to 96% (24/25) arm-related sites post-infarct (see Fig 5.14a and 5.14b).
Fig. 5.14. Surface map of microstimulation evoked motor outputs for right and left motor cortex from (a) pre-infarct and (b) post-spontaneous recovery period. Stimulation sites were 1-mm apart. Each electrode penetration is represented at its corresponding position by a color indicating the body territory activated at threshold current (see color legend). Shoulder/elbow representation area was circumscribed by 2-mm of other movements or non-responsive sites (black). Rostral and caudal boundaries are indicated by arcuate sulcus (AS) and central sulcus (CS); Middle Cerebral Artery and branches are represented by red lines. Dual response sites are represented by both body territory colors. ET-1 injection sites are indicated by the syringe symbol.

continued
5.4.2.2.2 Contralesional cortex changes

Most of the 64 sites explored in the contralesional (right) motor cortex (52%) evoked visible arm-related movement responses to stimulation pre-infarct. Similar proportions of sites evoked shoulder/elbow (39%) and wrist/hand/forearm responses (58%) ($\chi^2=1.13$, $P=0.29$). After the spontaneous recovery period, there was relatively little change from pre-infarct in the proportion of arm-related sites that elicited shoulder/elbow responses (39%:13/33) and wrist/hand/forearm responses (68%:19/28) ($\chi^2=0.168$, $P=0.68$) (See Fig. 5.14a and 5.14b).
5.4.2.2.3 Comparison of ipsilesional and contralesional cortex changes

Comparing the number of sites that evoked arm-related responses pre- and post-infarct for the ipsilesional and contralesional motor cortex, the overall proportion of sites in the ipsilesional cortex post-infarct was reduced, ($\chi^2=6.37$, $P=0.012$). This was expected because we intended to lesion the proximal arm area in the left motor cortex. Next, we compared the proportion of sites within the arm representation area that evoked shoulder/elbow and wrist/hand/forearm responses for the left and right cortex pre and post-infarct using a Chi-square test weighted by the number of arm-related sites (see Fig.5.15). The most significant difference found post-infarct was within the ipsilesional cortex with the absence of shoulder/elbow representation and expansion of wrist/hand/forearm representation into the pre-infarct shoulder/elbow territory (see Fig. 5.14b). In contrast to subject one (A01183), no complex whole arm or bilateral arm responses were evoked from the contralesional cortex after recovery for this subject.
Figure 5.15 Represents the change in proportion of sites that evoked shoulder/elbow and wrist/hand/forearm related responses for the ipsilesional (left) and contralesional (right) cortex pre- and post-infarct. Results are presented as a percentage of the total number of arm-related sites for each motor cortex. Blue bars = shoulder/elbow sites; Red bars = wrist/hand/forearm sites.

5.4.2.2.4 Arm representation threshold current comparison

We explored whether changes in threshold current levels (µA) were required to evoke the specified responses within the arm representation areas for pre- and post-spontaneous recovery maps for the ipsilesional and contralesional motor cortex (see Table 5.3). Since only one site in the ipsilesional cortex evoked a response in the shoulder/elbow representation after infarct, we could not compare these current thresholds. The threshold stimulation current to elicit shoulder/elbow movements for the contralesional cortex or wrist/hand/forearm movements for the ipsilesional and contralesional cortex remained comparable pre- and post-infarct ($\chi^2 = 8.69$, $P = 0.192$).
Table 5.3 Arm representation threshold current levels

<table>
<thead>
<tr>
<th></th>
<th>Left Cortex</th>
<th>Right Cortex</th>
<th>Left Cortex</th>
<th>Right Cortex</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>Pre</td>
<td>29.12 ± 10.19</td>
<td>36.54 ± 13.6</td>
<td>23.93 ± 9.24</td>
<td>29.47 ± 13.63</td>
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<tr>
<td>Post</td>
<td>41.25 ± 17.47</td>
<td>25.83 ± 11.58</td>
<td>28.33 ± 8.04</td>
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</tbody>
</table>

Mean current threshold levels (µA) ± SD pre versus post-infarct for ipsilesional (left) and contralesional (right) cortex subcategories of arm representation area. Proximal = shoulder/elbow responses; Distal = wrist/hand/forearm responses

5.4.2.3 Behavioral Results

5.4.2.3.1 General Behavior

Initially after awaking from anesthesia, the monkey postured in whole limb flexion of the shoulder and elbow of the upper limb contralateral to the lesion (right arm). For the first seven hours she made no attempt at volitional movements with the affected upper limb (right arm). In the first day following surgery, the subject showed signs of subtle right sided weakness, and reduction of voluntary use of the right arm. She was able to move about the cage using all four limbs, but when offered food she made no attempt to reach with the right arm. By day two post-infarct, she continued to show signs of proximal right shoulder weakness. This was especially evident with reaching out in front or away from the body for food. She kept the right arm tucked at her side and reaching movements were in a whole limb flexion pattern. If given the choice,
she would reach for food with the left arm. Then, three days post-stroke, she developed a sudden onset of left-sided motor deficits. Attempts to use the left side of her body were arduous and resulted in exaggerated associated responses of right arm whole limb extension with left arm whole limb flexion. Subtle proximal shoulder weakness was still observed in the right arm, and she fatigued easily as she now was forced to use the right arm for feeding. By 7 days post-infarct, she was initiating reach attempts again with the left arm, though unsuccessful. When at rest, the left arm was kept tucked at her side in a flexed posture. During the next five days, she began using the left arm, though weak, with feeding, grooming and hygiene activities. After the second electrophysiological mapping procedure, (day 15 post-infarct) she continued to show improvements with using the left arm for typical cage behaviors. Even though she had essentially been forced to use her right arm for feeding activities, 19 days post-infarct she was unsuccessful at retrieving food from the feeder box attached to the outside of her cage using her right arm, requiring that she turn her body around to enable her to reach with the left arm.

5.4.2.3.2 Behavioral Reach Task Performance

Behavioral reach performance was assessed 3 days post-infarct. The monkey was unsuccessful at performing reach trials with either arm. She postured in whole limb extension of the right arm with whole limb flexion of the left arm that was exaggerated with head rotation. She looked at the start switch after the ready cue was illuminated, but made no attempt to press the start
switch. Once it became evident that she was not going to perform the full task, the task was modified to allow her to begin to succeed. Food rewards were placed in the wells and feeder doors were opened in a random order to assess whether she would perform any reach attempts, but she was not required to begin at the start switch. She kept the left arm in a flexed posture against her side and made no attempt to reach. She was able to reach into the wells with her right arm, but required multiple attempts due to overshooting target openings and hitting her fingers on the outside of the well upon entry. Once she entered the well, she had difficulty manipulating the food reward, requiring that she scrape the food out of the well onto the table, and then pull the food closer to her body before bringing hand to mouth. By 7 days post-infarct, she was capable of placing the left and right hand on the start switch, but still made no attempt to press the start switch to begin reach trials. Therefore, in order to collect behavioral reach data, a consistent, preferred starting position for each arm was determined, and the food door was opened when the hand reached that position. For the left arm, she performed 4 of 12 reach trials. She demonstrated poor hand dexterity and appeared to have sensory deficits in the left arm. For example, she repeatedly dropped food rewards, had difficulty picking up food from the table, and often did not behave in response to food in her hand. For the right arm, she performed 21 of 24 reach trials. She continued to display errors of overshooting target openings and hit the outside of the wells upon entry.
As described above, left arm reach performance was unexpectedly impaired immediately post-infarct, and she regained purposeful use again about one week post-infarct, as evidenced by the slow movement time measured 7 days post-infarct (4281 ± 1987 (se) ms). A repeated measures ANOVA was carried out to determine whether the arm used or target location had any effect on behavioral reach performance (movement time, dwell time, transport time) comparing pre-infarct (baseline) with two post-infarct measures (14 days and 20 days post). Her 7 day post-infarct times were not included in the analyses due to the low number of successful trials for the left arm (n=4).

When we compared the time required to successfully reach into each feeder well (movement time) a main effect of session was found, with slowest reach performance at 20 days post-infarct (F=176.98, P<0.0001; Fig. 5.16). Overall, target location had an effect on movement times (F=6.536, P<0.0001), with the largest differences found for high and low targets away from the body for both arms.
Figure 5.16 Represents the mean time (milliseconds) required for successful reaches into feeder wells for left (blue bars) and right (red bars) arms at each assessment period. (error bars = standard error). No volitional reach attempts were made at 3 days post-infarct for either arm.

Next, the time required for retrieval of food rewards (dwell time) was compared at each assessment period. Although not included in the statistical analyses due to the low number of attempts, her performance was slowest 7 days after infarct. By 14 days post-infarct she was successful at entering all 4 wells with the left and right arm, but as mentioned above, her continued difficulty manipulating food with the left hand caused dwell times to be slower (Fig. 5.17; F=174.5, P<0.0001). As with movement times, the greatest difference for left
arm was to high targets away from the body. For right arm, she was slowest to both high and low targets away from the body throughout recovery.

Figure 5.17 Represents the mean time (milliseconds) to retrieve food from wells for right (red bars) and left (blue bars) arms at each assessment period error bars = standard error). At 3 days post-infarct there were no successful trials.
Figure 5.18 Represents mean time (milliseconds) to transport food from well to mouth for left (blue bars) and right (red bars) for each assessment period (error bars = standard error). At 3 days post-infarct there were no successful trials.

She had difficulty transporting food to mouth for left and right arm trials post-infarct, dropping food during transport ~ 10-16% of time (Fig. 5.18). There was a significant interaction of target location and arm on time post-infarct for transporting food to mouth (F=4.027, P=0.002). For the left arm, transport time was slowest at 14 days post-infarct for low targets down and across the body and then gradually returned toward pre-infarct levels. Despite the
unanticipated “forced use” of the right arm that resulted from the left arm motor impairments, transport time for right arm at 20 days post-infarct had still not recovered to pre-infarct levels, especially to high targets up and away from the body.

