CORTICAL PLASTICITY AND BEHAVIORAL RECOVERY FOLLOWING FOCAL LESION TO PRIMARY MOTOR CORTEX IN ADULT RATS

By

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ABSTRACT

Acquired brain injuries, such as ischemic stroke and traumatic brain injury, are the leading causes of physical disabilities. It is estimated that over five million Americans live with resulting disability and the economic burden is said to be over $56 billion annually. Previously, scientists have shown that damage of the primary motor cortex induced neural plasticity in the premotor area in human and non-human primate studies. Neural plasticity, particularly within the same hemisphere of the lesion (ipsilesional), is thought to contribute to and account for functional recovery. It is not yet known to what extent plasticity mediates recovery and how to take advantage of neural plasticity to maximize the functional outcome.

Rodent models are most often used not only for studying the role of motor cortex in motor skill learning but also in neurodegenerative research. To further elucidate the role of adaptive plasticity in the ipsilesional hemisphere during the recovery of upper limb function, we aimed to establish the baseline neural changes after a focal cortical injury. Therefore, we took advantage of two separate cortical motor areas, in the *Rattus norvegicus,* from which the corticospinal tracts terminate in the motor nuclei of the cervical level spinal cord (C6-C8), controlling upper extremity musculature—the first, a more caudally located subregion of M1, often referred to as the caudal forelimb area (CFA), and the second, a more rostrally located non-primary area, referred to as the rostral forelimb area (RFA).

The objective of this dissertation work was to characterize physiological changes in RFA during the complex and lengthy process of recovery using rat models of focal cortical trauma and cortical ischemia restricted to CFA.

In the first study (Chapter 2), we demonstrated that the ipsilesional RFA remained intact and the forelimb representations extensively reorganized during spontaneous recovery after a focal contusion to CFA. We suggested that the post-injury cortical plasticity in the remote structure may play a role in functional recovery.

In the second study (Chapter 3), we showed differential effects of rehabilitative training on ipsilesional RFA plasticity after CFA ischemic injury. Extensive physiological changes were evident past rehabilitative training. Thus, neural plasticity in RFA appeared to be dependent both on post-lesion motor experience and time.

Finally in Chapter 4, we demonstrated differential effects of ischemic and contusion in CFA on ipsilesional RFA plasticity. The RFA physiological integrity was more compromised by the contusion. The results suggest that secondary degeneration may have been more severe in remote areas after the contusion.

The dissertation work supports the hypothesis that cortical plasticity within the spared RFA after restrictive damage to CFA mediates use-dependent physiological reorganization, which provides a substrate for sustaining rehabilitation-aided motor functional recovery.

DEDICATION

To all the lab rats, cats, dogs and monkeys used in Neuroscience experiments, especially those that made this dissertation possible

Know what to look for

–Noam Chomsky

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LIST OF ABBREVIATIONS

AGl……lateral agranular cortex

AGm……medial agranular cortex

APOE……. apolipoprotein E

BDNF……....brain-derived neurotrophic factor

CBF……cerebral blood flow

CCI……controlled cortical impact

CFA……..….caudal forelimb area

CIMT……constraint-induced movement therapy

CM ……corticomotoneurons

CNS…….....central nervous system

CPu...……...caudate putamen

CSD……cortical spreading depression

CST……...corticospinal tract

DAI……...……diffuse axonal injury

DF……dorsal funiculus

DFL……distal forelimb area

ET-1……...……endothelin- 1

ICMS……..intracortical microstimulation

$GABA_A$…… γ -aminobutyric acid A

LRN……lateral reticular nucleus

LTP…….long-term potentiation

MCAO…… middle cerebral artery occlusion

M1……..primary motor cortex

MN……motoneurons

NDT……neuro-development treatment

NMDA……...….N-Methyl-D-aspartate

PLD……post-lesion day

PM.……...…premotor cortex

PMv……..……ventral premotor cortex

PN……...propriospinal neurons

PNS……peripheral nervous system

SMA…….……..supplemental motor area

RFA……..…….rostral forelimb area

RN……red nucleus

PT……...……pyramidal tract

S1……..……primary somatosensory area

S2……...…secondary somatosensory area

TBI……....…..traumatic brain injury

VA……ventroanterior thalamus

VL.……ventrolateral thalamus

VM……ventromedial thalamus

6-OHDA……6-hydeoxydopamine

CHAPTER ONE

Introduction

Organization of Motor Cortex

Cortical maps reveal how specific body parts are topographically represented and elucidate the functionality of the corticospinal neurons that terminate in the motor nuclei of the spinal cord. Thus, the maps reflect, at least to some extent, the brain support system for producing motor movements. Cortical maps may be derived by delivering small amount of electrical stimulation either to the cortical surface (Leyton and Sherrington, 1917) or intracortically (Asanuma and Sakata, 1967; Asanuma et al., 1968; Jankowska et al., 1975; Donoghue and Wise, 1982; Asanuma and Keller, 1991). In this study, cortical maps were created by the latter method by introducing a stimulating microelectrode into the cortex which activates a small collection of corticospinal cells in its vicinity (a technique called intracortical microstimulation, ICMS). Using such a technique, scientists have discovered that motor cortical representations depicted in the classical homunculus, delineated with orderly, sharply defined borders are oversimplification; rather the representations give a mosaic appearance because each muscle representation overlaps locally by divergent projections of the corticospinal neurons and because the representations of distal and proximal muscles are interspersed in primary motor (M1) and non-primary motor areas (Cheney, 1996).

The *Rattus norvegicus* species was used in the present graduate work. In rats, the electrophysiological studies (Hall and Lindholm, 1974; Donoghue and Wise, 1982; Sanderson et al., 1984; Gioanni and Lamarche, 1985; Neafsey et al., 1986) and tracer injection studies (Miller, 1987b; Li et al., 1990; Nudo and Masterton, 1990; Akintunde and Buxton, 1992) show that the corticospinal tract descends to the motor nuclei of the cervical level spinal cord, to control forelimb musculature from two separate cortical regions (Fig. 1 and 2). The first, the more

caudally located M1 contains a complete map of the skeletal musculature, including fore- and hindlimb (Woolsey, 1958; Hall and Lindholm, 1974). A subregion of M1 controls the forelimb, often known as the caudal forelimb area (CFA). Second, a more rostrally located non-primary area contains a map on upper extremity musculature only and is referred to as the rostral forelimb area (RFA) (Hicks and D'amato, 1977; Wise and Jones, 1977; Neafsey and Sievert, 1982; Nudo et al., 1990). The two motor forelimb representations, CFA and RFA, are segregated at an early postnatal stage (Uozumi et al., 1988), often by neck and vibrissae representations (Neafsey and Sievert, 1982; Sanderson et al., 1984).

Cytoarchitectonically, CFA is found in the lateral agranular cortex (AGl) and RFA is located in the medial agranular cortex (AGm) Fig 2 (Donoghue and Wise, 1982; Wang and Kurata, 1998). While hindlimb movements can readily be evoked within AGm, caudal and medial to CFA, no hindlimb movements are typically evoked within AGI. The threshold to evoke movements using ICMS are generally said to be lower in AGI than in AGm, though not substantially (Donoghue and Wise, 1982; Neafsey et al., 1986).

As shown in primates (Huntley and Jones, 1991) and humans (Ramón Y Cajal, 1900; Brodmann, 1903), the motor cortex in rats exhibits lamina I to VI (Fig 2). Rouiller et al. conducted an extensive study on connectional properties of CFA and RFA afferent and efferent projections in adult rats (Rouiller et al., 1993), as briefly summarized below.

Afferent and efferent connectivity of CFA and RFA in rats

CFA receives projections from the deep layers (layers V and VI) of RFA, and equally-distributed projection from all layers of S1and S2. CFA also receives bilateral projections from the perirhinal cortex but *no* projections from the insular cortex, a major difference from the RFA

connectivity. CFA receives uniformly spanned projections from the CFA of the opposite hemisphere, and projections from deep layers of the opposite RFA. Neither CFA nor RFA receives projections from the opposite S1 and S2.

Fibers projected to RFA originate from upper layers of CFA, above where the cluster of corticospinal neurons lies, and from upper layers of S1 and S2 areas of the somatosensory cortex. Projections also originate from all layers of the agranular insular cortex. RFA receives projections from deep layers and slightly more dense projections from upper layers of the opposite hemisphere RFA as well as moderate projections from the deep layer of the opposite CFA.

The origin of reciprocal corticothalamic inputs to CFA and RFA is partially segregated; CFA primarily interconnects with the ventro-lateral thalamic nucleus and RFA primarily interconnects with the ventro-medial thalamic nucleus.

More than 90% of the corticospinal projections from CFA and RFA are located in the contralateral dorsal funiculus of the spinal cord. 10% or less of the total fibers are located in the zones of ipsilateral dorsal, contalateral intermediate, contralateral lateral, and ipsilateral ventral in the spinal cord. Similar patterns of axonal arborization are emitted by corticospinal tracts originating from CFA and RFA. Axon ramifications are observed covering Rexed laminae I to X, though the terminal fields are most dense in laminae V, VI and VII. A few axonal raminifications can be seen to reach the motoneuronal pools of the ventral horn (lamina X), where boutons are observed in close apposition to motoneurons (Liang et al., 1991). A few axonal raminifications were present ventrolaterally in the grey matter, a location where they are clearly superimposed on the dendrites of the motoneurons (Babalian et al., 1993). It should be briefly mentioned that

corticalspinal projections densely terminate into lamina IX in macaque monkeys (Armand et al., 1997) and humans (Schoen, 1964).

CFA projects more densely and widely to the ipsilateral caudate putamen (CPu) than to the contralateral CPu, whereas RFA projects almost equally to the CPu on both sides. The difference in the projections from CFA and RFA to substantia nigra, subthalamic nucleus, red nucleus, tectum pointine nuclei and inferior olive is not distinct, in contrast with the summarized corticocortical, corticothalamic, and corticostriatal connections.

Taken together, the study by Rouiller concludes that the structural arrangement of the two forelimb motor areas shows that the two areas function not only in series but also in parallel—meaning neurons in RFA can control movements separately from CFA.

Differential role of RFA and CFA

Rouiller et al suggest that RFA can be considered "hierarchically superior to CFA". Shown for the visual (Coogan and Burkhalter, 1990) and auditory (Rouiller et al., 1991b) cortical areas, feedforward projections (ascending) typically originate from neurons in upper cortical layers, and feedback projections (descending) originate from neurons in deeper layers. The relations of laminar distribution between CFA and RFA connectivity appear to indicate that CFA to RFA is ascending and RFA to CFA is descending. Similarly, their connectivity to S1 and S2 also supports the notion that RFA may play a role of feedforward.

The rat sensorimotor cortex partially overlaps M1 and S1 cortices (Hall and Lindholm, 1974; Wise and Jones, 1977; Donoghue and Wise, 1982). This M1/S1 overlap, which obliquely runs in a 1.0mm rostromedial strip edge of S1, shares sensory representations of the glabrous skin on the

palmar surface and motor representations prominently of the shoulder flexion and wrist extension (Chapin et al., 1987). Hence, those neurons particularly discharge upon paw contact and are likely to mediate the forelimb control that includes paw contact, just before footfall, for example (Chapin and Woodward 1986). Further, the CFA dysgranular region receives deep touch and proprioception of the tendon and joints (Chapin and Lin, 1984). Barth et al found that lesion in CFA and not RFA induces deficits in footfault task. The authors described that the animals exhibited difficulty in inhibiting the inaccurate placing response (Barth et al., 1990).

The M1/S1 rough mirror-image connectivity particularly plays a crucial role in the movements presumably necessary for single-pellet reach and retrieval task. Rats first locate a singly presented pellet by sniffing within one to three respiratory cycles and then reach out for the pellet, with the motivation to eat it. The rat's performance is unchanged without vision, but he goes "blind" without proper olfactory bulb function (Whishaw and Tomie, 1989). Thus, layer II/III granular region in the sensory barrel cortex receives olfactory information as well as cutaneous inputs from the forelimb via the ventral posterior nucleus of the thalamus. The S1 dysgranular region receives deep inputs from muscle and joint (Chapin and Lin, 1984), which is contiguously bordered by granular barrel fields representing various parts of the body surface. The information is processed to layer II/III of the motor cortex. Then, it is carried down to the layer V and used to evaluate the error of the forepaw and digits positions relative to the pellet for a correct motor output. Pyramidal neurons from layer V make connections locally within other cortical areas or descend to the spinal cord (Leong, 1983; Miller, 1987a), 5-10% ipsilaterally (Brosamle and Schwab, 1997). Further, corticospinal tracts project to the red nucleus, pons (Mihailoff et al., 1985; Legg et al., 1989), reticular formation (Valverde, 1966) striatum (Donoghue and Kitai, 1981), and thalamus (Rouiller et al., 1993). Thus, the cortical evaluation

and correction for motor executions may occur at the levels of layer II/III, connections between layer II/III and V and connections within layer V. Hence, inducing a focal damage in CFA but not in RFA produces significant impairments in forelimb motor function that can readily be detected in the performance at the single-pellet reach and retrieval task (Gharbawie et al., 2007). It is thought that motor cortical lesions not only disconnect the normal sensorimotor movements but also alter the behavioral strategies compatible with success (Erickson et al., 2007; Alaverdashvili et al., 2008a).

Though CFA mostly mediates skilled motor learning (Kleim et al., 1998a; Gharbawie et al., 2007) and overlaps with cutaneous receptive fields, it is important to note, as described above, that RFA also maintains connections with S1 and S2 in a feedforward manner (Chapin et al., 1987; Rouiller et al., 1993). RFA focal lesion produces more enduring deficits in a bilateral stimulation test, showing heavier neglect in the contralateral forelimb than in the case of CFA lesion (Barth et al., 1990). The authors suggest that the presence or absence of cutaneous receptive fields does not solely predict the areal mediation of tactually-guided behavior.

Moreover, following some injuries RFA exhibits more plasticity than CFA. For example, hemidecortication at the neonatal stage exhibits relative normal forelimb movements on the contralesional side through reorganized corticospinal fiber termination from the undamaged hemisphere, more densely from RFA than CFA (Umeda and Isa, 2011). In this study, contra- and ipsi- corticospinal fibers were widely distributed equally in the undamaged RFA whereas ipsi-corticospinal fibers were found at the periphery of the cluster of contra-corticospinal fibers in CFA.

Although I have so far focused on differences between CFA and RFA, the two forelimb regions share some physiology. For example, both regions exert a prominent influence on motor neurons with similar latency and elicit a response in the wrist and digit muscles (9.7msec from CFA and 9.6msec from RFA to motoneurons) (Liang et al., 1993) Cortical field potential, the presumed "readiness potential", is detected approximately within 1.2s from RFA and 1.0 s from CFA prior to the onset of the self-initiated forelimb movements (Seki et al., 2005). Thus, it seems reasonable to propose that one forelimb region can vicariously take over the function of the other.

