ROLE OF REMOTE MOTOR CORTEX IN RECOVERY FOLLOWING AN EXTENSIVE MOTOR CORTICAL LESION IN A NON-HUMAN PRIMATE

By

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ABSTRACT

The ultimate goal in this research was to study the role of remote motor cortex in recovery from stroke in a non-human primate model in order to complement existing behavioral and anatomical findings from rodent studies and aid in the interpretation of human neuroimaging data. The notion that functional reorganization occurs in the intact (i.e. uninjured) hemisphere during recovery from stroke stems from functional imaging studies showing increased activation of the intact hemisphere in stroke patients. Supporting neuroanatomical studies in the intact cortex of the rat following focal ischemic lesions in the sensorimotor area demonstrated increased dendritic arborization and neurite growth followed by synaptogenesis in a pattern corresponding both spatially and temporally with behavioral recovery. However, the relationship of plasticity in the intact hemisphere to motor recovery is still not clear. It has been mostly related to poor motor outcome but also suggested to participate early or transiently during the recovery process.

Transcranial magnetic stimulation (TMS) has been used extensively to study excitability of the motor cortex in normal subjects and changes in motor evoked potentials (MEPs) and in intracortical excitability (ICI) in stroke patients. The presence of ipsilateral MEPs (iMEPs) and changes in the uninjured hemisphere have been related both to good and poor motor recovery. This technique has also offered insight into the mechanisms underlying iMEPs and plasticity in the injured hemisphere in terms of the participation of ipsilateral pathways in stroke recovery.

Recent neuroimaging data seems to point to the involvement of spared ipsilesional motor areas (in the same hemisphere as the lesion). It has also been suggested that regain of function of the paretic hand occurs as a consequence of a dynamic, bihemispheric reorganization after stroke onset and that the premotor areas are especially suited to reorganize following injury to the corticospinal tract. Given that lesion location and size determine the outcome and degree of cortical plasticity after stroke, reorganization of the motor cortex may follow different mechanisms depending on whether primary motor, premotor or supplementary motor areas in the ipsilesional hemisphere are spared and depending on whether subcortical structures are included in the lesion.

In summary, there is ample evidence of plasticity in both hemispheres following stroke. However the relationship to recovery is not clear. The present studies intend to clarify whether plasticity in the injured cortex is related to recovery of function and whether there is a change in ipsilateral electromyographic (EMG) activity (from the uninjured cortex) following an extensive cortical lesion.

The aim of the present study was to address three experimental hypotheses. The first hypothesis focuses on plasticity in the ipsilesional (injured) hemisphere, stating that p*hysiological changes in the ipsilesional SMA would be positively correlated to recovery of function*. The lesion was induced by electrocoagulation of the blood vessels in the physiologically defined primary motor (M1), premotor ventral (PMv) and premotor dorsal (PMd) upper extremity representation areas. Prior to and three weeks (early time point) and three months (13 weeks, late time point) after the lesion,

intracortical microstimulation (ICMS) was employed in the ipsilesional distal forelimb (DFL) supplementary motor area (SMA) to derive detailed output maps of this region at pre-lesion, early and late time points. Physiological changes were correlated with behavioral outcome. The ipsilesional DFL SMA was significantly enlarged three months post lesion. This enlargement was positively correlated to the lesion size and to the improved motor performance.

The second hypothesis stated that *after an ischemic lesion in the motor cortex, maintenance of recovered motor function would depend on activity in spared motor cortex in the ipsilesional hemisphere*. A secondary focal lesion in the ipsilesional SMA DFL area was performed three months after the initial lesion. Motor performance was monitored for the subsequent thirty days to determine if the original deficits were reinstated. Secondary lesions in the DFL SMA representation failed to show a significant effect on the behavior.

The third hypothesis is related to changes in the uninjured hemisphere, stating *that descending control of ipsilateral muscles does not change with recovery from a lesion in the motor cortex*. ICMS was employed in the uninjured cortex in M1, PMv and SMA forelimb motor areas. EMG signals were recorded from the proximal and distal ipsilateral (affected) forelimb muscles. Although ipsilateral EMG activity has been observed in normal subjects this generally requires active target muscle contraction and high stimulation intensities. Pre-lesion ipsilateral EMG activity detected in the normal squirrel monkeys was compared to contralateral activity. Post-lesion, there was an increase in the number of ipsilateral EMG events (affected arm)

at both time points compared to pre-lesion. The latency of these facilitation effects increased three weeks post-lesion and decreased to pre-lesion values by three months post-lesion. Ipsilateral suppressive effects also increased at both time points, but this change was paralleled by the same pattern of changes in the contralateral side.

This study of plasticity in both hemispheres at the early and late stage in a primate model of stroke is novel and should offer insight into the mechanisms of functional recovery following stroke.

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LIST OF COMMONLY USED TERMS

Injured (ipsilesional) hemisphere: same hemisphere as where the lesion is located.

Uninjured (intact, contralesional) hemisphere: hemisphere opposite to where the lesion is located.

Ipsilesional SMA: SMA located in the same hemisphere as the lesion.

Contralesional M1, PMv or SMA: M1, PMv or SMA representations on the opposite hemisphere to where the lesion is located.

Ipsilateral forelimb: forelimb on the same side of the body to the hemisphere being stimulated. When the unijured hemisphere is being stimulated the ipsilateral forelimb is the one on the opposite side to the injured hemisphere. Ipsilateral forelimb then refers to the affected forelimb.

Contralateral forelimb: forelimb on the opposite side of the body to the hemisphere being stimulated. When the uninjured hemisphere is being stimulated the contralateral forelimb is the one on the same side as the injured hemisphere. Contralateral forelimb then refers to the unaffected forelimb.

LIST OF ABBREVIATIONS

CC Callossal connections

CM corticomotoneuronal

CST Corticospinal Tract

DFL Distal Forelimb

EMG elecromyographic

EPSP excitatory postsynaptic potential

fMRI Functional Magnetic Resonance Imaging

GABA Gamma Aminobutyric Acid

ICI Intracortical inhibition

ICMS intracortical microstimulation

MCA Middle Cerebral Artery

MCAo Occlusion of the Middle Cerebral Artery

M1 primary motor area

NMDA N-methyl-D-aspartic Acid

PET Positron Emission Tomography

PMd dorsal premotor area

PMv ventral premotor area

PN Propriospinal neurons

SMA supplementary motor area

TCI Trancallossal inhibition

TMS Transcranial Magnetic Stimulation

I. BACKGROUND

a. Animal models of stroke

Both global ischemia, resulting from cardiac arrest, and focal ischemia, resulting from stroke, are common in the human population and are major causes of death and disability worldwide. Ischemic stroke (also called occlusive, due to closure of a blood vessel) is the most common type of stroke, accounting for 88% of all strokes according to 2005 American Heart Association statistics. However strokes can also be hemorrhagic, due to bleeding from a vessel. There are three causes of ischemic stroke: atheroembolism, in situ thrombosis, and lacunar stroke. Most occlusive strokes are due to atherosclerosis and thrombosis (Kandel, 2000).

The Middle Cerebral Artery (MCA) is the artery most commonly occluded in human stroke (Caplan, 1985). Occlusion of the MCA (MCAo) in its origin results in a reduction of cerebral blood flow in both the striatum and cortex (Marinkovic, 2001). Ischemic lesions in the territory of the MCA cause contralateral motor and sensory deficits as well as spatial neglect. Total MCA infarcts are common clinically, however, their recovery is poor. More distal MCAo results in an exclusively cortical infarct but the precise incidence of these lesions is unknown. Lacunar infarcts, small deep infarcts that result from occlusion of a penetrating artery, account for about a quarter of all ischemic strokes (Norrving 2003; Lie, 2004).

A large number of animal studies of recovery have employed models that attempt to create cortical injuries similar to clinical stroke. Each of the animal models is a compromise between the approach that will best mimic the clinical

condition, while controlling as many extraneous variables as possible. Different methods of inducing experimental brain lesions can result in distinct neuropathological sequelae. Differences in the progression of experimental lesion pathology may have an impact on the magnitude and rate of recovery of function. For example, studies comparing photochemically induced lesions (produced by thermocoagulation of pial blood vessels) or aspiration have revealed that they differ in the severity of behavioral deficits induced (Napieralski, 1998) and the structural and physiological changes they promote (Carmichael, 2002; Voorhies, 2002). Numerous models incorporating MCAo at one or more points along its length have emerged in an attempt to produce a more reproducible lesion (Tamura, 1981; Shigeno, 1985). However, the photochemical MCAo model for example, can result in microvascular injury, which is extremely relevant as studies in humans have shown that angiogenesis is an important mechanism underlying recovery from stroke (Krupinski, 1994). While most of the embolic techniques (including injection of autologus clots) avoid the invasive features of craniotomy, they provide less control over the location and extent of the resulting cerebral infarction (Molinari, 1974).

For this study we initially attempted the use of a model of permanent, distal MCAo by occlusion of the distal branches of the MCA. However, variability in the lesion precluded the use of this model. The degree and distribution of cerebral blood flow reduction following MCAo depends on the duration and site of occlusion, the amount of collateral blood flow into the territory and variability in the bifurcating pattern of the MCA (Gibo, 1981; Marinkovic, 2001). Instead, we used

electrocoagulation of the surface vessels of physiologically defined primary motor and premotor upper extremity representation areas. This resulted in a more reliable method, which in turn, excluded sensory and motor deficits in the leg and face representation areas, which would have confounded interpretation of results.

b. The squirrel monkey as a model to study recovery from stroke

Although rodent species have been very valuable in furthering our understanding of the cascade of events that follow acute stroke, and have been used for studies of recovery mechanisms (e.g. Jones et al. 1999), the need for nonhuman primate models of stroke has received increasing attention. The advantages and disadvantages of nonhuman primate models for stroke are reviewed in detail by Fukuda and del Zoppo (Fukuda, 2003).

Nonhuman primate species share a more recent common ancestry with humans, increasing the probability that neural processes related to stroke-induced deficits and recovery are similar to those of human stroke. Certain features of the primate motor system, especially in cortical motor structures, are more highly differentiated and elaborated compared with those of other mammalian species (Heffner and Masterton 1983; Nudo et al. 1995).

The adult squirrel monkey is especially suited for studies involving the motor system primarily because the motor hand area, referred hereafter as the distal forelimb (DFL) area, is contained within a relatively flat, unfissured sector of frontal cortex, allowing direct access for neurophysiological examination and infarct induction.

Cortical and spinal damage is generally more devastating in humans than in animal models (Porter R 1993.) and it is speculated that this is due to the difference in

the anatomy and physiology, especially of the corticospinal tract (CST). There are a number of lines of evidence supporting a special, but not exclusive role for corticomotoneuronal (CM) connections in dexterous performance. This is of particular interest since ultimately it is loss of this function that mostly affects humans with strokes involving the upper limb.

In the following paragraphs we will discuss differences of the squirrel monkey as compared to higher order primates and humans. It is important to keep these issues in mind since caution will be needed in the interpretation and extrapolation of results in this study for the benefit of stroke patients. This topic is extensively revised by Lemon and Griffiths (2005).

The thumb in the squirrel monkey does not rotate and is not capable of true tip-to-tip thumb-index finger opposition as in the precision grip of the human. CST projections into the ventral horn in the squirrel monkey are much sparser, and absent from the dorsolateral regions of lamina IX. The most numerous projections from the CST are focused on the segments in the lower cervical cord which innervate the hand (Armand 1997, Bortoff 1993), with relatively few direct projections to lamina IX in the more rostral segments (Bortoff 1993). In humans, however, there is evidence for dense projections into lamina IX (Schoen 1964) and this is reflected in large monosynaptic effects on hand-muscle motoneurons (Baldissera 1993, de Noordthout 1999, Hess 1987, Palmer 1992) as well as on many other upper-limb muscles, even those acting at proximal segments (Colebatch 1989, Palmer 1992, Turton 1999). The strength of the CM input can be gauged by estimating the size of the compound

monosynaptic excitatory postsynaptic potential (EPSP) evoked from supramaximal stimulation of the CST. For intrinsic and extrinsic hand muscles, this was estimated at 1.9 mV in the macaque and 4.2 mV in humans. In distinct contrast, the CM input to hand and forearm motoneurons in the squirrel monkey is weak. The mean amplitude of CM EPSPs was only 0.6 mV, and these EPSPs often had rather slow rise times (Maier 1999, Nakajima 2000). These data suggest that a dense CST projection into lamina IX is essential to underpin the large, fast monosynaptic input to hand motoneurons recorded in macaques, baboons, and humans (de Noordhout 1999, Lemon 2004, Nicolas 2001) and is believed to underlie differences in the ability to perform skilled motor tasks in these species.

Since all CM connections are excitatory, all of the important inhibitory control exerted by the CST must be mediated through oligosynaptic (more than one synapse) connections. The significance of indirect excitatory pathways is of particular interest in those species in which the CM system is present to only a limited extent such as the squirrel monkey. CM inputs to motoneurons supplying hand and finger muscles are rather small and slow. However, repetitive stimulation of the CST, via an electrode in the contralateral

medullary pyramidal tract (PT), produces large non-monosynaptic EPSPs (Maier 1993). Such responses are characteristic of oligosynaptic pathways (Illert 1976 1&2). These non monosynaptic responses that are present in over 80% of hand and finger motoneurons, may well be mediated through CST inputs to the propriospinal system. These propriospinal neurons (PN) are located in the upper cervical segments (C3-C4).

It has been speculated that there is a relative increase in the importance of the CM system for upper-limb motor control in the macaque compared to the squirrel monkey and the cat, and that this change is paralleled by a relative decline in the significance of transmission through a C3-C4 system (Nakajima 2000). If this hypothesis were correct then in humans, where the CM system controlling the upper limb is best developed, one might predict that transmission of corticospinal excitation through C3-C4 PNs is relatively weak (Lemon 2004). However, an alternative interpretation, is that corticospinal transmission through the PN system is more complex in the macaque than in the squirrel monkey, with a significant increase in the level of feedforward inhibition from the CST onto C3-C4 PNs (Alstermark, 1999, 2002). PN transmission in humans may also be under inhibitory control from the cortex and it is possible that relatively focused outputs activated under voluntary control (but not by gross stimulation with transcranial magnetic stimulation (TMS) escape such inhibition. Behavioral work on the C_3-C_4 propriospinal system has shown that it can mediate the command for target reaching and not for food-taking (which involves digit movements, wrist flexion and supination). Therefore, it is not logical that this particular system would need to be replaced with the monosynaptic corticomotoneuronal connections. It is possible that after spinal injury or stroke, the C3-C4 system may be disinhibited, which would be highly significant for neurorehabilitation (Pierrot-Deseilligny 2002).

c. Time course of recovery after stroke

The three weeks and three months (or 13 weeks) post-lesion time points utilized for the present studies have been selected mainly based on previous data obtained on the squirrel monkey following M1 lesions. Although the lesion model used for the present studies involves a more extensive injury and thus a maybe different recovery profile, choosing similar time points makes it easier to compare and interpret results. In addition, available data on behavioral recovery and evolution of brain activation patterns in stroke patients, and physiological and anatomical data obtained from post-injury animal models support the relevance of these specific time points. Although differences exist between human and the animal models, taken together the results suggest an early time point, within the first month after stroke onset, in which there are extensive anatomical and physiological changes simultaneous to behavioral improvement. At a later stage, somewhere between three and six months, behavioral improvement reaches a plateau and physiological and neuroimaging parameters tend to normalize.

Most stroke survivors show some improvement in motor function, however recovery follows different profiles depending upon the severity of the deficit, lesion location, primary endpoint, post-infarct experience, etc. Although in the past it was believed that recovery did not extend further than a month to maybe three months, now the prevailing view is that it can occur for months to years following the initial ischemic event. Thus it is difficult to establish specific time points relevant to the

recovery process. Nevertheless, there are a few indications of this division in early and late stages in animal models. Behavioral recovery after focal primary motor area (M1) lesions in squirrel monkeys occurs mainly during the first month post-infarct, and is difficult to detect after two months. However deficits persist for at least three months (Nudo, 1992). On the physiological aspect, a reduction in the hand representation area of the ventral premotor area (PMv) has been shown to occur three weeks after a focal M1 infarct in the squirrel monkey(Plautz 2005). A separate study, also following an M1 lesion showed an enlargement of PMv distal forelimb representation three months after the lesion (Frost, 2003). These same time points were chosen for the present studies.

Several imaging studies in stroke patients suggest that the selected time points represent two important stages in the recovery process. Important differences in brain activation during performance of a motor task, at similar time points to the ones selected for the present studies (early—10-14 days, and late—at least 3 months, phases after first-ever stroke), were compared and correlated to behavioral outcome using functional Magnetic Resonance Imaging (fMRI) (Ward, 2004). Another set of patients was studied, also with serial fMRIs within the first few days and at three to six months after stroke onset (Marshall, 2000). TMS and the study of intracortical inhibition (ICI) has also been performed in patients with disease duration of less or more than four months (Shimizu, 2002).

Time points used to study stroke patients in different stages of recovery

correspond with the same time points in which valuable anatomical data was obtained in animal models. Widespread areas of cortical hyperexcitability shown by means of electrophysiological methods in rat brain slices appear days after cerebral infarction, reducing over subsequent months (Buchkremer-Ratzmann, 1996). These changes in cortical excitability were shown to occur in regions structurally connected to the lesion in both hemispheres as a consequence of down-regulation of the $\alpha 1$ Gamma Aminobutyiric Acid (GABA) receptor subunit and a decrease in GABAergic inhibition using RT-PCR in a rat stroke model (Neumann-Haefelin, 1999). Time points selected in different studies and models are extremely important for the interpretation and extrapolation of results. Thus, as an example, physiological changes in the motor representation maps derived with ICMS in squirrel monkeys and changes in ICI shown with TMS in stroke patients can be explained by changes in cortical excitability shown in rats with physiological and molecular techniques.

Understanding the mechanisms of recovery from stroke is essential for providing a pathophysiological basis for developing improved rehabilitation techniques. Several new approaches to treatment in chronic stages after stroke have been proposed within the past several years. Based on recent pre-clinical and clinical studies, it may be possible to optimize interventions focusing on those strategies that maximize neuroplasticity mechanisms such as pharmacological agents, constraint-induced movement therapy and treatments based on stimulation of the cerebral cortex e.g (Taub, 1993; Goldstein 2000; Plautz, 2003). Understanding the time course of

events is of extreme importance in the effort to optimize intervention following stroke. Previous results suggest that there are differences in the cerebral implementation of action in patients with poor outcome that are dependent on the time since stroke. Thus, in those patients with the most to gain from rehabilitation, different therapeutic approaches may be required at different stages after stroke (Ward, 2004).

d. Plasticity in the remote cortex during recovery from stroke

After stroke in humans, widespread changes occur in activation patterns, associated with movement of the paretic limb, in both the injured and uninjured (or intact) hemispheres. Recent data points towards the existence of an early disinhibition of the intact cortex with increased activation of ipsilateral pathways. As recovery proceeds, activation of the various regions in the injured cortex increases.

i. Plasticity in the injured hemisphere

There is extensive data supporting the notion that recovery of function lost due to cortical injury is attributable to adaptive plasticity in the remaining motor network. Initial experiments studying plasticity following injury have shown the peri-infarct tissue undergoes physiological changes correlated to recovery. After small, subtotal lesions in a portion of the DFL representation in squirrel monkeys, the remaining DFL was reduced in size, giving way to expanded proximal representations (Nudo, 1992) when the animals were allowed to recover spontaneously (i.e., without the benefit of rehabilitative training) for several weeks. However, in animals that underwent rehabilitative training with the impaired limb, the DFL was preserved or expanded (Nudo, 1996). Comparative studies in human stroke patients suggest that

the intact, peri-infarct cortex may play a role in neurological recovery (Cramer, 1997; Jaillard, 2005; Teasell, 2005). Using TMS after stroke, a reduced excitability was shown in the motor cortex near the injury, with a decreased cortical representation of the affected muscles (Traversa, 1997; Butefisch, 2006). It was suggested that this effect occurs from a combination of diaschisis-like phenomena and disuse of the affected limb (Liepert, 2000). After several weeks of rehabilitation, motor representations in the injured hemisphere have been shown to be enlarged relative to the initial post-injury map(Traversa, 1997; Carey, 2002). Also, when goal-directed movement with the impaired hand was encouraged, a significant enlargement of the representation of the paretic limb was produced, closely paralleling results in non-human primates.

The notion that reorganization of peri-infarct tissue has a role in functional recovery following stroke was further extended by evidence showing plastic changes in remote cortical areas such as PMv following M1 lesions (Frost, 2003). Similar findings were obtained in the somatosensory cortex. Damage to the primary somatosensory cortex resulted in topographic reorganization in the second somatosensory area paralleling sensorimotor skill recovery from stroke in adult macaque monkeys (Pons, 1988). Numerous neuroimaging studies have also suggested that plasticity occurs in remote motor areas after stroke (for review see e.g. Cramer and Bastings 2000) (Cramer, 2000). Metabolic and hemodynamic changes have been documented in the premotor (PM) cortex (Weiller, 1992), SMA (Weiller, 1993) and M1 (Cao, 1998; Seitz, 1998; Nelles, 1999) in the injured hemisphere. Disruption of

activity in the premotor cortex of the lesioned hemisphere by TMS resulted in degraded behavior in chronic stroke patients with focal subcortical lesions (Fridman, 2004).

However, the relationship of ipsilesional activation to recovery is not yet well understood. In one longitudinal study, increased activation of SMA was correlated with better recovery(Loubinoux, 2003). Stroke survivors with MCA strokes that included lateral PM areas had poorer recovery (Miyai, 1999), while increased lateral PM activity was associated with better recovery(Miyai, 2003). In another study PMd in the injured hemipshere of human stroke survivors was inactivated temporarily with repetitive TMS. This procedure resulted in reaction time delays that were not generated by inactivation of the PMd in the uninjured hemisphere or the PMd of healthy subjects (Fridman, 2004).