5.4.3 Lessons learned from this subject

This was the second subject used for determining the effectiveness of ET-1 in inducing focal ischemia in the macaque. Injecting every cortical site that evoked a shoulder/elbow response with two rounds of ET-1 was effective at producing paresis of the contralateral upper limb proximal muscles. The unforeseen complications of left sided motor impairments that developed in this monkey appeared to be the result of brain swelling and brain herniation of the contralesional cortex that was further exacerbated by poor fit of the craniotomy cap. The important finding to note here is that even though she had unanticipated left-sided motor deficits that created a “forced-use” situation for the intended impaired arm, at 20 days post-infarct without specific training she had still not recovered pre-infarct reach performance in the right arm.

5.4.4 Plans implemented for next subject

For this subject we had made a single craniotomy cap to cover both craniotomy openings. The poor fit of the cap probably further exacerbated the problems with brain swelling and herniation. Therefore, for the next subject we developed two separate craniotomy caps that better matched the thickness of the
cortical bone removed to help minimize brain herniation. We also implemented a plan that if onset of unusual neurologic changes developed such as a sudden onset of weakness of the ipsilesional extremity (unimpaired) or worsening of neurologic deficits, we would immediately check fit of craniotomy cap and for presence of fluid on the brain.

5.5 Experiment three (subject A03354): full blown study with pre, post ischemia, and post rehabilitation measurements

5.5.1 Methods

5.5.1.1 Electrophysiological Map Procedures

Motor outputs from right and left cortical motor areas were mapped at three time points, pre-infarct, post spontaneous recovery and post-rehabilitation. Upper limb motor outputs for right and left PMRF were also mapped after completion of rehabilitation.

5.5.1.2 Cortical Infarct Procedure

No changes were made to the ET-1 injection protocol. Each site that evoked shoulder and elbow responses to stimulation was injected with two rounds of ET-1, for a total of 28 1µl injections. The depth of injections ranged from 3.0 to 5.5 mm (Fig. 5.19).
5.5.1.3 Magnetic Resonance Image Acquisition

MRI scanning procedures were as described for the previous subject. Multiple MRI scans (1mm slice thickness, contiguous slices) through cortical and brainstem structures were acquired in coronal, axial and sagittal planes spanning the brain. MRI scans were performed pre-infarct and then at set time points of 10 days post-infarct and after completion of rehabilitation to document ischemic injury.

5.5.1.4 Assessment of motor performance on the behavioral reach task

After several months of training on the behavioral reach task, three pre-infarct (baseline) reaching performance assessments were taken. Then, reaching performance was tested on days four, seven, and twelve post ischemia. After the second electrophysiological mapping procedure reaching performance was assessed prior to initiation of rehabilitation and then weekly measurements of reach performance were measured throughout rehabilitation.

5.5.1.5 Post-infarct rehabilitation procedure

The monkey received 60 to 90 minutes of daily intensive rehabilitation of the impaired upper limb. The non-impaired limb was restrained at her side. During the first five days, rehabilitative training began with reaching activities at table top level within the immediate workspace of the impaired arm, increasing the level of difficulty by having her reach away from the body, across midline towards the opposite shoulder, and up toward targets at shoulder and chin
heights. Once her reaching ability had progressed to the point where
performance of the task was possible, the behavioral reach task was re-
introduced and used as therapy for the remainder of rehabilitation sessions. She
performed over 300 reaching trials per training session. Each reach condition
(ie. across body, far reaches, full task) was practiced in blocks of 50 repetitions,
alternating between high and low targets for each condition.

5.5.1.6 Detection and analysis criteria for PMRF responses

Detection and acceptance criteria for PMRF responses were as described
for subject A01183. The sensitivity of the acceptance criteria was good, at 64 to
75%. The specificity of our acceptance criteria was strong, at 94%. Visual
inspection was still required to omit spurious responses due to stimulus artifact.

5.5.1.7 Histological procedure

After completion of the post-rehabilitative training map the subject was
deeply anesthetized with a lethal dose of sodium pentobarbital and then perfused
transcardially with phosphate-buffered saline followed by phosphate buffered
para-formaldehyde. This was followed by a perfusion with 10% sucrose in
phosphate buffer, followed by 30% sucrose in phosphate buffer. The brain was
removed and soaked in 30% sucrose for cryoprotection and then frozen using
isopentane at -20 degrees. Coronal sections were cut at 50 µm on a freezing
microtome and every tenth section (every ½ mm) was mounted and stained with
cresyl violet (CV). Fig. 5.22 is representative of the ischemic lesion.
5.5.2 Results

5.5.2.1 Anatomical results: Ischemic infarct size and location

As stated in the METHODS, 28 1µl ET-1 injections were made in the shoulder/elbow representation of left primary motor cortex; 2 injections were made at each arm movement-related site. As for the previous subjects, coregistration of MRI scan sections and neurophysiological map was used to reconstruct ET-1 injection site locations. Based on this reconstruction, injections were made in both the superficial and deep layers of motor cortex including sites deep in the bank of central sulcus (Fig. 5.19).

At 10 days post-infarct, volumetric analysis was carried out as described for the previous subjects. With 29 image slices affected, the volume of signal intensity changes measured on MRI was quite extensive (4741.89 ± 179.54 mm$^3$), which would correspond to a cube that is ~ 16-mm per side. Abnormal cortical tissue extended throughout the sensorimotor cortex. No apparent signal intensity changes were measured in subcortical structures (Fig. 5.20).

Final measurement of ischemic infarct volume and location was calculated from MRI scans acquired after completion of rehabilitative training (16 weeks). The ET-1 induced ischemic lesion characteristics were similar to other ischemic lesion models captured with MRI scans of a necrotic core and a rim of signal intensity changes in the surrounding tissue. The lesion volume was 480.72 ± 29.45 mm$^3$ involving both gray and white matter of the sensorimotor cortex, which would correspond to a cube ~ 8-mm per side. The lesion included the central
sulcus and extended 1-2 mm rostral to the most anterior injection site into pre-central gyrus and ~4 mm caudal to the most posterior injection site into post-central gyrus (See Fig 5.21 and 5.22 for area of infarction and representative histological section of infarction).
Figure 5.19. Coregistration of MRI and neurophysiological map used to reconstruct ET-1 injection site locations (indicated by white markings) in left motor cortex with respect to stereotactic coordinate records. **A.** T1-weighted MRI scans (1 mm slice thickness, contiguous slices) acquired in coronal plane (TR = 500, TE = 5, flip angle = 30 degrees). Images ordered in columns from rostral (top left) to caudal (bottom right). SPCD, superior precentral dimple; IPS, intraparietal sulcus; CS, central sulcus, LS, lateral sulcus, STS, superior temporal sulcus, CGS, cingulate sulcus; M1, primary motor cortex; S1, primary sensory cortex; SMA, supplementary motor cortex. Dashed line represents arbitrary demarcation of M1 and S1 boundary. Scale bar = 4 mm. **B.** Schematic diagram representing target area for ET-1 injection sites indicated by dark grey box. AS, arcuate sulcus; PRCS, superior precentral sulcus (dimple); TS, superior temporal sulcus; PCS, postcentral sulcus.
Figure 5.20. Represents the ET1 induced lesioned tissue in left motor cortex. T2-weighted MRI scans (1mm slice thickness, contiguous slices, 0.43mm x 0.43 mm pixel spacing) acquired in the coronal plane 10 days post-infarct were used to manually trace the region of interest. Region of interest (ROI) was defined as the area of abnormal signal intensity change (outlined in yellow) ROI was manually traced on each individual section using Image-J software. The estimated lesion volume was then calculated by multiplying the total ROI area (mm$^2$) by slice thickness. Coronal slices through the image are arranged by rows from rostral (top left) to caudal (bottom right).
Figure 5.21. Represents the ET-1 induced lesioned tissue in the left motor cortex. T2-weighted MRI scans (1 mm slice thickness, contiguous slices, 0.43 mm x 0.43 mm pixel spacing) acquired in the coronal plane 104 days post-infarct (final MRI) were used to manually trace the region of interest (ROI); defined as abnormal signal intensity changes (outlined in yellow). ROI was manually traced on each individual section as for the previous measurements at 10 days post-injury and the lesion volume calculated from multiplying the total ROI area (mm$^2$) by slice thickness. Coronal sections are ordered in rows from rostral (top left) to caudal (bottom).
5.5.2.2 Physiological Results

Again, the mapping procedures were balanced, and there was no significant difference in the total number of sites explored in the left versus right motor cortex. The numbers of sites explored within each motor cortex were also similar for all three time points, pre-infarct, post-spontaneous recovery and after rehabilitation ($\chi^2=0.24$, $P=0.89$).

5.5.2.2.1 Ipsilesional cortex changes

Of the 70 sites explored pre-infarct in the ipsilesional (left) cortex, 51% evoked arm-related movement responses to stimulation. Of these 36 arm-related sites, 36% evoked visible shoulder and elbow movements, 64% evoked
wrist/hand/forearm movements. Compared with the pre-infarct map, the shoulder/elbow representation in the affected M1 (left) remained completely absent after recovery (see Fig. 5.23). In fact, no visible movements were evoked to stimulation throughout the right arm representation area. Interestingly, there was an expansion of the right leg movement representation area, which was also severely impaired functionally immediately following the infarct, from 4% of sites (n=3) stimulated in the pre-infarct mapping study to 28% of sites (n=26) stimulated in the final mapping study 16 weeks post-infarct.

5.5.2.2.2 Contralesional cortex changes

The proportion of sites that evoked arm-related movements to stimulation within the contralesional (right) cortex was similar for all three time points, pre-infarct (55%; 46 of 83 sites), post-spontaneous recovery (56%; 54 of 96 sites) and post-rehabilitation (50%; 52 of 103 sites). Pre-infarct, there were similar proportions of visibly evoked shoulder/elbow responses (48%) and wrist/hand/forearm responses (52%) from arm-related sites ($\chi^2=0.087$, $P=0.77$). After recovery, there was a strong shift in the proportion of specified movements within the arm representation area ($\chi^2=35.33$, $P<0.0001$). After two weeks of spontaneous recovery 35% of arm-related sites (19 of 54) evoked shoulder/elbow movements, 63% of sites (34 of 54) evoked wrist/hand/forearm and 2% of arm-related sites (1 of 54) evoked movement at the elbow and hand (Fig. 5.23). Following 12 weeks of intensive rehabilitation, 29% of the arm-related sites (15 of 52) continued to evoke shoulder and elbow movements, 67%
(34 of 52) evoked wrist/hand/forearm movements, and 2% (1 of 52) evoked movement at the elbow and wrist. One site (2%) evoked bilateral shoulder elevation. No ipsilateral extremity-only or complex responses were observed.