Analogy to non-primary motor area and M1 in primates

The presence of a secondary motor area in rodents seems to parallel the differentiation of motor areas in primates. However, not enough evidence has been provided to conclude whether RFA is a homologue of the primate premotor cortex, the supplementary area (Neafsey and Sievert, 1982), or a combination of the two (Rouiller et al., 1998). For instance, the RFA predominantly makes connections with the ventromedial thalamus, whereas primate PM and SMA make connections with the ventrolateral thalamus, the structure to which CFA is reciprocally connected in rats. The RFA interconnects with the insular cortex in a much similar way to PM and SMA. No cutaneous receptive fields exist in RFA, nor in the primate SMA (Fig 2). (Nudo and Frost, 2007). Also, the lack of evoking hindlimb movements are commonly observed within AGm in rats (Neafsey et al., 1986) and SMA of monkeys (Luppino et al., 1991; Matsuzaka et al., 1992). Finally, CFA and primate M1 mainly send projections to the ipsilateral caudate putamen, and RFA and primate nonprimary motor areas send equally-dense striatal projections bilaterally.

CFA gives rise to the most prominent corticospinal projection and it exhibits more pertinent neural adaptation, than RFA, in acquiring the motor skills to reach and retrieve pellets (Kleim et al., 1998a; Remple et al., 2001), the relation similar to the primary motor cortex in non-human primates (Nudo et al., 1996a; Friel et al., 2005).

An early study indicated that neurons in CFA discharge in conjunction with forelimb movements on an isometric bar pressing task while neurons in RFA discharge prior to and during forelimb force changes (Donoghue, 1985). A more recent study also showed that cortical field potential, the presumed "readiness potential", can be detected approximately within 1.2s from RFA and 1.0 s from CFA prior to the onset of the self-initiated forelimb movements (Seki et al., 2005). Thus, neurons in RFA fire slightly earlier than neurons in CFA during the motor control, suggesting RFA may mediate motor planning and initiation. Such implications seem to agree with those from non-human monkey studies. Some cells in SMA change their firing rate during the waiting period before the "go" signal for the forthcoming movement (Mushiake et al., 1991). If RFA is functionally homologous to the non-primary motor areas of primates, then it can be speculated that RFA is involved in higher-order motor control such as motor anticipatory programming or sensorimotor integration, while CFA is involved in generating and sending final motor commands to lower motor centers.

Although homologies between primate and rodent motor areas are not conclusive, the connectional properties support the notion that the CFA and RFA of rodents are homologous to the M1 and the premotor hand areas of primates respectively, at least in the context of function and neurorehabilitation (Nudo and Frost, 2007), ableit most likely not in terms of evolution (Lemon and Griffiths, 2005).

Corticomotoneural connections vs oligosynaptic connections across species

Corticomotoneural (CM) actions are generally considered stronger in the species with higher dexterity (Lemon et al., 1998). All CM actions are excitatory; and hence, all the inhibitory controls by the corticospinal tract (CST) are mediated through oligosynaptic connections, such as segmental inhibitory interneurons, found in all species. For the species with limited CM actions, the indirect excitatory pathway mediates excitatory controls as well (Lemon and Griffiths, 2005). For example, the squirrel monkey (*Saimiri sciureus*) has both direct and indirect pathways of motoneural control. CM inputs to motoneurons of the hand and finger muscles were found to be weak and located on the remote dendrites of the motoneurons. In contrast, repetitive simulation of CST (in the contralateral medullary pyramidal tract) produced large EPSP that is unique to oligosynaptic pathways (Maier et al., 1997). Nakajima and Lemon showed that in squirrel monkeys non-monosynaptic fibers, such as the propriospinal neurons located in the upper cervical segments C3-C4, account for over 80% of the hand and finger motoneural control Fig 8 (Nakajima et al., 2000). The CST mediations by the reticulospinal tract have also been demonstrated in cats and rats, where they are thought to be more developed than in primates (Alstermark and Ohlson, 2000). In fact, no evidence of monosynaptic CM effects has been found in cats (Illert et al., 1976) or in rats (Alstermark et al., 2004). It appears that the C3-C4 propriospinal system supports the dexterous behavior exhibited by rats (Whishaw et al., 1998a; Whishaw et al., 1998b).

Rodent model of CNS injury

The cerebral cortex is required for learning and performing skilled movements that demand independent control of finger muscles. A motor cortical lesion induces quantifiable impairments

in performing tasks that require skilled movements in animals; thus, it is useful for assessing the neural substrate for motor control and for recovery after CNS injuries (Whishaw and Kolb, 2005). In contrast to their primates' counterparts, the rat rubrospinal and medial systems (reticulo- and vestibulospinal tracts) are larger in size (Drew et al., 2002). Thus, the rat cerebral cortex plays a lesser role than the primate forebrain in mediating movements necessary for voluntary locomotion and grooming.

While non-human primate models may often be used to study brain-behavior relations and for clinical applications as primates share the closest common ancestry with humans, high forepaw manipulative ability and the well-studied motor neural system in rats make the rat a suitable model for studying cortical control of movements and recovery from cortical injuries (Whishaw et al., 1991; Whishaw et al., 1992; Jones et al., 2003). In fact, rotatory movements in control of the limb and digits are mediated by the motor cortex in rats, similar to the digit use in non-human primates (Alaverdashvili and Whishaw, 2008).

Motor Cortical Functional Plasticity

The term *plasticity* is used for any form of observable remodeling in structure or change in function of the nervous system; for example, it may range from neurochemical changes from drug addiction to experience-induced memory storage. Changes in topographical organization as a result of competitive motor map interaction are manifestations of a phenomenon often called cortical reorganization or cortical plasticity, which occurs throughout life. The inherent anatomical substrates that allow for competitive cortical motor map interactions, for example, in the hand area include the following (Nudo, 2006): 1) individual corticospinal neurons project to

innervate several motoneuron pools Fig 7 (Cheney and Fetz, 1985; Park et al., 2004); 2) the corticospinal neurons that innervate a particular muscle are distributed across the hand cortical areas which overlaps with corticospinal neurons innervating other motoneurons (Cheney, 1996) Fig 10; and 3) local intracortical neurons densely connects across regions within the hand area (Huntley, 1997).

Recent observations indicate that there are two forms of topographical reorganization—1) Immediate, short-term map reorganization that occurs in a matter of minutes or hours, for example, following a facial nerve cut (i.e., PNS injury) (Sanes et al., 1988; Donoghue et al., 1990; Huntley, 1997) or more directly by repetitive cortical stimulation (Nudo et al., 1990); and 2) long-term reorganization which occurs in a matter of weeks to months following behavioral interventions (Jenkins et al., 1990; Nudo et al., 1996a; Xerri et al., 1996) or digit amputation (Wu and Kaas, 1999; Qi et al., 2000). This is not to say the type of reorganization corresponds to any one specific type of endogenous or exogenous stimulus. Depending on the post-injury time lapse, a facial nerve cut may induce both kinds of map reorganization depending on the time following the nerve cut (Sanes et al., 1988). Thus, different mechanisms are responsible for the temporally associated neural responses to altered stimulation in the cortex.

Short-term reorganization is mediated and constrained by existing horizontal connections (layer II/III and V) which traverse representation borders. That is, the spatial extent of border shifts cannot take place outside the pre-existing anatomical horizontal connectivity (Huntley, 1997). Thus, the reorganization mechanisms include triggering the pre-existing silent excitatory connections by disinhibition (Jacobs and Donoghue, 1991; Huntley, 1997) and long-term

potentiation of synaptic transmission in local horizontal cortical connections (Hess and Donoghue, 1994).

Long-term reorganization, on the other hand, is supported by the formation of new, functional afferent or intrinsic connections; hence, it requires structural changes through protein synthesis (Kleim et al., 2003a; Luft et al., 2004)—synaptogenesis (Kleim et al., 1996) and sprouting of thalamocortical or cortico-cortical connections (Carmichael 2003). In both types of reorganization, the spatial changes–the competitive map borders—are determined by the extent of synaptic recruitment within the horizontal cortical connections across the localized groups of pyramidal tract cells (Cheney et al., 2000; Monfils et al., 2005).

Extensive cortical plasticity occurs in sensory and motor cortices particularly when the input or output activity of the cortex is affected. In other words, cortical plasticity is activity-dependent. For instance, environmental enrichment (Hebb, 1974) affect both S1 (Xerri et al., 1996) and M1 (Volkmar and Greenough, 1972; Diamond et al., 1976; Turner and Greenough, 1985; Sirevaag and Greenough, 1987). Corresponding to the neural changes, behavior is also affected by environmental enrichment, and rats outperform less stimulated rats in mazes (Forgays and Forgays, 1952; Hymovitch, 1952; Greenough et al., 1972; Juraska et al., 1984).

Cortical plasticity can also be considered “maladaptive”. For instance, amputation of the upper-limb has a profound effect on cortical plasticity. The phantom limb is painful because the mouth representations invade the hand areas of S1 and M1 in upper limb amputees (Karl et al., 2001; Mercier et al., 2006; Reilly and Sirigu, 2008). Earlier work with animal models has indeed shown that amputation or the damage to peripheral nerves of digits silences the S1 cortical activity immediately after the injury. Over the weeks and months, the silenced area regains

representation, but only of the adjacent body regions (Wall and Egger, 1971; Merzenich and Jenkins, 1993).

Learning induced cortical map plasticity

Leaning induced plasticity refers to the alteration of the cortical areal representations in response to use/demand. In Braille readers, for example, the cortical areas of reading digits are larger in comparison to the areas representing non-reading digits (Pascual-Leone and Torres, 1993). Musical instrument players (Classen et al., 1998; Meister et al., 2005; Lappe et al., 2011) golf players (Bezzola et al., 2011) or subjects who performed a two finger opposition task (Karni et al., 1995) show a larger representation size in their activity-relevant cortical areas, i.e. sensorimotor, auditory or parieto-occipital regions, in comparison with non-proficient players. When rats learn a skilled walking task, the number of synapses within CFA and cerebellum increases (Kleim et al., 1996; Kleim et al., 1998b). The contralateral motor cortex (CFA) to the trained forelimb typically shows long-term potentiation-like effects (Rioult-Pedotti et al., 2000), apical dendrite growth (Greenough et al., 1985; Withers and Greenough, 1989), synaptogenesis (Kleim et al., 2002; Kleim et al., 2004), and an expansion in distal and wrist representations (Kleim et al., 1998a). Non-human primates that learned a skilled reaching task also show expansion of M1finger representations (Nudo et al., 1996a). The unskilled motor use, such as in bar-pressing, by contrast does not drive cortical plasticity in rats (Kleim et al., 1998a; Remple et al., 2001) or in monkeys (Plautz et al., 2000).

Cortical lesion-induced map plasticity in somatosensory cortex

Many lines of evidence have demonstrated that cortical plasticity in spared, functionally-relevant areas follows an injury to a cortical or subcortical structure. In an earlier study, Pons and

associates removed the entire S1 hand representations (including the central sulcus areas 3a, 3b, 1 and 2) and showed that S2 hand representations (in the lateral sulcus) selectively became silent in macaque monkeys (Pons et al., 1987b; Pons et al., 1988). After 6-8 weeks, the unresponsive large areas were then occupied by foot representations. The study not only showed that the cutaneous activation of neurons in S2 was primarily determined by input from the S1 rather than on a direct projection from thalamus but also that the robust reorganization occurs in S2 cortex. It is noteworthy that an S2 lesion, on the other hand, does not induce changes in neural activity in S1 (Pons et al., 1987a). In comparison to the degree of S1 reorganization after peripheral nerve lesions shown in the previous studies (Merzenich et al., 1983; Wall et al., 1983; Merzenich et al., 1984), the authors suggested that higher-order sensory areas may exhibit even a larger degree of cortical plasticity after injury (Pons et al., 1988).

Cortical lesion-induced map plasticity in motor cortex

Similar cortical relations are found in the cortical motor system. Recent squirrel monkey studies indicated that damage in the M1 distal forelimb area (DFL) induces reorganization in the DFL representation of the ventral premotor cortex (PMv) (Frost et al., 2003; Dancause et al., 2006b). Further, lesions to both M1 and PMv DFLs induced cortical plasticity in the SMA DFL(Eisner-Janowicz et al., 2008). Middle cerebral artery occlusion (MCAO) that spares the rat CFA and RFA and damages the lateral frontal cortex including S1 and S2 diminishes the cortical motor forelimb representations (Gharbawie et al., 2005b).

Subcortical lesion-induced map plasticity in somatosensory cortex

In macaques, a *complete* section of the dorsal columns at a cervical level results in deactivation of neural activity in forelimb, trunk, and hindlimb representations in S1 (areas 3b

and 1) through interrupting the afferents from the forelimb and the rest of the body below the forelimb (Jain et al., 1997; Qi et al., 2011). With a *partial* section of the dorsal columns, the remaining afferent inputs appear to play a role in recovery. After 6-8 months, the authors observed that afferents remaining from the arm expanded to mediate neural activation of the deprived (formerly hand) area. Further, afferents from the chin reactivated the former hand, trunk and hindlimb areas. In a following study, they suggested that the restored reactivations were secondary to the sprouting of afferent to new cortical territories in the trigeminal-dorsal column complex in the brain stem (Jain et al., 2000). Similarly in rats, the contralateral corresponding somatosensory representations are silenced after a transaction of the upper cervical dorsal columns (Onifer et al., 2005; Massey et al., 2006) or of the thoracic dorsal columns (Jain et al., 1995; Schlag et al., 2001). However, the capacity to reorganize and reactivate the deprived portions was less extensive in rats than in non-human primates (Jain et al., 1995). That is, the remaining afferents restored the formerly connected representations but did not reconnect with areas beyond their typical borders.

Subcortical lesion-induced map plasticity in motor cortex

Many studies have shown the effect of a subcortical lesion on the cortical motor topographical integrity. Subcortical damage induced by MCAO diminished or eradicated motor cortical forelimb representations ipsilateral to the occlusion. In this study, although the tissue stayed intact within motor cortex, with the diminished integrity of the map behavioral deficits were severe (Gharbawie et al., 2008). Removal of afferent cholinergic innervation from nucleus basalis of Meynert to motor and sensory cortices can disrupt the cortical map (Conner et al., 2005). A complete lesion at the medullary pyramids eradicated the motor representations, and a

partial lesion reduced the distal forelimb area of CFA (Kartje-Tillotson et al., 1987). Hemicerebellectomy within 3 weeks of age in rats can affect the cortical motor representations (O'donoghue et al., 1986). Bilateral nigrostriatal degeneration by intrastriatal 6-hydroxydopamine (6-OHDA) induces both RFA and CFA areal reduction (Brown et al., 2011). Divergent thalamic projections appear to affect cortical representations directly. Sectioning less than 30% of the ventral posterior thalamic nucleus leads to no change in the map; 35% induces partial alteration; more than 40% completely abolishes the motor map (Jones et al., 1997). It appears that cortical map integrity can be compromised by damage at many subcortical levels.

On the contrary, a unilateral 6-OHDA lesion preserves the ipsilateral map (Metz et al., 2004); and neither lesions to the medial or lateral caudate-puatmen affect motor representations (Karl et al., 2008). Those studies indicate that not all types of lesion to subcortical pathway that connects muscle and cerebral motor cortices result in cortical map representations.

Neural Consequences of Cortical Injury

Traumatic Brain Injury.