In the present studies, plasticity in the injured hemisphere was studied by monitoring changes in the ICMS maps of the spared SMA DFL representation of the injured hemisphere following an extensive cortical lesion of the other frontal motor areas. The relevance of these changes to functional recovery was established by correlation to post-injury motor performance scores. To further investigate whether the spared SMA DFL area was underlying maintained motor function, a secondary lesion was induced in this area three months after the initial lesion.

ii. Plasticity in the uninjured hemisphere

Several neuroimaging clinical studies have shown that plastic changes take place in the intact (or uninjured) hemisphere after stroke. These studies vary considerably in the technique used, including those employing Positron Emission Tomography (PET) to measure changes in regional cerebral blood flow (Chollet, 1991; Weiller, 1993; Nelles, 1999) and functional Magnetic Resonance Imaging (fMRI) to document changes in activation patterns (Cao, 1998; Seitz, 1998; Cramer, 2001). However, the interpretation of results from these imaging studies is complicated by the influence of such factors like the difficulty of the task and the need to control for unwanted and mirror movements.

Transcranial magnetic stimulation (TMS) has proven a more valuable tool to study changes in the activation patterns observed with the neuroimaging techniques mentioned above. TMS has been used extensively to study the excitability of the motor cortex both in normal subjects (Wassermann, 1991; Muellbacher, 1999; Ziemann, 1999) and in stroke patients. Changes in motor evoked potentials (MEPs) and in intracortical excitability (ICI) have been documented post-injury (Boroojerdi, 1996; Turton, 1996; Netz, 1997; Bastings, 2002; Mazevet, 2003) (Butefisch, 2003). However, the presence of ipsilateral MEPs (iMEPs) and changes in the uninjured hemisphere have been related both to good (Caramia, 1996; Muellbacher, 1999) (Manganotti, 2002) and poor (Netz, 1997) (Turton, 1996) motor recovery. Nevertheless, this technique has offered insight into the mechanisms underlying

ipsilteral MEPs (iMEPs) (Alagona, 2001) (Ziemann, 1999) and plasticity in the injured hemisphere in terms of the possible participation of ipsilateral pathways in stroke recovery (Alagona, 2001) (Mazevet, 2003).

To address the controversial issue of the meaning of the change in activation patterns shown with neuroimaging techniques and the changes observed in the excitability of the uninjured hemisphere with TMS, the present studies used ICMS to stimulate the uninjured hemisphere. Surface EMG signals were simultaneously recorded from both the ipsilateral (affected) and contralaeral (unaffected) forelimbs of the squirrel monkey.

e. Mechanisms underlying post-lesion functional and structural plasticity

Several neuroanatomical and structural changes have been shown to occur in the peri-infarct cortex and in the remote areas, including the uninjured hemisphere following stroke. Some of these changes will be discussed in view of understanding the mechanisms underlying the above-mentioned evidence for functional and structural plasticity.

Primate brains are endowed with a rich intracortical network that allows reciprocal communication among the various sensory and motor areas. Injury to the motor cortex results in a potent disruption of integrated sensorimotor networks, (Nudo, 2003). Upregulation of NMDA receptors and downregulation of $GABA_A$ receptors occurs in both the ipsilesional and contralesional hemispheres (Redecker, 2000). It follows that disruption of the cortical motor network, which results in loss of fine motor control, triggers a major reassembly of inter- and intra-areal cortical networks. This neuroanatomical remodeling in the neocortex provides a mechanism for recovery of function. This is supported by the occurrence of neurite growth followed by synaptogenesis and pruning in the neocortex, ipsilateral and contralateral to neocortical ischemia, in a pattern that corresponds both spatially and temporally with behavioral recovery (Stroemer 1995).

Axonal sprouting is an important component of response to injury and may be critical for functional plasticity and recovery (Carmichael, 2002). Sprouting of axons

into the denervated striatum has been shown to occur after ischemic lesions in the sensorimotor cortex in adult rats (Napieralski, 1996). However, functional changes in projection areas are not necessarily homogeneous (Witte, 2000). Although similar changes might occur in lower levels of the CNS, these have not been yet demonstrated to occur.

Recent studies examining the intracortical connections of PMv after M1 injury have demonstrated the emergence of a novel target of PMv neurons located in S1 (Dancause 2006). This study presented evidence of a major alteration of intracortical wiring patterns between different cortical fields. These new connections from Pmv to S1 were interpreted as a possible means by which the remaining cortical motor system could gain access to somatosensory information more effectively. Thus, at least after focal injuries to the cortex, the surviving brain tissue may be substantially altered in its basic anatomical connectivity and functional interactions among its constituent areas.

Several structural changes have been reported in the intact hemisphere following unilateral sensorimotor lesions in rats. Modifications in the GABAergic and Glutamatergic systems have been related to changes in the excitability of the contralesional cortex (Que, 1999). Hyperexcitability observed both in rat studies and in stroke patients using paired-stimulation paradigms (TMS) is thought to occur as a consequence of disinhibition due to loss of interhemispheric connections from the affected motor cortex (Witte, 1997; Liepert, 2000). Reduced intracortical inhibition (ICI) only in patients with cortical lesions and presence of transcallosal inhibition

(TCI) only in patients with subcortical lesions suggests ICI to be a result of the disruption of TCI (Shimizu, 2002).

The fact that a prominent remote hyperexcitability only occurs if the lower cortical layers (in which the output is situated) are affected suggests that electrophysiological diaschisis results from deafferentation (Buchkremer-Ratzmann, 1997). The time course of remote alterations in cortical physiology supports the assumption that secondary damage of nerve fibres which have their origin in the lesion, is the cause for the observed alteration in excitability (Schiene, 1996). Destruction of callosal connections may reduce the activity of the inhibitory neuronal network. This loss of input might, in turn, stimulate reactive synaptogenesis.

In summary, stroke is a leading cause of disease and disability worldwide. Although some recovery occurs, patients are often left with hemiplegia and difficulties with the activities of daily living. The study of stroke recovery has important implications for the development of new and improved treatment strategies.

Many animal models of stroke have been developed showing differences in the neuropathological sequelae and recovery profiles. Animal models have to be chosen in order to best serve the specific purpose of the experiments at hand. Ultimately the best model is the one that mimics the clinical condition as closely as possible and yet allows for the control of as many extraneous variables. The stroke model used for the present studies involves the induction of an ischemic lesion in an extensive portion of the frontal motor cortical representations of the upper extremity of the squirrel monkey. The areas included in the lesion (M1, PMv and PMd) are those that are irrigated by the MCA, the artery most commonly occluded in human stroke.

Several characteristics of the squirrel monkey make it a good species in which to study stroke recovery. The squirrel monkey is a New World non-human primate sharing a common ancestry with humans, with more highly differentiated motor cortical structures compared to other mammals. The relatively flat unfissured frontal cortex allows for the use of neurophyiological techniques and infarct induction.

There are differences in the CST of the squirrel monkey compared to higher order primates such as the Macaque. The squirrel monkey's CST has less monosynsaptic connections in the spinal cord, and these have less direct connections

with the motoneurons. A large proportion of the descending control is mediated through oligosynaptic pathways. These differences make this model especially suited for the study of plasticity in the uninjured cortex and the post-lesion changes in ipsilateral projections to the affected forelimb.

The time points used for the present studies, three weeks and three months post-infarct, are mainly based on previous behavioral and physiological data on this same species. However, available data on behavioral recovery and evolution of brain activation patterns in stroke patients, and physiological and anatomical data obtained from post-injury animal models support the relevance of these specific time points. Taken together the results suggest an early time point, within the first month after stroke onset, in which there are extensive anatomical and physiological changes simultaneous to behavioral improvement. At a later stage, somewhere between three and six months, behavioral improvement reaches a plateau and physiological and neuroimaging parameters tend to normalize.

Many neuroimaging studies in stroke patients have demonstrated post-injury changes in both hemispheres. However their relationship to functional recovery is not yet clear. There seems to be a pattern of early disinhibition of the intact hemisphere with consequent increase activity in the ipsilateral pathways. At a later phase there is an increase in the activation and involvement of the spared cortex in the injured hemisphere. This pattern has been associated with the temporal evolution of motor development.

The mechanisms underlying this plastic reorganization during stroke recovery include post-lesion hyperexcitability, induced by reduced intracortical inhibition, which in turn is due to disruption of transcallosal inhibition following deafferentation of the remaining cortical areas. This environment promotes neuroanatomical remodeling and axonal sprouting. The neuroanatomical remodeling includes neurite growth and synaptogenesis followed by pruning. The establishment of new connections at late stages might have important implications for the implementation of new and improved therapeutic approaches.

Results from the present studies are expected to shed light into the controversial issue of whether spared motor areas in the injured hemisphere have a functional role in stroke recovery and whether there is a change in ipsilateral descending control of the affected arm muscles following stimulation of the uninjured hemisphere.

II. PURPOSE

The purpose of this study was to analyze a) the correlation between behavioral and physiological changes in the ipsilesional cortex and, b) the contribution of ipsilateral pathways to spontaneous recovery following an extensive lesion comprising the M1 and ventral and dorsal premotor (PMv and PMd) distal and proximal forelimb representations in a non-human primate.

a. Correlation between behavioral and physiological changes in the ipsilesional SMA during spontaneous recovery following an extensive motor cortical lesion

The motor cortex is composed of several cortical areas that are reciprocally interconnected (Stepniewska, 1993) including M1, PMv, PMd, SMA and the cingulate motor areas (CMA). It is likely that damage to any one motor area will affect the function of the other areas. It has been proposed that since premotor areas have independent corticospinal projections, restitution of motor function following injury may depend on the extent to which these other efferent systems can compensate for damaged motor areas (Strick, 1988). Given that lesion location and size determine the behavioral outcome after stroke (Shimizu 2002), it has been proposed that these factors may also influence the degree (or location) of plasticity. The fact that SMA receives its blood supply from the anterior cerebral artery, its location close to the infarct area, its direct corticospinal projections, its reciprocal intrahemispheric connections with M1 and extensive interhemispheric connections make it an interesting target for studying ipsilesional cortical plasticity following stroke.

Prior to and three weeks and three months after the ischemic lesion, ICMS was employed to derive detailed output maps of the ipsilesional SMA forelimb motor area. Using microelectrode stimulation techniques it is possible to elucidate the function of specific cortical motor areas by deriving high-resolution functional maps

of topographic motor representations (Donoghue1992, Gould 1986, Nudo 1992, 1996a, Strick and Preston 1982). Then physiological changes in the SMA DFL area were correlated to behavioral performance. At post-lesion week 13 the monkeys received a secondary lesion in the SMA DFL area. This was followed by a four-week period of spontaneous recovery and weekly behavioral assessment. The main goal was to test the hypothesis that physiological changes in the ipsilesional SMA are correlated to recovery of function.

b. Contribution of ipsilateral pathways to recovery from an extensive motor cortical lesion.

The notion that functional reorganization occurs in the intact (i.e. non-affected or contralesional) hemisphere during recovery from stroke stems from functional imaging studies showing increased activation of the contralesional hemisphere in stroke patients. However, the meaning of increased activation as studied by fMRI is still not clearly understood and is confounded by detection of mirror movements, increased attention, increased difficulty in performing a determined motor task, and by associated non-specific cognitive components (Johansen-Berg, 2002), (Rouiller, 2004) (Cao, 1998). Thus it is questionable whether contralesional activity indicates an involvement of ipsilateral projections for recovered function after stroke. That disruption of contralesional motor areas by TMS does not influence performance in motor tasks (Werhahn, 2003) and that ipsilateral MEPs obtained with TMS of the contralesional hemisphere have been related to poor motor recovery (Turton, 1996; Netz, 1997) suggest that contralesional motor areas are not responsible for improved function in the paretic arm. There is also ample support for the notion that changes in the contralesional cortex are related to increased intracortical inhibition rather than to a recruitment of ipsilateral projections (Shimizu, 2002). Only 10% of the fibers in the CS tract are uncrossed and descend as the ventral corticospinal tract. Moreover, some might cross at a segmental level or recross at the spinal level (Galea, 1997). Although non-primary areas send prominent bilateral projections to the spinal cord, these do not terminate in areas corresponding to distal forelimb muscles (He, 1993). Consistent

with this anatomical data, ipsilateral responses have been obtained in normal subjects (Wassermann, 1991; Ziemann, 1999) but this required active target muscle contraction and high stimulation intensities.

Prior to and three weeks and three months after the ischemic lesion, ICMS was employed in the contralesional cortex in the M1, PMv, and SMA forelimb areas. EMG signals were recorded in the proximal and distal ipsilateral (affected) forearm muscles. Four surface EMG electrodes were placed on both forelimbs at distal (forearm) and proximal (upper arm) locations, using the contralateral limb (unaffected) electrodes as controls.

The main goal was to test the hypothesis that following an extensive lesion in the motor cortex, ICMS of contralesional motor areas would not result in EMG responses in the musculature of the ipsilateral (affected) forelimb.

III. METHODS

ICMS techniques were used to: a) derive detailed maps of ipsilesional SMA and b) to stimulate contralesional M1, PMv and SMA upper extremity representations and record forelimb EMG activity in nine (n=5 and 4, respectively) adult squirrel monkeys, (*Saimiri* species) before and after focal ischemic infarcts in physiologically defined cortical motor areas. First, hand preference and manual dexterity measurements were assessed for each animal using a pellet retrieval task that required skilled use of the hand. Then, intracortical microstimulation (ICMS) mapping techniques were used to physiologically identify the M1, PMv and PMd forelimb representations contralateral to the preferred hand, i.e. the target for the infarct. For SMA mapping, the SMA forelimb representation in the same hemisphere was also examined using the same stimulation techniques to obtain a pre-lesion comparison to post-infarct maps. For EMG recording, the contralesional M1, PMv and SMA areas were stimulated to obtain pre-lesion EMG data. Then, an ischemic infarct of the electrophysiologically defined M1, PMv and PMd forelimb area was induced. After the infarct and a post-surgical monitoring period, animals were returned to their home cages. During a subsequent three-month follow-up period, neither pharmacologic nor rehabilitative intervention (spontaneous recovery) was provided. Weekly assessment of hand preference and manual dexterity was conducted. Three weeks and 13 weeks (three months) after the lesion a second and third set of ICMS maps and EMG data were acquired in each animal. At post-lesion week 13, the five animals used for SMA mapping received a secondary lesion in the SMA DFL area. This was followed by a four-week period of spontaneous recovery and weekly behavioral assessment. At the

end of the experiment, animals were humanely euthanized and perfused for histological examination of the lesions. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Kansas Medical Center.

a. Ischemic infarct

Of the nine monkeys, two monkeys received the cortical lesion in two stages: First, the arterial branches of the MCA that supply the frontal cortex were occluded as they emerged from the lateral sulcus (distal MCA branch occlusion). We found this procedure to be ineffective due to reperfusion, and substantial recovery of behavioral performance. Thus, a second, more permanent lesion was made three weeks or eight weeks after the MCA branch occlusion (in monkey 684 and 424D, respectively). All five monkeys received the permanent lesion, described below. Because the results of these two monkeys after the permanent lesion did not differ substantially from the other three monkeys, the results of all five monkeys were considered as a single group.

For the permanent lesion, ICMS was used to derive maps of the cortical representations of the forelimb areas of M1, PMv and PMd. The surface vasculature within this area was permanently occluded by microforceps connected to a bipolar electrocoagulator (Codman and Shurtleff, Inc.). This technique produces a well-defined, focal infarct, confined to the targeted area of cortex, and sparing underlying white matter (Nudo and Milliken, 1996, Stowe et al 2007). The outline of the lesion was delineated according to the physiological representation areas, but respecting also a general rule regarding the major blood vessel pattern. Thus, the lesions were delimited by: -caudally: the central sulcus, bordering but not including the distal branch of the Rolandic artery irrigating the sensory cortex; -laterally: the lateral

border of the M1 representation, bifurcation of the main vein draining the M1-PMv area, exclusion of face representations and lateral border of PMv; -rostrally: inclusion of distal branch of the Pre-rolandic artery and the rostral border of PMv and PMd forelimb representations; -medially: distal fine branches of Rolandic and Pre-rolandic arteries ("border" between middle and anterior cerebral artery territories) and , border between proximal and leg representations of M1 more rostrally, and border between proximal distal forelimb and trunk representations of M1 more caudally. In some cases anastomoses between the distal branches of the middle cerebral artery and the anterior cerebral artery distal branches could be clearly seen. These anastomoses were electrogoagulated to prevent reperfusion of the lesion target area.

The area of the infarct was visually inspected for reperfusion 10 min after electrocoagulating the targeted vasculature. If reperfusion was observed, the electrocautery procedure was repeated over the reperfused vasculature to create a permanent occlusion of the targeted region. Laser Doppler analysis of blood flow before and 1 hr after the infarct corroborated elimination of blood flow within the defined lesion area but not in adjacent areas. This method produced a reliable, non-variable lesion avoiding reperfusion by eliminating all blood flow at once and by eliminating anastomoses and venous drainage from the territory of interest.

Four months after the lesion, animals were deeply anesthetized with ketamine (20 mg/kg i.v.) followed by euthasol (1ml; 390mg pentobarbital sodium/ml; i.p.) and then perfused through the left ventricle with 0.1 mol/L sodium phosphate buffer, pH7.25 and 4% paraformaldehyde. 44 sequential 50 μm thick coronal sections were collected

across the rostro-caudal aspect of the lesion. 11 sections, each 20 sections apart, were stained for Nissl substance following standard protocols. Serial sections were cleared and rehydrated, placed in cresyl violet (15 to 20 mins), then rinsed and dehydrated through graded alcohols. Sections were placed in xylene, then coverslipped with DPX mounting medium. Slides were then photographed at 1.25X.

b. Behavioral assessment

Monkeys were trained to retrieve small, banana flavored food pellets (45 mg), delivered one at a time, from wells drilled into a Plexiglas board (Klüver board) attached to each monkey's home cage. The board was attached to the cage so that the monkey had unrestricted access to each food well. Five wells were distributed across the Klüver board. All wells were 5 mm deep, but differed in diameter (well 1 = 25 mm, well 2 = 19.5 mm, well 3 = 13.5 mm, well 4 = 11.5 mm, and well 5 = 9.5 mm) and thus, varied in difficulty for the monkey to retrieve a food pellet. To remove food pellets from the smaller wells (wells 3 to 5), the monkeys had to learn to use their digits differentially whereas on the larger wells (wells 1 and 2), synergistic movements of the digits and a prehensile grip were adequate to remove the pellets.

Prior to pre-infarct training, each monkey was tested for hand preference on the Klüver board task. In this procedure food pellets were randomly placed in each well for a total of 25 trials (5 trials per well) with the board in one position (e.g., well 1 to the right of the monkey) and 25 trials with the board reversed to counterbalance any possible position bias (well 1 repositioned to the left of the monkey). One session was conducted per day, over two consecutive days (a total of 100 trials). This will be referred to as the "Open Board" task.

During the pre-infarct motor-skill training period, a single one hr session was

conducted per day in which the monkeys were trained to become increasingly efficient in retrieving pellets from a designated well. The criterion for monkeys to complete training on a particular well was successful retrieval of 100 consecutive pellets on a given well. Successful retrieval was defined as grasping of the pellet and successful placement in the mouth. Monkeys were introduced to the task with the largest well and once the monkeys met criterion performance on this well they were trained on the next successively smaller well the following day. This procedure was carried out until the monkeys could meet criterion performance on the smallest well (well 5) on two consecutive days. The monkeys were kept under food restriction and trained on each hand individually by use of a barrier (a translucent Plexiglas block placed in front of the target well, which forced the use of either the left or right hand). This will be referred to as the "Board with Barrier" task.

Probe trials (Open Board and Board with Barrier) were conducted weekly at pre-lesion, during the 13 wk spontaneous recovery period and the four wk recovery period following the secondary SMA lesion. All trials were video taped with a Hi 8 video recorder (24 frames/sec) for later performance analysis. The data from the probe trials were used for statistical analysis of weekly performance. These data consisted of a scoring system from 0 to 5 for the impaired hand. No value was assigned if the monkey would not perform the task with the non-impaired hand on the same well for which the impaired hand was being tested. A score of "0" was assigned to a monkey which would do nothing with the impaired hand when a food pellet was placed in a well, "1" when reaching across the cage door bars, "2" when

touching the board in an attempt to retrieve the pellet, “3” when placing the fingers inside the well, “4” when flexing the digits inside the well and “5” when successfully retrieving a pellet from a well.

c. Surgical techniques

Each monkey was initially anesthetized with ketamine (25 mg/kg i.m.), shaved (head and arm opposite the hemisphere to be mapped) and then intubated with a tracheal tube (O.D. = 2.5 mm). The monkey was then placed on a gas mixture of 75% nitrous oxide: 25%oxygen with 1.5 to 2 % halothane. Intravenous fluids (3% dextrose in Ringers solution) were then administered and maintained throughout the procedure. Mannitol (8 ml) was administered intravenously to control for possible edema. The monkey's head was stabilized in a stereotaxic frame, and under aseptic conditions, the scalp and the temporal muscle were reflected, exposing the skull. A craniectomy was then performed and the portion of the dura overlyingf the areaof interest was removed to expose M1, PMv, PMd and SMA, and the surrounding cortex. The segment of skull that was removed (skull cap) was maintained in sterile saline at 4°C during the ICMS procedure (see below). A sterile plastic chamber was secured over the opening and filled with sterile silicone oil to protect the cortex from desiccation. Upon completion of the surgical opening, gas anesthesia was withdrawn and small doses of ketamine (~5 mg/15 min as needed) were delivered intravenously to maintain a stable anesthetized state. Ketamine was occasionally supplemented with Valium (0.01 mg as needed) to control excessive muscle tone. Vital signs (respiratory rate, expired CO_2 levels, oxygen saturation in blood, and heart rate) were monitored and recorded every 15 min. At the conclusion of the ICMS procedure, the monkey was again placed on the nitrous oxide and halothane gas mixture. Silicone film was placed over the cortex as a dura replacement and to minimize the growth of tissue over the surface of the

cortex and blood vessel anastomoses. The skull cap was then replaced and secured to the skull with dental acrylic. The scalp was then sutured and the incision anesthetized with lidocaine. A topical antibacterial spray (Furazolidone) was applied to the scalp, followed by a subcutaneous injection of 0.1 ml penicillin (30,000 units). Dexamethasone (0.5 mg i.v.) was given before and after surgery.

d. Neurophysiological mapping techniques

For each monkey, ICMS techniques were used to define the forelimb representation in M1, PMv, PMd and SMA contralateral to the preferred hand. Movements were evoked by delivering a small electrical current into the brain through a stimulating microelectrode. The microelectrode consisted of a glass micropipette (1.5 mm O.D.) filled with a 3.5 M NaCl solution. The pipette was tapered so that the electrode measured about 10–25 μm O.D. at the tip and about 100 μm O.D. 2 mm from the tip. The tip was then beveled to an angle of 25° to aid in penetration through the pia. The ICMS stimulus was delivered as a train burst of 13, 0.2 ms cathodal monophasic pulses, delivered at 350 Hz. This pulse train was triggered through a pulse generator (Master-8; A.M.P.I., Israel) and delivered by a stimulus isolator (Model BSI-2, BAK Electronics Inc.) at a rate of one train per second. The microelectrode was held perpendicular to the cortex, suspended from a stereotaxic micropositioner (Narishige SM-11) and then lowered to ~1,750 μm below the surface with a hydraulic microdrive (Kopf Model 650) targeting layer V. Stimulation was increased in increments of 1 μA. The M1, PMv, PMd and SMA forelimb areas were delineated by defining the borders between sites at which stimulation evoked digit or wrist–forearm movements and sites at which stimulation evoked more proximal (elbow, shoulder, and face) movements or by sites at which stimulation evoked no movement at the maximum current level (30 μA for M1, PMv and PMd and 60 μA for SMA).