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**Fig. 5.23.** Surface map of microstimulation evoked motor outputs for right and left motor cortex (a) pre-infarct, (b) post-spontaneous recovery and c post-rehabilitative training. Each electrode penetration is represented at its corresponding position by a color indicating the body territory activated at threshold current (see color legend). The shoulder/elbow representation was circumscribed by 2-mm of other movements or non-responsive sites (black). Rostral and caudal boundaries indicated by arcuate sulcus (AS) and central sulcus (CS); Middle Cerebral Artery and branches represented by red lines, green line represents pre-central dimple. Dual response sites are represented by both body territory colors. ET-1 injection sites are indicated by the syringe symbol. Scale bar for stimulation grid = 1mm
Fig. 5.23, continued

B  2 weeks Post-Infarct

RIGHT

LEFT

1 mm
5.5.2.2.3 Comparison of ipsilesional and contralesional cortex changes

We compared the number of sites that evoked the specified shoulder/elbow and wrist/hand/forearm movements for the ipsilesional and contralesional cortex at the beginning of the study using a Chi-square test.
weighted by the number of arm-related sites explored for each side of the cortex (Fig. 5.24). No significant difference was found between the lesioned and non-lesioned cortex for proportion of shoulder/elbow sites or wrist/hand/forearm sites ($\chi^2=3.32, P=0.19$). Since no arm-related responses were evoked from the ipsilesional cortex during recovery, no further comparisons could be made.

**Change in Arm Representation Area**

![Bar chart showing change in arm representation area](image)

Figure 5.24 Represents change in proportion of sites that evoked shoulder/elbow and wrist/hand/forearm related responses for the ipsilesional (left) and contralesional (right) cortex pre-infarct, 2 weeks post-infarct and post-recovery (final). Results are presented as a percentage of the total number of arm-related sites for each motor cortex. Blue bars = shoulder/elbow sites; Red bars = wrist/hand/forearm sites.
5.5.2.2.4 Arm representation threshold current comparison

We explored whether a change in threshold current levels (µA) was required to evoke the specified shoulder/elbow and wrist/hand/forearm movements at the spontaneous recovery or post-rehabilitation time points for the contralesional cortex. The threshold stimulation current to elicit shoulder/elbow responses remained comparable pre and post-infarct ($\chi^2 = 5.68$, $P = 0.058$). However, slightly less current was required to elicit wrist/hand/forearm responses at the two week spontaneous recovery period mapping compared with baseline and final mapping (Table 5.4; $\chi^2 = 10.81$, $P = 0.004$). Since the ipsilesional cortex arm-related sites were non-responsive to stimulation post-infarct, no further comparisons could be made.

Table 5.4 Arm representation threshold currents (µA)

<table>
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<tr>
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<td>Proximal</td>
<td>Distal</td>
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<td>Distal</td>
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<tr>
<td>Pre</td>
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Represents mean threshold current levels (± se) required to elicit shoulder/elbow (Proximal) and wrist/hand/forearm (Distal) responses for the left (lesioned) and right (non-lesioned) cortex. Pre = baseline map; Post-1 = spontaneous recovery map; Post-2 = final map. NR=non-responsive
5.5.2.2.5 Effectiveness of PMRF stimulation

Stimulation was applied at 111 sites from 16 electrode penetrations (8 left, 8 right). After the anatomical reconstruction, 13 sites were found to be outside the predetermined boundaries of the PMRF and were excluded. This left 88 stimulation sites for the study. The number of left (46/88) and right (42/88) PMRF sites explored was balanced ($\chi^2=0.18$, $P=0.67$). Of these, 75 (85%) were effective for evoking 216 responses. The number of responses evoked from left (99/216) and right (117/216) brainstem sites were similar ($\chi^2=1.34$, $P = 0.25$). For each effective stimulation site in right PMRF, the number of muscles responding per site ranged from 0 to 8, with a median of 1 and a mean of $2 \pm 2$ (SD). A similar number of muscles responded per site for left PMRF, with a range of 0 to 7 muscles per site, a median of 0 and a mean of $1 \pm 2$ (SD). In the current injury model, bilateral responses were evoked from 57% of all effective sites; 24% of sites evoked only contralateral responses; 19% of sites evoked only ipsilateral responses. As previously reported in awake behaving animals, repetitive stimulation had a similar capacity to affect ipsilateral and contralateral muscles, with a greater tendency toward bilateral outputs.
Figure 5.25 Representative example of responses for stimulus trains from a left and right PMRF site. The two vertical lines for each panel represent the 105-ms stimulation period.
5.5.2.2.6 Response pattern of PMRF following recovery

Following 12 weeks of intensive rehabilitative training, overall there were more right arm responses (141/216) than left (75/216) ($\chi^2=20.17$, $P<0.0001$). There was greater right arm (impaired) representation from left PMRF sites (Fig. 5.26; $\chi^2=36.38$, $P<0.0001$). As for right PMRF sites, the right arm representation remained strengthened, although no statistical difference was found in the proportion of ipsilateral versus contralateral responses ($\chi^2=0.115$, $P=0.73$). Based on anatomical findings from intact animals,\textsuperscript{173-177} we expected the proportion of ipsilateral to contralateral responses evoked from each side of the brainstem to be approximately a 60:40 split. Therefore, the results suggest strengthening of contralateral (right arm) motor outputs for left PMRF in this chronic recovered animal.

5.5.2.2.7 Agreement of PMRF upper limb motor output pattern

As in experiment one, we compared agreement of PMRF motor outputs associated with upper limb recovery after the severe cortical injury with the typical motor output pattern previously described for the awake behaving monkey,\textsuperscript{1-3} of ipsilateral flexor with contralateral extensor facilitation and suppression of the antagonists. Overall, 40% of the 216 evoked responses
matched the expected response pattern for a given muscle, with the greatest frequency of muscles matching the response pattern being right arm muscles (76%). Comparing percentage of agreement by side of brainstem stimulation, more left PMRF evoked responses (77%) matched the expected output pattern (Table 5.5). Next, we compared the agreement for ipsilateral and contralateral responses evoked from right and left PMRF sites. Within left PMRF, agreement was strongest for contralateral (right arm) responses (81%) ($\chi^2 = 25.1$, $P < 0.0001$). Within right PMRF, although no statistical difference was found, agreement was slightly stronger for ipsilateral (right arm) responses (60%) ($\chi^2 = 0.8$, $P = 0.37$).

When we expanded the analysis to specifically compare flexor and extensor muscle response agreement for each side of the brainstem, no difference was found in the number of flexor versus extensor muscle responses matching the expected output pattern for left PMRF stimulation ($\chi^2 = 1.23$, $P = 0.27$). For the right PMRF, agreement was higher for ipsilateral flexors ($\chi^2 = 5.29$, $P = 0.021$).

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<tr>
<td><strong>Total</strong></td>
<td>67</td>
<td>20</td>
<td>87</td>
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Table 5.5 Agreement with expected PMRF response pattern

Table represents aggregate number of flexor and extensor muscle responses from right and left PMRF sites for subject A03354 that matched the expected sign of response (facilitation versus suppression) from typical awake behaving animals. Ipsilateral, same side as brainstem stimulation; Contralateral, opposite to side of brainstem stimulation.
5.5.2.3 Behavioral Results

5.5.2.3.1 Clinical observations

The infarct initially resulted in posturing in whole limb flexion of the shoulder and elbow of the upper limb contralateral to the lesion (right arm) with whole limb extension of the ipsilesional limb (left arm) and head rotation towards the left when the monkey first awoke from anesthesia. Initially, she demonstrated significant functional deficits of the right upper and lower limbs. In the first 2 days, there was little voluntary use of the affected limb (right arm) for food manipulation, hygiene or movement about the cage. She usually held the right arm in a flexed posture at her side or in her lap, and no attempts at grasping for food with the right arm were observed. When she stood on the perch to drink, the right upper and lower limbs would draw up into whole limb synergistic flexion pattern. By the third day, voluntary use of the right arm was observed as she began attempting to use the limb to navigate about the cage. However, movements were clumsy, and she frequently used her left hand to reposition the right arm. By the start of rehabilitation (18 days post infarct), the monkey was consistently using the right arm to move about the cage, but continued to rely on the left arm for grooming, hygiene and food manipulation. By the fourth week of rehabilitation she had recovered right leg function.
5.5.2.3.2 Behavioral reach task performance

Left arm reach performance, as measured by the behavioral reach task was slow immediately post-infarct (4 days post), but quickly recovered by the start of rehabilitation. Reach performance with the right arm was markedly diminished. At day four post-infarct no reach attempts were made with the right arm. By one week post, she was initiating reaching but was unsuccessful at the task. More specifically, she made up to 25 reach attempts per trial to enter each well, but was unsuccessful at entering any of the feeder wells. Reach performance errors included large gross circling motions around the well opening, especially when reaching towards low targets away from the body. She also overshot the target by several inches when reaching towards high and low targets medially across the body. After one week of rehabilitative training she successfully completed the movement and transport phases on 6 trials. A repeated measures ANOVA determined there was a difference for reach performance (movement time), with slower performance for right arm reaches regardless of target location (F=6.046, P = 0.002). Movement time performance gradually improved in accuracy and speed over the 12 weeks of intensive rehabilitative training, but did not fully return to pre-infarct performance levels by the end of the study (Fig. 5.27).
Figure 5.27. Represents successful reach attempts into feeder wells for left (blue bars) and right (red bars) arms at each assessment period expressed in milliseconds. (error bars = standard error). Pre-test 1 through 3 measured before ischemic infarct; pre-rehab, before start of intensive rehabilitative training; weekly assessments throughout rehabilitative training; final, at end of study. *Vertical dashed lines* represent time points for electrophysiological mapping studies.
The subject developed new compensatory motor strategies with the right arm that enabled her to successfully retrieve food rewards from wells (dwell time) and transport food to her mouth (transport time). Keeping the fingers flexed, she used her right hand as an extension of the arm, scooping the food from wells to the table and then manipulating the food using a gross grasp technique to bring food to her mouth. As a result, dwell time with her right arm was significantly slower (F=6.388, P<0.0001), and does not approach pre-infarct levels until week 9 of rehabilitation training (Fig. 5.28).