Traumatic brain injury occurs in approximately 1.4-2 million Americans each year, and an estimated 5.4 million people currently live with resulting disabilities (Selassie et al., 2008; Zaloshnja et al., 2008; Corrigan et al., 2010). A severe primary mechanical impact can fracture the skull, forcefully rupturing the brain parenchyma with shearing and tearing of blood vessels and brain tissue. The event triggers a cascade of molecular and cellular responses that lead to secondary injury, often termed diffuse axonal injuries (DAI).

Ischemic Brain Injury.

Of the 795,000 people afflicted by stroke each year in the U.S., approximately 400,000 survive with long-term disabilities that include upper extremity impairments. Impairment in cerebral blood flow leads to deprivation of oxygen and glucose, and, when persistent (for a matter of minutes), results in neural death.

Despite the difference between the causes of a traumatic versus an ischemic brain injury, mechanisms of acute injury formation are shared, particularly the mechanisms that lead to secondary cellular death. For instance, trauma induces early ischemic events secondary to physical damage to arteries and local vasospasms of arteries (Dirnagl et al., 1999; Leker and Shohami, 2002; Bramlett and Dietrich, 2004).

In both types of lesions, energy failure due to the primary injury first leads to depolarizations through uncontrolled glutamate release (also referred to as excitotoxicity). Influx of calcium and sodium into cells of the lesioned area then follows (making depolarization inside the cells) which sequences to swelling, ionic imbalance, enzyme activations and mitochondrial damage in the ischemic penumbral region and the traumatized region (Globus et al., 1995; Mikawa et al., 1996; Chan, 2001; Lewen et al., 2001). Intracellular calcium inevitably produces oxidative stress (nitric oxide and free radicals) and activates various enzymes that may damage DNA and cell membrane (Siesjo et al., 1995; Kim et al., 2002).

Meanwhile, neurotransmitters and potassium leak out of the cells, causing cortical spreading depression (CSD) or peri-infarct depolarization, which comes as a repetitive wave. Using electrocorticographic methods, Strong and associates detected CSD across the cortex in a propagating manner near the foci of the cortical damage (Strong et al., 2002). The CSD adds to

the peripheral growth of the lesion and has been shown not only in experimental ischemia and TBI (Sunami et al., 1989; Back et al., 1994; Dietrich et al., 1994) but also in human parenchymal damage (Mclachlan and Girvin, 1994; Mayevsky et al., 1996). CSD causes metabolic stress and mediates the cell-destructive cascade in remote areas not structurally immediate to the lesion itself (Witte and Stoll, 1997; Kawahara et al., 1999; Shen and Gundlach, 1999; Dietrich et al., 2000).

Deafferentation is another mechanism that affects the neural survival of remote structures. This disconnection of neurons within the same networks may result in a significant dysfunction of the remote structures that are functionally-related but structurally-remote to the damaged region (Luhmann et al., 1996; Buchkremer-Ratzmann and Witte, 1997; Witte, 1998). Such mechanisms that affect remote structures are often referred to as diaschisis.

Diffuse axonal injury (DAI) that results from stretch injury to the membrane of an axon also activates cell-destroying cascades. Eventually, the axon breaks (Henderson et al., 2005). Thus, areas distant from the injury core may additionally be damaged; basal ganglia, superior cerebellar peduncle, corpus callosum, and midbrain are often affected in clinical TBI, and the extent of DAI reflects the functional outcome. For instance, physically well recovered patients may have persistent impairment in balance, agility and in coordination (Rinne et al., 2006).

Behavioral Consequences of Experimental Cortical Injury

First shown in rats by Peterson, reaching is controlled by highly localized contralateral motor cortical area (Peterson, 1934). The direct corticospinal tract controls fine movements of the digits

and isolates the proximal joints through a mechanism in which successive cortical stimuli produce progressively larger excitatory postsynaptic potentials in the motor neurons. Thus, the fine motor control during single-limb reaching for objects is affected by a cortical lesion, or pyramidal tract sectioning at the medulla which eliminates the projection of corticospinal axons from the M1 and premotor areas (Lawrence and Kuypers, 1968b, a). More specifically, individuated movements of digits often are lost permanently and extensor or flexor synergies become linked over the wrist, elbow and shoulder joints. If not for indirect access of cortical commands to motor neurons through the descending system of the brain stem, partial recovery may not be observed.

In rhesus monkeys, Kuypers et al suggest that residual ability of grasp and retrieve object after pyramidal tract (PT) section is mediated by the rubrospinal projections (Kuypers, 1964). In monkeys, the PT has dense direct projections to motor neurons in layer XIII and IX of the cervical spinal cord (Lawrence and Kuypers, 1968a; Lawrence and Hopkins, 1976; Bortoff and Strick, 1993) whereas similar connections in rats are anatomically sparse in comparison (Casale et al., 1988; Rouiller et al., 1991a). Single cell recording studies have demonstrated that the corticospinal tract may be more involved in the scaling of targeted responses than in the rubrospinal tract in monkeys (Cheney et al., 1988) and in cats (Martin and Ghez, 1988).

In rats, pyramidal tract lesions decrease the performance success during skilled and spontaneous reaching more than red nucleus (RN) lesions do. Though a restricted lesion to RN induces impairments on the reaching measures, lesioned rats with spared PT can guide the limb to grasp an object (Whishaw et al., 1998a). The RN lesion impairs arpeggio movement, in addition to the rotatory impairments by the PT lesion. An RN lesion subsequent to a cortical damage reduces qualitative accuracy in paw opening (Whishaw et al., 1990). Combined RN and

PT lesions produced more severe motor deficits than either lesion alone and induced impairments both in rotatory and arpeggio movements, however, they do not abolish the ability to reach for and grasp food or hold a food item (Whishaw et al., 1998a). Hence, some components of skilled limb use must be supported by other descending pathways and spinal cord circuits other than PT and RN tracts.

Lesions in contralateral caudate-globus pallidus (Gharbawie et al., 2006; Karl et al., 2008) or substantia nigra dopamine-depletion, 6-OHDA rat Parkinson's model (Hwang et al., 2006; Hosp et al., 2011) cause behavioral deficits in reaching similar to the deficits secondary to a motor cortical lesion. That is, reaching is supported by the corticofugal pathways and not solely by pyramidal control.

Cortical ablation should not produce profound deficits in locomotion in the rat model because reticulo-vestibulo-spinal tracts are larger in non-human primates, cats and rodents than in humans. While abnormal positioning of the joint of the more affected side has been detected to some degree (Metz et al., 1998; Ueno and Yamashita, 2011), intralimb coordination does not manifest deficits, suggesting that locomotion is mediated by intraspinal tracts and that, unlike in humans, the descending pathway is nonessential for gait control in rat. Another fine motor movement such as grooming is also stereotypically integrated with subcortical areas, and, therefore; *not* extensively affected by a cortical lesion (Grill and Norgren, 1978).On the other hand, the motor cortex and the descending fibers are necessary in *skilled* walking (e.g. horizontal ladder walk) in rodents (Metz and Whishaw, 2009).

Resolution of Diaschisis as a Mechanism of Functional Recovery

Remote functional changes have been proposed to mediate recovery after brain damage. Von Monakow first proposed that recovery from a brain lesion is caused by an attenuation of depressed function in remote brain areas—which he called diaschisis (from the Greek, "shocked throughout") (Von Monakow, 1914). Glassmann subsequently suggested that the peri-infarct area suffers a temporary loss of function and then regains its original excitability during the course of recovery (Glassman, 1971).

Diaschisis occurs in distant but connected areas. Feeney points out that von Monakow described three types occurring simultaneously, but one form may predominate depending upon the "injured connections" (Feeney and Baron, 1986).

> *a) Diaschisis cortico-spinalis—progression of functional depression from a motor cortex injury to the spinal cord along pyramidal tract fibers.*
>
> *b) Diaschisis commisuralis—functional contralateral cortical depression via axons of the corpus collosum following injury to the cortex of one hemisphere.*
>
> *c) Diaschisis associative—intracortical fiber-mediated depression of function in intact cortical areas neighboring the locus of injury. (p819)*

Present-day research has expanded this concept to a wider range of neurophysiological phenomena, including any remote effects that are initiated by a focal lesion caused either through neural projections, as listed above, or systemically (e.g. cortical spreading depression or edema) (Witte et al., 2000). Though the remote consequences of a lesion occur simultaneously, and the causal or beneficial relationships to behavioral manifestation are difficult to isolate, studies have tested variables including neurotransmitter levels, synaptic receptor densities, cerebral blood

flow (CBF), metabolism and electrophysiological activity. For example, a non-lesioned hemisphere exhibits reduced blood flow in an acute clinical stroke (Slater et al., 1977). In a recent rat study, hypometabolism was detected in areas S1, S2 and CFA after a focal ischemic lesion induced in the barrel cortex (Carmichael et al., 2004).

The remote effects are not necessarily depressive. The excitability of the projection area remote from the lesion increases (Reinecke et al., 1999), largely by downregulated $GABA_A$ inhibition (Schiene et al., 1996; Qu et al., 1998; Que et al., 1999; Reinecke et al., 1999) and NMDA receptor upregulation (Humm et al., 1999). Hagemann et al found a propensity for long-term potentiation in the ipsilesional cortex, unique to post-lesioned animals (Hagemann et al., 1998). Such hyperexcitability is speculated to increase spontaneous cellular activity and may thus facilitate cortical plasticity (Witte et al., 1997). For example, following a focal lesion to the rat hindlimb S1 area, the vibrissa S1 (B3) area shows—as B1 is being stimulated—an enhanced intercolumnar transmission compared with control animals (Schiene et al., 1999).

Soblosky et al. suggested that considerable functional recovery occurs in rats without specific intervention, supporting the theory that recovery in function may be secondary to alleviation of perilesional dysfunction (Soblosky et al., 1997). It is important, however, to note that the theories of diaschisis attenuation and adaptive post-lesion brain plasticity are not mutually exclusive in the recovery process. For example, cortical representations adjacent to a lesion may be silenced unless animals receive physical intervention (Nudo et al., 1996b), suggesting that not all brain dysfunctions are resolved spontaneously. In fact, the degree of behavioral dysfunction and lesion volume can be worsened with extreme overuse or complete disuse of the affected limb (Schallert

and Jones, 1993; Leasure and Schallert, 2004). Thus, it is likely that functional recovery cannot be determined without experience and experience-induced brain plasticity.

Lastly, the diaschisis effect as well as subsequent brain plasticity (i.e. axonal sprouting) in remote structures may differ by lesion type (Napieralski et al., 1998). Boyeson et al. showed that an elevation of threshold to elicit bodily movements in the perilesion areas after contusion and laceration, but not after the ablation type of cortical injury (Boyeson et al., 1991).

Adaptive Plasticity (Vicariation) as a Mechanism of Functional Recovery

First described by Munk in 1881, the vicariation hypothesis states that, after cortical lesions, adaptive plasticity occurs in other regions within the same area or in other cortical areas that may not have been originally involved in the function to the same degree as the injured area. Recent studies have shown that such adaptive plasticity can be enhanced robustly through rehabilitative intervention. Hence, post-lesion experience-driven plasticity is an important concept in neurorehabilitation as the process of post-injury functional recovery may be regarded as a relearning of movement skills lost secondary to brain damage both in sensory and motor cortical areas.

Extensive cortical plasticity occurs during spontaneous recovery from CNS injury (Frost et al., 2003; Nishibe et al., 2010); however it often occurs to a greater extent in the presence of a post-lesion physical intervention. Studies have shown that the directed and skilled use training of the limb allows for the reemergence of representations once lost to cortical damage, reflecting successful recruitment of cortical activity for the sensation or for the movement. For example,

after a focal lesion to the S1 3b area in squirrel monkeys, Xerri and colleagues showed that post-lesion training on the object-retrieval task promoted skill recovery associated with the emergence of new representations of the cutaneous fingertips in area 3a, the zone formerly connecting the proprioceptive inputs (Xerri et al., 1998). Further, the restricted use of the less affected forelimb and the intense rehabilitation of the affected forelimb after lesion to the M1 hand area induce preservation of the hand area in the spared residual M1, which otherwise would be lost during spontaneous recovery (Nudo et al., 1996b). In this case, the rehabilitative effects appear to reinforce the preservation of the forelimb area within its normal territory against the injury-induced compromise in neural integrity. Rats exhibit robust post-lesion cortical reorganization that is directly correlated with behavioral recovery. After being entirely destroyed, the forelimb area of M1 (i.e. CFA) reemerged in the caudolateral peri-lesion area where the hindlimb movements were formerly represented (Castro-Alamancos and Borrel, 1995). Additional lesions to the newly emerged representations reinstate the motor deficits but have no effect on the forelimb function of intact rats. Thus, it is likely that the reorganized area is necessary for the recovery to persist.

Not only CNS injuries, but also following PNS injury, such as median nerve crush, behavioral recovery is said to depend on the return of normal topography through accurate reinnervation of injured nerves into the original receptive fields in the somatosensory cortex (Wall et al., 1983). Several lines of evidence since then have shown that regeneration errors occur after nerve crush injuries. Such errors can distort topographical representations, suggesting an adaptive value for topographical organization of sensory input (Wall and Cusick, 1986; Kawakami et al., 1989; Korodi and Toldi, 1998; Kis et al., 1999). Indeed, Florence showed that in young macaque monkeys, rehabilitative training on a sensory enrichment task promoted

functional recovery of fine motor skills that was associated with normalization of cutaneous receptive fields including the area 3b topographical reinstatement (Florence et al., 2001).

Functions that are mediated unilaterally, such as language or fractionated finger manipulations, do not show substantial recovery when damaged. Further, a shift of motor representation is not found to substitute for lost sensory representations. The vicariation of a function becomes increasingly difficult when the lesion is extensive so much so that the neurons which may substitute for the functional connections are not adequately spared.

Behavioral Compensation as a Mechanism of Functional Recovery

While cortical plasticity is implicated in adaptive motor function, it is important to note that post-injury functional recovery is, at least partially, attributed to compensation or substitution of function, using different muscle groups, rather than solely to behavioral capacity prior to brain damage in animal models. Self-taught compensatory movements occur during spontaneous as well as therapy-aided recovery. For instance, after a motor cortical lesion, rats use their mouth or upper body and not forepaws to collect food (Whishaw et al., 1981; Gharbawie and Whishaw, 2006; Alaverdashvili and Whishaw, 2008). Difficulty in recovering the thumb-digits grasping pattern is shown in rhesus monkeys, who instead develop "scooping" movements of the digits (Moore et al., 2011). A sensory cortical lesion also produces compensatory movements during the early stage of recovery (Xerri et al., 1998).

Indeed, some studies indicate that the majority of behavioral improvement occurs through effective compensatory strategies, enabling functional restitution, and that true recovery is

minimal (Whishaw, 2000; Krakauer, 2006; Knieling et al., 2009; Alaverdashvili and Whishaw, 2010). That is, successful reaching was primarily representing the usage of effective compensation, most notably the rotatory upper body movements to compensate for the seemingly-permanent lost forelimb rotation (Whishaw et al., 1991; Gharbawie et al., 2005a; Metz et al., 2005; Moon et al., 2009).