A digital picture of the blood vessel pattern over the cortex was taken using a

video frame-capture card (Scion Corp., Frederick, MD, USA) and an image analysis program (NIH Image). The file was saved and re-opened in an illustration program (Canvas, Deneba Inc.) where a computer-generated grid scaled to 1 mm for M1, PMv and PMd, or 250 μm for SMA, was superimposed onto the digital image of the blood vessel pattern. This image was used to guide microelectrode penetrations with reference to the surrounding vasculature while accurately measuring the distance between the microelectrode penetrations (inter-penetration distance). Movements associated with stimulation at each of the penetration sites were designated on the digital image. One experimenter, who was blind to the location of the electrode, was primarily responsible for defining evoked movements, which were subsequently confirmed by a second experimenter.

e. Movement analysis

Digit movements included all extensions, flexions, abductions, and adductions of fingers and thumb. Wrist–forearm movements included all wrist extensions, wrist flexions, wrist radial deviations, wrist ulnar deviations, forearm supinations, and forearm pronations. Shoulder, elbow and oro–facial movements, evoked by stimulation of cortical regions that border the distal forelimb area, were not included in the data analysis. Movements of two independent joint categories within the distal forelimb area (e.g., digits and wrist–forearm) or between the distal forelimb and proximal arm (e.g., wrist–forearm and shoulder) evoked at the same stimulus threshold (or within 2 μA) were separately identified and are herein referred to as dual responses. For analysis purposes, the terms "digits" and "wrist–forearm" refer to all digit responses and all wrist–forearm responses, respectively, including those that are associated with dual responses. For example, areal measurements for digit/wrist–forearm and digit/proximal dual responses were added to the areal measurements of exclusive digit responses. Stimulus thresholds were also defined and recorded at each stimulation site as the minimum intensity of stimulation between 1 and 60 μA necessary to evoke a movement in at least 50% of the pulses.

f. Electromyographic data collection and analysis.

For collection of surface Electromyographic (EMG) data, the monkeys were instrumented with bipolar surface EMG sensors (Noraxon, Scottsdale, AZ). The EMG signal along with the stimulation was synchronized and sampled at 5000 Hz through a 16-bit data acquisition station controlled with LabView custom software (National Instruments, Austin, TX).

Data sets were analyzed using MATLAB (Mathworks, Natick, MA). The EMG signals were filtered using a second order low-pass Butterworth filter with a cutoff frequency of 200 Hz using forward and backward reflection to minimize initial and final time artifacts. Phase shift was eliminated using forward and backward passes. The surface EMG (SEMG) signals were then passed through a notch filter to remove electrical noise in the 58 to 62 Hz range and rectified.

Each trial consisted of twenty-five stimulation trains. The EMG responses to those stimulations were added together by electrode for each stimulus site. Each one of those EMG responses was time locked to the stimulation onset. A composite record per stimulation site was created by addition of the SEMG signals.

To detect the onset of EMG activity, the baseline was determined by taking the mean and standard deviation of the SEMG signal for 100 msec prior to the stimulus onset. The threshold for muscle activation was set at two standard deviations above the mean and the threshold for suppressive activation was set at two standard deviations below the mean. A 50 sample point sliding window was used to search for muscle activation and onset was determined when 50 consecutive sample

point were above the threshold. Similarly, for the suppressive effect, if the signal was below the threshold for 50 consecutive sample points the suppressive effect was marked as the first point when that occurred. Latency times for both the suppression and activation were calculated relative to the stimulus onset. The mean SEMG signal from a 300-sample window after muscle activation was defined as the amplitude of the SEMG response. This window was small enough to ensure that the SEMG signal was active during that time.

g. Statistical analysis

Since motor performance scores are ordinal data, and thus, not normally distributed, a nonparametric statistical test (Wilcoxon signed-ranks; Wilcoxon statistic = "T"; lowest p value of 0.031 with n=5) was employed for analysis of selected pre- and post-lesion comparisons. As such multiple comparisons can increase the probability of Type I error, these results should be considered with caution. However, since the comparisons were limited to the most critical time points (3 wks and 13 wks post-lesion), we have limited this possibility to the extent possible with non-parametric tests with this small sample size.

SMA DFL area measurements of the maps at post-lesion wk 3 and 13 were compared to pre-lesion measurements using repeated measures ANOVA using $p<0.05$. Physiological mapping results were correlated to lesion size and behavioral outcome using Correlation Z test analysis.

Differences in the ipsilateral and contralateral number of EMG facilitaion effects were compared using Chi Square analysis. Differences in magnitudes and latencies were analyzed using unpaired T-tests and ANOVA.

IV. RESULTS

A total of nine monkeys were used for the present studies. Five animals were used for the ipsilesional SMA experiments and four for the contralesional EMG experiments. Behavioral results are based on the five monkeys used for the SMA experiments.

a. Verification of cortical infarcts

Electrocoagulation lesions targeted the ICMS-defined upper extremity representations in M1, PMd and PMv, and intervening representations within this territory. This included the entire distal and proximal forelimb representations in M1, PMd and PMv and a portion of the face representation along the M1/PMv border. Lesions were matched as closely as possible based on these neurophysiological criteria. As described in methods, in three animals, cortical lesions were made in a single stage, while in two animals cortical lesions were conducted in two stages. Since results were similar after the two lesion types, the five animals were treated as a single group for the subsequent behavioral and neurophysiological analyses. Time refers to weeks following the 2nd (permanent) lesion in the cases with 2-stage lesions. Thus, pre-lesion behavioral and neurophysiological data were derived prior to the permanent lesion. The lesion areas averaged 86.8mm^2 ±12.7 SD, ranging from 70.5 to 99.9 mm^2 based on measurements from the scaled digital picture of the blood vessel pattern over the cortex. The variation was due to the normal anatomical differences in the blood vessel patterns and spatial configuration of the forelimb representation (**Figure 1).**

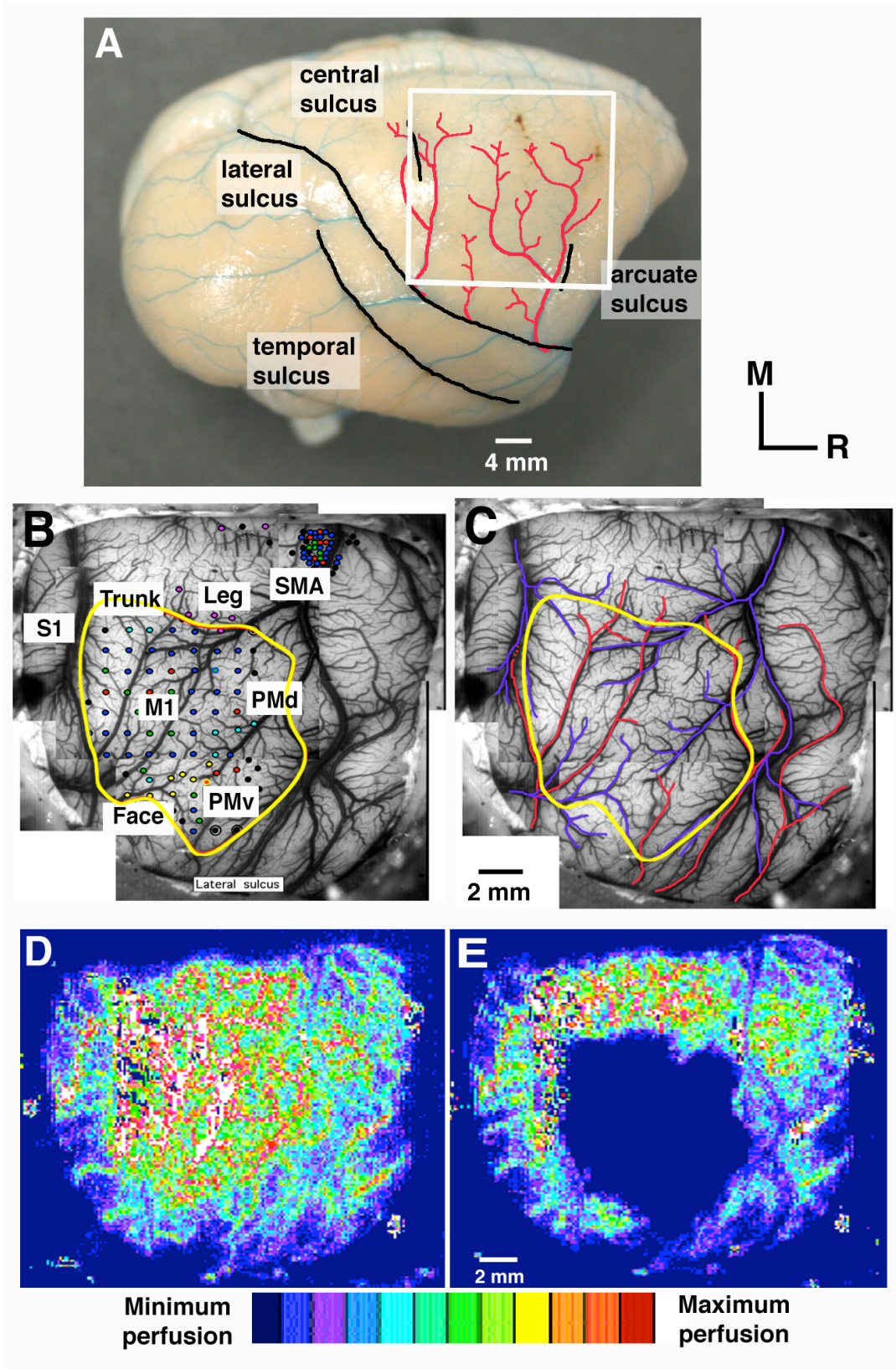

A
central sulcus
lateral sulcus
arcuate sulcus
temporal sulcus
4 mm
M
R
B
Trunk
Leg
SMA
S1
M1
PMd
PMv
Face
Lateral sulcus
C
2 mm
D
E
2 mm
Minimum perfusion
Maximum perfusion

Figure 1. Cortical infarct.

A. Lateral view of blue pigment casted squirrel monkey brain. An additional animal was used to study the brain arterial vascular pattern of the squirrel monkey by injection of a plastic mixture through the ascending aorta. Highlighted in black are the main sulci. . The pre-Rolandic and Rolandic branches of the middle cerebral artery that emerge from the depths of the lateral sulcus are highlighted in red. All craniectomies (outlined in white) in the five monkeys included the central sulcus on the caudal aspect, and the arcuate sulcus on the rostral aspect, bordered the lateral sulcus on the lateral aspect, and the sagittal sinus on the medial aspect.

B, C. ICMS mapping techniques were used to determine the extent of upper extremity motor representations in frontal cortex. In each case, the target of ischemic lesions was defined by a contiguous area bounded by the physiologically defined M1, PMv and PMd upper extremity representations, including digits, wrist/forearm and proximal representations, and excluding M1 leg and trunk representations and S1. Most of the face representation was excluded, except for an area on the border between M1 and PMv. The outline of the lesion was delineated according to the physiological representation areas, but respecting also a general rule regarding the major blood vessel pattern shown in **C**.

D, E. Laser-Doppler blood flow images of the area of interest before (D) and 1 h after (E) the ischemic lesion. The scale depicts relative perfusion.

Histological examination of coronal sections of the lesion stained with Cresyl violet confirmed that the injury comprised all cortical layers. Towards the center of the lesion some of the white matter was lost presumably due to the degeneration of axons of neurons within the infarct zone. Histological sections verified that the lesion was confined to the intended targeted areas (**Figure 2).**

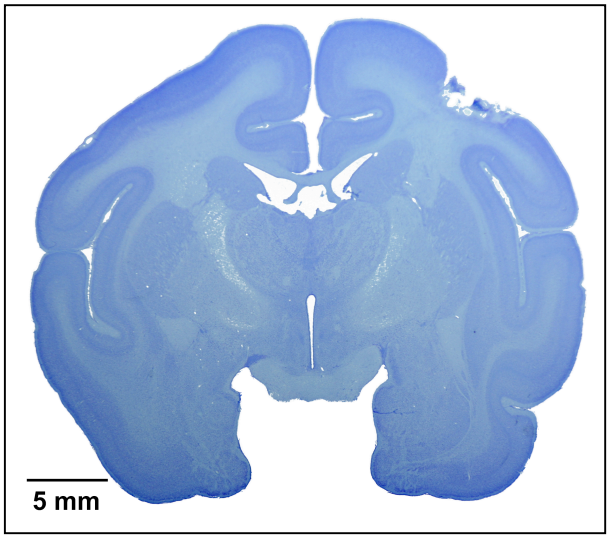


Figure 2. Representative 50 μm thick coronal section through the lesion stained with Cresyl violet, confirming the cortical location of the injury. Histology was performed following perfusion four months post-lesion.

b. Post-lesion behavioral outcome

i. General post-lesion behavioral observations

Prior to the lesion, monkeys took approximately four weeks to meet criterion performance on the smallest well (well 5). The day following the permanent lesion, and after return to their home cages, all five monkeys showed a clear monoplegia of the forelimb contralateral to the cortical infarct. Their affected arm was flaccid. The shoulder adopted a medial rotation and declined position with the elbow turned away from the midline; the hand adopted a neutral position with the fingers extended. The unaffected arm showed no obvious impairments when compared to the affected arm. Weakness of the leg on the affected side was evident only during the first post-lesion day.

After the first two or three days post lesion, the monkeys were able to feed themselves normally with standard monkey chow from the food bins. They used the unaffected arm to scratch and groom but not the affected one. No spatial neglect was observed as evidenced by retained ability to retrieve food rewards from the contralateral space. They presented no sensory deficits as evidenced by tactile stimulation and reflex withdrawal with both hands with no delay.

During the first two weeks post-lesion the monkeys were somewhat inactive compared with their pre-lesion behavior. The monkeys were able to climb and jump from different locations within the cage but clearly exhibited postural abnormalities

indicated by occasional slips of contralateral forelimbs off the perch and sliding down of the forelimb on cage front rails (denoting weakness). They were able to use their affected hand for climbing and posturing but not for fine coordination.

Beginning approximately in post-lesion wk 3 and during the subsequent nine weeks (post-lesion wk 4-12) general behavior was variable for each monkey. Some improved rapidly and maintained a high level of home cage activity, while others were less active. Some sat on the perch, and emitted normal vocalizations. Others kept their heads faced down, rested on the perch or on floor on the back of the cage and did not emit many vocalizations. In general, however, when considering the five monkeys as a group, by post-lesion wk 13 there was an increase in the general activity and use of the dominant hand during testing sessions as compared to post-lesion wk 3 and especially compared to the first days immediately after the lesion. During the 13 wk period there was an increase in compensatory use of the non-dominant hand during normal cage activities.

ii. Effect of lesion on hand preference

Prior to the permanent lesion, all monkeys were tested during two consecutive days to establish baseline Klüver Board performance. They performed 50 trials per session on the Open Klüver Board (Open Board) to assess spontaneous forelimb use and hand preference. The monkeys demonstrated a clear asymmetry in hand use, as they preferred one hand over the other on approximately 75% of the 50 trials. After the lesion, when presented with the Open Board, monkeys showed a change in hand preference, retrieving the pellets almost exclusively with their non-impaired arm (i.e., the previously non-preferred hand), and using the affected arm an average of 3% of the 50 trials. This change in hand preference persisted throughout the 13 wk period of observation (**Figure 3**). ANOVA for number of pellets retrieved with the dominant hand revealed a significant difference ($F=17.09$, $p<0.0001$) for the main effect of Week. Pre-lesion was significantly different from each of the post-infarct weeks in post-hoc analysis (Dunnett's test; $p<0.0001$).

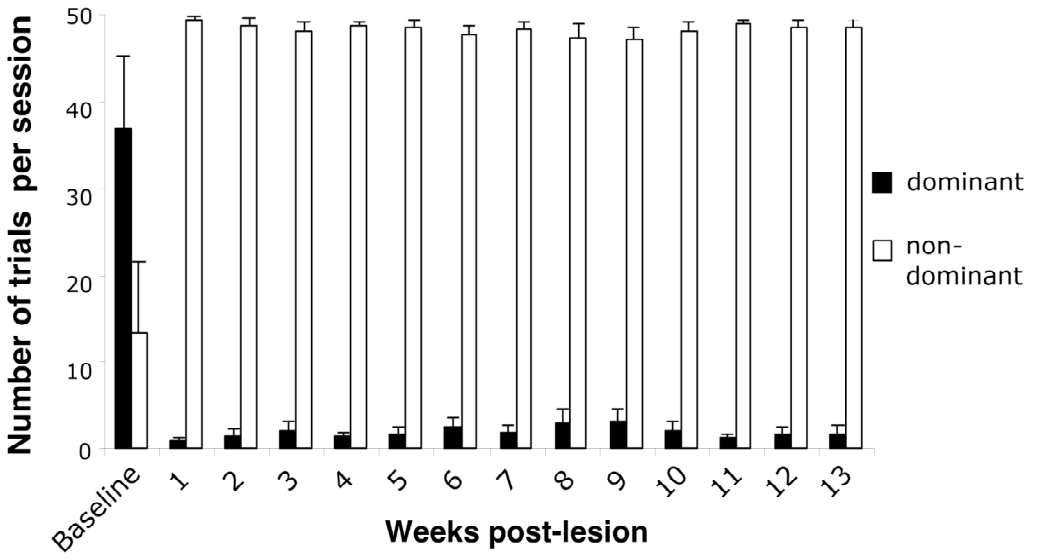


Figure 3. Change in hand preference.

Prior to the lesion, all monkeys (n=5) were tested during two consecutive days for baseline Klüver Board performance to assess spontaneous forelimb use and hand preference. The monkeys demonstrated a clear asymmetry in hand use, as they preferred one hand over the other on approximately 75% of the 50 trials. After the lesion, when presented with the Open Board monkeys showed a change in hand preference, retrieving the pellets almost exclusively with their non-impaired arm (i.e., the previously non-dominant hand). This change in hand preference persisted throughout the 13 week-period of observation.

iii. Effect of lesion on motor performance scores

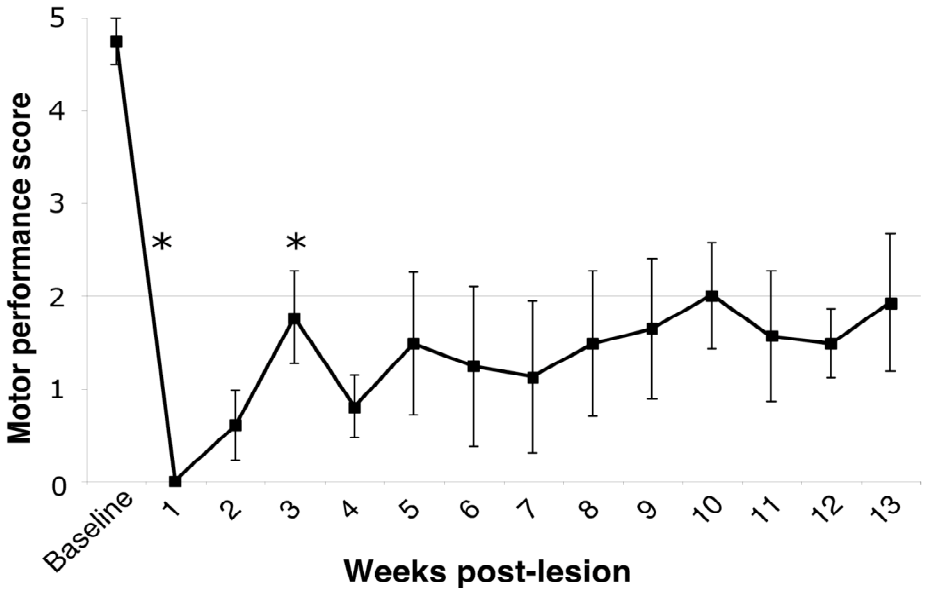
In previous studies employing more restrictive lesions in M1, manual dexterity was assessed by tallying finger flexions per pellet retrieval on the Klüver Board, or the time to retrieve pellets (e.g. Frost et al. 2003). However, with the more extensive lesions employed here, retrievals were typically not successful, and a more general motor performance assessment was needed. Mean Klüver Board performance scores for the five monkeys during the pre-lesion assessment was 4.8±0.5 SD. Mean scores for all monkeys throughout the 13 wk post-lesion recovery period on the Open Board with the dominant (impaired) hand was 0.6±0.2 SD, ranging from 0.2 to 1.0. Monkeys attempted to use their impaired hand on average 1.8±1.3 SD times out of the 50 trials (3.6%), ranging from 0 to a maximum of 9 attempts (18%) for each session.

To encourage monkeys to use the impaired hand, and thus, to more adequately test motor abilities, a clear Plexiglas block was used as a barrier (Board with Barrier). The monkeys could see through the barrier, but could only reach with one hand according to the barrier's position with respect to the well and hand being tested. This allowed assessment of motor performance with each hand independently. In general, throughout the 13 wk time period, monkeys were able to reach and attempt pellet retrieval with the impaired arm but unsuccessfully. They extended their arm, reached through the bars of the cage front and directed their hand towards the board, touched the board but were not able to retrieve the pellet. This appeared to be due to an

inability to place the fingers inside the well. Throughout this period however, their performance progressed from not moving their arm at all in post-lesion wk 1, to only reaching through the cage bars in post-lesion wk 2 to being able to actually reach far enough to touch the board in post-lesion wk 3 (**Figure 4)**. No independent use of their fingers was observed and no forearm pronation and/or wrist flexion/extention was used, in contrast to pre-lesion behavior.