Her transport time also remained slower than pre-infarct levels throughout rehabilitative training. After one week of rehabilitative training the transport phase required 7093 ± 1453 ms (se). After 5 weeks of intensive training, transport time remained slow, especially from low targets away from the body (2557 ± 273 (se) ms). As illustrated in Fig. 5.29, after intensive rehabilitation she regained success at task performance but never fully recovered pre-injury performance levels by the end of the study.
Figure 5.28. Represents time required to retrieve food from wells with left (blue bars) and right (red bars) for all assessment periods expressed in milliseconds. (error bars = stand errors). Pre-test 1 through 3, baseline measures prior to lesion; pre-rehab, before start of rehabilitative training; weekly assessments throughout rehabilitative training; final, at end of study. Vertical dashed lines indicate time points for electrophysiological maps.
Figure 5.29. Represents time required to successfully transport food from well to mouth with the left (blue bars) and right (red bars) arms for all assessment periods expressed in milliseconds. (error bars = standard errors). Pre-test 1 through 3, baseline measures prior to lesion; pre-rehab, before start of rehabilitative training; weekly assessments throughout rehabilitative training; Final, at end of study. *Vertical dashed lines* indicate time points for electrophysiological maps.
5.5.3 Lessons learned from this subject

A comparable number of sites, depths of injections and total volume of ET-1 was injected in the left motor cortex for this subject as in the previous subject. The intended level of severity of proximal upper limb motor deficits was produced in this subject. However, this came at the cost of profound whole right sided motor impairments. MR images of the cortex 10 days after the infarct showed expansive damage throughout the left hemisphere. Reconstruction of ET-1 injection site depths and characteristics of the motor cortex measured from coronal MRI scans suggested that many of the ET-1 injections made at depths ≥ 4-mm (especially more medial sites) may be at or near the border of the grey-white matter and a few sites deep within the bank of central sulcus appeared to be near the motor-sensory cortex boundary (see Fig. 5.19). If injections were made directly into the CST fiber tracts, this may have accounted for the significantly greater right sided functional deficits for this subject.¹⁸³ Therefore, for the next subject, alterations were made to the cortical infarct protocol, and ET-1 injections were made 1-mm superior to the site of stimulus evoked shoulder/elbow movement responses.

We have demonstrated through work with this subject with considerable motor deficits, that extensive cortical infarcts in macaque monkeys are survivable. We also established animal care procedures for significantly impaired animals for natural cage behaviors, especially feeding that will be used for the next subject and future experiments.
An interesting finding was the reduction of shoulder/elbow representation and expansion of the distal hand area in the contralesional M1 post-recovery. Rehabilitative training consisted of daily 90 minute sessions of intensive reaching activities with the impaired arm. The non-impaired arm was restrained at her side throughout the training session. However, the rest of the day, she was free to move about her cage, and behaviorally, she was free to use the right arm for all natural cage behaviors. Observations of natural cage behaviors were made 3 times a day, typically at meal times. Based on observations, even though she was able to use the right arm for gross reaching activities within the cage, her preference was to use the left arm for feeding and hygiene tasks.

5.6 Experiment Four (subject A01074): full blown control study

5.6.1 Methods

This monkey was meant to be used as a control (no rehabilitative training after infarct) for measuring changes in cortical and PMRF arm-related motor outputs, matching the 16 week timeline for the monkey that received intensive rehabilitation after infarct (subject A03354). Unfortunately, she sustained a tail injury eight weeks post-infarct that required surgery to repair. During recovery from that procedure, she suffered a neurologic injury requiring early removal from the study. Likely causes of this injury may have been air embolus, thrombus, or hemorrhage associated with the tail surgery. Upon autopsy, evidence of a brainstem stroke was found.
5.6.1.1 Electrophysiological map procedure

No changes were made to the microstimulation mapping procedures. Motor outputs for right and left cortical motor outputs were mapped pre-infarct and post-spontaneous recovery.

5.6.1.2 Cortical Infarct Procedure

Two changes were made to the procedure for inducing the ischemic brain injury was as previously described. A 26 gauge Hamilton syringe needle was used instead of the 22 gauge hypodermic needle used for the previous three subjects, and ET-1 injections were made at depths 1-mm superior to stimulation sites. Each site that evoked a shoulder or elbow response to stimulation was injected with two rounds of ET-1, for a total of 36 $1\mu$l injections (Fig. 5.30 and 5.32). Each injection was made at depths of 2 to 2.2 mm.

5.6.1.3 Magnetic resonance imaging

MRI scanning procedures were as described for previous subjects. MR scans were acquired pre-infarct and then 10 days post-infarct to document ischemic injury.

5.6.1.4 Assessment of motor performance on the behavioral reach task

After several months of training on the behavioral reach task, four pre-infarct reach performance assessments were taken. The timeline was originally scheduled to be in close proximity to subject A03354, thus the first pre-infarct assessment was conducted in early September. However, the subject
developed behavioral issues requiring psychoactive medication to control. The experiment was delayed several months, therefore additional pre-infarct measurements were taken to gauge stability of performance prior to moving forward with the experiment. Then, reaching performance was tested on days four, seven, twelve and nineteen after ischemia and then weekly throughout the rest of the experiment. Comparison of performance of the behavioral reach task included all data up until removal from the study (7 weeks post-infarct).

5.6.2 RESULTS

5.6.2.1 Anatomical Results: Ischemic infarct size and location

As mentioned in METHODS, 36 1µl ET-1 injections were made within the shoulder/elbow representation of left primary motor cortex; 2 injections to each movement-related site. As for previous subjects, coregistration of MRI scan sections and neurophysiological map was used to reconstruct ET-1 injection site locations (see Fig. 5.30). Based on this reconstruction, ET-1 injections appeared to be within the more superficial layers of the motor cortex.

At 10 days post-infarct, the volumetric analysis of left motor cortex lesioned tissue was carried out using the Image-J software as previously described. 19 image slices (1mm thickness each) were affected, with a volume of 893.25 ± 32.76 (SD) mm³ extending through all layers of grey matter and into the white matter, which corresponds to a cube ~ 9.6-mm per side (Fig 5.31). The area of signal intensity changes corresponded with the stereotaxic coordinates
for intended ET-1 injection sites in the pre-central gyrus, but also appeared to extend rostrally into the pre-motor areas. The subcortical structures remained intact.
Figure 5.30. Coregistration of MRI scan section and neurophysiological map used to reconstruct ET-1 injection site locations (indicated by white markings) in left motor cortex with respect to stereotactic coordinate records. **A.** T1-weighted MRI scan (1mm slice thickness, contiguous slices) acquired in coronal plane (TR=500, TE = 5, flip angle = 40 degrees). Images are ordered in columns from rostral (top left) to caudal (bottom right). SPCD, superior precentral dimple; CS, central sulcus, LS, lateral sulcus; STS, superior temporal sulcus; CGS, cingulated sulcus; M1, primary motor cortex; S1, primary sensory cortex; PMA, pre-motor cortex; SMA, supplementary motor cortex. Scale bar = 4 mm. **B.** Schematic diagram representing target area for ET-1 injection sites above indicated by the dark grey box. AS, arcuate sulcus; TS, temporal sulcus; RPCD; rostral precentral dimple; PCD, post-central dimple.
Figure 5.31. Represents the ET-1 induced lesioned tissue within the left cortex. T2-weighted MRI scans (1 mm slice thickness, contiguous sliced, 0.43 mm x 0.43 mm pixel spacing) acquired in the coronal plane 10 days post-injury were used to manually trace the region of interest (ROI) on each individual section; defined as area of abnormal signal intensity change (outlined in yellow). The lesion volume was calculated by multiplying ROI area (mm$^2$) by slice thickness. Coronal images) are in order by rows from rostral (top left) to caudal (bottom last).
5.6.2.2 Physiological Results

In general, this monkey’s right motor cortex appeared larger than the left, as evidenced by the greater number of sites required to adequately explore the arm representation area of right motor cortex ($\chi^2=8.82$, $P=0.003$). However, the number of sites explored within each motor cortex (right and left) did not differ between pre- and post-infarct mapping procedures (right: $\chi^2=0.038$, $P=0.85$, left: $\chi^2=0.025$, $P=0.87$).

5.6.2.2.1 Ipsilesional cortex changes

A majority of the 78 sites explored in the ipsilesional (left) cortex (58%) pre-infarct evoked arm-related movement responses to stimulation. Of these arm-related sites, 38% evoked visible shoulder and elbow movements, 49% evoked wrist/hand/forearm movements, 9% evoked dual joint movements (ex. shoulder with trunk shift) and 2 sites (4%) evoked complex whole arm movements (see Fig. 5.32a). After the two week spontaneous recovery period nearly half (48%) of the 84 sites explored continued to evoke arm-related responses. The prevailing changes found were the reduction of the shoulder/elbow representation to 18% of arm-related sites and the expansion of wrist/hand/forearm representations into the pre-infarct shoulder/elbow territory. 78% of arm-related sites now evoked wrist/hand forearm responses to stimulation (see Fig. 5.32b).
5.6.2.2.2 Contralesional cortex changes

A majority of the 121 sites explored in the contralesional (right) cortex (53%) pre-infarct also evoked visible arm-related responses to stimulation. Of these arm-related sites, 39% evoked visible shoulder and elbow related movements and 45% evoked wrist/hand/forearm related movements. There was very little change observed in the electrophysiological map derived after the two week spontaneous recovery period, with similar proportion of sites evoking visible arm-related responses (56%). Within these arm-related sites, there was also very little change in the proportion of shoulder/elbow (36%) and wrist/hand/forearm (56%) sites from the pre-infarct map (see Fig. 5.32a and b).
Fig 5.32. Surface map of microstimulation evoked motor outputs for right and left motor cortex **a** pre-infarct, **b** post-spontaneous recovery. Each electrode penetration is represented at its corresponding position by a color indicating the body territory activated at threshold current (see color legend). The shoulder/elbow representation was circumscribed by 2-mm of other movements or non-responsive sites (black). Rostral and caudal boundaries indicated by arcuate sulcus (AS) and central sulcus (CS); Middle Cerebral Artery and branches represented by red lines, green line represents pre-central dimple. Dual response sites are represented by both body territory colors. ET-1 injection sites are indicated by the syringe symbol.