Age influences how animals reach and retrieve. After a focal injury to the motor cortex, young subjects and aged ones develop different compensation. Young animals developed compensation in pronation, supination I and II whereas the old compensated in aim and supination II. This shows that the aged subjects are able to develop strategies to meet the ends of reach and retrieval performance (Alaverdashvili and Whishaw, 2010).

Environmental enrichment (Knieling et al., 2009) or rehabilitative therapy (Friel and Nudo, 1998) adds to the effective compensatory movements but not to true motor recovery when fulfilling the motor tasks. As compensatory strategies develop on the less-affected limb, apical dendrites sprouted in the contralesional cortex, the cortical plastic changes that were unique to animals that used the less-affected limb (Jones and Schallert, 1992a; Schallert et al., 2000).

Other studies, however, have indicated that behavioral habits that would compete with successful movements or that would hyper-rely on the intact body side may be problematic for a long-term recovery. For example, learned bad use, often observed in rats following the motor cortex damage, describes an outcome with a prominent increase in repeated proximal forelimb advance gestures without accurate aiming or grasping, resulting in reduction of the functional level (Alaverdashvili et al., 2008a). This type of compensatory behavior may not be detected in the end-point quantitative assessment and thus, without the detailed behavioral assessment, interpretation of brain plasticity would be limited (Whishaw et al., 2008). Further, hyper-reliance

on the less-affected limb helps develop learned non-use (Erickson et al., 2007). This learned non-use of the more impaired side dismisses its potential to regain function for which condition constraint-induced movement therapy becomes effective in clinical strokes (Mark and Taub, 2004). In addition to non-use, the repetitive use of the less-affected forelimb has a detrimental effect on the use of the impaired forelimb and reduces the neural activity in the ipsilesional remaining cortex (Allred et al., 2005; Allred and Jones, 2008). It is suggested that early post-lesion training of the less-affected forelimb may result in neural plasticity that produces a maladaptive functional outcome (Allred et al., 2010), but having the less-affected limb anesthetized may attenuate the abnormality in the more-affected forelimb use (O'bryant et al., 2007).

Within the same experimental settings, using an M1-ischemic lesion model, Friel found that two squirrel monkeys out of 5 preferred to use the same movement pattern as before lesion and that three monkeys exhibited alternative strategies that the usage increased further after rehabilitative training (Friel and Nudo, 1998). Studies suggest that the degree of compensatory behavior may depend on the tissue properties of spared, intact brain structures (Metz et al., 2005) or on the specific injury location within M1 representations (Friel and Nudo, 1998), but not on mere lesion size (Metz et al., 2005; Gharbawie et al., 2007). Whishaw stated that “unless the lost innate cortical engram can be replaced, recovery occurs through compensation” (Whishaw, 2000). In an attempt to elucidate what neural changes correspond to which behavioral changes, the qualitative analysis of the reach and retrieval task is important.

Neurorehabilitation after TBI and Stroke

TBI and stroke prognosis.

A biomarker S-100B (a protein of astroglial cells) has so far been recognized as a prominent predictor of not only primary brain injury representative of neural death but also of the functional outcome. A review by Raabe et al. of 18 clinical studies determined that this biomarker is independent of extracranial injury among multitrauma patients (Raabe et al., 2003). The baseline or sustained levels of S-100B from the blood serum samples have been found to significantly correlate with failure in returning to work (Stranjalis et al., 2004) and a poor outcome on Glasgow Coma Score at 1 month (Townend et al., 2002) and at 6 months (Vos et al., 2004).

Apolipoprotein E (APOE), a genetic factor responsible for the structural integrity of microtubules in axons, has been associated with prognosis after trauma. Patients with APOE 4 allele required a longer hospital stay and showed a poorer outcome (Friedman et al., 1999; Lichtman et al., 2000; Alexander et al., 2007). A long-term follow-up study, through 1.9 year post-injury, also showed that having the allele has a significant impact on the Glasgow Coma Score-Extended scores following TBI rehabilitation (Ponsford et al., 2011). Also APOE genotype is associated with the trajectory of lower cognitive function (Noe et al., 2010).

Polymorphism of the brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, also is a good predictor of functional outcome after frontal lobe TBI (Krueger et al., 2011; Rostami et al., 2011). The BDNF genetic factor appears to play a role in brain function and structures (Bath and Lee, 2006). Healthy adults with BDNF val66met allele shows reduced activations in the learning-associated motor cortex (Kleim et al., 2006; Mchughen et al., 2010), the memory-related hippocampus (Hariri et al., 2003; Hashimoto et al., 2008), or cognitive function (Miyajima et al., 2008). Thus, the authors suggest that the pre-disposed genetic factors may have implications during rehabilitative therapy after CNS damage. Animal

studies have demonstrated the beneficial role of BDNF in functional recovery after ischemic lesions (Zhang and Pardridge, 2006; Ploughman et al., 2009; Maclellan et al., 2011).

TBI and stroke rehabilitation

The current evidence suggests that any form of motor rehabilitation with a high-enough intensity, compared with no intervention, most probably reduces disability post- stroke or -TBI (Pollock A 2008). However, a recent survey shows that many physical programs for patients with either stroke or TBI did not reached the recommended intensity (i.e. the number of repetition) offered by neuroplasticity research (Dejong et al., 2011).

The Bobath concept, also known as neuro-development treatment (NDT), promotes motor learning by guiding patients through initiation and completion of intended tasks with proper postural alignment. However, it has been confirmed that the Bobath provides no superior outcomes, including the sensorimotor control of the affected upper limb, to other therapeutic approaches (Kollen et al., 2009). Constraint-induced movement therapy (CIMT) has received substantial attention from the rehabilitation community over the past decade. A study comparing CIMT to the Bobath approach in 24 subjects found no significant difference among the Wolf Motor Function Test "functional ability" and "performance time", the Motor Evaluation Scale for Arm in Stroke Patients; and the Functional Independence Measure scores (Huseyinsinoglu et al., 2012).

Early Initiation and High Intensity of Training

The literature agrees on the benefit of early intervention after TBI (Mazaux et al., 2001; Lipper-Gruner et al., 2002; Wagner et al., 2003) and stroke (Albert and Kesselring, 2011; Balaban et al., 2011), albeit with some associated negative consequences. Wagner et al reviewed an 1866 patient database, showing that acute care setting led to superior outcomes, including

locomotion and transfer, at the time of discharge and decreased length of stay (Wagner et al., 2003). Though another study demonstrated that the early initiation yielded excellent therapeutic response, it was associated with a greater risk of dropping out than the delayed rehab group (Paolucci et al., 2000). Immediate intervention after stroke also seemed more beneficial than a three month delay in intervention in a study by Wade and associates; however, the improvement was not retained beyond 3 months after discontinuation of the therapy in the early group (Wade et al., 1992). Nevertheless, training during the 1st three months of training is associated with increased motor excitability in the affected cerebral cortex assessed by TMS (Boake et al., 2007).

Wolf et al reported that with constraint-induced movement therapy after 24 months of therapy, subjects were able to achieve the endpoint recovery whether they start the training 3-9 or 15-21 months after stroke (Wolf et al., 2010). It may be that after the critical (acute or subacute) periods, the improvement no longer depends on the initiation time chronically.

The benefits of early training initiation have been supported by animal studies (Hsu and Jones, 2005). Biernaskie et al. found that animals receiving early (beginning post lesion injury day 5) rehabilitative training combined with an enriched environment exhibited greater behavioral improvements compared with animals that receive it at a later time point (day 30) (Biernaskie et al., 2004). Barbay et al. found that although delayed rehabilitative training (30 days post-lesion) improved motor skills, reorganization within the peri-lesion area was more limited compared with the early onset training show in another study by Nudo et al. (Nudo et al., 1996a; Barbay et al., 2006).

Second, literature suggests the benefit of a higher intensity or dose (Cifu et al., 2003). Following a severe TBI, subjects received intensive training of sit-to-stand and step-up exercises either 2 hour or 4 hours per day for 4 weeks. Subjects who received 4 hours achieved statistically

better performance, not in exercise capacity but, in sit-to-stand (Canning et al., 2003). During chronic stroke, 3 hours 4 times/week therapy produced *more* gain than a) 1.5 hour 1 time/week therapy or b) 0.75 hours 3 times/week in functional independence, sensory discrimination and fine motor skills (Byl et al., 2008).

Too early and too intensive therapy

However, earlier initiation and higher doses of motor training cannot be assumed (more) beneficial. It has been reported that too intensive CIMT training administered at a very early stage after stroke hinders potential long term recovery (Dromerick et al., 2009). In this study, the higher dose CIMT consisted of 3 hours of shaping and wearing the constraint mitten for 90% of the waking hours while the standard CIMT consisted of 2 hours of shaping and 6 hours of wearing the mitten per day. The higher dose CIMT resulted in reduced motor improvement in the upper extremity function at day 90.

Similarly, in a rodent ischemic model, immediate casting of the unaffected forelimb and overuse of the affected forelimb have been shown to enlarge the lesion volume (Kozlowski et al., 1996), increase excitotoxicity (Humm et al., 1999), and decrease motor function (Bland et al., 2000).

Importance of Neural Plasticity in Rehabilitation after CNS Injuries

Studies that produced promising preclinical and clinical results were often designed to test the principal hypothesis: "synaptic plasticity mediates rehabilitation-dependent functional reorganization within the brain and therefore interventions enhancing plasticity should improve functional outcome" (Kleim, 2011). Understanding what brain structures are engaged during motor recovery allows us not only to identify the timing and intensity required to instate the enduring neural changes in the structures of the interest but also enhance such neural engagement

to maximize the motor performance. The increasing understanding of brain plasticity has facilitated evidence-based rehabilitative approach and will do so further.

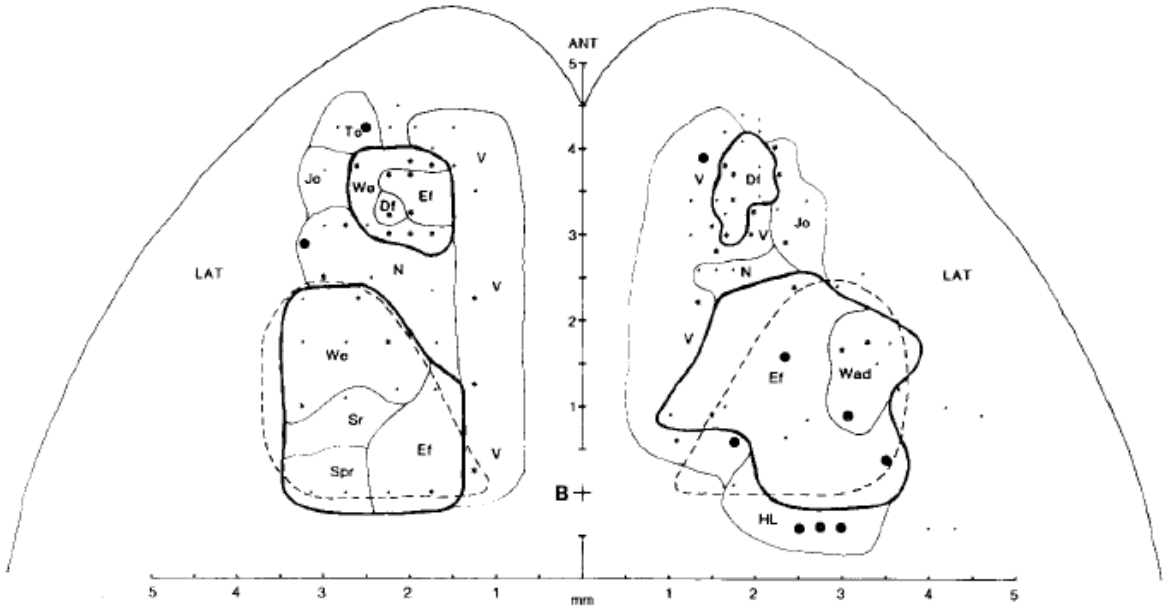

Fig 1 Stimulation mapped on the dorsal surface of the rat frontal cortex from Fig 1, p152 *Brain research* (Neafsey and Sievert, 1982). B denotes bregma. Heavy lines surround forelimb areas. Dashed line indicates forelimb area by Hall and Lindholm.

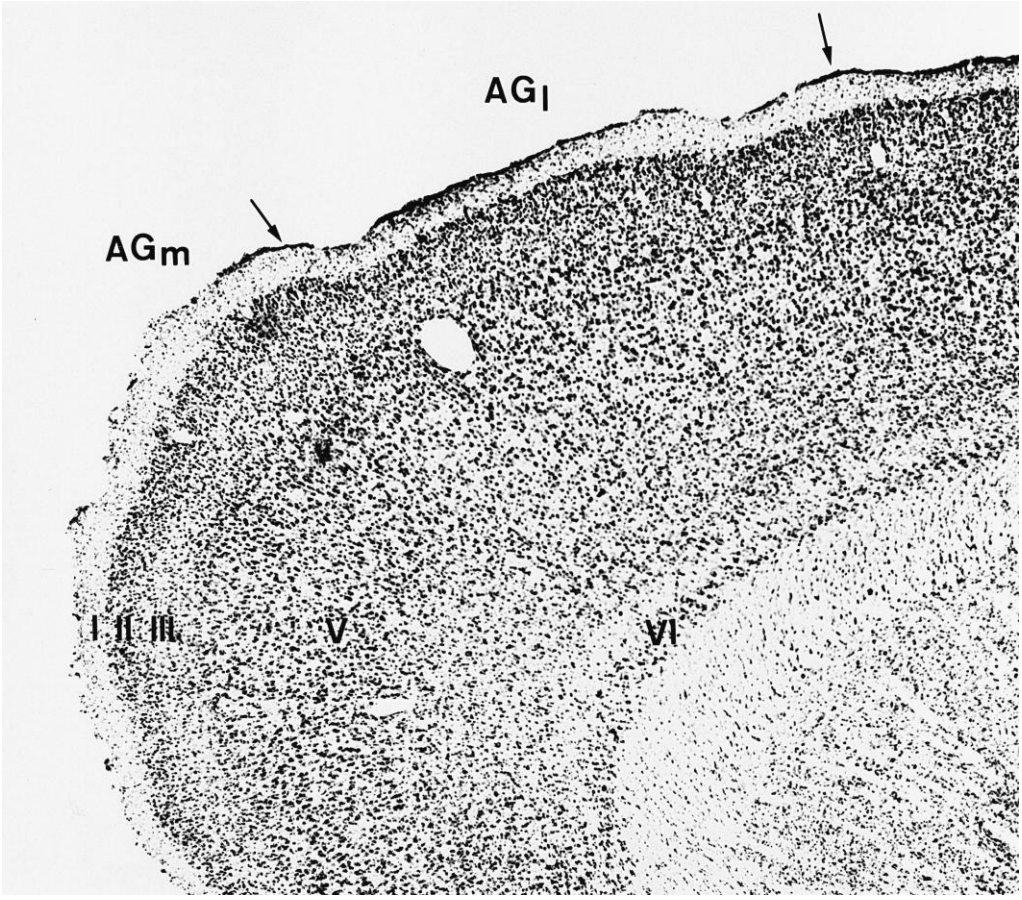

Fig 2 A Nissl-stained sagittal section from Fig 1, p138 *Brain Research* (Wang and Kurata, 1998). Arrow indicates boundaries between the medial agranular cortex (AGm) and the lateral agranular cortex (AGl).