Motor performance scores for all monkeys averaged across all five wells declined from a mean of 4.8±0.3 SE pre-lesion to 0.0±0.0 SE on post-lesion wk 1. The motor performance score increased to 1.8±0.5 SE on post-lesion wk 3, and was 1.9±0.8 on post-lesion wk 13. Wilcoxon signed-ranks test (lowest p value of 0.031 with n=5) was employed for analysis of selected pre- and post-lesion comparisons. This test revealed a significant difference between pre-lesion and post-lesion wk 1 (T=7.5; p=0.031) confirming the effectiveness of the lesion. Following the lesion there was an improvement in motor performance scores from post-lesion wk 1 to post-lesion wk 3 (T=7.5; p=0.031), although scores at post-lesion wk 3 remained significantly different from pre-lesion (T=-7.5, p=0.031). No difference was found between post-lesion wk 3 and 13 (T=1.5, p=0.375), suggesting that further recovery did not occur **(Figure 4A).** Klüver Board performance scores for individual wells showed a trend in the degree of recovery from well 1 to 5 reflecting the increasing level of difficulty in retrieving pellets from the smaller wells. The average scores across post-lesion wks 1-13 for each well were: well 1 = 1.9±0.3 SE; well 2 = 1.3±0.2 SE; well 3 = 1.2±0.2 SE; well 4 = 1.1±0.2 SE; well 5 = 1.0±0.1 SE (**Figure 4B).**

A


Motor performance score
0
1
2
3
4
5
*
*
Baseline
1
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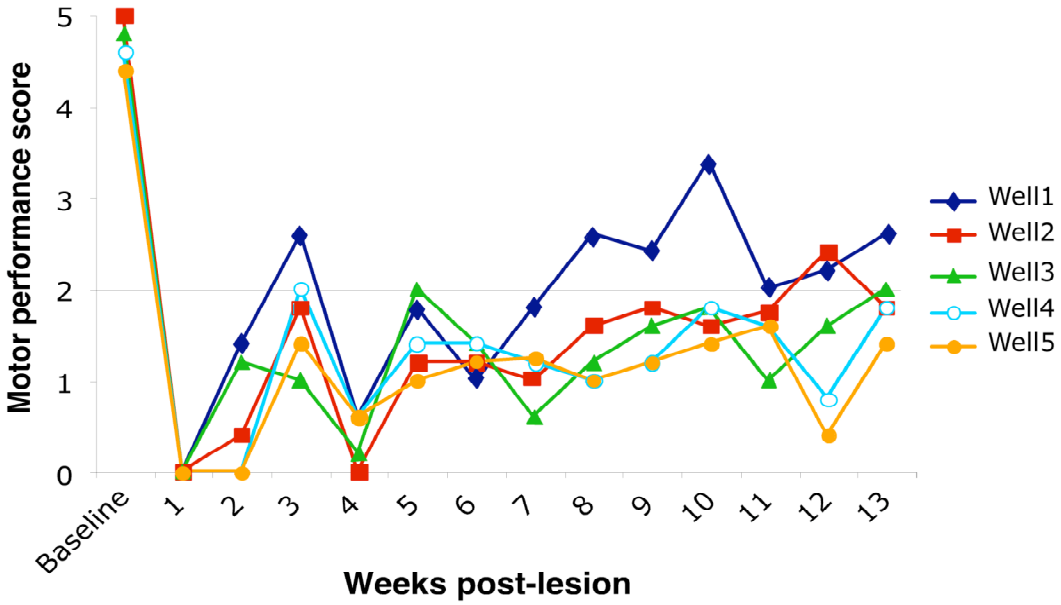
11
12
13
Weeks post-lesion

B


Motor performance score
0
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Baseline
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10
11
12
13
Weeks post-lesion
Well1
Well2
Well3
Well4
Well5

Figure 4. Klüver Board performance.

A. Throughout the 13 wk post-lesion period, when the barrier was employed on the Klüver Board to encourage the use of the impaired hand, the monkeys were able to reach and attempt pellet retrieval but mostly unsuccessfully. B. Motor performance scores for individual wells showed a trend in the degree of recovery from well 1 to 5 reflecting the increasing level of difficulty in retrieving pellets from the smaller wells. For example, compare performance on well 1 (largest well; blue lines) to well 5 (smallest well; orange lines). For clarity, error bars are not shown.

c. Pre-lesion physiological measures

i. Pre-lesion SMA ICMS maps

A representative ICMS map of the DFL SMA area is shown in **Figure 5.** Pre-lesion ICMS SMA mapping results are discussed together to the post-lesion data below.

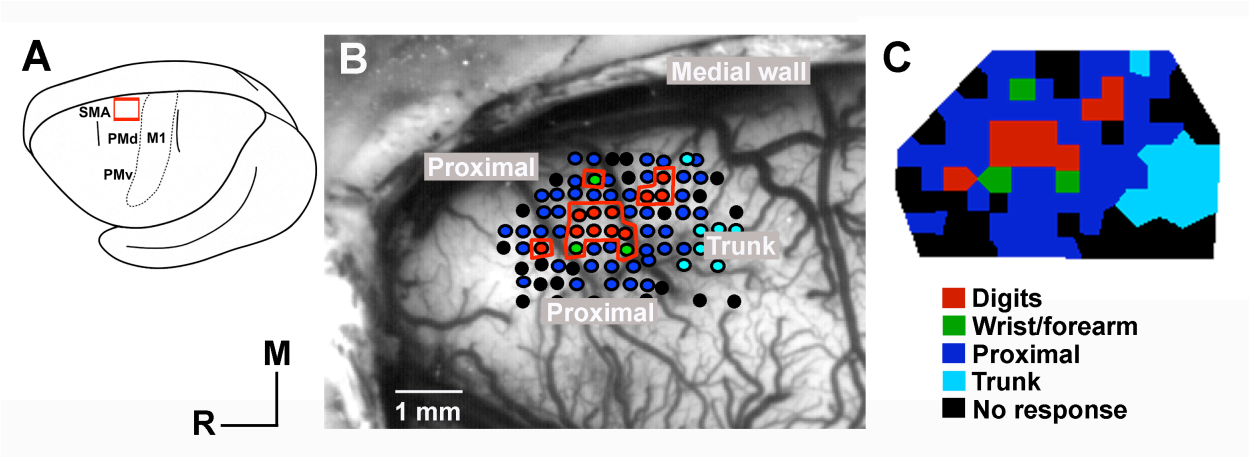

Figure 5. Representative ICMS map of DFL in SMA. A. In the squirrel monkey, this area is exposed on the dorsal surface near the midline. B. SMA digit and wrist/forearm representations, outlined in red, are surrounded by proximal representations. Small dots represent microelectrode penetration sites. C. Two-dimensional color-coded reconstruction of an SMA DFL map.

ii. Pre-lesion EMG activity

EMG activity was recorded following ICMS conducted on four animals. The M1, PMv and SMA areas on the contralesional hemisphere were stimulated. Ten sites were stimulated on each area (**Figure 6**). EMG was recorded on the ipsilateral and contralateral forelimbs. Thus, results were divided according to the side of surface EMG response (ipsilateral or contralateral to the stimulated hemisphere) for a first analysis, and further analyzed according to the specific area being stimulated (M1, PMv or SMA) and recording electrode location (distal—forearm, or proximal—upper arm). As an example, the term "ipsilateral SMA" will be used to refer to results obtained on the ipsilateral (affected) forelimb following stimulation of SMA on the contralesional (non-lesioned hemisphere). This section refers to pre-lesion data only and thus the post-lesion contralesional hemisphere is the non-dominant hemisphere.

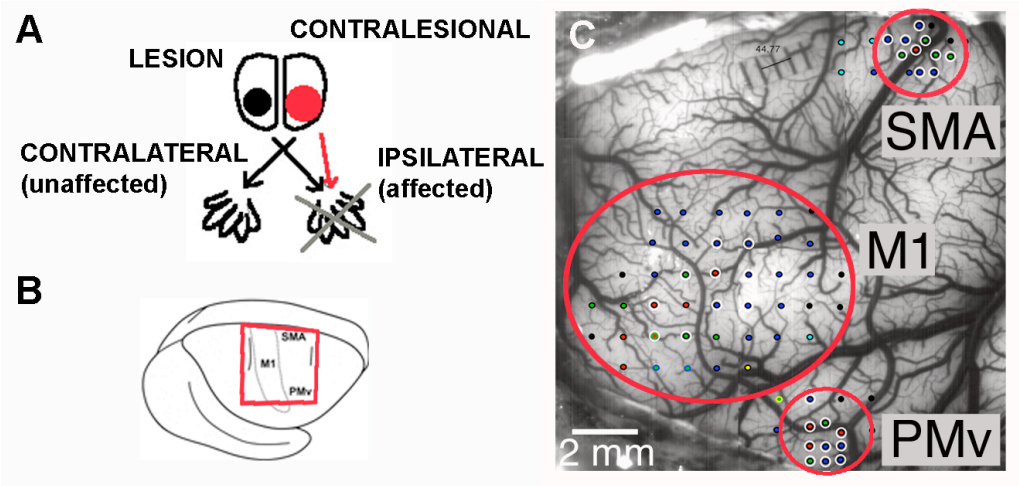

Figure 6. Methodological details for EMG data recording.

A. Cartoon depicting technical details. ICMS was conducted on the contralesional hemisphere. EMG data was collected from ipsilateral (affected) and contralateral (unaffected) forelimbs. B. Brain cartoon showing craniectomy area (red outline) and the three areas studied. C. ICMS map of M1, PMv and SMA areas surveyed with a site to site interpenetration distance of 1mm in M1 and 500μm in PMv and SMA. Five distal and five proximal representation sites were stimulated on each area for EMG recording (white bordered sites).

Two types of effects were noted: 1—facilitation effects, and 2—suppressive effects. For facilitation effects we noted the number of effects obtained, their magnitude and latency. For suppressive effects we noted the number of events and their latency.

1. Facilitation effects (Table 1)

Considering all animals together (n=4), a total of 146 facilitation responses were obtained on the contralateral side and a total of 25 on the ipsilateral side. Thus, ipsilateral responses were obtained on 17% of cases in which contralateral responses were obtained.

Table 1. Summary of pre-lesion results.

		CONTRA			IPSI		
		M1	PMv	SMA	M1	PMv	SMA
FACILITATION	# OF EVENTS	55	53	38	15	5	5
	MAGNITUDE	0.128	0.068	0.027	0.013	0.014	0.012
	SE (mV)	0.019	0.012	0.006	0.004	0.005	0.003
	LATENCY	19.4	22.9	34.8	54.2	35.7	28.8
	SE (msec)	1.5	1.4	2.2	5.1	5.2	4.4
SUPPRESSION	# OF EVENTS	1	1	6	3	3	3
	LATENCY	13	62.4	39.7	28.3	74.1	50
	SE (msec)			10.8	3.7	2.7	17.8

The mean±SE magnitudes were 0.083±0.009 mV for contralateral responses and 0.014±0.003 mV for ipsilateral responses (**Figure 7 and 8**). Thus, magnitudes for ipsialteral responses were about 20% of the magnitudes for contralateral EMG responses.

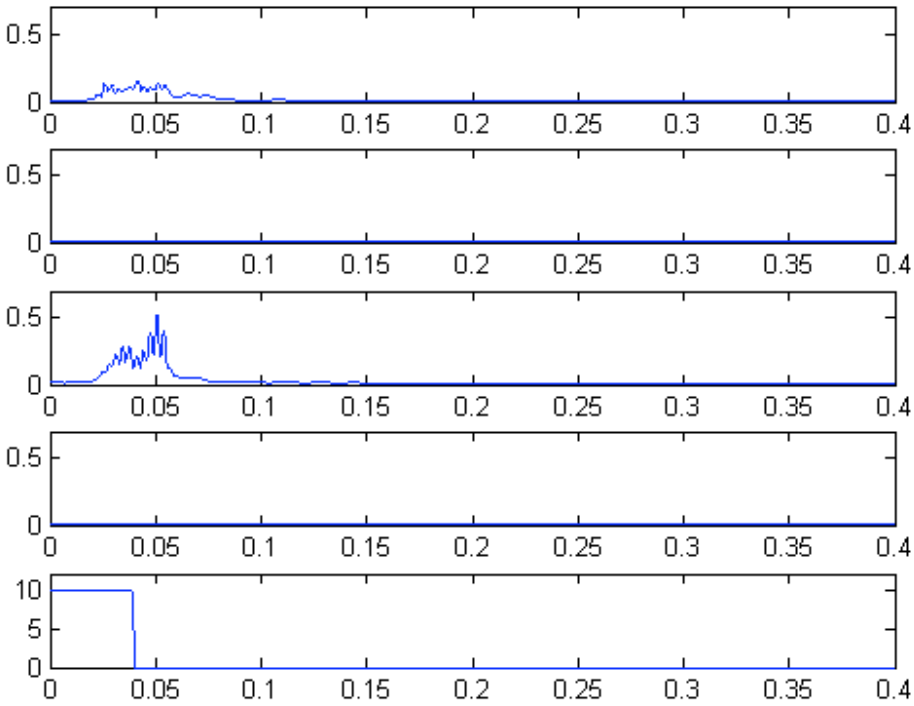

Figure 7. Example of averaged and rectified EMG traces. Time is represented on the x-axis and magnitude (mV above baseline) is represented on the y-axis. The four upper traces represent EMG traces for the following surface electrode locations, from top to bottom: contralateral distal, ipsilateral distal, contralateral proximal and contralateral distal. The fifth trace illustrates the duration of the stimulus train.

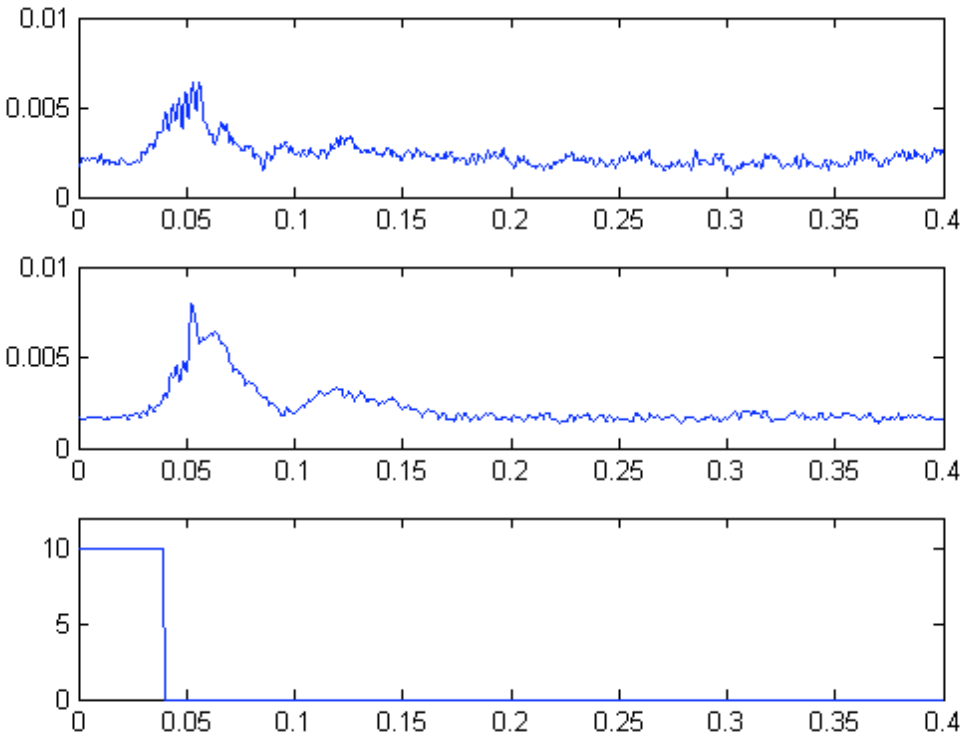

Figure 8. Magnification of ipsialteral effects shown in Figure 7. Time is represented on the x-axis and magnitude (mV above baseline) is represented on the y-axis. The two upper traces represent EMG traces for the following ipsilateral electrode locations: top, distal and.bottom, proximal. The third trace illustrates the duration of the stimulus train.

Mean ± SE magnitude was 0.128±0.019 mV for contralateral M1, 0.068±0.012 mV for contralateral PMv and 0.027±0.006 mV for contralateral SMA. ANOVA for magnitude of effects in contralateral areas showed significantly higher magnitudes for M1 as compared to both PMv and SMA ($p<0.0001$, Fisher's M1 contra vs. PMv contra, $p=0.0035$; M1 contra vs. SMA contra, $p<0.0001$). PMv and SMA showed statistically similar magnitudes (PMv contra vs. SMA contra, $p=0.0815$).

Further analysis of each area, comparing magnitudes at contralateral distal and proximal electrode locations shows greater magnitudes for distal contralateral PMv (0.1±0.02 mV) as compared to proximal (0.04±0.07 mV) (unpaired t-test for distal vs. proximal PMv, values $p=0.01$) but not for distal vs. proximal contralateral M1 or SMA.

Mean ± SE magnitude for ipsilateral M1 was 0.013±0.004 mV, for ipsilateral PMv 0.014±0.005 mV, and for ipsilateral SMA 0.012±0.003 mV. The magnitudes of ipsilateral facilitation effects were not different when stimulating the three different areas (ANOVA for M1vs. PMv vs. SMA, $p=0.96$) and were not different according to the EMG electrode location.

Comparison of mean magnitudes recorded at both forelimbs for each area revealed a statistical difference between contralateral and ipsilateral M1 (unpaired t-

test, p=0.003) but not between contralateral and ipsilateral PMv (unpaired t-test, p=0.174) or SMA areas (unpaired t-test, p=0.333). **Figure 9**.

In summary, the magnitude of facilitation effects is greater in contralateral M1, which accounts for the difference between contralateral and ipsilateral effects. There is no difference in the magnitude of facilitation effects between distal and proximal recording electrodes locations except for greater magnitudes when stimulating PMv and recording on the contralateral distal forelimb.

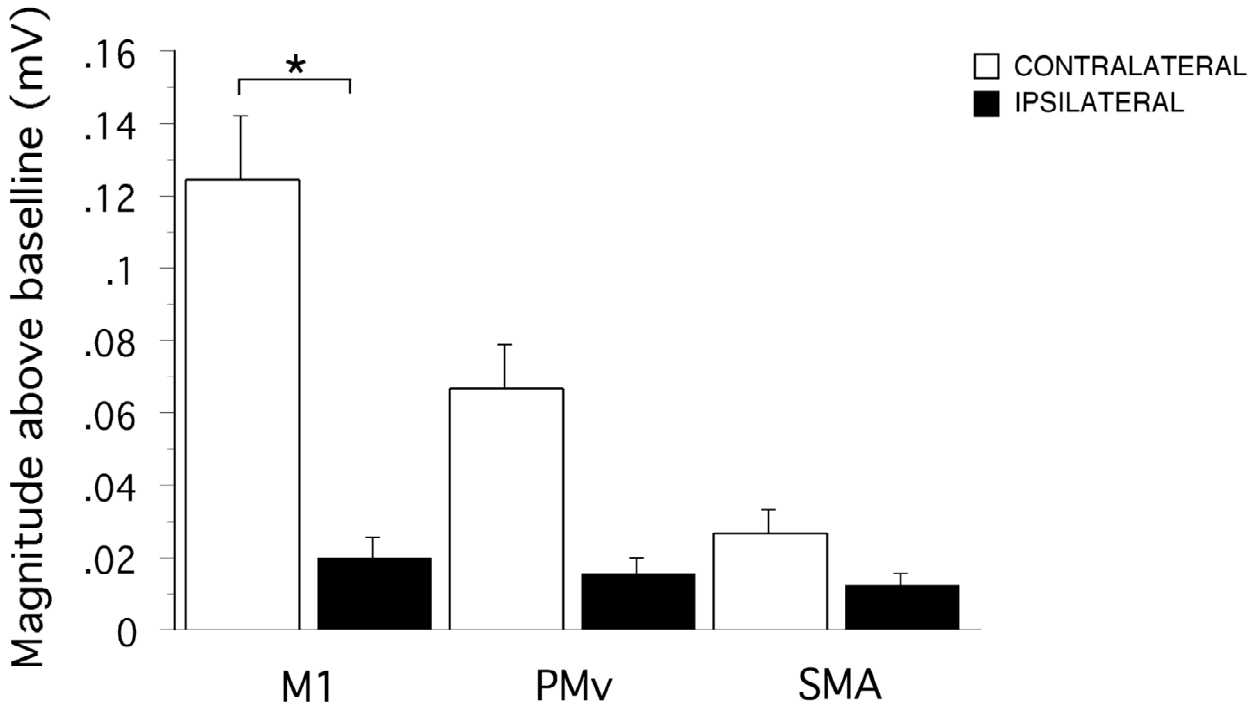

Figure 9. Pre-lesion mean ±SE magnitudes above baseline in mV for contralateral and ipsilateral M1, PMv and SMA areas.

Mean±SE latencies were 24.9±1.1 msec for contralateral effects and 44.8±3.8 msec for ipsilateral effects (unpaired t-test, $p<0.0001$; **Figure 10**). Ipsilateral latencies showed a wider range of distribution compared to contralateral latencies.

Mean±SE latencies for contralateral M1 were 19.4±1.5 msec, for contralateral PMv 22.9±1.4 msec, and for contralateral SMA 34.8±2.2 msec. Analysis of latencies when stimulating the different areas and recording on the contralateral forelimb revealed SMA latencies to be longer from those of M1 and PMv (ANOVA, $p<0.0001$; Fisher's for contra M1 vs. contra PMv, $p=0.09$; contra M1 vs. contra SMA, $p<0.0001$; contra PMv vs. contra SMA, $p<0.0001$).