*continued*
Fig. 5.32, continued

B POST-RECOVERY

RIGHT

LEFT

Subject A01074

Color Legend
- SHOULDER
- ELBOW
- FOREARM
- WRIST
- DIGITS
- LEG
- TRUNK
- FACE
- NO RESPONSE
- COMPLEX ARM
- ET-1 INJECTION SITE
5.6.2.2.3 Comparison of ipsilesional and contralesional cortex changes

Overall, we explored comparable numbers of sites that elicited arm-related responses pre- and post-infarct for the ipsilesional and contralesional cortex ($\chi^2=1.40$, $P=0.237$). A Chi-square test weighted by number of arm-related sites for each cortex side was used to compare changes in shoulder/elbow and wrist/hand/forearm movement representations between the ipsilesional and contralesional cortex pre- and post-infarct. As expected, the only significant difference was found post-infarct within the ipsilesional cortex in the expansion of the wrist/hand/forearm movements into the pre-infarct shoulder/elbow territory (see Fig. 5.32b and Fig. 5.33) ($\chi^2=15.84$, $P=0.001$).

![Change in Arm Representation Area](image)

Figure 5.33. Represents change in the proportion of sites that evoked shoulder/elbow and wrist/hand/forearm related responses for the ipsilesional (left) and contralesional (right) motor cortex pre- and post-infarct. Results are presented as a percentage of the total number of arm-related sites for each motor cortex. Blue bars = shoulder/elbow sites; Red bars = wrist/hand/forearm sites.
5.6.2.2.4 Arm representation threshold current comparison

The mean threshold current levels (µA) required to evoke the specified shoulder/elbow and wrist/hand/forearm responses for pre- versus post-infarct maps for the ipsilesional and contralesional cortex were compared (see Table 5.6). The threshold stimulation current to elicit shoulder/elbow responses remained comparable pre- and post-infarct for both the ipsilesional and contralesional cortex ($\chi^2 = 2.07$, $P = 0.559$). Less current was required to evoke wrist/hand/forearm responses post-infarct for both the ipsilesional and contralesional cortex (Table 5.6; $\chi^2 = 12.34$, $P = 0.006$).

Table 5.6. Arm representation threshold current levels

<table>
<thead>
<tr>
<th></th>
<th>Left Cortex</th>
<th>Right Cortex</th>
<th>Left Cortex</th>
<th>Right Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>Pre</td>
<td>29.72 ± 14.8</td>
<td>38.89 ± 15.65</td>
<td>34.55 ± 19.08</td>
<td>35 ± 16.82</td>
</tr>
<tr>
<td>Post</td>
<td>29.38 ± 9.04</td>
<td>35.83 ± 20.2</td>
<td>25.54 ± 13.49</td>
<td>24.61 ± 13.97</td>
</tr>
</tbody>
</table>

Mean threshold current levels (µA) ± SD pre versus post-infarct for ipsilesional (left) and contralesional (right) cortex subcategories of arm representation area. Proximal = shoulder/elbow responses; Distal = wrist/hand/forearm responses.

5.6.2.3 Behavioral Results

5.6.2.3.1 Clinical Observations

Early during recovery from anesthesia the monkey demonstrated asymmetrical synergistic posturing similar to that observed in the other three monkeys of whole limb extension of left arm, whole limb flexion of right arm and rotation of the head toward the left. This right arm flexor synergy pattern
worsened with head rotation toward the left, she lost her balance posteriorly and was unable to right herself. By early day one post infarct, she was moving about the cage using all four limbs, although the right arm was not as useful as the other three. She would brace herself on an extended right arm for balance in sitting when reaching for food with the left. She recovered functional use of the right arm fairly quickly. By day two, she was able to reach with either arm for food. During observation of natural cage behaviors it was difficult to discern differences in motor performance between right and left arms; proximal right shoulder weakness was subtle. Four days post-infarct she was independent with all cage behaviors, and was using both hands for hygiene, grooming and feeding behaviors.

5.6.2.3.1 Behavioral reach task performance

Four days post-infarct, the monkey was able to complete all components of the behavioral reach task with the left arm (press start switch, reach into wells, reward retrieval, transport food to mouth, etc). However, for right arm trials she would place her right hand on the start switch but would not depress the switch to start the trial. She was able to reach into all four target wells with the right arm successfully after each well door opened. Therefore, in order to collect performance data for right arm reach trials, the well door was opened after she faced forward and placed her hand on the start switch. By one week post-infarct, she was able to perform all components of the task with her right arm.
For movement time, she showed the greatest difference in performance immediately after infarct (4 days post). Right arm reaches were slower ($F=42.98$, $P <0.0001$) regardless of target location. Her reach performance gradually improved over the time span of the study, approaching pre-injury performance levels by final behavioral assessment measures (Fig. 5.34). At 3 weeks post-infarct she became very disinterested in food, which negatively impacted performance on all phases of reach performance (see days 21 and 28 post-infarct). She was restarted on Fluoxetine to stimulate her appetite. After 2 weeks on the medication, in addition to the notable improvement in her affect and appetite, there was direct carryover to performance on the behavioral reach task (see figure 35 days post movement time). Therefore, the measures for these two sessions were not considered valid and were excluded from all analyses of reach performance (movement time, dwell time, transport time).
Figure 5.34. Represents mean time (in milliseconds) required to reach from start switch into wells with left (blue bars) and right (red bars) arms. (error bars = standard error). Pre-test, baseline measures prior to lesion; sub-acute, measures during spontaneous recovery period; control period, weekly assessments – no rehabilitative training. Days 21 and 28 post infarct times were not included in analyses due to disinterest in food determined to be unrelated to the experimental infarct. (pre-test 3 not included in analysis due to uneven number of observations for ANOVA). Vertical dashed lines indicate time points for electrophysiological cortical maps.

Impairments in performance for retrieval of food from wells (dwell time: F=2.55, P=.028) and transporting food to mouth (transport time: F=8.54, P<0.0001) with the right arm were similar, with mean times being slowest 4 days post-infarct (see figures 5.35 and 5.36). Although no statistically significant interaction was
found, target location influenced her ability to retrieve and transport food with the right arm, with the slowest times to retrieve food from high targets medially up and across the body (1041 ± 187 (SE) ms), and slowest times to transport food to mouth from high targets laterally up and away from the body (1679 ±198 (SE) ms).

**Figure 5.35.** Represents mean time (milliseconds) required to retrieve food reward for left (blue bars) and right (red bars) arms. (error bars = standard error). Pre-test 1 through 4, baseline measures prior to lesion; sub-acute, measures during spontaneous recovery period; control period, weekly assessments – no rehabilitative training. Pre-test 3 not included in analysis due to uneven number of observations for ANOVA. *Vertical dashed lines* indicate time points for electrophysiological cortical maps.
Figure 5.36. Represents mean time (milliseconds) required to transport food from well to mouth for left (blue bars) and right (red bars) arms. (error bar = stand errors). Pre-test, baseline measures prior to lesion; sub-acute, spontaneous recovery period; control period, weekly assessments – no rehabilitative training. Pre-tests 2 & 3, days 19, 21 and 42 days post not included in analysis due to uneven number of observations for ANOVA. Vertical dashed lines indicate timeline for electrophysiological cortical maps.

Interestingly, although no formal kinematic measures were collected during behavioral reach performance assessments subtle proximal right shoulder weakness was observed as a change in arm posture for depressing the start switch to begin a trial. Initially, holding her right shoulder abducted away from the
body with the hand perpendicular to the switch was her preferred posture. Early post-infarct, she kept the arm tucked at her side with the hand directly in line with the switch. By 6 weeks post-infarct she had resumed her pre-infarct posture.

5.6.2.4 Lessons learned from this subject

Initially, we were unclear whether the experiment would be successful at producing any measurable change in cortical motor output following ET-1 induced infarct in this subject due to her rapid recovery of function and very subtle deficits during in-cage behaviors. Several factors were different for this subject from the other three subjects that could potentially confound the results. First, her long time use of Fluoxetine (Prozac) for behavioral modification secondary to stress anxiety was a concern based on literature that it may have an effect on vascular smooth muscle tone, thereby modulating cerebral blood flow and counteracting the effectiveness of the ET-1.\textsuperscript{184-186} Second, we changed the type and gauge of needle used for delivering the ET-1. Third, we decided to inject ET-1 one millimeter superficial to the depth of stimulation that evoked movement responses. Finally, the same batch of ET-1 was used for all four subjects and when this subject presented with such subtle deficits it raised questions of how long the drug could be kept once in solution. At the second electrophysiological mapping session, it was evident that there was a change in the left cortical motor output map. The shoulder/elbow area was significantly reduced, and to evoke these responses we had to increase the stimulation depths from pre-infarct testing.
5.7 Discussion

A series of unilateral intracortical ET-1 injections targeting the M1 shoulder/elbow representation were effective at producing a lesion, and resulted in impaired reaching performance of the upper limb contralateral to the site of the lesion in the macaque monkey. As described in the METHODS, ET-1 lesion-induced behavioral changes in reach performance were measured through observation of natural cage behaviors and the behavioral reach performance data. Deficits in reach performance were confirmed by increased time to complete components of the behavioral task, and errors in path trajectory during the movement and transport phases of the reach. The monkey with subtle proximal arm deficits (A01183) recovered use of the impaired limb for natural cage behaviors (ie. hygiene and feeding) fairly quickly.

In agreement with previous studies in squirrel monkeys and rats, in our subacute recovery experiments, the subjects with mild lesions demonstrated the ability to perform natural cage behaviors at near pre-stroke levels, but exhibited motor deficits on the skilled behavioral task. After the spontaneous recovery period, it was expected to see the reduction in shoulder/elbow representation since that was the intended target of the lesion. However, for subject A01183, the expansion of sites in the contralesional M1 that post-injury elicited complex whole arm and bilateral arm responses was unexpected. Enrichment activities were selected that minimized foraging and bilateral play, but behaviorally, the monkey was free to move about the cage; using both arms for natural cage
behaviors. Observations of cage behaviors during the spontaneous recovery period indicated that although the subject (A01183) was able to use the impaired arm, if given the choice, preferred to use her left arm for feeding and hygiene activities.