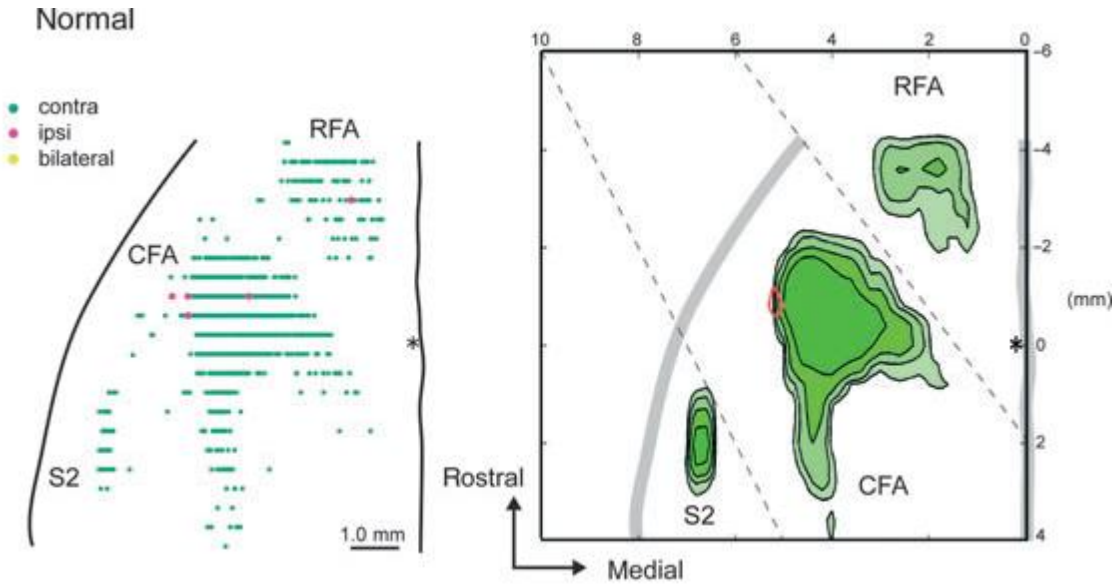

Fig 3 Distributions of corticospinal neurons retrogradely labeled in normal rats from Fig 3, p1457 *European J. of Neuroscience* (Umeda and Isa, 2011). (Left) Each dot represents one labeled neuron. Contra-corticospinal neurons are in green, and ipsi-CSNs in magenta. (Right) Density maps of labeled neurons on dorsal plane * indicates the bregma.

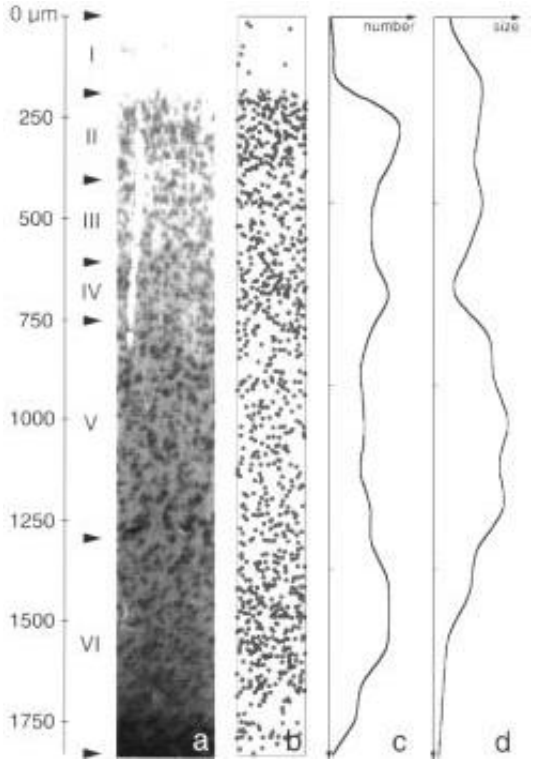

Fig 4 from Fig 2, p180 *Cerebral cortex* (Skoglund et al., 1997) a) Micrograph reconstruction of rat primary motor cortex, from a sagittal section stained in Richardson's solution b) Reconstruction of the positions of the neurons within a cortical prism. Each neuron is shown as a sphere. c) Linear plot showing the relative distribution of neurons versus the distance from the pial surface. d) Linear plot showing the medium size (volume) of the neurons vs. the distance from the pial surface.

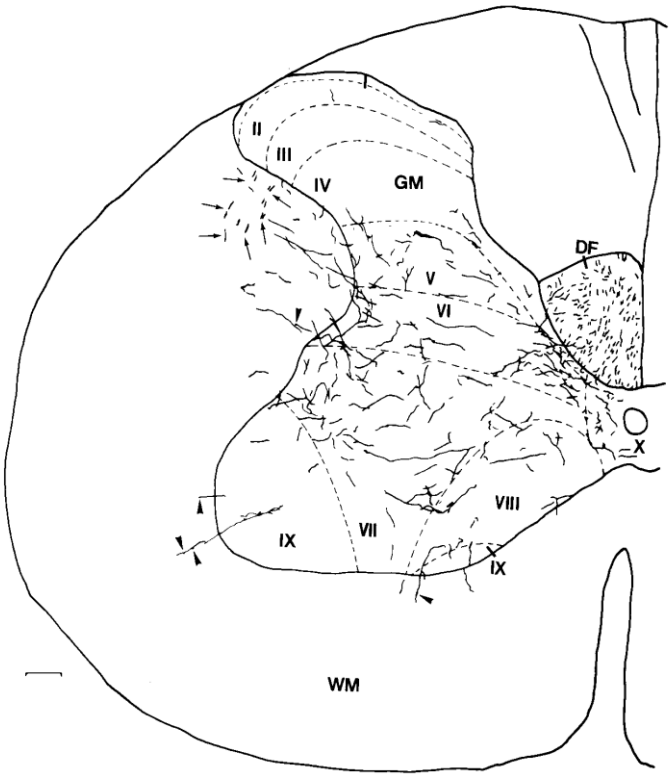

Fig 5 Hemisection at C5, showing the distribution of labeled corticospinal axonal arbors after anterograde tracer in the contralateral RFA from Fig 8, p282 *Somatosensory and motor research* (Rouiller et al., 1993). Scale bar 100µm. More than 90% of the total number of labeled axons was located in the contralateral dorsal funiculus (DF). Axonal arborization was observed covering Rexed laminae I to X, though the terminal fields are most dense in laminae V, VI and VII. A few axonal ramifications were present ventrolaterally in the grey matter, a location where they were clearly superimposed on the dendrites of the motoneurons.

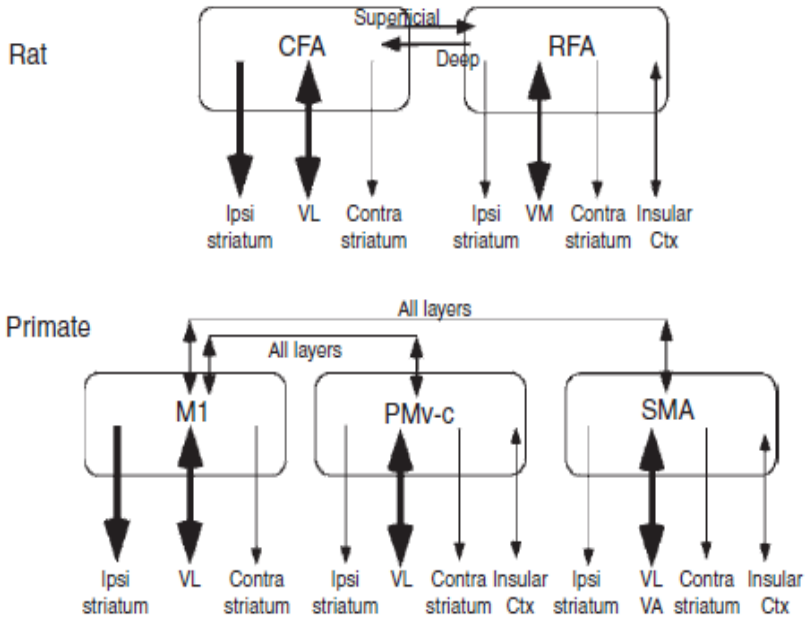

Fig 6 Comparison of secondary motor areas from Fig 4, p741 in: *Evolution of Nervous Systems* (Nudo and Frost, 2007). It demonstrates the major projections of the cortico-thalamo and the cortico-striatal in rats (top) and in primates (bottom). Ventrolateral thalamus (VL), Ventromedial thalamus (VM), Ventroanterior thalamus (VA).

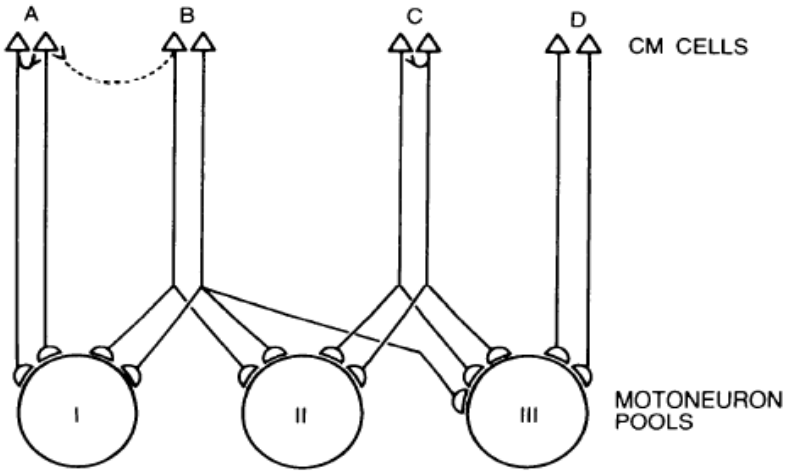

Fig 7 Anatomical substrate for cortical map competition from Fig 12, p803 *J of Neurophysi*ology (Cheney and Fetz, 1985) The corticospinal axons diverge to motoneurons innervating more than one muscle. It is important to note that there are few, if any, monosynaptic connections to motor neurons from corticospinal fibers in rats (Alstermark et al., 2004).

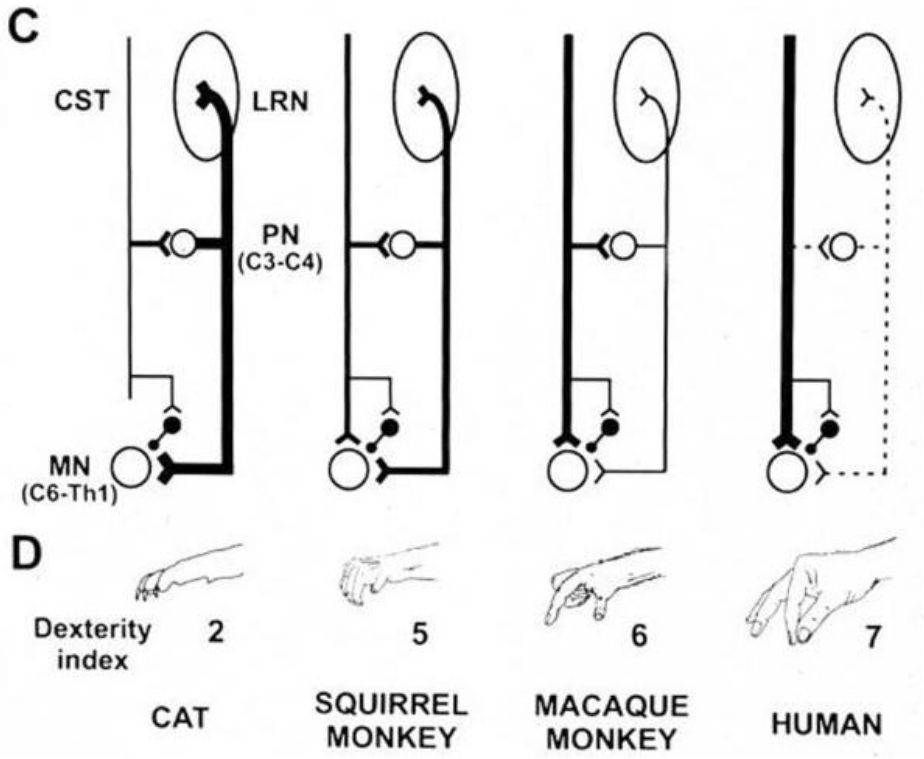

Fig 8 Diagram of propriospinal neurons (PN) and corticospinal inputs to upper-limb motoneurons from Fig 12, p707 *J of Neurophysiology* (Nakajima et al., 2000) In cats, there is a strong projection from C3-C4 PN to motoneurons, transmitting much of the corticospinal excitation. This same pathway is still present in the squirrel monkey, although to a lesser extent, and it exists in parallel with CM connections. The PN system becomes progressively weaker from squirrel to macaque monkey, and this change is accompanied by a progressive increase in the strength of the CM connection, which is stronger in humans. By extrapolation, these changes predict that the PN system is unlikely to play a major part in transmitting corticospinal excitation in humans (dashed lines in C). Corticospinal activation of segmental inhibitory interneurons (filled symbol) is present in all species. (D) Index of dexterity for the four species shown. Lateral reticular nucleus (LRN) Propriospinal neuron (PN) Motoneuron (MN)

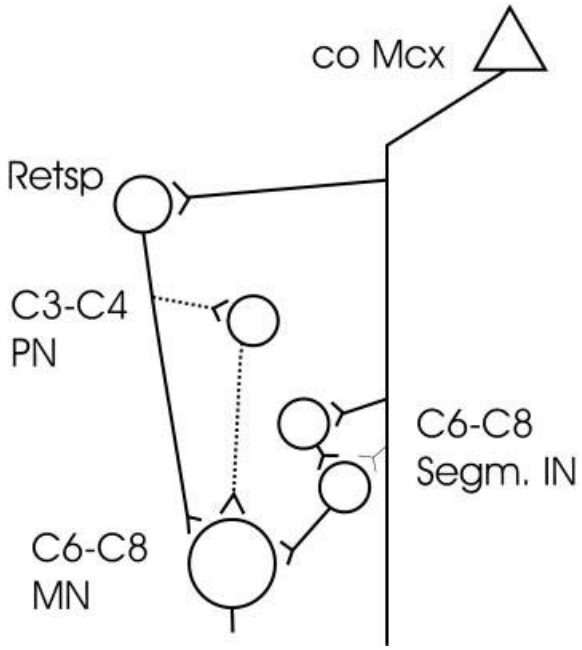

Fig 9 Excitatory corticospinal projection in rats from Fig 7, p1838, *J of Neurophysiology* (Alstermark et al., 2004) The circuit represents a population of neurons. The dotted line from Retsp to C3-C4 is tentative and the weak line represents that connection that has not yet been proven or disproven.