Analysis of latencies for distal vs. proximal contralateral electrode locations at each area showed statistical difference for SMA (unpaired t-test, $p=0.02$), but not for M1 or PMv. Distal mean±SE latencies when stimulating SMA were 30.4±2.5 msec and proximal 40.9±2.5 msec.

Mean ±SE latencies for ipsilateral M1 were 54.2±5.0 msec, for ipsilateral PMv 35.7±5.2 msec and for ipsilateral SMA 28.8±4.4 msec. Ipsilateral M1 latencies were statistically longer than those of ipsilateral PMv and SMA (ANOVA, $p<0.01$; Fisher's for ipsi M1 vs. ipsi PMv, $p<0.05$; ipsi M1 vs. ipsi SMA, $p<0.005$; ipsi PMv vs. ipsi SMA, $p=0.5$)

Analysis of latencies for distal vs. proximal electrode locations, for each area, did not show statistical difference for ipsilateral muscles.

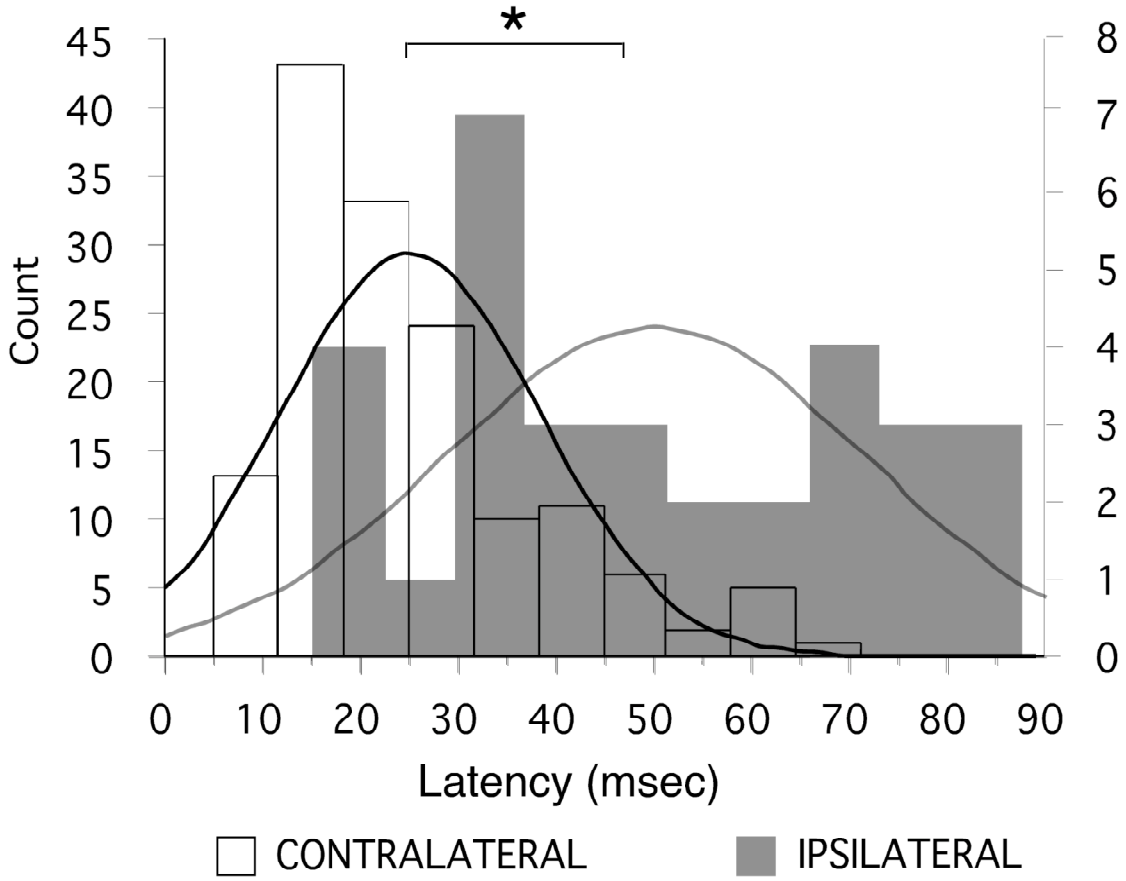

Figure 10. Histogram showing the distribution of contralateral and ipsilateral latencies. There is a significant difference between mean±SE contralateral (24.9±1.1 msec) and ipsilateral (44.8±3.8) latencies (unpaired t-test $p<0.0001$). The curves represent the normal distribution.

Comparison of mean latencies at each forelimb for each area revealed a statistical difference when stimulating M1 (unpaired t-test M1 contra vs. M1 ipsi, $p<0.0001$) and PMv (unpaired t-test PMv contra vs. PMv ipsi, $p=0.01$) but not SMA ($p=0.31$). **Figure 11**.

In summary, the longer ipsilateral latencies of facilitation effects are due to differences in latencies the ipsilateral forelimb when stimulating M1 and PMv. Latencies were not different between electrode locations except for longer latencies at contralateral proximal electrode locations when stimulating SMA.

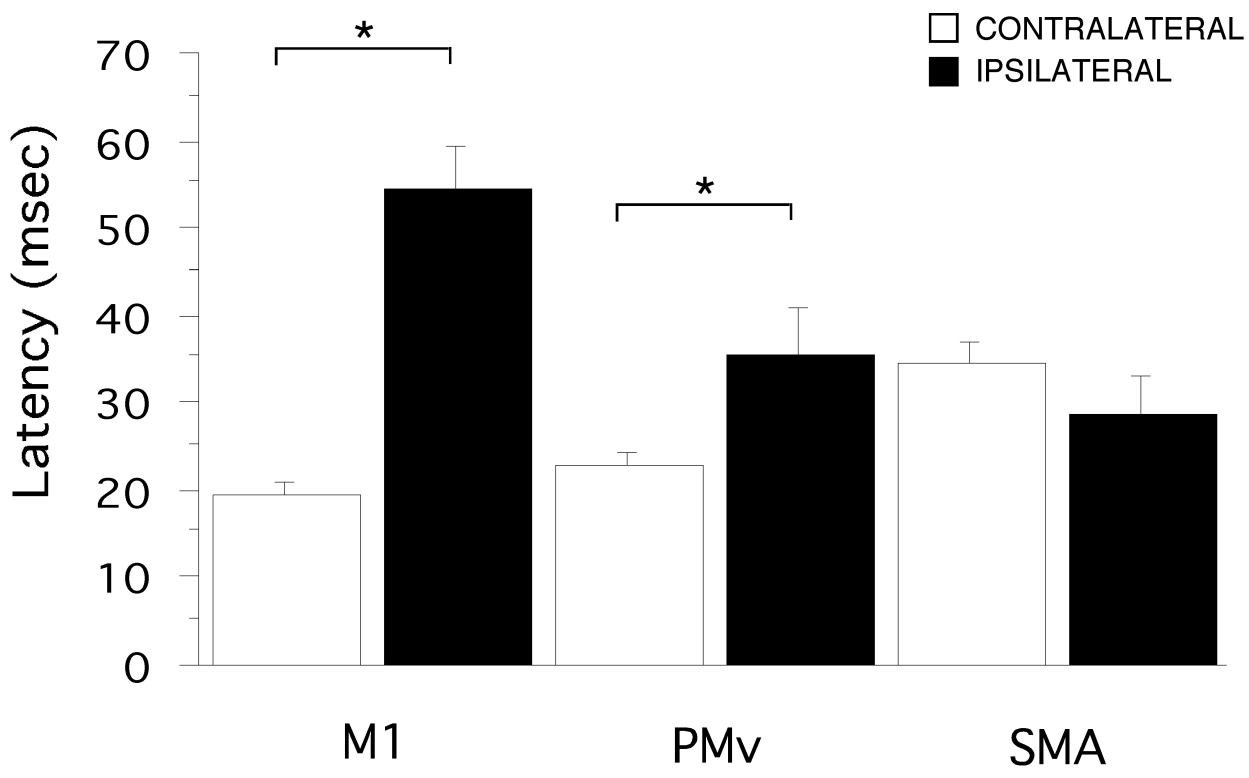

Figure 11. Mean ±SE latencies in msec for contralateral and ipsilateral M1, PMv and SMA areas.

2. Suppressive effects

A few suppressive effects **(Figure 12)** were obtained pre-lesion (eight contralateral and nine ipsilateral), with mean±SE latencies of 50.1±8.2 msec for ipsilateral effects and 34.3±8.4 msec for contralateral effects, for stimulations at all areas pooled together. Six of the suppressive effects on the contralateral forelimb were obtained when stimulating SMA. The mean latency was of 39.7±10.8 msec. The other two effects were obtained when stimulating M1 and PMv.

The nine ipsilateral suppressive effects were distributed across all three areas with three effects with a mean latency of 28.3±3.7 when stimulating M1, three effects with a mean latency of 74±2.7 msec when stimulating PMv and three effects with a mean latency of 50.0±17.8 msec when stimulating SMA. Thus, although average suppressive effects had longer latencies ipsilaterally, the small number of effects did not allow for detailed analysis of suppressive latencies according to the stimulated area.

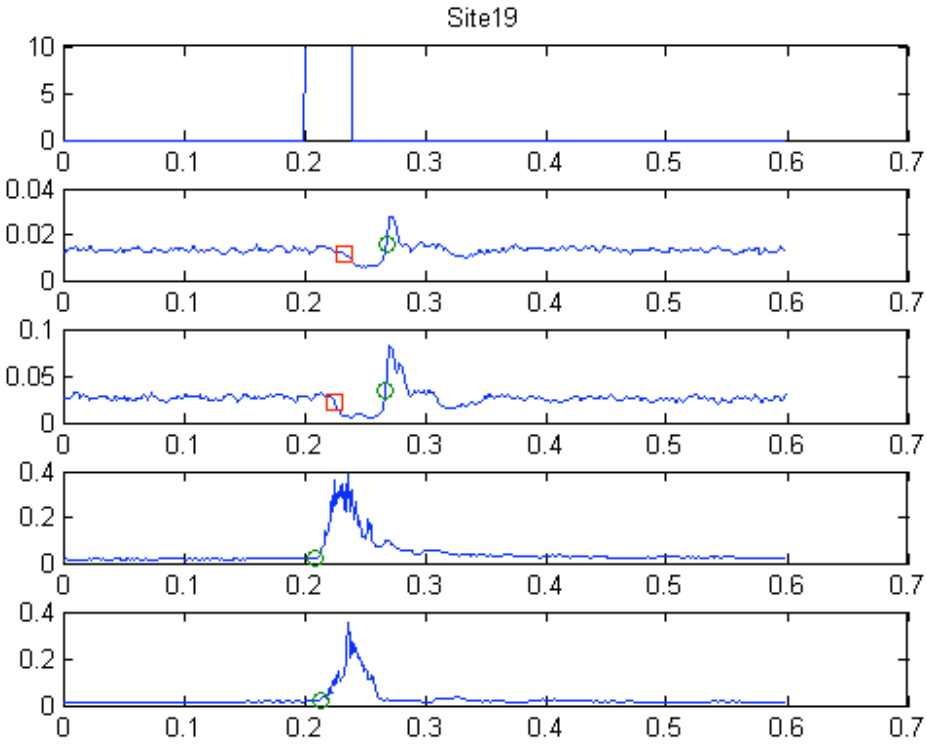

Figure 12. Example of suppressive effects at the ipsilateral distal and proximal electrode locations.

d. Post-lesion physiological outcome

i. Effect of lesion on ICMS maps of motor representations in SMA

Pre-lesion neurophysiological maps of the SMA distal forelimb representation were compared with maps at the end of post-lesion wk 3 and 13 (**Figure 13**). The total SMA DFL averaged 0.83±0.05 SE mm^2 pre-lesion, 0.56±0.30 SE mm^2 on post-lesion wk 3 and 1.41±0.23 SE mm^2 on post-lesion wk 13. Thus, on average, DFL area decreased relative to pre-lesion at wk 3 and increased relative to pre-lesion at wk 13, though there was some variation across cases.

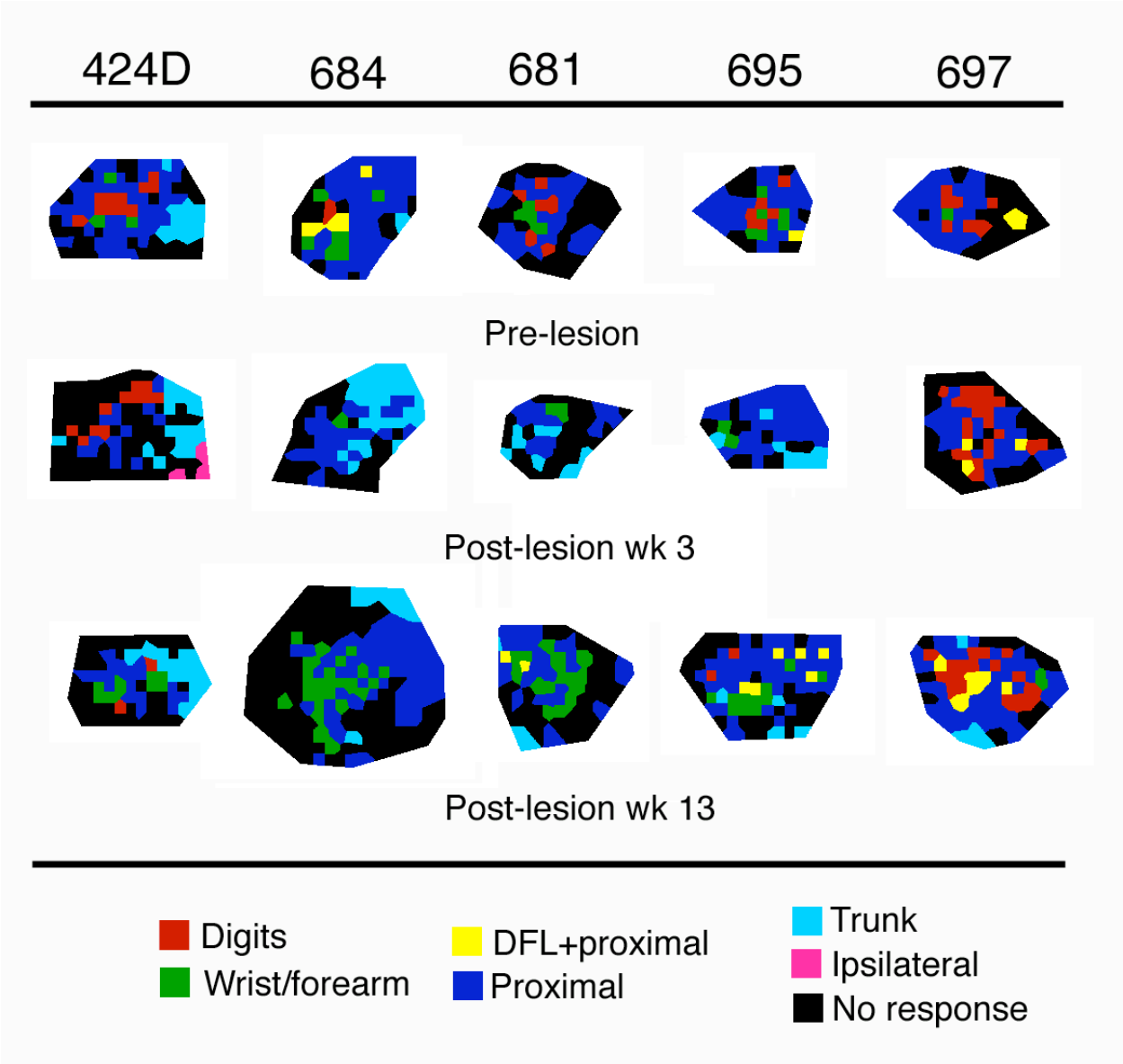
424D
684
681
695
697
Pre-lesion
Post-lesion wk 3
Post-lesion wk 13
Digits
Wrist/forearm
DFL+proximal
Proximal
Trunk
Ipsilateral
No response

Figure 13. ICMS maps of SMA DFL before and after cortical lesion.

Colors represent the movement(s) evoked by electrical stimulation (60 μA) at that site.

Pre-lesion neurophysiological maps of the DFL representation were compared with maps at post-lesion wk 3 and 13. The total SMA DFL representation averaged 0.83±0.05 SE mm^2 at baseline, 0.56±0.3 SE mm^2 on post-lesion wk 3 and 1.41±0.23 SE mm^2 on post-lesion wk 13. The total DFL area included digit and wrist/forearm representations, which contributed 61% and 39%, respectively, at baseline, and then in different proportions on post-lesion wk 3 and 13. The DFL area was further analyzed for individual digit and wrist/forearm representations. Sites where ICMS evoked combined distal and proximal movements were coded separately but grouped with either DFL or proximal representations according to the movement evoked at the lowest threshold.

Area measurements for total DFL (and the distribution of digit and wrist/forearm components) are shown in **Table 2**. Area measurements at post-lesion wk 3 and 13 were compared to pre-lesion values. These changes are expressed as percent change from pre-lesion to describe general trends. The DFL area included digit and wrist/forearm representations, which comprised 61% (0.51 mm^2) and 39% (0.32 mm^2), respectively in pre-lesion maps, and then in different proportions at post-lesion wk 3 and 13. Compared with the pre-lesion map, the wk 3 post-lesion map demonstrated a decrease in total DFL area in four of the five animals. The percentage decrease ranged from 22% to 89%. In the fifth monkey (697), the total DFL area increased 37%. At post-lesion wk 13, an increase in DFL area (relative to pre-lesion area) was seen in four of five animals. The percentage increase ranged from 29% to 73%. In the fifth monkey (424D), the total DFL area decreased by 19% compared to the pre-lesion map. The main effect of week (pre-lesion, 3 wk, 13 wk) on total DFL area approached statistical significance (repeated measures ANOVA, $F=4.234$, $p=0.056$).

Table 2. Area measurements for total DFL, absolute and percent change from pre-lesion and the distribution of digit and wrist/forearm components.

Time point	Monk#	Area (mm2)			Change from pre-lesion (mm2)		
		Total DFL	Digits	Wrist/ forearm	Total DFL	Digits	Wrist/ forearm
	424D						
Pre-lesion		0.88	0.67	0.21			
PL wk 3		0.69	0.69	0	-0.19 (-22%)	0.02 (3%)	-0.21 (-100%)
PL wk13		0.71	0.15	0.56	-0.17 (-19%)	-0.52 (-78%)	0.35 (167%)
	684						
Pre-lesion		0.95	0.46	0.49			
PL wk 3		0.1	0	0.1	-0.85 (-89%)	-0.46 (-100%)	-0.39 (-80%)
PL wk13		1.85	0	1.85	0.9 (95%)	-0.46 (-100%)	1.36 (278%)
	681						
Pre-lesion		0.75	0.46	0.29			
PL wk 3		0.17	0	0.17	-0.58 (-77%)	-0.46 (-100%)	-0.12 (-41%)
PL wk13		1.48	0	1.48	0.73 (97%)	-0.46 (-100%)	1.19 (410%)
	695						
Pre-lesion		0.87	0.5	0.37			
PL wk 3		0.18	0	0.18	-0.69 (-79%)	-0.5 (-100%)	-0.19 (-51%)
PL wk13		1.12	0.17	0.95	0.25 (29%)	-0.33 (-66%)	0.58 (157%)
	697						
Pre-lesion		0.7	0.46	0.24			
PL wk 3		1.66	1.66	0	0.96 (137%)	1.2 (261%)	-0.24 (-100%)
PL wk13		1.91	1.81	0.19	1.21 (173%)	1.35 (293%)	-0.05 (-21%)
	Mean±SE						
Pre-lesion		0.83±0.05	0.51±0.04	0.32±0.05			
PL wk 3		0.56±0.29	0.47±0.33	0.09±0.04	-0.31±0.33 (-30%)	-0.04±0.32 (-7%)	-0.27±0.04 (-94%)
PL wk13		1.41±0.23	0.43±0.35	1.01±0.30	0.55±0.24 (71%)	-0.19±0.36 (-26%)	0.76±0.26 (231%)

Further analysis of the digit and wrist/forearm movements revealed no statistically significant changes in digit representations across the three maps, due to substantial variation across animals. Three of the five animals lost all digit representations at post-lesion wk 3, while digit area for monkey 424D was largely unchanged, and digit area in monkey 697 increased substantially. Two of the three monkeys who had lost all digit sites on post-lesion wk 3, did not recover digit representations on post-lesion wk 13. In the third monkey the digit area was reduced compared to the pre-lesion map. The digit area of monkey 424D also decreased in post-lesion wk 13. However, the digit area for monkey 697 further increased at post-lesion wk 13. Overall, the mean digit representation was 0.51±0.041 SE mm^2 pre-lesion, 0.47±0.33 SE mm^2 on post-lesion wk 3 and 0.43±0.35 SE mm^2 on post-lesion wk 13 (repeated measures ANOVA, n=5, F=0.042, p=0.96).

Changes in wrist/forearm representations were more consistent across animals. Wrist/forearm representations decreased in all animals on post-lesion wk 3 ranging from a 41% decrease to a total loss of wrist/forearm representations. On post-lesion wk 13 wrist/forearm representations increased in four of the five animals, ranging from a 57% to 310% increase compared to pre-lesion maps. The wrist/forearm representation in the fifth monkey (697) decreased 21% compared to the pre-lesion map, although this was an increase from post-lesion wk 3. Mean values for wrist/forearm representations were 0.32±0.5 SE mm^2 pre-lesion, 0.09±0.39 mm^2 on post-lesion wk 3 and 1.01±0.3 SE mm^2 on post-lesion wk 13 (ANOVA, F=9.27,

$p<0.01$; Fisher's post-hoc test for pre-lesion vs. post-lesion wk 3: $p=0.33$; pre-lesion vs. post-lesion wk 13 $p=0.01$; post-lesion wk 3 vs. post-lesion wk 13 $p=0.003$) (**Figure 14)**.