For the subject with a severe lesion (A03354), based on previous work by Nudo and others,\textsuperscript{43, 55} to have no visible arm movements evoked to stimulation throughout the right arm representation area in the lesioned M1 post-rehabilitation was unexpected. Perhaps one reason for this difference may be due to the size of the intended lesions. Nudo’s model\textsuperscript{55} targeted a small area within the distal hand area, whereas in the current model all shoulder/elbow sites were targeted. Based on the behavioral data in the current study for subjects with subtle motor impairments, we believed targeting all proximal arm sites was needed to acquire the intended severity of proximal limb motor impairments. For the subject with a severe lesion, the reduction of shoulder/elbow representation and expansion of the distal hand area in contralesional M1 post-recovery was a novel finding. Since the non-impaired arm was restrained at her side during the rehabilitative training sessions, these changes were not the result of intensive left arm rehabilitative training. A possible explanation for the changes in the homologous regions of the contralesional M1 in the absence of left M1 influence may be attributable to strengthening of PMRF motor outputs via increased collateral branches from uncrossed corticospinal pathways with reticulospinal neurons as well as strengthening existing corticoreticular
connections. Future anatomical studies are needed to investigate these changes in corticoreticular projection patterns following stroke recovery.

However, as mentioned earlier, subjects were free to move about the cage and arm use for natural cage behaviors was not restricted. Observations of natural cage behaviors were made 3 times a day, typically during meal times. She was less able to incorporate the paretic limb into natural cage behaviors throughout the spontaneous recovery period. As seen in human clinical stroke cases, the more severely impaired monkey developed increased reliance on the unimpaired limbs to perform hygiene and feeding activities. In fact, as she progressed with rehabilitation, her right arm reach performance improved on the behavioral reach task, and although she was capable of using the impaired limb for natural cage behaviors, she preferred to use the left arm (non-impaired). She developed compensatory strategies with feeding behaviors, using her hindfeet as an assist for holding and placing biscuits instead of incorporating her right arm into the task. This may be a limitation of using a monkey model because by being quadrupedal animals, unlike humans, monkeys can compensate with their hindlimbs. As we move forward with future projects, we need to develop methods for monitoring and quantifying the animal’s use of the impaired and non-impaired limb during natural behaviors.
5.7.1 ET-1 induced neurophysiological changes

After the two-week recovery period, cortical tissue where ET-1 was injected either became non-responsive or demonstrated a change in the representation of shoulder/elbow movements. This is consistent with an ischemic injury resulting in some combination of neuronal death and disruption of neuronal circuitry. However, unlike Nudo and others\textsuperscript{36, 42, 55, 189} who demonstrated expansion of the hand representation into intact cortical areas adjacent to the lesioned zone (peri-lesional) after small infarcts within the hand representation, in the current model, the proximal arm representation did not expand into the peri-lesional zone after a large lesion within the proximal arm representation area. A contributing factor to this difference may be because our objective was to create an infarct that covered the entire M1 shoulder/elbow movement representation, whereas Nudo’s model creates smaller infarcts targeting approximately 30\% of the hand representation area.

The nature of the deficits varied according to the methods used for the ET-1 injection. In the first experiment, very few ET-1 injections were made and injections were confined more to the surface of the cortex grey matter. The deficits were less severe and some responsive sites could still be obtained from the injected region; the changes in the body part represented were evident. However, in the second experiment with the ET-1 injected deeper, at or near the border of the grey and white matter, the deficits were severe, and evidence of plasticity in the PMRF was noted.
After the spontaneous recovery period, the subject with a mild lesion demonstrated evident changes in the shoulder/elbow representations of both the affected and contralesional M1. No substantial changes were noted in this subject's pattern of PMRF output. In the subject with a severe lesion, reaching was severely impaired immediately after the lesion. With intensive rehabilitation, gross reaching recovered in a few weeks, and reach times were slow but comparable to pre-injury levels after 16 weeks. Surprisingly, however, the shoulder/elbow representation in the affected M1 remained completely absent after recovery, and there was very little change evident in the contralesional M1. Put another way, this subject developed the ability to perform the reaching task with the right arm despite the fact that there were no sites in the left motor cortex that evoked right arm responses, and there were no sites found in the right motor cortex that evoke right arm movements, either. The novel result was that there was greater right arm (impaired) representation from left PMRF sites in the subject. This indicates that plasticity may occur in the PMRF in association with recovery from a severe cortical injury, and this in turn suggests that increased reliance on PMRF outputs for control of voluntary movement may be associated with upper limb recovery after a severe cortical injury.

5.7.2 Mechanisms for plasticity

After cortical injury, the surviving brain tissue in the lesioned and contralesional cortex may be substantially altered in its anatomic connectivity. One mechanism for plasticity could depend on strengthening of the uncrossed,
ipsilateral CST projections to ipsilesional (right side) spinal motoneurons influencing right arm muscles, which are less active than the contralateral projections in the intact nervous system.\textsuperscript{187, 188} By the removal of the stronger, contralateral CST connections, the ipsilateral CST neurons would now have greater influence over the target motoneurons. Increased ipsilateral CST control may result from ipsilateral neurons sprouting and forming new synapses at sites vacated by the injured axons, or by strengthening the synaptic effectiveness of the existing ipsilateral synaptic connections,\textsuperscript{28, 187, 188} thus creating the potential for replacement of the contralateral actions of CST neurons lost after injury. In the current study, in the subject with the mild lesion (A01183) one right arm-only response was evoked from contralesional cortex stimulation. No right arm responses were evoked from contralesional (right) cortex stimulation in the subject with the severe lesion (A03354). This suggests strengthening of ipsilateral CST projections was not a major contributor to recovery.

In addition to the direct CST projections to the ipsilesional (right) side of the spinal cord, the contralesional motor cortex can also influence these motoneurons indirectly through connections with RST neurons and interneurons at the spinal cord level.\textsuperscript{139, 162, 187} Structural changes of the postsynaptic terminals may be key substrates to triggering this brain rewiring, enhancing efficiency of existing synapses and signaling axonal sprouting from uninjured neurons.\textsuperscript{84, 190} Therefore, another mechanism for plasticity in concert with representational reorganization could depend on strengthening of existing
contralesional corticoreticular connections or increasing the density of
corticoreticular projections to right or left PMRF, or both. Findings of enhanced
right arm representation from ipsilesional (left) PMRF stimulation in the subject
with a severe lesion supports the hypothesis that recovery of arm function may
depend on increased corticoreticular activation from the contralesional motor
cortex.

It is established that RST neurons have the capacity to influence muscles
of the ipsilateral and contralateral limb both directly through monosynaptic
connections onto spinal motoneurons and indirectly through excitatory, inhibitory
and commissural interneurons within the intermediate zone and medial regions of
the ventral horn of the spinal cord. ¹¹, ¹⁶, ¹⁸-²⁰, ²², ¹¹⁷ Thus, neuroanatomical studies
could provide an important adjunct to these electrophysiological experiments.
Recovery of right arm function for the severely injured subject could have
resulted from expansion of the direct ipsilateral (right) RST projections to
ipsilesional spinal cord motoneurons, or stronger indirect RST projections via
commissural interneurons, or both. Enhancement of corticoreticular connections
from the contralesional cortex and/or RST projections onto interneurons
influencing right arm muscles would indicate recovery is the result of both cortical
and subcortical plasticity. Still, there is no guarantee that tracing would occur
effectively after injury, and careful controls would be required.
5.7.3 Alternative motor areas and recovery

Although not part of the current study, changes in premotor cortex (PMA and SMA) movement representation organization may contribute to recovery of arm function for subjects with smaller M1 lesions. The premotor areas share extensive connections with M1, send collateral branches to brainstem structures, and have direct access to the spinal cord through corticospinal projections, making these areas well positioned to compensate for the loss of neuronal function resulting from M1 lesions. Expansion of ventral premotor cortex (PMv) and SMA hand representation areas in chronic recovered animals has been shown to be proportional to the amount of hand area damage in M1; the greater the amount of sparing, the less the expansion of premotor hand areas.

In the present study, low threshold dorsal premotor cortex (PMd) sites were included in our electrophysiological maps since we stimulated as far rostrally as the precentral dimple. We did note increased bilateral and complex responses from PMd in the contralesional cortex in the subject with the mild lesion (A01183), but not in the subject with the severe lesion (A03354). However, we did not explore the SMA in these subjects, and a future study should focus on possible contributions of changes in SMA to the recovery seen in the more severe lesion. Whether this occurs through expansion of the direct CST pathway from SMA or by stronger corticoreticular projections from SMA, or both would be an interesting subject of future investigation.
5.7.4. **Future alterations to the model**

In retrospect, basing the injection sites solely on electrophysiological results during mapping was only partially successful. In addition, an improvement would have been to use the MRI images to determine the coordinates of the motor cortex, especially places where grey matter could be found deep. The deeper parts of M1, in the central sulcus, are special in that this is where the majority of monosynaptic projections come from.\textsuperscript{54,103} In order to produce the severe deficits associated with a stimulus for plasticity in the PMRF, it may be necessary to lesion these deeper areas specifically. A 3-dimensional representation of the motor cortex reconstructed from the MRI images could be used to plan stimulation depths prior to the first mapping surgery, and then injections could be made more completely to cover deep and superficial cortical grey matter for the shoulder/elbow representation of M1. T2-weighted MRI scans provided the necessary details for reconstruction of ET-1 injection sites and verification of the extent of lesions. Therefore, we can also reduce the time the animal is required to be anesthetized by eliminating the T1-weighted scans and focusing on scans that provide more details about the anatomy and the infarct for analyses.

Conducting post-mortem histochemical analyses on the first subject prior to moving forward with the other subjects may also have provided valuable information regarding electrode penetration depths and characterization of the lesion that could have helped control for the variability of lesion size and
behavioral deficits in subsequent animals. For example, the ET-1 concentration used in the current study was based on previous published results in the literature on rats.\textsuperscript{96} Perhaps in addition to injection every movement-related site with two rounds of ET-1, the concentration of ET-1 needed to be greater. Maybe to generate the desired behavioral deficits in monkeys, ET-1 injections needed to be made at 0.5 mm interpenetration intervals throughout the proximal arm area instead of only injection sites that evoked proximal arm responses.