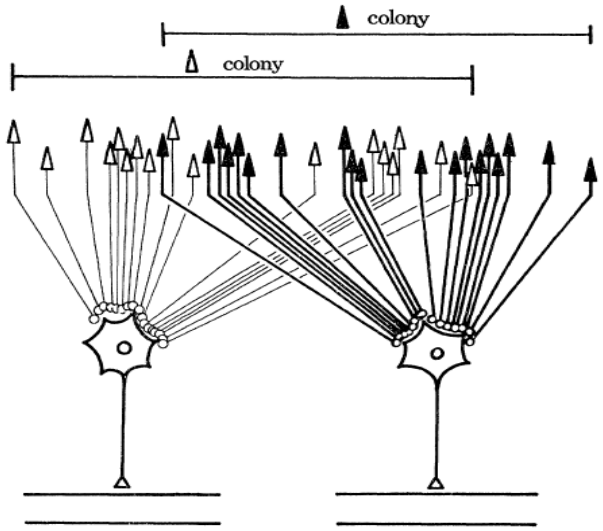

Fig 10 Anatomical substrate for cortical motor map competition from Fig12 *Proceedings of the Royal Society of London* (Andersen et al., 1975) The figure illustrates convergent cortical spinal neurons to motoneuron pools.

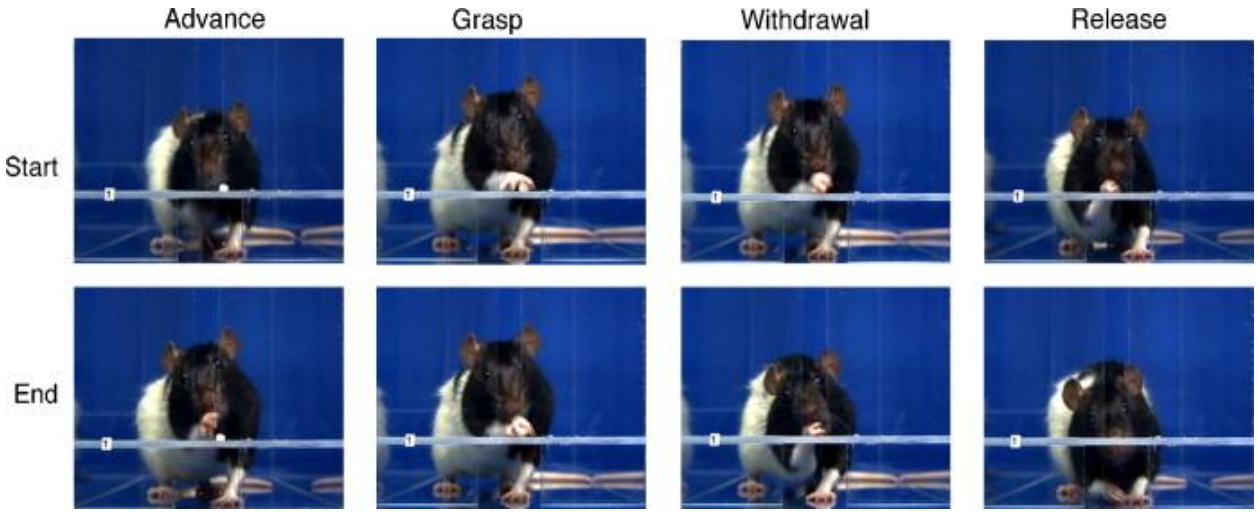

Fig 11 Single-pellet reach and retrieval task (Whishaw et al., 1992) from Fig 1, p283 *Behavioral Brain Research* (Alaverdashvili et al., 2008a) The task detects sensormimotor functional deficits and allows assessment of both quantitative success and movement quality. This figure illustrates gestures: advance consists of the rat lifting its reaching limb, moving it forward through the slot, and orienting the paw over the pellet; grasp starts with extending and raising the paw over the pellet and ends with closing and flexing the digits on the pellet; withdrawal starts with the grasping of the pellet and ends with placing the pellet into the mouth, or bringing the paw to the mouth; release starts with opening the digits to place the food in the mouth and ends with moving the forelimb into a relaxed posture.

CHAPTER TWO

Reorganization of Motor Cortex after Controlled Cortical Impact in Rats

and

Implications for Functional Recovery

ABSTRACT

We report the results of controlled cortical impact (CCI) centered on the caudal forelimb area (CFA) of rat motor cortex to determine the feasibility of examining cortical plasticity in a spared cortical motor area (rostral forelimb area, RFA). We compared the effects of three CCI parameter sets (groups CCI-1, CCI-2 and CCI-3) that differed in impactor surface shape, size and location on behavioral recovery and RFA structural and functional integrity. Contralesional forelimb deficits were evident in all three CCI groups assessed by skilled reach and footfaulttasks that persisted throughout the 35 day post-CCI assessment period. Hindlimb deficits were detected in the footfault task for 28 days post-CCI. Nissl-stained coronal sections revealed that RFA was structurally intact. Intracortical microstimulation experiments conducted at 7 weeks post-CCI demonstrated that RFA was functionally viable. However, the size of the forelimb representation decreased significantly in CCI-1 compared to the control group. Redistribution of RFA movement representations (significant reduction in distal forelimb area and non-significant enlargement in proximal forelimb area) was induced in CCI-2 and CCI-3. The RFA areal reduction and reorganization are discussed in relation to possible diaschisis and to compensatory functional behavior, respectively. Also, an inverse correlation between the anterior extent of the lesion and the size of RFA was identified and discussed in relation to corticocortical connectivity. The results suggest that CCI can be applied to rat CFA while sparing RFA. This CCI model can contribute to our understanding of neural plasticity in premotor area as a substrate for functional recovery.

INTRODUCTION

The adult cerebral cortex is organized in a way that allows for substantial recovery of lost functions after acquired brain injuries. Various mechanisms underlying functional recovery are embodied in the theory of vicariation—the ability of one part of the brain to substitute for the function of another (Slavin, 1988). Since modern views of brain organization recognize that the cerebral cortex is arranged in a distributed, hierarchical fashion,we assert (as do Slavin et al.) that vicariation does not necessarily require that a function lost after damage is taken over by a totally unrelated structure, as suggested by early interpretations (Finger, 1982; Finger, 2009), rather that other related components of the distributed network reorganize to support the recovered function. A number of studies supportive of this theory have demonstrated that the motor cortex of adult mammals changes its activation patterns in response to cortical injuries. Rat and non-human primate studies using intracortical microstimulation (ICMS) to derive detailed maps of the functional representations in the motor cortex have suggested that the neural substrates mediating recovery reside within the peri-infarct cortex (Glees, 1949; Castro-Alamancos and Borrel, 1995; Nudo et al., 1996b),spared motor areas in the injured hemisphere, such as the premotor cortex (Frost et al., 2003; Dancause et al., 2006b) and the supplementary motor area (Eisner-Janowicz et al., 2008), as well as the cortex of the uninjured hemisphere (Reinecke et al., 2003; Rema et al., 2003). Neural reorganization within these spared motor regions of the injured and uninjured hemisphere is thought to be necessary for post-injury recovery of motor function (Castro-Alamancos et al., 1992; Rouiller et al., 1998; Liu and Rouiller, 1999; Conner et al., 2005).

Non-human primate studies in premotor cortex following ischemic damage in primary motor cortex (M1) are especially relevant to this issue of plasticity in related areas within the motor

cortex hierarchical network. The hand representation in the ventral premotor area (PMv) expands after an ischemic lesion in the M1 hand area (Frost et al., 2003; Dancause et al., 2006b). In addition, corticocortical axons from the spared PMv hand area sprout and form novel connections with parietal somatosensory hand areas (Dancause et al., 2005).

It is less clear whether similar changes occur in rat motor cortex. Homologies between primate and rodent motor areas are not straightforward. For example, there are at least seven separate hand representations in the primate motor cortex whereas only two have been identified in rodents. It is thought, however, that the caudal forelimb area (CFA) and the rostral forelimb area (RFA) of rodents are equivalent to the M1 hand area and the premotor area of primates, respectively (Nudo and Frost, 2007). Also, rat cortical motor areas exhibit not only intrinsic and intracortical connections comparable to those of non-human primates but also similar structural relations among cortical and subcortical motor areas (Neafsey et al., 1986; Keller, 1993; Fang et al., 2005; Stepniewska et al., 2006). Taken together, it is reasonable to use rodent models of plasticity in spared cortical motor regions to investigate mechanisms of functional motor recovery after cortical injury.

A substantial body of evidence supports both structural and functional plasticity in spared motor structures after cortical lesions. However, the type of injury induction is critically important in the subsequent neural reorganization (Voorhies and Jones, 2002; Gonzalez and Kolb, 2003). For example, in a study by Gonzalez and Kolb comparing three models of permanent ischemia as well as aspiration lesions, behavioral effects were similar. But examination of dendritic branching and spine density demonstrated atrophy in some lesion models and hypertrophy in others (Gonzalez and Kolb, 2003). Neurophysiological changes in spared cortical areas also have been documented at least since the pioneering work of Glees and

Cole who used surface stimulation techniques in monkeys to show that the thumb representation re-appeared in the adjacent cortical location after focal damage performed by undercutting (Glees, 1949). Similar results have been demonstrated after focal ischemic lesions, though post-injury use of the impaired hand was necessary for the neurophysiological changes to occur (Nudo et al., 1996b). Expansion of premotor hand representations after ischemic lesions in the primary motor cortex hand area has also been shown (Frost et al., 2003).

After traumatic brain injury (TBI), functional plasticity, especially in the injured hemisphere, may be more limited due to the potential for more widespread disruption of axonal and dendritic processes, especially in corticocortical networks. However, neurophysiological studies of plasticity in spared areas after TBI have been relatively rare. In one notable study, Boyeson et al., used ICMS techniques in rats, similar to those used in the present study, after undercutting, suction ablation or contusion injuries of limb representations and found no evidence for re-emergence of the damaged representation (Boyeson et al., 1991). In fact, after 30-290 days post-contusion, hindlimb movements could not be evoked at all, suggesting that contusion injuries result in a slowly-evolving lesion, and that the substrates for recovery were located elsewhere in the brain.

The present study was designed to determine the feasibility of examining plasticity in spared cortical motor structures in the same hemisphere after a controlled cortical impact (CCI) in adult rats. Our primary goal was to develop a set of CCI parameters that results in restricted damage to CFA, sparing neurons in RFA, but producing chronic motor deficits. Since RFA, or any other remote motor representation, was not specifically examined in the earlier work by Boyeson et al., this represents a unique test of the hypothesis that spared motor regions in the same hemisphere are still intact, and may functionally reorganize after contusion injuries. Determining the

relationship between behavioral recovery and neurophysiological reorganization in spared motor structures in this model may provide insight into the underlying neural mechanisms for recovered functions. To this end, three sets of CCI parameters comparing the effects of subtle differences in impactor tip surface, size and stereotaxic position were analyzed. Specifically, we assessed the behavioral performance before and for 35 days after CCI, using contact placing (reflex) tests, the skilled reach task (single pellet retrieval) and footfault tests, and subsequently examined movement representations within RFA using ICMS.

The study confirmed chronic deficits in forelimb skilled behavior using each of the three CCI parameters and the functional integrity of RFA. No obvious structural damage within RFA was evident. The resulting size of the RFA representation area varied according to lesion proximity to RFA.

MATERIALS AND METHODS

Subjects and group assignments

Long-Evans hooded rats (n = 24; 300-400g; Charles River Laboratory) were procured at 4 months of age. All animal use was in accordance with NIH regulations and approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center. During the first month after arrival, rats were handled daily to acclimate them to human touch, and to habituate them to the new cage environment. Each rat was singly-housed in a transparent cage and provided with food and water ad libitum. The room was kept on a 12hr:12hr light:dark cycle, and ambient temperature was maintained at 22 °C.

Skilled reach (single pellet retrieval) task

A pellet-retrieval task (Whishaw et al., 1991) was used to assess the effects of CCI and subsequent recovery on forelimb motor behavior. The testing chamber was constructed of Plexiglas and measured 25 cm (length) X 25cm (width) X 35 cm (height). A 1 cm wide reaching slot, extending from the bottom of the chamber to 20 cm in height, was centered at the front of the chamber. A food shelf (4 cm deep X 25 cm wide) was secured to the outside of the chamber 3 cm from the bottom. A pair of indentions was made in the food shelf 2 cm from the front and 0.5 cm to the left and right side of reaching slot, to hold 45 mg food pellets (Bioserve, Frenchtown, NJ). Rats were restricted to 15-20 grams rat chow at the end of each shaping or training day to increase their motivation for retrieving pellets (Bury and Jones, 2002; Hsu and Jones, 2006a). All sessions were videotaped with a Sony Handycam camcorder.

Behavioral Shaping. Shaping consisted of having rats reach out for single food pellets placed centrally (between the indentations), 1 cm from the front of the chamber. Thus, the rats could retrieve pellets using either forelimb. They usually become proficient within 5 days. Forelimb dominance then was evaluated during 20 reaches in each of two consecutive daily 20- min sessions (Hsu and Jones, 2006a). The dominant forelimb was defined in each rat as the limb used on more than 70% of the trials (Maldonado et al., 2008). A total of 18 rats displayed left dominance while 6 displayed right dominance.

Skilled Reach Training. Once forelimb dominance was established, we began baseline training by compartmentalizing the chamber with a removable wall in order to constrain reaches to the dominant forelimb only. Each training session consisted of a maximum of 60 trials or 20 min per day (whichever came first). Training sessions were conducted 5 days per week for 2

weeks. A trial was counted as successful when the rat grasped and transported the pellet to the mouth. A failure was tallied when a) a pellet was successfully grasped, but dropped before reaching the mouth, b) a pellet was dislodged from the food shelf, or c) the rat failed to contact the pellet after 5 reaches (e.g., rat grasping air without contact with pellet).

Pre-CCI Baseline Probe Sessions. After training was completed, five probe sessions (two in the 1st week; three in the 2nd) were conducted to define baseline skilled reach performance. Lasting no longer than 15 min, probe sessions were identical to training sessions except only 20 trials were administered. The scores were reported as the number of successfully retrieved pellets.

Post-CCI Probe Sessions. After CCI, performance was assessed during probe sessions conducted weekly beginning on post-CCI day 7 and continuing through week 5.

Footfault task

The footfault task was used to measure locomotor ability separately for the forelimb and hindlimb. The apparatus consisted of a plastic platform grid (57 cm length X 44 cm width) elevated 30 cm from the laboratory table. The grid had openings of 3.5 cm X 3.5 cm and grid rung diameter was 2 mm. The task required no prior training. In a probe session, each rat was placed on the platform and was videotaped from below for a minimum of 2 min (Gilmour et al., 2005) as it traversed the platform grid. Video recordings of forelimb and hindlimb footfaults were analyzed in slow-motion. Footfaults were defined as the placement of the limb through grid opening. We tallied: a) the number of footfaults by each limb and b) the total number of forelimb or hindlimb steps. A step was defined as the sequential movement of both left and right

limbs. We analyzed the dominant limb only (i.e., the impaired limb after CCI). Forelimb performance was quantified by the percent forelimb footfaults, the percentage of footfaults by the dominant forelimb divided by the total number of forelimb steps (Hsu and Jones, 2006b). Hindlimb performance was calculated in the same manner.Baseline performance was defined during four pre-surgery probe sessions (two in the 1st week; two in the 2nd). Post-CCI performance was assessed during weekly probe sessions beginning on post-CCI day 7 and continuing through week 5.