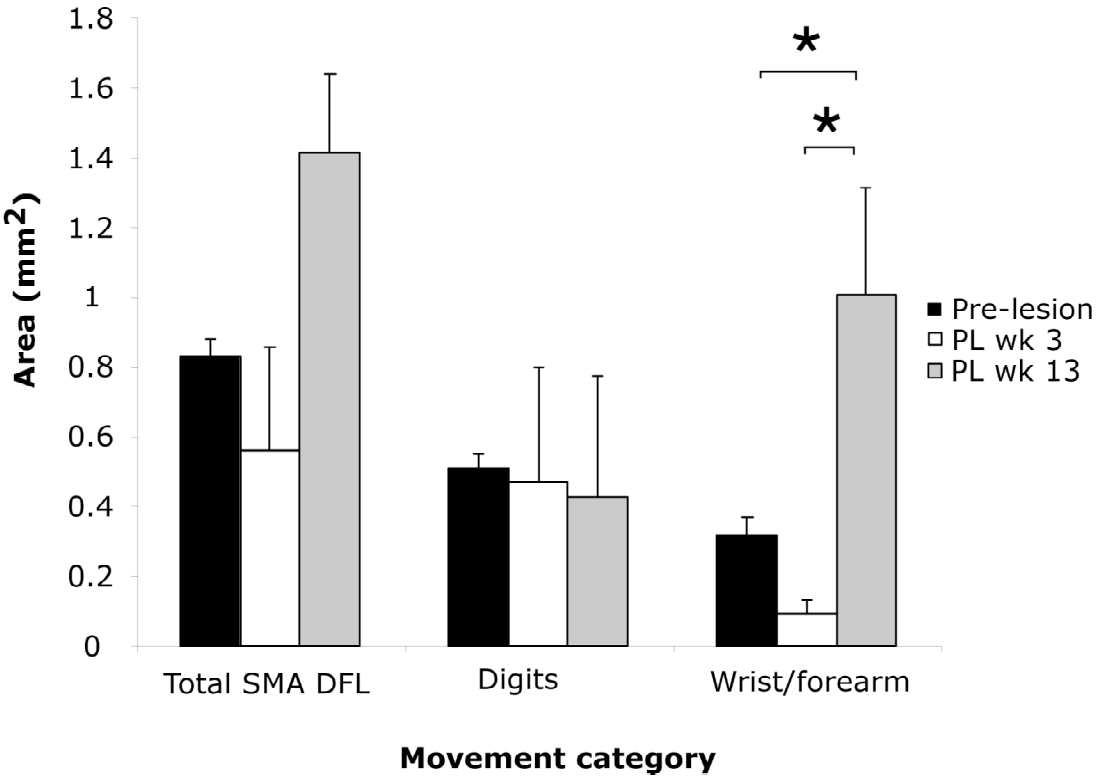

Figure 14. Total SMA distal forelimb area (mm^2) for pre-lesion and post-lesion (PL) wk 3 and13.

The mean digit representation at pre-lesion was 0.51±0.041 SE mm^2 , 0.47±0.33 SE mm^2 on post-lesion wk 3 and 0.43±0.35 SE mm^2 on post-lesion wk 13 (ANOVA, F=0.042, p=0.96). Mean wrist/forearm representations showed a statistically significant increase on post-lesion wk 13. Mean values were 0.32±0.5 SE mm^2 pre-lesion, 0.09±0.39 mm^2 on post-lesion wk 3 and 1.01±0.3 SE mm^2 on post-lesion wk 13 (ANOVA, F=9.27, p<0.01; Fisher's for pre-lesion vs. post-lesion wk 3 p=0.33; pre-lesion vs. post-lesion wk 13 p=0.01; post-lesion wk 3 vs. post-lesion wk 13 p=0.003

1. Relationship between lesion size and physiological changes in SMA

To test the hypothesis that lesion size determines the degree of cortical plasticity, we then examined the relationship between physiological changes in the SMA DFL and lesion size. At post-lesion wk 3, there were no correlations between physiological changes in the SMA DFL areas and lesion size (R^2=0.105, p=0.63). However, at post-lesion wk 13, the change in the total SMA DFL area positively correlated with lesion size (R^2=0.973, p=0.0004; **Figure 15**). The larger the lesion, the greater the change in SMA total DFL area compared with pre-lesion maps. The changes in the individual movement categories (digit and wrist/forearm) were not correlated to lesion size (R^2=0.328, p=0.36 for change in digit area and R^2=0.03. p=0.81 for change in wrist/forearm area).

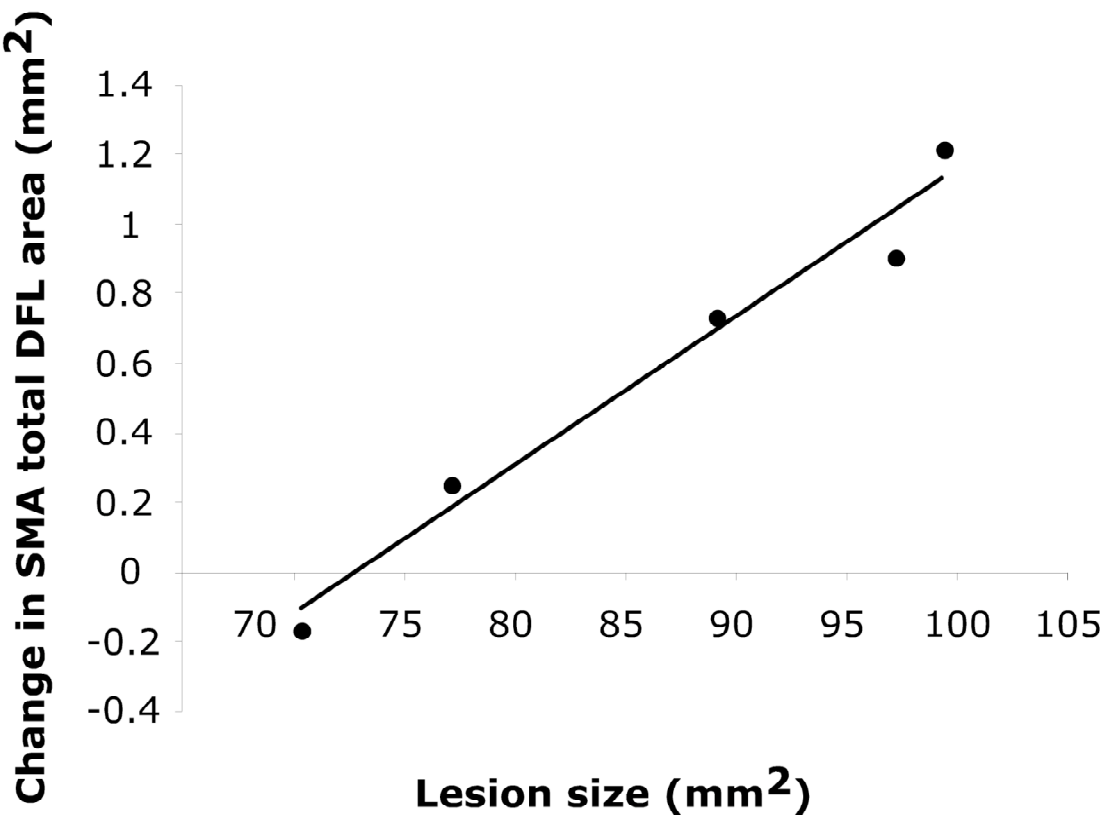

Figure 15. Correlation between change in SMA total DFL representation and lesion size at post-lesion wk 13. The changes in DFL representation areas from pre-lesion to post-lesion wk 13 positively correlated with lesion size (R^2=0.973, p=0.0004).

2. Relationship between motor performance and physiological changes in SMA

To test the hypothesis that the level of motor performance is a function of the SMA area we then examined the relationship between the absolute SMA DFL area and motor performance scores at post-lesion wk 3 and 13. At post-lesion wk 3 these two variables were not correlated (R^2=0.191 p=0.51). At post-lesion wk 13, motor performance scores were linearly related to the SMA total DFL area (R^2=0.925, p=0.005; **Figure 16**). The larger the SMA total DFL area, the higher the Klüver Board performance score achieved. Digit and wrist/forearm areas were not correlated to motor performance (R^2=0.241, p=0.45 for digit area and R^2=0.036, p=0.79 for wrist/forearm area). The change in behavior from post-lesion wk 3 to post-lesion wk 13 was not related to the change in area during this same time period (R^2=0.247, p=0.44) indicating that the level of motor performance is a function of the final state of the SMA DFL representation rather than a function of the changes this area undergoes.

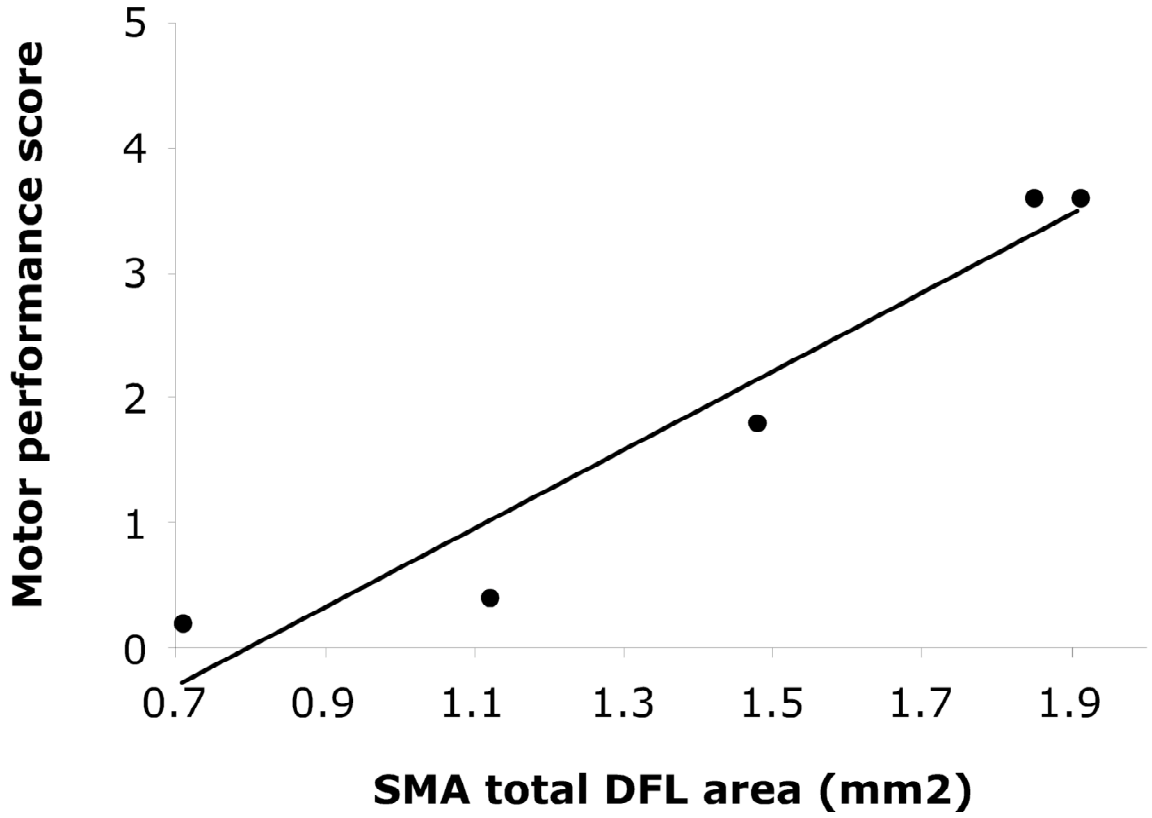

Figure 16. Correlation between SMA total DFL representation and Klüver Board performance score at post-lesion wk 13. Behavioral performance was positively correlated to the SMA total DFL representation on post-lesion wk 13 (R^2=0.925, p=0.005).

3. Effect of secondary lesion in SMA DFL motor representation

Once established that lesion size determines the degree of cortical plasticity in SMA, and that the level of motor performance is a function of the spared SMA area, it followed to test the hypothesis that maintenance of recovered motor function depended on activity in the spared SMA. Thus, three months following the initial lesion, a secondary lesion was induced in the DFL SMA area. Behavioral evaluation was conducted weekly during the following four weeks following the same behavioral protocol used following the initial lesion.

The average±SE lesion size for the SMA DFL area for the five monkeys was 3.9±0.6 mm^2. The average of the motor performance scores at weeks 12 and 13 post-initial lesion were used as pre-secondary lesion values. Motor performance scores for all monkeys averaged across all five wells declined from a mean±SE of 1.7±0.4 pre-secondary lesion to 1.0±0.3 on post-secondary lesion wk 1. The motor performance score increased to 1.7±0.5 on post-secondary lesion wk 2, was 1.8±0.6 on post-secondary lesion wk 3 and 2.8±1.0 on post-secondary lesion wk 4.

Figure 17 illustrates motor performance scores following the SMA DFL area lesion for each monkey. Wilcoxon signed-ranks test (Wilcoxon statistic = "T"; lowest p value of 0.031 with n=5) was employed for analysis of pre- vs. post-lesion comparisons. This test did not reveal a significant difference between pre-secondary lesion and post-secondary lesion wk 1 (T=1.8; p=0.079). Thus, although there is a decline in motor scores following the secondary SMA lesion, followed by an apparent recovery, we could not confirm that maintenance of recovered motor function depended on activity in the spared SMA.

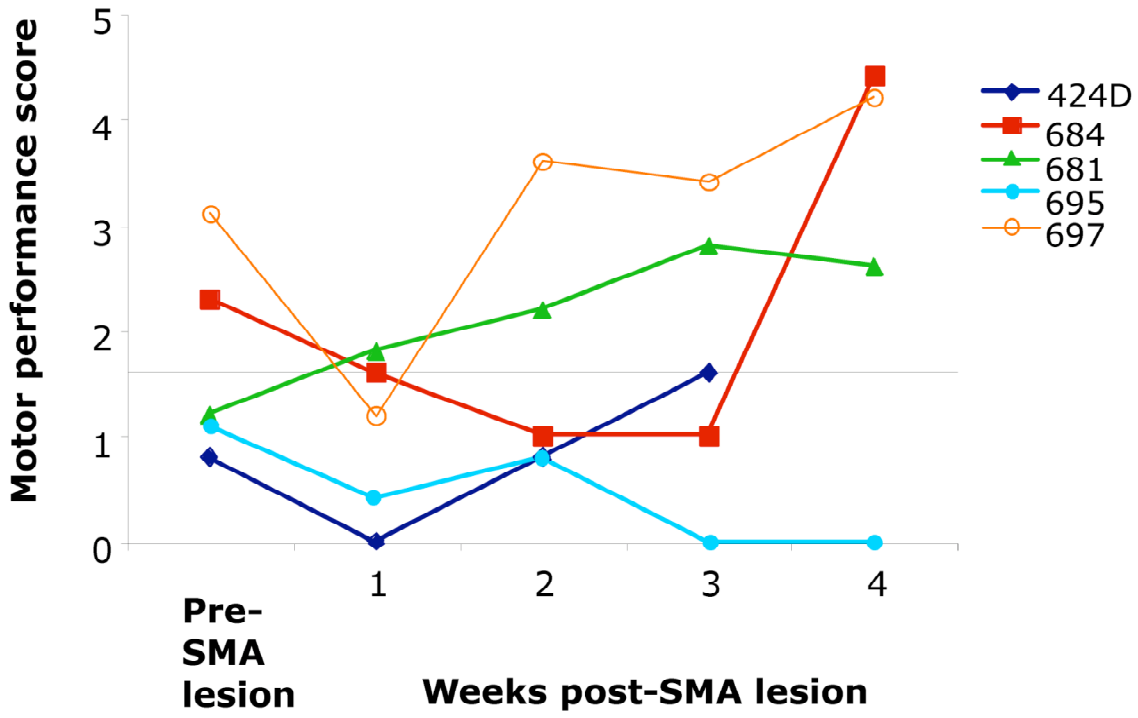

Figure 17. Motor performance scores following SMA DFL area lesions for each monkey.

b. Effect of lesion on ipsilateral EMG activity

The same two time points used in the study of changes in ipsialesional SMA DFL representations were used for the study of changes in EMG activity following stimulation of the contralesional cortex. Ipsilateral effects at three weeks and 13 weeks post-lesion (three months) were compared to pre-lesion values and are summarized in **Table 3**.

Table 3. Summary of changes in EMG measures compared to pre-lesion.

		3 WEEKS	3 MONTHS	
FACILITATION	# OF EVENTS	increase	increase	IPSI
		decrease	same	CONTRA
	MAGNITUDE	no change		IPSI
				CONTRA
	LATENCY	increase	same	IPSI
		no change		CONTRA
SUPPRESSION	# OF EVENTS	increase	increase	IPSI
				CONTRA
	LATENCY	decrease	same	IPSI
		no change		CONTRA

Ipsilateral measures that change differently to the corresponding contralateral measures are shaded in grey. The number of facilitation events increases ipsilaterally three weeks post-lesion and is still increased three months post-lesion. Contralateral facilitation events decrease at three weeks and go back to pre-lesion values at three months. The magnitude of facilitation effects does not change post-lesion. Latencies of facilitation effects do not change contralaterally but increase at three weeks and then return to pre-lesion values at three months post-lesion. The number of suppression events increases on both sides three weeks post-lesion and is still increased at three months post-lesion. However there is a decrease of suppression latencies three weeks post-lesion and a return to pre-lesion latencies ipsilaterally, but not contralaterally.

1. Ipsilateral increase in number and latency of facilitation effects

The number of facilitation events increased ipsilaterally from a total of 25 effects noted pre-lesion to a total of 55 effects three weeks post-lesion and a total of 47 three months post-lesion (**Figure 18**). Chi square analysis of the change in the number of facilitation effects shows a different pattern of changes for ipsilateral and contralateral forelimbs (Chi square P value=0.0002 comparing ipsilateral and contralateral results). While the number of effects increased ipsilaterally, they decreased contralaterally from a total of 146 pre-lesion, to 108 at post-lesion wk 3 and 150 on post-lesion wk 13. Analysis of this change in facilitation effects following stimulation of each area separately shows that variations in both forelimbs (ipsilateral and contralteral) are the same throughout time following stimulation of M1 but different following stimulation of PMv and SMA (Chi square P-value=0.01 for PMv and 0.05 for SMA).

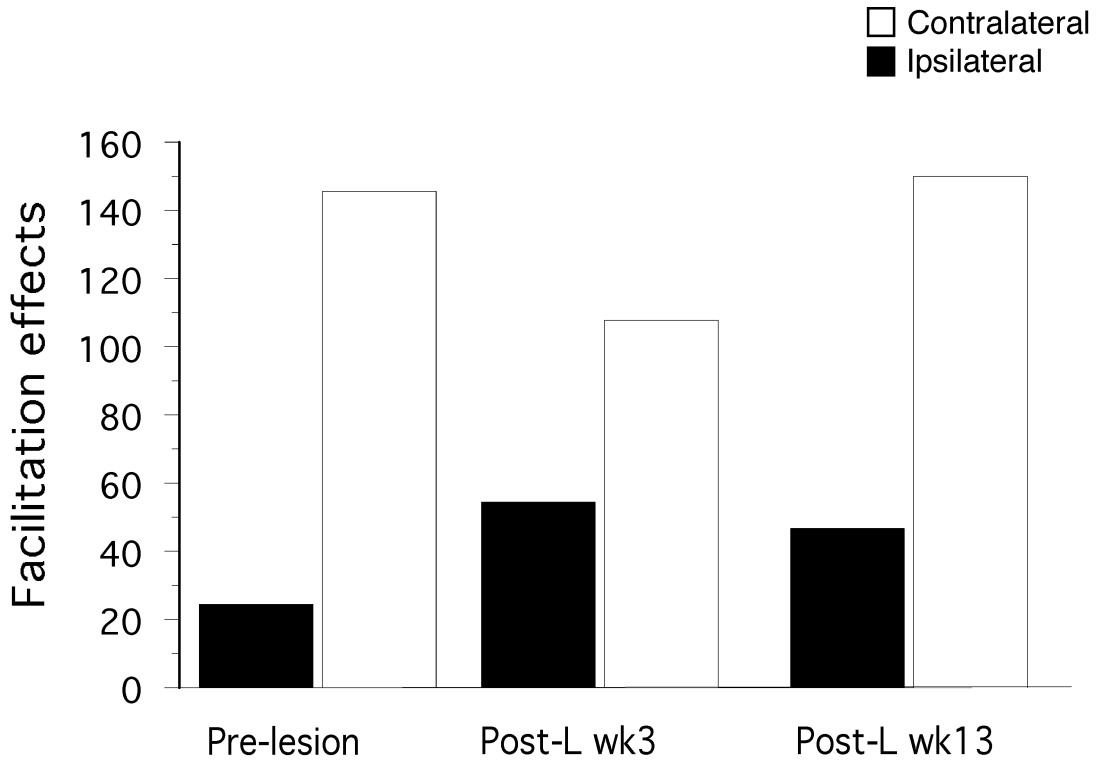

Figure 18. Post-lesion change in number of facilitation effects. Ipsilateral facilitation effects increased at post-lesion wk 3 and remained increased on post-lesion wk 13. In contrast, contralateral facilitation effects decreased at post-lesion wk 3 and returned to pre-lesion values by post-lesion wk13.

The magnitude of ipsilateral and contralateral facilitation effects did not change post-lesion. The latency of ipsilateral facilitation effects increased three weeks post-lesion, paralleling increases in facilitation effects, but returned to pre-lesion values by post-lesion wk 13 (ANOVA, $p=0.0008$; Fisher's pre vs. wk3, $p=0.0005$; pre vs. wk13, $p=0.02$; wk3 vs. wk13, $p=0.006$). Contralateral latencies do not change post-lesion. The changes in ipsilateral latencies when stimulating each of the three areas are shown in table **Table 4** and illustrated in **Figure 19**. Ipsilateral latencies following stimulation of M1 do not change significantly post-lesion. Following stimulation of PMv latencies increase at post-lesion wk 3 and are still increased at post-lesion wk 13 (ANOVA $p=0.001$; pre vs. wk3 $p=0.0003$; pre vs. wk13 $p=0.001$). Following stimulation of SMA, pre-lesion latencies increase at three weeks too but they decrease to pre-lesion values by post-lesion wk 13 (ANOVA $p=0.03$; pre vs. wk3 $p=0.008$). Facilitation latencies following stimulation of SMA also show an effect of the muscle group being recorded from. Significantly shorter latencies are obtained on distal muscles at post-lesion wk3. The mean±SE latency on distal muscles is 33.4±5.5 msec and on proximal muscles 64±1.7 msec at post-lesion wk 3 (T-test SMA distal vs. prox at wk3, $p<0.0001$).