5.7.5 Behavioral changes

The behavioral task itself was adequate for measuring changes in performance times for the movement and transport phases of the reach. However, greater improvement in performance times for the movement phase of the reach compared with the transport phase may have been inadvertently built into the design of the task. Subjects were trained to reach into the well within 3 seconds to retrieve the food reward or the feeder well door closed. Whereas, for the transport phase, once the food was retrieved there was no incentive for the subject to move quickly. Redesigning the study to report number of dropped rewards during transport attempts may provide data to further illustrate changes in behavior that contributed to the transport times across recovery. Another option could be to change the design of the table top so that after food retrieval from the well, if the subject drops the food to the table and does not pick up the food from the table after a pre-set amount of time, the food is removed and the task is reset for a new trial.
Changing the start switch to a flat button, requiring the monkey rest her hand on it to activate the switch may alleviate the problem all four subjects developed after infarct of not depressing the start switch to begin reach trials with the impaired arm. Redesigning the study to add recording EMG activity from shoulder girdle and forelimb muscles (elbow and wrist) during reach performance may provide data that would enhance our understanding of the subject’s post-infarct motor performance, delineating between unsuccessful attempts to reach and poor behavior. For example, video analysis without EMG can not discern between reach trials where the subject attempted to recruit upper limb muscles but was unsuccessful at moving the arm to reach and trials where the subject made no attempt to reach. Analysis of the EMG activity during reach performance may also provide further evidence of selected muscle recruitment patterns for reaching during recovery. With a chronic EMG implant, however, this would come at the cost of MR imaging, because the EMG wires are not MRI-compatible.

5.7.6 Translational benefits of the model

Naturally occurring lesions in humans are seldom as neatly restricted as those created in animal models. Experimental models enhance scientist’s and clinician’s understanding of specific motor tracts injured by stroke and changes in motor output patterns for these tracts, as well as interconnected motor areas associated with recovery. Primate models of stroke involve some aspects of Middle Cerebral Artery occlusion (MCAO) (ie. embolization, aneurysm clips,
ligatures, etc). These methods produce variable sized infarcts often involving cortical and subcortical structures. The lesion data of the current primate model provides new evidence that ET-1 may be an effective alternative to MCAO methods for ischemic injury in the macaque monkey, and adds further support for previous studies that ET-1 is effective at inducing focal ischemic lesions in the motor cortex when injected directly into the brain tissue.

Electrophysiology studies, like the current study, provide important findings to aid the decision making process in instituting rehabilitative intervention trials; optimizing basic therapeutic strategies in clinical trials. Although the corticospinal system is often the first system affected by stroke, multiple descending systems work in cooperation to produce coordinated voluntary control of arm movements. The corticospinal system enables independent movements of the fingers and performance of skilled hand functions. The medial systems, such as the reticulospinal system, provide control over integration of movements of the body and limbs, and synergistic whole limb movements. Postural and proximal limb muscle control provides the underlying muscle tone needed to initiate movement and sustain the resultant position of the limb. Thus, rehabilitation requires the recovery of postural and proximal control for whole limb/whole body movements, such as gross reaching, shifting the trunk in preparation for standing, and moving in bed as precursors to regaining fine motor skills requiring distal control of the hand and digit. For example, regaining adequate control of the hand/fingers to grasp the cup.
becomes pointless if the person is unable to reach to obtain the cup and transport the cup to his/her mouth to drink. Therefore, in addition to the lateral systems, it is important to understand the systems for proximal motor control, such as the reticulospinal system.

The current study has revealed that after a severe lesion, recovery of arm function was associated with significant changes in PMRF upper limb motor outputs. Now that changes in PMRF motor outputs can be shown to occur after severe injury, further studies need to be designed to investigate how these changes are influenced by therapeutic interventions, and the impact these changes in PMRF reorganization may have on functional outcomes. Several questions could be addressed. Does the mode of therapeutic intervention, unilateral versus bilateral, impact PMRF reorganization? Does level of recovery (good versus incomplete) influence these PMRF reorganization patterns? How do cortical and PMRF plasticity interact? Future research can highlight the importance of applying the appropriate rehabilitation interventions at the appropriate time to enhance adaptive cortical and subcortical plasticity for recovery of arm function.

5.7.7 Conclusion

For the first time, we have demonstrated ET-1 induced ischemia in the macaque monkey motor cortex, and functional reorganization of upper limb motor outputs from the ipsilesional and contralesional cortex and PMRF associated with upper limb recovery after a severe cortical injury. This opens a
new line of investigation to compliment plasticity research to understand reticulospinal contributions to functional recovery of reaching after stroke.
Chapter 6: Discussion and Conclusions

Compared to the contributions of the corticospinal pathways, very little is known about the reticulospinal system's role in recovery of upper limb motor function after cortical injury. Single cell recording and stimulation studies in the intact animal have demonstrated that the PMRF has the capacity to reciprocally activate flexor and extensor muscles of both arms.\(^5, 6, 8-10, 23\) Clinical inferences have been made that after cortical stroke, movements of the impaired arm become constrained to whole limb flexor or extensor patterns (synergies), resembling the motor output patterns of the PMRF.\(^49-51\) These altered co-activation patterns are indicative that increased reliance on PMRF activation is the reason these abnormal synergies exist. However, to date there is no direct PMRF stimulation data in the human brain to confirm these theories. The data presented in this dissertation have helped fill this void of information by providing possibly one of the most extensive studies to date of the non-human primate descending motor systems during recovery from ischemic cortical injury. This report is the first to describe the use of Endothelin-1 (ET-1) to induce a focal ischemic injury in the macaque motor cortex, and map the changes in motor output effects for the ipsilesional and contralesional cortex and both sides of the PMRF.
6.1 Neurophysiological techniques

Repetitive microstimulation (stimulus trains) is a common neurophysiological method used for studying motor outputs from the motor cortex in the normal and injured brain. The advantages of this method versus single pulse stimulation (StimulusTA) is that stimulus trains can be used in the anesthetized as well as awake behaving animal, results are obtained quickly and evoked movements are apparent. The physiologic difference between stimulus trains and StimulusTA is that stimulus trains are considered more likely to recruit motor pools through polysynaptic connections via temporal summation, and may reveal indirect pathways, complicating results. However, in the motor cortex, adjacent cells have similar motor outputs. Hence, engagement of polysynaptic pathways within adjacent regions of the motor cortex may not produce much of a difference in motor output effects. For the reticulospinal system, however, with no apparent topographical organization of motor output effects within the PMRF (sites evoking facilitation vs. suppression), engagement of polysynaptic pathways may produce mixed effects.

Overall, the results from StimulusTA and stimulus trains in the PMRF in the awake behaving monkey revealed a common pattern of bilateral reciprocal motor outputs to the upper limbs: ipsilateral flexor with contralateral extensor facilitation with suppression of the antagonists. Since StimulusTA are thought to be better able to reflect monosynaptic and disynaptic pathways, these similarities between the two methods indicate that more mono and disynaptic
pathways are being activated well by both methods. One difference found was the excessive facilitation from stimulus trains. The design of the study could have contributed to this difference. With relatively no background level of EMG, suppression of the EMG was hard to detect. Perhaps if stimulus trains had been delivered during the reaching phase of the task, more suppression could have been detected. Even controlling for this limitation, however, there was still an obvious tendency for stimulus trains to evoke facilitation where the StimulusTA response was suppression. Therefore, we believe at least some of the over-representation of facilitation may be attributed to more polysynaptic pathways being activated by stimulus trains. The second difference was the fairly equal distribution of effects among limb girdle, proximal and distal muscles for stimulus trains versus the prevalence of effects for limb girdle muscles for StimulusTA. This tendency for stimulus trains to have more effects in distal muscles may also indicate that stimulus trains engage more polysynaptic connections. Despite these differences, the general map of PMRF motor output effects evoked with stimulus trains was representative, suggesting this method could accurately capture the output patterns of the PMRF after brain injury.

6.2 Ipsilateral corticospinal control of upper limb motoneurons

Anatomical and physiological studies of the corticospinal system have traditionally focused on the contralateral effects. One reason for this is the predominance of contralateral projections. Another reason is that injury
to the motor cortex or the corticospinal tract results in impairments in movements on the opposite side of the body. However, studies in humans using neuroimaging and trans-cranial magnetic stimulation have shown changes in the undamaged motor cortex after unilateral stroke when attempts are made to move the impaired limb, suggesting enhanced ipsilateral corticospinal involvement may contribute to recovery. Based on the anatomical evidence, uncrossed corticospinal projections from the intact motor cortex influence upper limb motoneurons through direct and indirect connections, making it plausible for ipsilateral corticospinal activation to contribute to recovery of motor function after injury.

Our electrophysiological results support previous anatomical evidence that the primary (M1) and premotor cortical areas related to ipsilateral upper limb movements exist in the macaque monkey. Ipsilateral effects were equally prominent for trunk/shoulder and limb muscles for all three cortical areas. A greater proportion of ipsilateral effects were produced from premotor areas. These findings were expected based on previous evidence of deficits in proximal limb motor control after damage to premotor cortical areas. The premotor areas are more known for preparation and selection of movements, and can influence muscles directly or indirectly via interneuronal connections. Since both M1 and premotor areas give rise to corticospinal projections, the premotor areas have the ability to influence the generation and control of movements independent of M1. Therefore, increased reliance on intact ipsilateral
corticospinal projections from SMA may add to the novel muscle co-activation patterns of the upper extremity seen after stroke.

Ipsilateral corticospinal projections from M1 and premotor cortex terminate predominantly in the medial or intermediate zone, influencing primarily proximal limb muscles.\textsuperscript{27, 29, 56} Therefore, our findings of equally prominent ipsilateral effects for trunk/shoulder and limb muscles were expected. The design of our study may have contributed to the greater percentage of ipsilateral effects than previous studies.\textsuperscript{56, 146, 148} Perhaps if we had included the intrinsic hand and finger muscles, the balance of ipsilateral and contralateral corticospinal projections, as well as the distribution of ipsilateral effects for proximal versus distal limb muscles would have changed. Future studies should investigate this possibility.