Contact placing

A contact placing (reflex) test was administered only during days 1 to 3 post-CCI. The rat was held in a suspended position facing the corner of a flat surface. Then, it was gently moved closer to the corner from the lateral side until either the vibrissae (vibrissae-forelimb placing) or the forelimb (forelimb-forelimb placing) made contact with the surface (Whishaw et al., 2004). For normal performance (lifting forelimb ipsilateral to side being stimulated before forced contact), a score of "1" was recorded. When the lifting response was not elicited within 3 sec, an unsuccessful score of "0" was recorded. The test was repeated ten times with the maximum score of 10 for each side on each test day.

Controlled cortical impact (CCI) procedure

After the completion of baseline behavioral assessment, rats were randomized to one of three CCI groups (CCI-1, CCI-2 and CCI-3) differing in impactor tip configuration, and a control group, consisting of rats of similar size and age that were not subject to the surgical procedures (Table 1). Each CCI rat was anesthetized with an initial dose of 4% isoflurane and maintained

with 3.5% isoflurane/30% oxygen through a mouthpiece, remaining under aseptic conditions until the end of the procedure. The animal was placed prone with its head position fixed in a stereotaxic frame. The skin over the skull was incised and a small hole (3-4 mm diameter) was made in the skull using a dental drill with a trephine bit over the cortex contralateral to the dominant forelimb. A total of 14 animals received CCI in the right hemisphere and 4 on the left. The CCI device consisted of a linear motor, the impactor, (P01-23x80, LinMot, Inc., Zurich, Switzerland) and an electronic servo controller (E100-MT, LinMot, Inc., Zurich, Switzerland) as previously described (Bilgen, 2005; Onyszchuk et al., 2007; Onyszchuk et al., 2008). A stainless steel impactor tip was attached to the end of the linear motor. A three dimensional motion manipulator (Kopf Instruments, Tujunga, CA) was used to precisely align the impactor tip with the stereotaxic coordinates corresponding to CFA (see Table 1). In using the larger impactor tips (CCI-2, CCI-3), the stereotaxic coordinates were shifted posteriorly to avoid injury to RFA, the focus of the electrophysiological experiments. The area of craniotomy was constantly irrigated with sterile, physiological, room-temperature saline while the underlying dura was kept intact. The impactor tip was slowly lowered through the craniotomy hole perpendicular to the cortex until it was in contact with the dura surface, as examined under light microscopy. The motion profile of the impactor tip was programmed to withdraw from the surface a distance of 20 mm and then deliver a downward stroke of 22 mm (i.e., indentation depth of 2.0 mm) with a preset velocity of 1.5 m/s and indentation duration of 85 ms. The skin was pulled over the intact dura, and then, sutured back to complete the procedure and the anesthesia was terminated. Buprenorphine (0.6mg/kg, subcutaneously) was given as a post-surgical analgesic at least one hour after the rat regained consciousness.

Intracortical microstimulation (ICMS) procedure

Two weeks following the final post-CCI behavioral assessment (i.e., 7 weeks post-CCI), an electrophysiological mapping procedure was performed in the frontal cortex ipsilateral to the CCI. For the purposes of this procedure, the animal was anesthetized with an initial dose of ketamine hydrochloride (70 mg/kg ip) and xylazine (5.0 mg/kg ip). Additional doses of ketamine (20 mg/kg ip) were administered as necessary to maintain a stable anesthetic level. The animal was placed prone with its head stabilized in a stereotaxic frame. After a craniotomy over the frontal cortex corresponding to RFA and removal of the dura, a highly-magnified digital image of the cortex vasculature was taken to guide the points of microelectrode penetration. Using a computer graphics program (Canvas, ACDSee, Victoria, British Columbia, Canada), grid lines (250 μm apart) were overlaid on the image. Using a hydraulic microdrive, a NaCl-filled glass micropipette (tapered to 15-20 μm o.d. tip; impedence = 500-700 kΩ) was lowered perpendicular to the cortical surface at each grid intersection point until the tip reached the depths of cortical layer V (1700~1800 μm). A stimulus isolation unit (BAK Electronics, Mount Airy, MD) was used to deliver 200 μs monophasic, cathodal constant-current pulses at the rate of 350 Hz for 40 ms (~ 13 pulses). Joint and muscle movements elicited by the stimulation were inspected visually, and were considered reliable when observed in at least 50% of the ICMS trains. The movement elicited at the minimum required current was recorded. The absence of visually detectable movements at the maximum current level of 80 μA was recorded as "no response". Using custom and commercial software, the cortical surface areas representing each movement category (digit, wrist, elbow, shoulder, neck, face) were calculated. Average current levels required to evoke each of the movement categories were also determined. Procedures identical to the above have been used in a number of publications from our laboratory on both rodents and

non-human primates (Kleim et al., 2002; Kleim et al., 1998; Nudo et al., 1990; Nudo and Milliken, 1996).

Histology

Following the completion of the ICMS procedure, each animal was deeply sedated, then perfused via the left ventricle with 10% formalin solution. The brain was removed and stored in 10% formalin until it was ready for histology. To verify the lesion volume and location, the brain was first cryoprotected in 30% sucrose solution at 3ºC for over 48 hours. Serial 30 µm thick frozen coronal sections of the lesion vicinity—approximately from 3.7 mm anterior to bregma to 3.2 mm posterior to bregma were then collected using a cryostat (Paxinos and Watson, 2007). Sections were mounted on microscopic slides and Nissl-stained. The caudal and rostral extents of the lesion were determined. Reconstruction of the lesion was done indirectly by tracing the perimeter of the cortices using the Cavalieri method in Stereo Investigator (Microbrightfield, Inc., Williston, VT; also see supplemental information); the lesion volume was estimated by the difference of the cortical volume in the injured hemisphere subtracted from the cortical volume in the intact hemisphere (Tennant and Jones, 2009).

Statistical Analysis

SPSS version 17.0 (SPSS, Chicago, IL) for Windows software was used for statistical analysis. One-way ANOVAs were used to analyze the histological and ICMS data, followed by Fisher's LSD post-hoc test when appropriate. A non-parametric test (Chi-square test) was used to test the distribution of CCI severity with respect to callosal damage across groups. Due to lack of normality as well as unequal variance, a non-parametric test (Kruskal-Wallis H test) was used to

analyze the behavioral data for an overall group effect (two-tailed). In the case of significant group effects, Mann-Whitney U tests were used for pair-wise comparisons (two-tailed). A simple linear regression ANOVA was performed on ICMS results (RFA area) to determine if there was a significant relationship between the rostral and caudal extent of the impactor tip and the subsequent RFA area. Results for parametric tests are provided in mean ± SEM and nonparametric tests in median + 95% confidence interval. The minimum value for statistical significance was $p \leq 0.05$.

RESULTS

Histology

All animals were sacrificed approximately 7 weeks following CCI. Fig. 1 illustrates a dorsal view of the lesion and coronal sections at the levels of RFA (Fig 1B) and CFA (Fig 1 CE). Histological inspection at the level of CFA showed that in seven cases (CCI-1: n=1; CCI-2: n=3; CCI-3: n=3) all cortical layers were destroyed, with the corpus callosum partially or completely intact (Fig. 1C). In five cases (CCI-1: n=2; CCI-2: n=1; CCI-3: n=2), the deepest part of layer six was spared (Fig. 1D) and in another five cases (CCI-1: n=2; CCI-2: n=2; CCI-3: n=1), the corpus callosum appeared severed due to direct impact (Fig. 1E). The presence or absence of damage in the corpus callosum was not related to CCI group (chi-square=1.88, p=0.758). At the level of RFA, no cortical damage was evident under light microscopy in any of the cases. There was no statistical difference in lesion volume among the three impact groups ($F_{2, 10} = 0.64$, p=0.54; Fig. 3A). However, while not examined quantitatively, there appeared to be a difference in lesion shape based on impactor tip shape. The flat tip (Fig. 1C) tended to produce more consistent, columnar injuries compared with rounded tips (Fig. 1D and see supplemental data). Because the

rostrocaudal location of the impactor tip differed slightly between groups (CCI-1 was 0.5 mm more rostral, but its tip diameter was 0.25 mm smaller), the rostral and caudal extents of the histological damage relative to bregma were also estimated (Fig. 2B). The rostral extent of the lesion did not differ across groups ($F_{2, 10}=1.24$, $p=0.33$). However, the caudal extent differed significantly ($F_{2, 10}=4.20$, $p=0.047$), measuring more anterior in CCI-1 compared to CCI-2 and CCI-3. Although neurophysiological maps of CFA were not derived, in all cases, the lesion location corresponded to typical stereotactic coordinates for CFA (Neafsey et al., 1986; Nudo et al., 1990).

Behavioral performance

Skilled Reach Task. To eliminate outliers from the skilled reach task analysis, rats were required to have a baseline (pre-injury) skilled reach performance score of at least 10 successful trials out of 20). As a result, one rat from the control group and one rat from CCI-1 were omitted from the skilled reach analysis but retained for all remaining analyses. As illustrated in Fig. 3A, no group differences were detected in the mean number of successfully retrieved pellets on any of the pre-lesion assessment days. A significant group effect in forelimb function was evident on post-lesion days 7 ($H=11.4$, $p=0.009$), 14 ($H=10.0$, $p=0.018$), 21 ($H=11.3$, $p=0.01$) and 35 ($H=8.1$, $p=0.044$), but not on post-lesion day 28 ($H=6.9$, $p=0.072$). On post-lesion days 7, 14, 21 and 35, each of the three CCI groups displayed a lower number of successful retrievals than the non-lesion control group. There were no significant differences among the three CCI groups on any given assessment day.

Footfault Task. In baseline sessions, all groups maintained equivalent forelimb footfault performance levels traversing the elevated grid (Fig. 3B). Following CCI, a significant group

difference in performance was found on post-lesion days 7 (H=11.6, p=0.009), 21 (H=12.8,p=0.005) and 35 (H=11.6, p=0.009), but not on post-lesion days 14 (H=7.4, p=0.061) or 28 (H=4.5, p=0.215). On post-lesion days 7 and 35, post-hoc tests revealed that the percent footfaults in each CCI group was higher than that of the control group. On post-lesion day 21, the percent footfaults of CCI-2 was higher than that of the control group, whereas the percent footfaults of CCI-3 was higher than that of both the control and CCI-1 groups.

As was described for forelimb footfaults, in the baseline sessions, all groups maintained equivalent hindlimb footfault performance. After CCI, a significant group effect in hindlimb function was found on post-lesion days 7 (H=9.9, p=0.019) and 21 (H=8.4, p=0.038), but not on days 14 (H=2.8, p=0.422), 28 (H=6.2, p=0.10) or 35 (H=5.7, p=0.126; Fig. 3C). Post-hoc analysis revealed that on post-lesion day 7, each of the CCI groups made more footfault errors than the control group. On post-lesion day 21, CCI-2 and CCI-3 displayed higher percent footfaults than the control group, but CCI-1 showed no significant difference compared to the control group. On this assessment day, however, the three CCI groups did not differ significantly from one another.

Contact Placing Test. The vibrissae-forelimb/forelimb-forelimb test was used to assess sensorimotor reflex function through the first 3 days post-lesion prior to motor performance assessment which began on post-lesion day 7. Only the non-lesion control animals maintained contact placing with both limbs. Animals in all CCI groups showed a complete loss of contact placing for the limb contralateral to the cortical injury, scoring 0/10, and intact placing for the limb ipsilateral to the cortical injury, scoring 10/10, on each assessment day (not illustrated).

Microstimulation mapping in RFA

ICMS procedures were conducted in the intact RFA of the injured hemisphere in all animals 7 weeks following CCI. Digit and wrist movements evoked by ICMS were classified as "distal forelimb", and elbow and shoulder movements as "proximal forelimb" for both topographical area and movement threshold analyses. Topographic and threshold results were successfully obtained in 18 of 21 rats (5/5 control, 5/6 CCI-1, 4/4 CCI-2, 4/6 CCI-3. Three rats died during the ICMS procedure.). In the remaining 3 rats (one rat from CCI-1 and two rats from CCI-3), no evoked movements were observed from ICMS stimulation in RFA. Such outcomes are not uncommon in ICMS experiments, and are typically attributable to improper anesthetic depth that cannot be corrected during the course of the procedure. As a result, quantitative neurophysiological analyses were based on a total of 18 rats. Representative topographic maps of the distal and proximal forelimb representations are shown in Fig 4A. The total RFA (combined distal and proximal RFA topographical area) was significantly different among groups ($F_{3, 14}$=6.72, p=0.005). Post-hoc comparisons revealed that RFA was significantly smaller in CCI-1 compared to each of the other groups (Fig. 4B). RFA of neither CCI-2 nor CCI-3 was different from that of the control group or from each other. When the total RFA was subdivided into distal and proximal areas, the proximal forelimb area showed significant differences across groups. ($F_{3, 14}$=4.62, p=0.019). CCI-1 displayed a smaller proximal forelimb area compared to CCI-2 and CCI-3, but none of the CCI groups was different from the control group. However, while the mean distal area was smaller in each of the experimental groups compared with the control group, there was no significant group effect for the distal forelimb area ($F_{3, 14}$= 2.297, p=0.122). It should be noted that this result may have been attributable to a single outlier. In one of the control rats, no distal forelimb movements were evoked, an outcome that is rare in RFA maps.

When this single outlier was excluded from the analysis, the ANOVA showed a significant group effect ($F_{3, 13}$= 10.69, p=0.0008), and pair-wise analysis showed a significant difference between the control group and each of the experimental groups.

No significant differences were found in the threshold currents to evoke forelimb movements for the combined (distal + proximal) RFA ($F_{3, 14}$=2.25, p=0.128), distal forelimb ($F_{3, 13}$=2.07, p=0.154) or proximal forelimb ($F_{3, 13}$=1.02, p=0.414) movements. On average, however, CCI-1 required 20 μA, 11 μA and 13 μA higher currents than the control, CCI-2 and CCI-3, respectively (Fig. 5C). A simple linear regression ANOVA demonstrated a significant negative relationship between the rostral lesion extent (based on histology) and the size of RFA (F=5.828, p=0.036, r^2=0.368; Fig. 5). In other words, the closer the lesion was to RFA, the smaller the RFA topographical area. There was no relationship between the rostral lesion extent and the RFA threshold (F=0.012, r^2=0.001, p=0.914).

DISCUSSION

We aimed to develop a set of CCI parameters in rats that would result in long-term functional deficits in forelimb use while sparing the premotor forelimb area. We examined three sets of CCI parameters that differed in impactor tip surface shape, size and location, targeting the caudal forelimb area (CFA). The cortical injury created by the CCI device was consistently reproduced across rats, resulting in a tissue cavity in CFA and adjacent cortical areas, though some variability was observed in cortical depth and white matter involvement. We focused our neurophysiological studies on the rostral forelimb area (RFA), since it is a cortical region similar in many respects to premotor cortex in primates (Nudo and Frost, 2006). The main findings were

that a) all three CCI groups displayed motor deficits throughout the five-week post-CCI test period, b) RFA was structurally intact in every rat, c) RFA was functionally intact in most rats, though there were significant changes in movement representations, and d) the functional responsiveness of RFA to cortical microstimulation was related to the anterior extent of the injury.