Table 4. Ipsilateral vs, contralateral facilitation effects pre-lesion and at three weeks and three months post-lesion.

Case #		PRE LESION			3 WEEKS			3 MONTHS		
DISTAL		M1	PMv	SMA	M1	PMv	SMA	M1	PMv	SMA
673	CONTRA				10	10	8	9	9	8
	IPSI				2	1	2	0	1	0
537	CONTRA	10	10	10	5	4	6	10	8	7
	IPSI	2	1	2	6	3	3	4	2	1
600	CONTRA	10	7	2	0	0	0	5	0	9
	IPSI	1	2	0	4	0	1	3	0	3
683	CONTRA	10	10	9				8	5	8
	IPSI	0	0	0				0	1	2
TOTAL DISTAL	CONTRA	30	27	21	15	14	14	32	22	32
	IPSI	3	3	2	12	4	6	7	4	6
% of contra		10	11	10	80	29	43	22	18	19
PROXIMAL										
673	CONTRA				9	6	5	9	7	8
	IPSI				2	5	2	5	2	3
537	CONTRA	9	10	7	9	9	7	9	7	2
	IPSI	6	0	2	7	7	3	5	3	4
600	CONTRA	8	7	4	9	6	5	4	1	3
	IPSI	2	2	1	3	0	4	3	0	2
683	CONTRA	8	9	6				8	4	2
	IPSI	4	0	0				0	0	3
TOTAL PROX.	CONTRA	25	26	17	27	21	17	30	19	15
	IPSI	12	2	3	12	12	9	13	5	12
DISTAL & PROX.	CONTRA	**55**	**53**	**38**	**42**	**35**	**31**	**62**	**41**	**47**
	IPSI	**15**	**5**	**5**	**24**	**16**	**15**	**20**	**9**	**18**
Latency(±SE) in msec	CONTRA	**19.4 ±1.5**	**23.0 ±3.1**	**34.8 ±2.2**	**24.0 ±2.6**	**33.7 ±3.1**	**26.5 ±2.9**	**27.8 ±2.4**	**27.2 ±2.4**	**29.7 ±2.4**
	IPSI	**54.2 ±5.1**	**35.7 ±5.2**	**28.8 ±4.4**	**61.0 ±2.0**	**62.8 ±3.3**	**51.9 ±4.7**	**49.3 ±3.8**	**62.2 ±4.3**	**44.7 ±4.2**

Table 4 summarizing number of EMG responses obtained on distal and proximal musculature. Emphasis is placed on ipsilateral responses as compared to contralateral responses. Only facilitation responses are considered in this analysis. Percentages are based on all four animals.

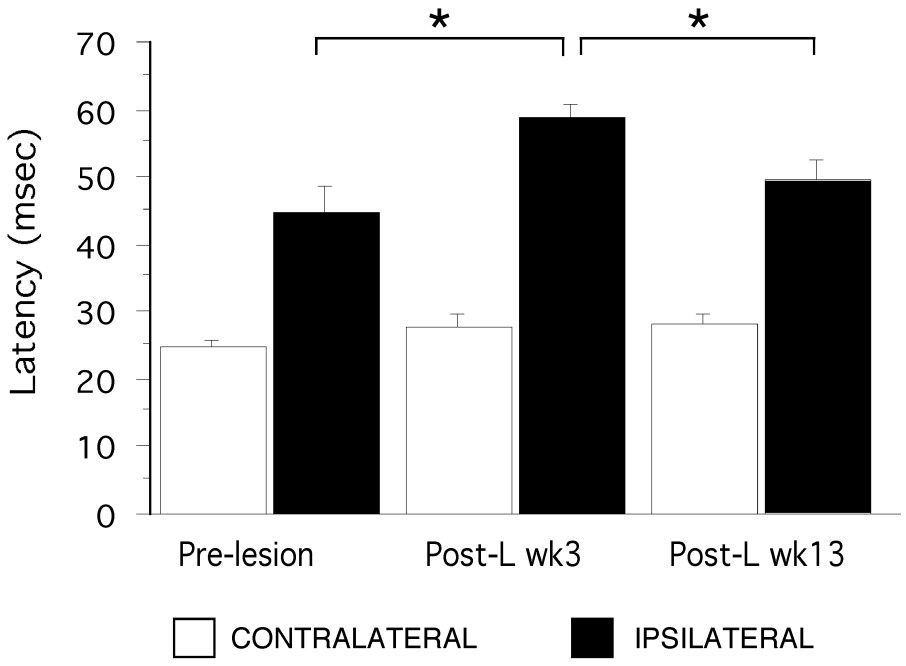

Figure 19. Post-lesion change in latencies for all areas.

2. Ipsilateral decrease in latency of suppressive effects

Post-lesion changes in suppressive effects and latencies are shown in **Table 5** and illustrated in **Figure 20**. Ipsilateral suppressive latencies decreased significantly three weeks post-lesion and returned to pre-lesion values three months post-lesion (ANOVA p=0.007; pre vs. 3w, p=0.004, 3w vs. 3mo p=0.04, pre vs. 3mo p=0.1). Contralateral suppression latencies however, do not change significantly post-lesion.

Table 5. Number of suppressive effects and mean latencies.

	PRE LESION		3 WEEKS		3 MONTHS	
Case #	IPSI	CONTRA	IPSI	CONTRA	IPSI	CONTRA
673			8	13	11	22
537	2	6	21	7	4	3
600	6	2	10	8	1	3
683	1	0			11	9
Mean latency ±SE	50.1 ±8.2	34.2 ±8.4	33.1 ±1.9	38.2 ±4.8	41.0 ±3.2	35.4 ±4.0

Number of suppressive events and mean latencies for all four animals.

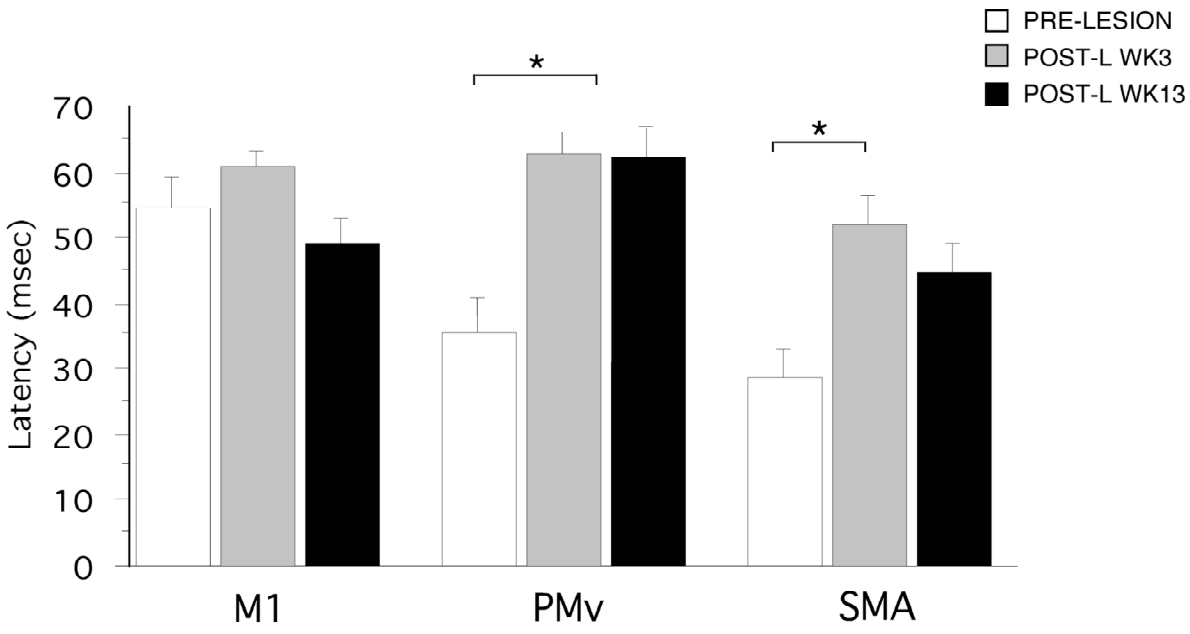

Figure 20. Ipsilateral mean latencies for each area, at each time point.

V. DISCUSSION

a. Effects of large cortical lesions on behavior

Motor behavior is controlled by a distributed network involving at the minimum the primary and secondary motor areas, the dorsal prefrontal cortex, the posterior parietal cortex, the striatum and the cerebellum. Although it may be overly simplistic to think in terms of compartmentalized units that have individual functions (Nudo 2007-Byrne book), specific tasks are attributed to each area. It is generally accepted that M1 mediates skilled voluntary movements, especially of the distal musculature (Phillips 1977). PMv is thought to be involved in visual- and somatosensory-motor integration for motor control of the upper extremity (Hoshi 2004, Murata 1997, Rizzolatti 1983, Schieber 2000), and provides prominent inputs to M1, exerting a powerful facilitatory effect, especially during visually guided movements of the hand (Shimazu 2004). PMd contains a separate representation of the forelimb, hindlimb and trunk, and is thought to be involved in visually guided tasks, since PMd neurons are active during a preparatory motor-set (Wise 1985) and in relation to visuomotor-association tasks (Kurata 1988).

The present study shows profound effects on the monkeys' behavior following an extensive cortical infarct affecting the forelimb representation areas of M1, PMv and PMd. Lesion of the forelimb representations of these three frontal motor areas produced a clear monoplegia of the entire forelimb contralateral to the cortical infarct similar to that seen in humans. No spatial neglect was observed as evidenced by retained ability to retrieve food rewards from the contralateral space. No sensory

deficits were observed since there was no delay in reflex withdrawal following tactile stimulation on both hands. Thus, this is a good model to study permanent damage following pure motor cortical lesions affecting the entire forelimb, comparable to the motor symptoms seen in stroke patients. It excludes sensory and spatial symptoms as well as impairments in the face and leg. It does not duplicate clinical stroke, but instead, creates an environment in which to study reorganization of the motor cortex independent of other confounding variables in order to understand plasticity properties of the remaining cortical network.

A large number of animal studies of recovery have employed models that attempt to create cortical injuries similar to those that occur in stroke. The MCA is the artery most commonly occluded in human stroke (Caplan 1985). However, occlusion of the MCA (MCAo) in its origin results in a reduction of cerebral blood flow in both the striatum and cortex (Marinkovic 2001). Ischemic lesions in the territory of the MCA cause contralateral motor and sensory deficits as well as spatial neglect. More distal MCAo results in an exclusively cortical infarct, however since the branching pattern of the MCA is variable, this produces variable lesions. Numerous models incorporating MCAo at one or more points along it's length have emerged in an attempt to produce a more reproducible lesion (Tamura 1981, Shigeno 1985, Marshall 2003). Two of the five monkeys used in this study received an initial distal MCA branches occlusion lesion. However this model produced variable results due to the amount of collateral blood flow into the territory and variability in the bifurcating pattern of the MCA (Marinkovic 2001, Gibo 1981). This model also produced motor

deficits in the face, trunk and leg areas. Sensory deficits also resulted from these lesions further complicating motor evaluation. Thus, these monkeys were allowed to recover and then received the permanent occlusion. Behavioral and physiological results from the two monkeys in which cortical lesions were conducted in two stages were not different from those that received cortical lesions in a single stage.

Despite the consistency in the lesion location defined eletrophysiologically, general outcome following the lesion was variable between the monkeys. Some improved rapidly and maintained a high level of home cage activity, while others were less active and did not show much improvement. However these differences did not translate into significant differences in their motor performance. One monkey's motor performance though, was clearly different because of engagement in a self-rewarding repetitive skilled use of both hands (stimulation of the genital organ achieved by manual contact).

Following the permanent lesion, there was a clear change in hand preference in all the monkeys, which did not reverse throughout the 13 wk period of observation. Behavioral evaluation required a more general motor performance assessment than those previously employed (eg Frost 2003, Barbay, 2006). The scoring system used in this study attempts to assess motor performance as a global motor functional measure of the entire forelimb rather than the skilled use of the hand. Motor performance scores following the lesion showed some improvement, however, these reached a maximum on post-lesion wk 3 and reached a plateau, with no further improvement being achieved up to three months post-lesion. The averaged highest score achieved

in the individual well analysis indicates no further improvement than being able to extend their arm through the front cage bars, touch the Klüver Board and occasionally placing the fingers inside the correct well. No independent use of the fingers and no forearm pronation/supination and/or wrist flexion/extension were observed. Although in a few occasions the monkeys were able to retrieve pellets from the wells, this was achieved by means of compensatory strategies such as change in body positioning and use of alternative strategies that did not demand the dexterous use of the hand.

b. Ipsilesional plasticity

Recent neuroimaging data seems to point to the involvement of spared ipsilesional motor areas (in the same hemisphere as the lesion) and suggests that regain of function of the paretic hand occurs as a consequence of a dynamic, bihemispheric reorganization after stroke onset. However, given that lesion location and size determine the outcome and degree of cortical plasticity after stroke, reorganization of the motor cortex may follow different mechanisms depending on whether primary motor, premotor or supplementary motor areas in the ipsilesional hemisphere are spared and depending on whether subcortical structures are included in the lesion.

i. SMA reorganizes after injury to other frontal motor areas

Previous studies following partial or total M1 cortical lesions have shown significant recovery during the first several weeks post-injury. It has been suggested that at least part of the recovery may be due to plasticity of spared brain regions in the peri-infarct region, in the somatosensory cortex (Pons, 1988, (Dancause 2006)) or in more remote motor cortical areas such as PMv (Frost, 2003). In the present study, the changes found in the DFL movement representation in SMA following ischemic infarct in the forelimb representation of M1, PMv and PMd indicate that neurophysiologic reorganization of more remote cortical motor areas occurs also in response to an extensive cortical infarct including other frontal motor areas. Thus it would appear that reorganization of secondary cortical areas is a general feature of injury-induced plasticity.

One possible mechanism for mediating functional changes in motor cortex is the modification of synaptic strength of horizontal connections (Hess 1994). Dendritic and synaptic morphology of motor cortex neurons have been shown to be altered by specific motor learning tasks (Greenough 1985, Jones 1999, Kleim 1996, 2002). These neurophysiologic and neuroanatomic changes during acquisition of motor skills may provide a substrate for altering the topography of motor maps (Rioult-Pedotti 1998). In the present study, the changes observed in the SMA DFL maps might be interpreted as a consequence of post-injury motor learning of new motor skills in the setting of a restricted motor capacity inflicted by the injury. It is thus

possible that physiological changes in the SMA DFL reflect reorganization in an attempt to optimize outcome following injury.

Several facts make SMA an optimal remote area for functionally relevant reorganization following an ischemic lesion. SMA receives its blood supply from the anterior cerebral artery and is thus commonly spared from MCAo, the most common form of clinical stroke. The SMA DFL representations are strongly interconnected via the corpus callosum (Rouiller 1994) and heavily interconnected ipsilaterally with the DFL areas of M1 (Stepniewska 1993). SMA has also been implicated in regulating synchronization between M1 of both hemispheres (Wiesendanger, 1994) since it is activated prior to M1 during movement production (Deecke 1994). Thus, although plasticity in the ipsilesional SMA might not be sufficient to substitute for function lost following extensive injury to the other major frontal motor representations, it might be specially suited to detect alterations in the normal connection pattern and direct reorganization of descending systems.

The extensive ipsilateral projections of SMA (23%, (Bortoff, 1993)) and its role in bimanual coordination has to be kept in mind when interpreting the neurophysiological changes observed in the DFL representation of SMA. It is conceivable to believe that these ICMS map modifications are related to the unaffected forelimb. Although these ipsilateral projections terminate mainly in laminae VII and VIII in the spinal cord (Dum, 1996), with greater influence on motor neurons pools controlling proximal rather than distal muscles (Kuypers, 1970; Colebatch, 1979; Nirkko, 2001) what really matters is the change in excitability of the

motorneuron pool controlling upper limb movements. In this sense, SMA might be specially accommodated to orchestrate post-injury compensatory strategies. The appreciation of the strong SMA homotopic connections have led to the speculation of SMA as having an important role in shifting of the attention or moderation between activation of both hemispheres (Brinkman 1984) and in reflecting which arm to use during execution of function (Hoshi 2004). These functions might become especially relevant following stroke, even if this does not necessarily involve recovery of function in the sense of regaining lost motor capabilities. Compensatory strategies include trunk and shoulder-girdle movements, especially in reach-to-grasp strategies (de Oliveira 2007) further supporting the role of SMA in recovery from stroke. The fact that ipsilateral terminations are found in the proximal and trunk zone of the spinal cord which, receive massive bilateral projections from reticulo- and vestibulo-spinal systems, (Kuypers 1981) suggests that ipsilesional SMA changes might be tightly related to simultaneous changes in the contralesional cortex.

From the results to date, it is not possible to determine if any one motor area is more important in the recovery of motor abilities after stroke. The present study did not monitor changes that might be occurring in CMA given that it is less accessible for neurophysiological mapping studies. Nevertheless, we hypothesize that the entire cortical and subcortical motor system that is spared by the injury participates to varying degrees depending upon the extent and location of the injury and behavioral demands. At least some of the functions of the injured region(s) are redistributed across the remaining cortical and subcortical motor network, yet recovery ultimately

depends on the integrity of the entire motor system.

ii. Remote reorganization is directly related to the reciprocal connectivity of the various motor areas

It has been previously demonstrated that lesion size determines the degree of cortical plasticity after stroke (Frost 2003). In that study ICMS techniques were used to analyze motor representations in PMv of adult squirrel monkeys before and after an ischemic infarct in M1 DFL. ICMS mapping at 3 mo post-infarct revealed substantial enlargements of the DFL in PMv suggesting that the disruption of cortical connectivity between M1 and PMv was responsible for PMv reorganization. A follow-up study tested this hypothesis of disruption of cortical connectivity underlying reorganization, by inducing restricted lesions in either subregion of M1. PMv projects almost exclusively to the rostral portion of M1 and only sparsely to the caudal portion of M1 (Dancause, 2006). Lesions resulted in a *reduction* of the PMv DFL (Dancause, 2006) at 3 mo post-lesion suggesting that following partial M1 lesions, plasticity in the peri-infarct cortex may be sufficient for recovery to occur. Plasticity (as evidenced by enlarged motor representations) can occur in connected remote regions, but only when the injury to the primary region is substantial. It follows that reorganization of the motor cortex may obey different mechanisms depending on whether primary motor, premotor or supplementary motor areas in the ipsilesional hemisphere are spared and whether subcortical structures are included in the lesion. In the present study we have found a positive correlation between lesion size and physiological changes in SMA 3 months post-injury. In this purely cortical

stroke model, a larger lesion promotes a greater change in the DFL representation of the SMA. This is another example that remote reorganization is directly related to the reciprocal connectivity of the various motor areas. It seems that the greater the damage to reciprocal intracortical pathways, the greater the plasticity achieved in the secondary, intact area.

Since neuroanatomical changes are known to occur in the peri-infarct area, and neuronal networks are densely interconnected, many of the functional changes that have been observed in cortical remote regions may have structural correlates similar to those observed in the peri-infarct region. Following M1 injury the emergence of a novel target of PMv neurons located in S1 has been shown in a recent study (Dancause, 2005). These results represent evidence for a major alteration in the intracortical wiring patterns between different cortical areas. It was thus suggested that the establishment of these new connections provide a means by which the remaining cortical motor system can gain access to somatosensory information more effectively. These changes in the connectivity pattern may be also underlying the observed modifications in the SMA DFL maps found in the present study.

iii. Relationship of post-lesion map changes to behavioral outcome

While many studies have shown changes in motor maps in remote regions using ICMS or fMRI in humans, the relationship of post-lesion map changes to behavioral outcome is poorly known. The physiological changes in the SMA DFL representation found in this study were linearly correlated with the change in motor performance scores on post-lesion wk 13. This suggests that the level of motor performance is a function of the DFL SMA area. The larger the SMA total DFL area, the higher the motor performance score achieved. It would seem that the cortex is responding to new behavioral demands. This suggests that the physiological changes underlie improvement in motor function. However there are several caveats to this interpretation.

The correlation between changes in the SMA DFL maps and motor behavior were present at post-lesion wk 13, but not at post-lesion wk 3. Analysis of motor performance scores throughout the 13 wk post-lesion spontaneous recovery period revealed a significant improvement from post-lesion wk 1 to post-lesion wk 3. However although changes in the SMA DFL maps were observed at post-lesion wk 3 these did not reach statistical significance. This suggests an initial and transient dissociation between improvement in motor performance and physiological changes. These two parameters seem to evolve at different paces. The changes in behavior do not translate or are not evidenced by changes in the maps.

Motor performance scores do not change significantly beyond post-lesion wk3 indicating no further improvement occurs during the rest of the spontaneous recovery period monitored in this study. However, it is at the end of this behaviorally silent period when the correlation becomes evident. This delay in physiological and behavioral correlation suggests that the mechanisms underlying the functionally relevant plasticity occur over a longer time period. Both anatomical and physiological data point to the existence of at least two different periods during recovery from stroke, which have been reviewed in the Introduction. The "late" time point varies according to different authors and the stroke model used, however, it is generally believed to be achieved once behavioral recovery reaches a plateau, after the first three months. This later stage is related to a return or normalization of several physiological parameters such as intracortical inhibition and hyperexcitablity.

The establishment of new connections between PMv and S1 have been detected as late as five months following M1 lesion in the squirrel monkey. Although it is unknown whether these new connections were present at an earlier time point, it suggests that these events occur over a longer period of time compared to the early changes in neuroanatomical events underlying axonal sprouting, synaptogenesis and dendritic arborization followed by pruning. Thus, if changes in long-distance intracortical networks underlie changes in the DFL SMA observed in the present study, it is reasonable to expect correlations with improved motor function at this later time period. The modification in connections of the spared motor areas following stroke may provide a means by which the remaining cortical motor system

can gain access to information more effectively. If substantial alterations in the connectivity of the surviving brain tissue are correlated to improvement in functional recovery following an extensive cortical lesion, this may yield important clues to neurorehabilitation approaches.