Interestingly, we found little evidence of ipsilateral CST effects for trunk/shoulder and limb muscles in our ischemic injury model. This was surprising given our findings of ipsilateral effects in intact animals. The differences in experimental designs probably contributed to the discrepancy in ipsilateral effects between the two studies. For the ischemic injury study, visibly evoked muscle contractions or movements were recorded from stimuli applied to arm-related sites within M1 and the dorsal premotor area in anesthetized animals. For the ipsilateral corticospinal study, EMG responses were recorded from flexor and extensor muscles of both arms during stimulation applied throughout M1 and premotor areas while subjects performed a reaching task.
Anesthetic agents may have varying effects on motor evoked potentials (MEP) depending on the mechanism of action.\textsuperscript{194} For example, isoflurane, a neuromuscular blocking agent, may suppress MEP amplitudes and even block responses completely.\textsuperscript{195} Careful consideration was used for developing the anesthesia protocol for the ischemic injury model in order to create the necessary surgical plane of sedation without blocking MEPs from stimulation. Ketamine\textsuperscript{196} and Dexmedtomidine\textsuperscript{197} were selected for their ability to produce dose-dependent sedation and analgesia with little to no interference with cortical evoked potentials.

As stated above, the cortical motor output maps for the ischemic injury model were derived by recording visible movements evoked without EMG analysis. However, EMG activity was also recorded from flexor and extensor muscles of both arms during stimulation. Thus, more ipsilateral CST responses may have been present after recovery, but simple observation of evoked movements may have underestimated and even missed potential responses. Further analysis of the EMG activity recorded with stimulation may be necessary to reveal a more accurate representation of ipsilateral and contralateral CST effects.

Finally, another contributing factor to the difference in ipsilateral CST effects may be the cortical motor areas explored for the two studies. Our findings for the ipsilateral CST study in the intact animal showed a greater proportion of ipsilateral CST effects from premotor areas compared with M1. Perhaps if we
had mapped the motor outputs for SMA and PMA after recovery in the ischemic injury study, we may have seen a change in the proportion of ipsilateral effects with recovery. Future studies should investigate this possibility.

6.3 Effectiveness of ET-1 induced ischemia in the macaque

We were able to demonstrate that ET-1 injections are effective for inducing focal ischemic lesions in the motor cortex in the macaque monkey. For three of the four subjects, the lesions were contained to the proximal arm area of the motor cortex. One subject presented with profound neurologic deficits encompassing the entire right side of the body. Human clinical stroke and animal stroke models all report variability in location and extent of lesion and neurologic deficits. Therefore, we were not surprised by the variability in lesions and behavioral deficits between the four subjects. It appears that the greatest damage to cortical function was produced by injections made within the deeper layers of the grey matter or with injections made in the white matter immediately below the grey matter. According to a study by Hughes et al, using ET-1 injections in the white matter immediately below the grey matter in rats resulted in extensive axonal disruption and neuronal death in the grey matter just above the targeted white matter. These findings indicate that perhaps the deeper injections for our subject created greater disruption in axonal integrity within white matter tracts, consequently resulting in her greater behavioral deficits. Another contributing factor to the variability in neurologic deficits for our subjects may
have been that the injection depths within the cortical grey matter targeted both the direct and indirect corticospinal connections to motoneurons influencing upper limb muscles.\textsuperscript{103}

6.4 Contributions of the reticulospinal system to arm recovery

In the subject with a mild lesion, reaching was mildly impaired after 2 weeks of spontaneous recovery. Changes were evident in the shoulder/elbow representations of both the lesioned and contralesional M1. No substantial changes were noted in the pattern of PMRF output. These findings indicate that there may be increased reliance on undamaged remote cortical regions including the ipsilateral corticospinal tract associated with arm recovery after a mild cortical lesion. It is beyond the scope of the present study, but there may also be structural changes in the indirect corticospinal connections (ie. corticocortical and corticoreticular) associated with arm recovery after a mild cortical lesion.

In a subject with a severe lesion, reaching was severely impaired immediately after the lesion. With intensive rehabilitative training, gross reaching recovered in a few weeks, and reach performance was slow but comparable to pre-injury levels after 16 weeks. Surprisingly, the shoulder/elbow representation in the lesioned M1 remained completely absent after recovery, and there was very little change in the contralesional M1. The novel result was that there was greater right arm (impaired) representation from left PMRF sites. These findings
indicate that there may be increased reliance on PMRF motor outputs associated with arm recovery after a severe cortical lesion.

Even though we explored a small subset of PMRF sites, we were able to demonstrate changes in right arm representation between the right and left PMRF. Again, with no apparent topographical organization of motor output effects within the PMRF, we believe the response pattern elicited for the selected subset of PMRF sites was representative, and had we performed a more complete motor output map the outcome would have been the same.

6.5 Future studies

The current findings indicate that recovery of arm function after severe stroke was reliant upon the contributions of both the corticospinal and reticulospinal systems. Further understanding of the reticulospinal system’s capacity for bilateral upper extremity coordinated movements in the intact and injured nervous system may provide beneficial insights that will enhance current rehabilitation practices and facilitate development of new treatment interventions for upper extremity recovery after stroke.

Electrophysiological techniques detect primarily functional changes within systems. Neuroanatomical studies are needed to investigate the structural changes within the PMRF that may contribute to the functional changes in motor output effects recorded during recovery. For example, although significant changes in right arm representation from PMRF stimulation were not found for
the subject with a mild cortical lesion, perhaps structural changes such as increased synaptic strength or axonal sprouting could be found. These changes would also be indicative of plasticity within the PMRF with recovery.

Neuroanatomical tract tracing studies can be utilized to investigate changes in corticoreticular and reticulospinal projection patterns, and provide us with valuable information about PMRF reorganization after cortical stroke. Retrograde tracer injections in the contralesional motor cortex paired with anterograde tracer injections in the ipsilesional side of the cervical spinal cord would allow us to chart corticoreticular projections onto both sides of PMRF and reticulospinal projections from the undamaged motor cortex. Anterograde tracer injections in the premotor areas of the ipsilesional cortex paired with retrograde tracers in the lesioned side of the cervical spinal cord would allow us to address questions about the influence of premotor connections on PMRF reorganization after stroke.

Nudo and others\textsuperscript{42, 55} have demonstrated that reorganization occurs in remote cortical regions after injury. Therefore, the next logical experiment would be to repeat the same study and map the motor outputs in SMA and PMRF. A second set of experiments would be to create focal ischemic lesions in the premotor and SMA proximal arm representation areas and explore whether the reticulospinal system undergoes plasticity associated with recovery.

Next, studies need to compare the effects of unilateral and bilateral rehabilitative training on both cortical and PMRF reorganization. Designing a
study that has the subject producing muscle recruitment patterns in and out of
the typical “synergies” for the PMRF would allow us to test the recruitment
patterns associated with recovery. With a better understanding of the neural
mechanisms that contribute to changes in functional recovery, more effective
therapeutic interventions and appropriate timelines can be developed. With the
delivery of appropriate therapeutic interventions throughout the acute, subacute
and chronic periods after stroke, perhaps movements of the affected arm would
not become constrained to abnormal synergistic movement patterns.49-51

6.6 Translation to clinical practice

What are the clinical implications of this research? Though clinical
theories suggest relationships between cortical and brainstem systems and arm
recovery exist, very little research has explored these relationships. However,
inferences have been drawn from the motor patterns presented in the paretic
limbs following stroke regarding the role the reticulospinal system plays in
recovery. Dewald and colleagues50 have shown that stroke survivors display a
predominance of whole limb movement patterns (synergies) of the paretic arm
after stroke, and suggested that increased reliance on the reticulospinal system
may be one explanation for this abnormal voluntary control. When subjects
studied by Dewald’s group50 elevate their paretic shoulder, they typically flex the
elbow and shoulder as a tightly coupled movement. This movement pattern is
consistent with the normal motor output from the PMRF reported from single
neuron recording and stimulation studies in the cat and monkey\textsuperscript{5, 6, 8-10, 23}

Microstimulation techniques in the current experiment enabled us to investigate the functional reorganization in upper limb motor outputs of the PMRF as well as reorganization in the ipsilesional and contralesional motor cortex during recovery. To our knowledge, this is the first time that an electrophysiological study has investigated the contributions of both cortical hemispheres and bilateral reticulospinal motor output effects to upper limb muscles during recovery.

Despite the complete absence of evoked responses to stimuli in the arm representational area of the lesioned cortex and minimal changes within the contralesional cortex, the subject with a severe lesion regained functional use of the impaired arm outside the constrained synergistic patterns of movement. So, what may have contributed to recovery of arm function for this subject? In addition to the cortical changes, there was an increase in right (impaired) arm representation from PMRF stimulation and asymmetries in the balance between left and right PMRF outputs for ipsilateral and contralateral upper limb effects. This suggests that there may be increased reliance on PMRF motor outputs associated with upper limb recovery after a severe cortical injury. This opens a new line of investigation to compliment cortical plasticity research to understand reticulospinal contributions to functional recovery of reaching after stroke.
6.7 Conclusion

With such diversity between clinical practices and the research evidence in the management of the paretic arm, restoration of full functional use after stroke remains a major problem in rehabilitation. Clinicians have been taught that time since stroke, extent of brain damage and severity of hemiparesis are important prognostic predictors for recovery, with the initial grade of paresis being the most important predictor for motor recovery. These studies indicate that stroke survivors with complete loss of hand function early after stroke typically recover very little functional use of the arm. Those with some muscle activity of the wrist and hand appear to do well if interventions were initiated early during recovery, and rehabilitation emphasized meaningful task-specific training. However, early in therapy, the emphasis of rehabilitation often shifts to general functional independence, such as activities of daily living, ambulation and balance, and not on improving function of the impaired arm. Following the principles of adaptive plasticity, intensive use of the affected arm is vital to cortical reorganization, and therefore recovery. This is an important concept for rehabilitation because by not specifically exercising the impaired arm, the movement representational areas will be further reduced by its disuse.

Clinical theories attribute deficits in voluntary motor control of the arm to loss of normal corticospinal control, thereby altering the available normal muscle co-activation patterns, and limiting the ability to generate movements outside fixed whole limb synergistic patterns. The results of our study provide further
confirmation that voluntary control of reaching requires the coordination of multiple motor systems,\textsuperscript{142} and adaptive plasticity occurs within brainstem structures as well as cortical structures with recovery.
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