One other apparent discrepancy in the results from this study is the lack of correlation between changes in the individual components of the DLF SMA and the improved motor performance. Since changes in the SMA DFL were largely due to observed modifications of the wrist/forearm representations, it would be expected that changes in this individual component of the DFL would be correlated to change in motor performance scores. However recent evidence suggests that behavior may have a specific influence on plasticity (Carmichael and Chesselet 2002) and we believe this mechanism explains results obtained for monkey 697, a clear outlier in this analysis. The SMA DFL representation maps for this monkey were notably different from the rest of the group: whilst the wrist/forearm area of the four animals increased, that of monkey 697 decreased and whilst the digit area of the four animals decreased, that of monkey 697 greatly increased. Paired to this discrepancy in motor maps, this monkey showed an important difference in the behavior during the recovery period, which the authors believe underlie the difference in the outcome of the representation maps.

iv. Functional relevance of ipsilesional plasticity

In macaques the amount of the SMA devoted to the DFL is nearly equal to that devoted to the more proximal body parts since the size of the SMA region which projects most densely to lower cervical segments is comparable to the region which projects most densely to upper cervical segments (He et al 1995). Although the majority of the corticospinal efferents from the SMA terminate in the intermediate zone of the spinal cord, some terminations are found within lamina IX where motoneurons are located. This observation suggests that efferents from the SMA make monosynaptic connections with motoneurons. Thus the anatomical substrate exists for the SMA to directly control arm movements independent of output from M1 (Dum and Strick, 1996). However, the density of projections is 13 times less (Maier, 1997) and the magnitude of effects 10 times smaller as compared to M1 (Boudrias, 2006). Thus, output from SMA to motoneurons is markedly weaker compared with M1 raising doubts about the role of SMA corticospinal neurons in the direct control of muscle activity (Boudrias, 2006). Results from this study indicate that even if measurements of plasticity and behavior are correlated, these changes in the SMA DFL area are not sufficient by themselves to drive recovery under the circumstances of an extensive cortical infarct comprising the rest of the frontal areas forelimb representations. This is further supported by results following the secondary lesion in DFL SMA inflicted three months after the initial lesion. Although the monkeys showed a slight disimprovement, motor prefromance scores at post-

secondary lesion wk 1 are not statistically different from those before the secondary SMA lesion. Thus it may be that the slight dip in behavior could be rather related to general post surgical effect. Moreover, motor performance scores during the following weeks show a gradual increase, suggesting a transient effect.

There are some issues to be taken into account in the interpretation of results following the secondary SMA lesions. The first one concerns the lesion itself. Secondary lesions are known to have less impact due to the pre-conditioning of the brain following the initial lesion. Second, these lesions were approximately 4mm^2 compared to approximately 80 mm^2 of the initial lesions. Although they still comprised the entire DFL SMA representation, the effects of these considerably smaller lesions might have been transient for our weekly evaluation. Third, the motor performance scale used for these studies was intended to detect gross changes in the performance of the whole upper limb and might have failed to detect subtle changes in the hand movements. Detailed analysis of digits flexions is necessary to detect deficits and monitor recovery following M1 lesions, which are approximately 15-20 mm^2 in the squirrel (Frost 2003). Taken together, these caveats suggest that caution needs to be exerted in extrapolating effects of these secondary lesions in the DFL SMA.

c. Degree of recovery and lesion location determine physiological measures following stroke

Two important variables influence results and their interpretation when studying recovery from stroke. One is the degree of recovery. There is an important difference in the literature depending on whether patients are left with severe disability or if they undergo substantial recovery. There seems to be a pattern in which those that have worse recovery activate more the contralesional hesmisphere areas (eg Johansen-Berg et al 2002, Ward, 2003). Patients with better recovery seem to follow a pattern of activation of the ipsilesional hemisphere motor areas (animal studies: Frost et al 2003, Liu and Rouiller, patient studies: Weiller 1992, Seitz 1998, Carey 2002). However, this is tied to the other variable, which is lesion location. Different results are reported according to whether M1 is spared from the lesion and the degree of damage to CS projections. Two examples are: one study including patients with lesions that spared M1 (Ward, 2003) and another which included patients with subcortical lesions (Loubinoux, 2003).

d. Contralesional plasticity

The notion that functional reorganization occurs in the intact (i.e. non-affected, less-affected or contralesional) hemisphere during recovery from stroke stems from functional imaging studies showing increased activation of the contralesional hemisphere in stroke patients. Supporting neuroanatomical studies in the contralesional cortex of the rat following focal ischemic lesions in the sensorimotor area demonstrated increased dendritic arborization and neurite growth followed by synaptogenesis in a pattern corresponding both spatially and temporally with behavioral recovery. However, the relationship of plasticity in the intact hemisphere to motor recovery is still not clear. It has been mostly related to poor motor outcome but also suggested to participate early or transiently during the recovery process.

Increase in perceived task complexity, problematic in all activation and task performance studies is how to control for the effort of subjects and the associated non-specific cognitive components. The outcome of increased effort may be enlarged activation volumes and nonspecific cognitive components may recruit newly activated regions. Suppression of unwanted movements and mirror movements further complicate the interpretation of results from these imaging studies.

Transcranial magnetic stimulation (TMS) has been used in stroke patients to study change in motor evoked potentials (MEPs) and changes in intracortical excitability (ICI) both in normal subjects and stroke patients. This technique has also

proven extremely useful in the study of descending pathways and how they change following injury. It has offered insight into the mechanisms underlying contralesional plasticity in terms of the participation of ipsilateral pathways in stroke recovery.

The pathways mediating ipsilateral responses and their relationship to the contralateral corticospinal projection have not yet been fully defined. One possibility involves interhemispheric connections such as the transcallosal projections to the contralateral motor cortex (ipsilesional plasticity). Another hypothesized mechanism is direct descending oligosynaptic pathways from the ipsilateral cortex (contralesional plasticity).

Contralesional plasticity may be mediated by pathways that are in part bilaterally organized, such as the reticulospinal tract, spinal interneuronal, the uncrossed corticospinal tract, and the propriospinal pathway. These are discussed below in more detail according to relevance of present results.

i. Ipsilateral EMG activity

The existence of ipsilateral connections from the motor cortex to the muscles has been established in normal humans and higher primates (Kuypers 1985). These connections are more exaggerated under some pathological conditions and in children between the ages of 5 and 10 years (Müller et al. 1997; Muellbacher et al. 1999; Staudt et al. 2002). In normal adults, ipsilateral responses can be demonstrated more readily in the proximal muscles. Only a few reports of ipsilateral responses in distal muscles have been shown under very strong background contractions and maximal intensity of stimulation using a transcranial magnetic stimulator (TMS) (Wasserman et al. 1991, 1994; Ziemann et al. 1999; Alagona et al. 2001).

In the present study surface EMG activity was recorded bilaterally in distal and proximal forelimb muscles of the normal adult anesthetized squirrel monkey following ICMS of the right (n=3) and left (n=1) M1, PMv and SMA cortical areas representing the upper limb. Both facilitatory and suppressive activity was detected. Ipsilateral EMG results were compared to contralateral responses. Ipsilateral facilitation was obtained in approximately 17% of cases in which contralateral EMG was simultaneously recorded in the same muscle groups. The magnitudes of these ipsilateral facilitatory responses were approximately 20% of the magnitude of contralateral responses. Latencies of ipsilateral facilitatory responses were significantly longer and broader compared to contralateral latencies. This is in accordance with results following TMS of normal men in which the ipsilateral motor

evoked potentials (MEPs) were delayed relative to the MEPs evoked by the same stimulus in the corresponding contralateral muscles (Wassermann et al 1991, Ziemann et al 1999, Alagona et al 2001, Bawa et al 2004). However, in contrast to results from human studies in which the production of ipsilateral MEPs required contraction of the target muscle, these ipsilateral responses were obtained in the anesthetized monkey.

Data from Ziemann et al (1999) suggest an ipsilateral oligosynaptic pathway, such as a corticoreticulospinal or a corticopropriospinal projection as the route for the ipsilateral MEP. They propose that the dissociation of the ipsilateral and contralateral MEPs at the cortical level through differences in map location, preferred current direction, the privileged input of the asymmetrical tonic neck reflex to the pathway of the ipsilateral MEP, and the presence of large ipsilateral MEPs in a patient with complete agenesis of the corpus callosum, indicate that corticofugal motor fibres other than the fast-conducting crossed corticomotoneuronal system can be activated by TMS in healthy adults. They thus exclude other pathways, such as branching of corticomotoneuronal axons, a transcallosal projection or a slow-conducting monosynaptic ipsilateral pathway.

TMS of motor cortex not only induces short latency responses (MEPs) but also gives rise to transient inhibition or "silent period" which is described as a long-lasting inhibitory phenomena of up to several hundred milliseconds duration, which are reflected in an electrical silence in tonically activated muscles. It has a cortical origin,

most probably mediated by inhibitory interneurons within the primary motor cortex acting on the large pyramidal output cells. In pathological conditions like hemispheric stroke it may be abnormally increased or decreased depending on the site of the lesion (Schnitzler, 1994; von Giesen, 1994). In the present study suppressive effects occurred in both contralateral and ipsilateral forelimbs on distal and proximal muscle groups. Ipsilateral suppressive effects were detected following stimulation of all three areas. These suppressive effects had longer latencies compared to contralateral suppression often without any preceding excitatory response. These results parallel those obtained in normal subjects by Wasserman et al. (1991). These authors assessed the excitability of the alpha motoneuron pool during the period of the ISP by eliciting H-reflexes, which showed no change, suggesting that the ipsilateral inhibition acts at a level above the alpha motoneuron. This in turn suggests that connections from motor cortex to ipsilateral muscles could be via the corpus callosum and contralateral hemisphere or via purely ipsilateral pathways.

In summary, the present results are in accordance with previous studies demonstrating the presence of excitatory and inhibitory ipsilateral activity, with similar characteristics to those described in humans. However, no active target muscle contraction was needed to obtain ipsilateral activity in the squirrel monkey. The important role of oligosynaptic pathways in this species might underlie this significant difference.

Anatomical data shows relatively sparse CST projection to lamina IX in the squirrel monkey (Bortoff and Strick 1993). Physiological data subsequently demonstrated that CM inputs to motoneurons supplying hand and finger muscles are small and slow whilst repetitive stimulation of the CST, via an electrode in the contralateral medullary, pyramidal tract (PT), produces large non-monosynaptic EPSPs (Maier et al 1997). This contrasts the dense CST projection into lamina IX found in macaques, baboons and humans, which underlies the large, fast monosynaptic input to hand motoneurons. Moreover, since all CM connections are excitatory, all of the important inhibitory control exerted by the CST is thought to be mediated through oligosynaptic connections. Thus, the study of indirect excitatory pathways is of particular interest in the squirrel monkey in which the CM system is present to only a limited extent. Pre-lesion ipsilateral EMG data obtained in the squirrel monkey is consequently especially situated for its comparison with data following destruction of an extensive portion of the contralateral CST. In addition, these non-monosynaptic responses have been found in over 80% of hand and finger motoneurons in the squirrel monkey may be mediated through CST inputs to the propriospinal neurons located in the upper cervical segments (C3-C4) (Nakajima et al 2000). Changes in this propiospinal system-mediated ipsilateral activity following the lesion might strongly suggest the participation of this pathway in stroke recovery mechanisms.

ii. Mechanisms underlying changes in ipsilateral EMG activity following cortical injury

Ipsilateral MEPs have been observed more frequently in patients than in healthy subjects. However, it remains to be established whether ipsilateral projections may be one of the substrates for functional restoration after stroke. Some authors suggested that the presence of ipsilateral responses to TMS is an indicator of poor motor recovery (Turton et al, 1996, Netz et al, 1997), others have implicated ipsilateral activity early but transiently in the recovery process (Basting 1997). Interpretation of studies of ipsilateral responses is further complicated by variability in the selection of patients regarding the lesion location (cortical or subcortical) and the stimulation protocol used. Alagona et al, for example (2001), found no ipsilateral MEPs in relaxed FDI or biceps of the paralyzed arm on TMS over any region of the unaffected hemisphere at 100% of stimulator output. However, they did find ipsilateral MEPs in the unaffected relaxed FDI and biceps by stimulation of premotor areas of the affected hemisphere. The aim of the present study was to specifically address the question of whether descending control of ipsilateral muscles changes following an extensive lesion in the motor cortex by comparing pre-lesion EMG activity in both forelimbs following ICMS of the contralesional hemisphere three weeks and three months post-lesion.

In the present study simultaneous EMG recordings obtained on the ipsilateral (affected) forelimb were compared to EMG recordings obtained on the contralateral

(unaffected) forelimb. The number of facilitation effects increased ipsilaterally three weeks post-lesion and was still increased three months post-lesion. Change in motor cortex excitability (intracortical inhibition; ICI) has been postulated as a putative mechanism underlying changes in ipsilateral motor responses following stroke. Data published by Shimizu et al (2002) are particularly relevant to these findings. They compared results following a paired conditioning-test TMS paradigm in two groups of patients; one with unilateral cortical stroke (cortical group) and another with subcortical stroke caudal to the corpus callosum (subcortical group). ICI was significantly less in the cortical group than in age-matched healthy control subjects. It was especially more marked in the cortical group patients with a disease duration of less than 4 months after onset. They suggest that the reduced ICI in the cortical group might have been a result of disruption of transcallosal inhibition (TCI) due to absence of TCI in the cortical group but not in the subcortical group. Reduced ICI has been shown also by other authors (Manganotti et al, 2002). The fact that the increase in facilitation effects detected in our study was observed following stimulation of PMv and SMA but not M1 implicate a callosal mechanism given the denser callosal connections between homotpic premotor areas compared to M1 (Rouiller, 2004).

Shimizu et al further showed that patients in the cortical group with a disease duration longer than 4 months showed a tendency for ICI to be normalized. In our group of monkeys the number of facilitation effects decreases somewhat but is still increased compared to pre-lesion values. This might be related to the fact that although their motor performance improves during the first few weeks following the

lesion, there is no further recovery at least not during the three-month period of evaluation.

It has been suggested that when patients have not yet recovered, a relatively greater component of the descending command is mediated through the propriospinal relay (Mazevet 2004). Paired to the increase in post-lesion ipsilateral facilitation effects, we have shown an increase in the latency of these effects. These results are consistent with a transiently increased efficacy of non-direct or oligosynaptic pathways such as descending (possibly reticulospinal) projections onto propriospinal neurons. Such increased efficacy could be responding to hyperexcitability of these neurons or unmasking and/or reorganization of the projections to them. On the other hand, the shorter latency of ipsilateral suppressive effects post-lesion may implicate the transient activation of more direct pathways such as the ipsilateral (uncrossed) corticospinal pathway (Alagona et al 2001). However, the fact that there was no change in the amplitude of effects indicates that the overall corticomotoneuronal excitability has not changed in patients in accordance with results from Butefisch et al (2003) who show a similarity of MT, mean test MEP and recruitment curves in patients and healthy volunteers.

Results from the present studies support the increased activity of ipsilateral pathways following an extensive cortical injury. Hyperexcitability, changes in ICI through callosal connections, increased output via direct or indirect descending pathways may all be implicated as mediating the observed post-lesion changes.

However, the dissociation between changes in the ipsilateral and contralateral facilitation and suppression effects following stimulation of the contralesional cortex suggests a dissociation of mechanisms underlying changes in the ipsilateral and contralateral forelimbs. This dissociation is promising in terms of rehabilitation possibilities through modulation of the different pathways to enhance recovery following stroke.

e. Early vs. late physiological changes

Prior ICMS mapping data have been collected in this laboratory at early time periods after M1 infarct in the squirrel monkey. Motor mapping procedures conducted in PMv and PMd in seven animals at 3 wks post-infarct showed decreased DFL representations in both areas (Plautz 2005—SFNAbstract). Comparable results from the present study show a decrease in the SMA DFL representation area on post-lesion wk 3. However this decrease is not correlated with motor performance. This points to a transient change in the physiology of SMA that does not necessarily drive the initial behavioral improvement. Widespread areas of cortical hyperexcitability appear days after cerebral infarction, reducing over subsequent months (Buchkremer-Ratzmann 1996). These changes occur in regions structurally connected to the lesion in both hemispheres as a consequence of down-regulation of the $\alpha 1$ GABA receptor subunit and a decrease in GABAergic inhibition (Neumann-Haefelin 1999). The plastic phenomena observed at this early time period could be pointing to a disruption of cortico-cortical connections from the damaged cortex.

Three weeks post lesion, the number of ipsilateral facilitation effects increased and suppressive latencies decreased in the ipsilateral (affected) forelimb following stimulation of the contalesional cortex. These results were found when stimulating PMv and SMA. Both PM (Marconi, 2003) and SMA (Jurgens 1984; Rouiller, 1994) hand areas have extensive homotopic and heterotopic connections. The most frequent type of postsynaptic response to activity of callosally projecting neurons consists of

an NMDA and non-NMDA-dependent, monosynaptic, EPSP followed by a prolonged, disynaptic, GABA-dependent IPSP mediated by interneurons (Toyama, 1974; Kawaguchi 1992). Loss of these connections result in an early disinhibition of the contralesional cortex, especially those more extensively connected. This, in turn, leads to an increase in the efficacy of descending pathways.

Three months after the lesion, the SMA DFL representation areas were enlarged compared to pre-lesion and post-lesion wk 3, and at this time there is a strong correlation with motor performance scores. This suggests that SMA is reorganizing in an attempt to compensate for damaged regions in the ipsilateral motor cortex.

Most of the parameters that showed changes in ipsilateral EMG activity three weeks post-lesion returned to pre-lesion values by three months. Although correlation with behavior was not conducted in this set of experiments, these monkeys received the same lesion and showed a similar recovery curve, with an initial improvement, which then reaches a plateau by three months. Thus, it would seem that there are long-term changes in the ipsilesional cortex, which are functionally relevant, whilst short-term changes in the contralesional cortex are transient, returning to pre-lesion values in the long term.

These results have important implications in the development of new or improved treatment strategies. There seem to be at least two time points with different motor reorganization scenarios that would require different treatment approaches. If

we think in terms of the affected forelimb, treatments during the early time point would be guided towards improving changes in the contralesional cortex, maybe enhancing ipsilateral descending control. Treatments at a later time period would be focused on the enhancement of the changes in the ipsilesional cortex and contralateral pathways.

f. Existing hypothesis on stroke recovery and contribution of the present studies

Several hypotheses have been generated so far concerning the mechanisms underlying stroke recovery. The ultimate goal is to find insights towards the development of new or more effective treatment strategies. The present studies focused on the participation and contribution of different pathways to recovery from an extensive cortical lesion. This section will discuss the contribution of the present findings to stroke recovery.

It had been previously shown that plasticity in the peri-infarct cortex participates in stroke recovery. It was also shown that the sensory cortex has an active role. More remote motor areas were then shown to involved with larger lesions. The present study shows that SMA reorganizes after injury to other frontal motor areas. Thus it can be hypothesized that the entire cortical and subcortical motor system that is spared by the injury participates to varying degrees depending upon the extent and location of the injury and behavioral demands. At least some of the functions of the injured region(s) are redistributed across the remaining cortical and subcortical motor network, yet recovery ultimately depends on the integrity of the entire motor system.

It has been previously demonstrated that lesion size determines the degree of cortical plasticity after stroke. However previous studies involved relatively small lesions (M1 or portions of M1). In this purely cortical stroke model, a larger lesion promotes a greater change in the DFL representation of the SMA. This is another

example that remote reorganization is directly related to the reciprocal connectivity of the various motor areas. It seems that the greater the damage to reciprocal intracortical pathways, the greater the plasticity achieved in the secondary, intact area. Reorganization of the motor cortex may obey different mechanisms depending on whether primary motor, premotor or supplementary motor areas in the ipsilesional hemisphere are spared and whether subcortical structures are included in the lesion.

An important contribution of the present studies is the finding that both the ipsilesional spared and contralesional motor areas change following an extensive cortical lesion. However the interesting fact about these plastic changes is their relation to functional outcome. Although behavior was monitored only in the group of monkeys that were used for the SMA mapping, all nine monkeys used for this study showed the initial recovery at the early time period, which then plateaus, with no further improvement. This is similar to what occurs in stroke patients. The enlargement in the SMA DFL representation was correlated to the improved motor function at the late time period. The changes in ipsilateral EMG activity were, in contrast, observed mainly in the early time period. There has been controversy respecting the role of observed changes in the contralesional and ipsilesional motor areas in recovery. Thus, "it is feasible that the intact hemisphere could play a contributing role when reorganized activity in the affected hemisphere is not enough to compensate for the motor deficits." (Werhahn, 2003). Spared contralateral motor networks seem to be in a better position to functionally react to the loss of motor abilities. However, since the ultimate goal is to find new or improved treatment

strategies, which are more necessary in the "poor outcome" population of stroke patients, the changes in the ipsilateral pathways become of extreme importance.

"Several studies support the hypothesis that after stroke, specific features of brain function revert to those seen at an early stage of development, with the subsequent process of recovery recapitulating ontogeny" (Cramer, 2000). Many clinical characteristics of stroke recovery resemble normal development, particularly in the motor system. Understanding the patterns of similarity between normal development and stroke recovery might be of value in its treatment.

VI. CONCLUSIONS

Extensive cortical lesions involving the upper extremity representations of M1, PMv and SMA in the squirrel monkey produce chronic deficits. There is an initial improvement during the first three weeks post lesion, reaching a plateau, with no further improvement up to three months post lesion.

Following these extensive motor cortical lesions plasticity occurs in both hemispheres. Enlargement of the DFL SMA representation three months post-lesion indicates ipsilesional SMA reorganizes after injury. Positive correlation of this plasticity in the ipsilesional SMA is correlated to lesion size, indicating that this remote reorganization is directly related to the reciprocal connectivity of the various motor areas. Positive correlation of post-lesion ipsilesional SMA with motor performance scores indicates a positive relationship with recovery.

Changes in ipsilateral EMG facilitation and suppression, following stimulation of contralesional motor areas, suggests the contralesional motor cortex undergoes disinhibition during the early post-lesion period. This disinhibition is probably mediated through transcallosal connections. The resulting increase in the efficacy of ipsialteral descending pathways is probably mediated by oligosynaptic pathways such as the propriospinal or corticoreticular projections.

Contralesional plasticity occurs early after injury and becomes less evident in the more chronic stage. Ipsilesional plasticity is less evident in the early stage and becomes functionally relevant in the chronic stage.

These findings are novel and support previous findings in other stroke models and patients. They provide insight into possible approaches in treatment strategies to be applied in different components of the motor system at different stages during recovery.

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