Effect of CCI parameters on lesion volume and behavioral deficits

The three sets of impactor tip parameters were statistically indistinguishable in lesion cavity volume and motor deficits. This result is not surprising in light of a previous study that found no group differences in lesion volume or behavioral deficits after contusion injuries, using an even wider range of impactor tip sizes, but with an impact depth similar to that used in our study (Whishaw et al., 2004) both rat and mouse CCI models, tissue damage and severity of behavioral deficits are generally related to impact depth (Feeney et al., 1982; Dixon et al., 1991; Saatman et al., 2006; Mao et al., 2010), rather than tip diameter. However, impactor tip shape is also thought to be an important factor (Mao et al., 2010). In the present data, while there was no difference in injury volume, the shape of the damage was somewhat non-uniform with the rounded tip, and a more consistent column of damage with the flat tip.

Behavioral deficits after CCI in rat motor cortex

Among the widely implemented assessments in rat TBI studies are tasks that measure postural and locomotor behaviors, such as beam walking and balancing (Feeney et al., 1982; Dixon et al., 1991; Goldstein, 1993; Soblosky et al., 1996). In the present study, we primarily assessed forelimb motor behavior since we were interested in characterizing deficits resulting

from a focal impact injury in CFA, the rodent equivalent of the primary motor hand area in primates. The skilled reach task is one of the most sensitive indicators of forelimb motor deficits even during the chronic post-injury period (Adkins and Jones, 2005). In addition, the footfault test is a sensitive measure of both forelimb and hindlimb locomotory function after CCI, though typically, only forelimb footfaults are examined (Grossman and Stein, 2000).

All animals sustained skilled reach (pellet retrieval) and forelimb footfault impairments for the entire 35 days of the post-CCI assessment timeline. A more transient hindlimb footfault deficit was observed through post-lesion day 21. This differential effect on forelimb function is probably due to the fact that CCI was centered on CFA, though it is possible that some damage to the hindlimb motor representation occurred as well, since the hindlimb representation is located in close proximity to CFA. While the impairments were significant, skilled forelimb behavior was not completely abolished. Even in the early stages after CCI, the rats could still achieve 20-40% success on this task and were able to traverse the grid. Even though the initial loss in forelimb performance steadily corrected, the CCI animals never reached baseline performance, indicating persistent and perhaps chronic forelimb deficits. Our results are similar to those of a CCI study by Whishaw et al. who also found chronic deficits in forelimb use (Whishaw et al., 2004). Based on their CCI parameters, the contusion likely included not only CFA but also RFA (see Fig. 1 in Whishaw et al., 2004). The similarity in outcome indicates that sparing RFA did not preclude the chronic deficits and that a more focal CCI that spares RFA, can result in a similar consequence for skilled forelimb use. There is a comparatively larger number of studies examining the effects of ischemic lesions. While a direct comparison between ischemic and contusive lesions has not been made, it is likely that deficits following CCI would be somewhat more severe. A recent study from our laboratory using endothelin-1 injections

(Fang et al., 2010) to produce focal ischemic infarcts in approximately the same cortical territory of CFA, resulted in a skilled reach success rate of about 50% one week after the lesion (lesion only control group) compared with about 20% success in the present study. Whether this difference in severity is related to greater neuronal death with CCI, axonal shearing of corticocortical fibers, or subcortical trauma has yet to be identified.

Reorganization of motor maps in RFA after CCI in rat motor cortex

The present results demonstrate that CCI injuries can be confined to CFA while leaving RFA structurally and functionally intact. No obvious structural damage was evident in any of the CCI rats, at least when examined at the light microscopic level using Nissl stains. Forelimb movements in RFA were evoked in response to ICMS with normal threshold currents that were comparable to previous ICMS studies in intact rats (Neafsey et al., 1986; Nudo et al., 1990; Kleim et al., 1998a). These results at 7 weeks post-CCI parallel those of Boyeson et al. (Boyeson et al., 1991) who demonstrated return of ICMS thresholds to normal values in the adjacent, intact cortex within 15 days. The present results suggest that this model can be effective in examining post-injury reorganization in the more remote premotor cortex following focal CCI. The size of the RFA representation, as defined by ICMS, was consistent across normal intact control rats and similar to the size of RFA reported in previous ICMS papers (*e.g.*, Kleim et al., 1998). However, CCI-1 showed a reduction in total (combined distal and proximal) forelimb area of more than 60%. This reduction occurred in both proximal (69% reduction) and distal (57% reduction, though not statistically significant) representations. In contrast, the combined forelimb representation was unaffected in CCI-2 and CCI-3. However, when a rare outlier was eliminated from the control group, CCI-2 and CCI-3 showed reduced distal representations. Thus, an overall

reduction in forelimb representation was evident in CCI-1 while a redistribution of distal and proximal representations may have occurred in CCI-2 and CCI-3. The smaller size of RFA in CCI-1 and possibly higher currents required to evoke movement were likely due to the difference in CCI parameters. The impactor tip in CCI-1 was placed 0.5 mm more rostral. Although the tip diameter was 0.25 mm smaller, more rostral lesions may have introduced more direct or indirect damage to RFA.

Redistribution of forelimb movement representations in spared motor areas: a case for behavioral compensation?

An important question for understanding mechanisms of recovery after CCI is whether altered movement representations in spared motor regions underlie functional recovery. Following a cortical injury, animals adjust the kinematics of forelimb movements to compensate for deficits in the affected musculature, often resulting in both proximal forelimb and postural compensation (Whishaw et al., 2004). Compensatory use of proximal musculature is also commonly observed in humans after stroke (Cirstea and Levin, 2000). Functional outcomes improve over time, but true recovery may be masked (Whishaw et al., 1991; Whishaw, 2000) or even hindered (Alaverdashvili et al., 2007; Alaverdashvili et al., 2008a) by use of alternative movement strategies (Levin et al., 2009).

In rat CFA, as well as in monkey primary motor cortex, motor skill training induces an expansion of distal forelimb representations at the expense of proximal representations (Nudo et al., 1996a; Kleim et al., 1998a). In the present study, after CCI in CFA, it is reasonable to hypothesize that improved motor behavior on the skilled reach task was related to functional changes in RFA. While there was substantial variability in the component movement

representations in the ICMS forelimb maps, the results suggest a redistribution of movements in CCI-2 and CCI-3 from distal to proximal. Therefore, if RFA plasticity formed the basis for motor recovery after CCI in the present study, and if motor skill acquisition drives the topography of motor maps, RFA plasticity likely supported compensatory motor strategies, rather than recovery of the original movement patterns. A more detailed behavioral analysis of pellet retrieval strategies would be required to resolve this issue.

Structural and functional reorganization is not limited to spared regions of the injured hemisphere, but may occur in homotopic regions of the intact hemisphere as well (Jones and Schallert, 1992b). However, Jones and colleagues have provided substantial evidence that structural changes in both homotopic and heterotopic areas of the intact, contralateral cortex are related to hyper-reliance on the intact limb, rather than recovery of the impaired limb (Allred (Chu and Jones, 2000; Bury and Jones, 2002; Allred and Jones, 2008) et al., 2008; Bury and Jones, 2002; Chu and Jones, 2000). Human neuroimaging studies have also repeatedly shown bihemispheric changes in activation patterns after stroke. However, the functional significance of increased activity in the intact hemisphere is still subject to intense debate. It is not yet clear whether changes in fMRI patterns represent an adaptive, maladaptive or ephiphenomenal effect (Nowak et al., 2009).

Role of corticocortical circuitry in remote effects after CCI

Diaschisis, classically defined, is reduced function in an area remote from, but connected to, an injured brain area (Von Monakow, 1914; Feeney and Baron, 1986) . To ascertain whether changes in RFA topography could be the result, at least in part, of diaschisis, it is important to understand normal corticocortical connectivity patterns in rats. Similar to the relationship

between PMv and M1 in primates (Dancause et al., 2006a), RFA and CFA are reciprocally interconnected (Rouiller et al., 1993) and, more importantly, CFA is the principal target of corticocortical fibers from RFA. Our preliminary anatomical studies suggest that projections are not equally distributed across CFA, as the projection from RFA to the more rostral portion of CFA is particularly dense (Bury and Nudo, unpublished data). Thus, a focal CCI in CFA should damage a significant proportion of corticocortical axons originating in RFA (and reciprocal connections), but more rostrally located lesions would result in differentially more damage. In the present study, pooling the CCI cases demonstrated a significant inverse relationship between the rostral extent of the injury and the resulting size of the RFA map. As discussed earlier, the RFA maps were smallest in CCI-1, the group with a more rostral impactor tip. In 3 of 6 cases (all groups) in which the rostral extent of the injury was between 1.5 and 2.5 mm (relative to bregma), no movements could be evoked using ICMS. These physiological results suggest that while RFA remained structurally and functionally intact in general, its integrity was increasingly compromised as lesions became more proximate. While a quantitative assessment of cell loss in RFA after CCI in CFA has not yet been done, it is reasonable to assume that as lesions edged closer to the rostral forelimb representation, some proportion of RFA neurons underwent retrograde degenerative changes and may not have survived. Previous studies assessing axonal (Yaghmai and Povlishock, 1992; Hall et al., 2008) and dendritic (Posmantur et al., 1996) cytoskeletal structures after CCI found that the acute degenerative effect of CCI spread beyond the contusion's immediate surroundings in a rostralcaudal direction within the injured hemisphere. Hall et al. documented a continuous increase in the volume of degenerating axons that emanate at least 3 mm rostrally and caudally from the contusion epicenter, between 24 hours and 7 days post-CCI (Hall et al., 2008). Morphological observations showed that a number of

swollen, bulbous and disconnected axons became widespread in the injured hemisphere on day 7 post-CCI (Chen et al., 2003). In addition, Posmantur et al. found MAP2 immunoreactivity losses in the ipsilateral cortex, indicating widespread sublethal responses resulting in disassembly of apical dendrites from pyramidal layers and, as such, affecting the function of dendrites leading to the synaptic transmission dysfunction (Posmantur et al., 1996).

Spared motor areas as targets for therapy after TBI

Sprouting of axons in the peri-lesional cortex has been documented during early post-CCI (Harris et al., 2010) and post-ischemic (Carmichael et al., 2005) periods. In addition, it has been suggested that spared motor areas participate in recovery (Liu and Rouiller, 1999). At least in non-human primates, corticocortical axons sprouting from spared premotor areas after M1 ischemic lesions form novel connections with parietal somatosensory hand areas (Dancause (Dancause et al., 2005). While CFA and RFA play different roles in movement control in intact rats (Barth et al., 1990), it is possible that RFA is particularly plastic in its structure and function following CFA injury. After electrolytic lesions in CFA, RFA plasticity has been demonstrated with a form of ICMS that uses long-duration trains of stimulation to elicit complex movements (Ramanathan et al., 2006). In rats that were not rehabilitated, the RFA map of complex movements was similar to that of intact rats. Meanwhile, in rats that received rehabilitative training on a pellet retrieval task, the RFA map significantly expanded. Combined with the present results, it would appear that RFA may be an important substrate for restorative therapies after TBI in rats.

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Table 1. Impactor Tip Characteristics

Group	*Diameter (mm)*	*Shape*	*Stereotaxic coordinates (mm)*
Control (n = 6)	–	–	–
CCI-1 (n = 6)	3.00	Rounded	3.0 L, 0.0 P
CCI-2 (n = 6)	3.25	Flat	3.0 L, 0.5 P
CCI-3 (n = 6)	3.25	Rounded	3.0 L, 0.5 P

L, lateral to midline; P, posterior to bregma.

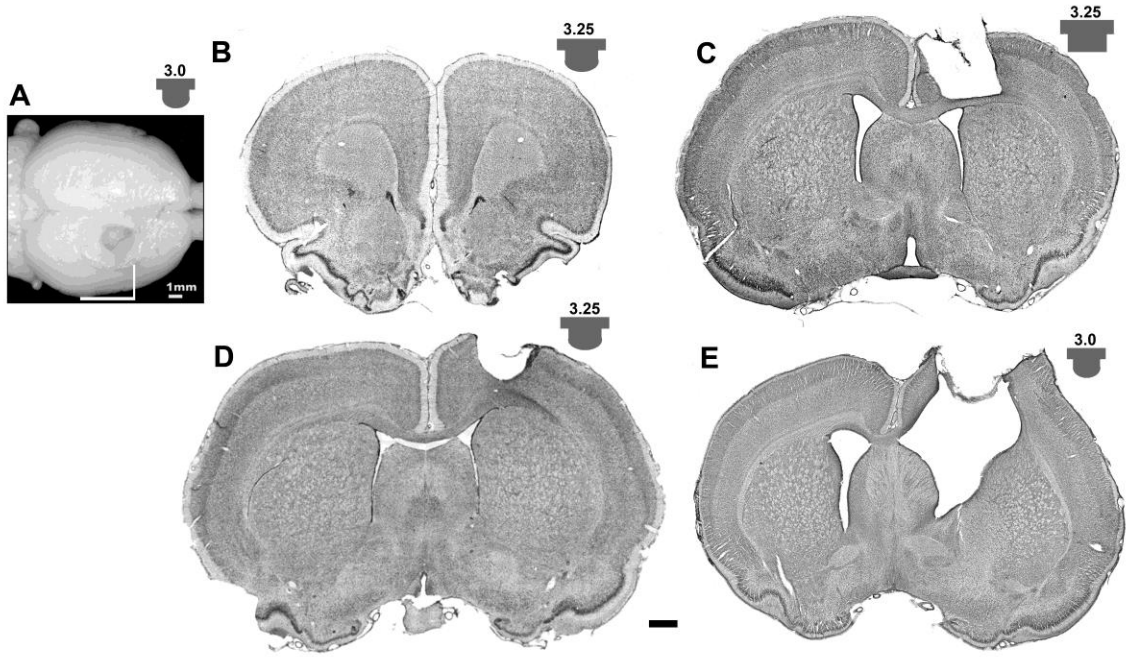

Figure 1

Fig 1 (A) Dorsal view of the lesion (group CCI-1). Coronal sections at (B) 2.7 mm from bregma at the level of RFA (group CCI-3; representative of all cases); (C) ~ 0.2 mm from bregma at the level of CFA (group CCI-2; representative of majority of cases); (D) ~ 0.2mm from bregma at the level of CFA (group CCI-3; representative of 5 cases); (E) ~ 0.2mm from bregma at the level of CFA (group CCI-1; representative of 5 cases). Dorsal view in (A) corresponds to injury severity shown in (E). Scale bars =1mm. Impactor tip shape (rounded or flat) and size (mm) indicated by inset associated with each panel (not to scale). The rounded tip often resulted in some sparing in deep cortical layers at the boundary of the lesion, while the flat tip typically resulted in a more uniform column of damage. No histological evidence of damage was found at the level of RFA (B) in any of the cases

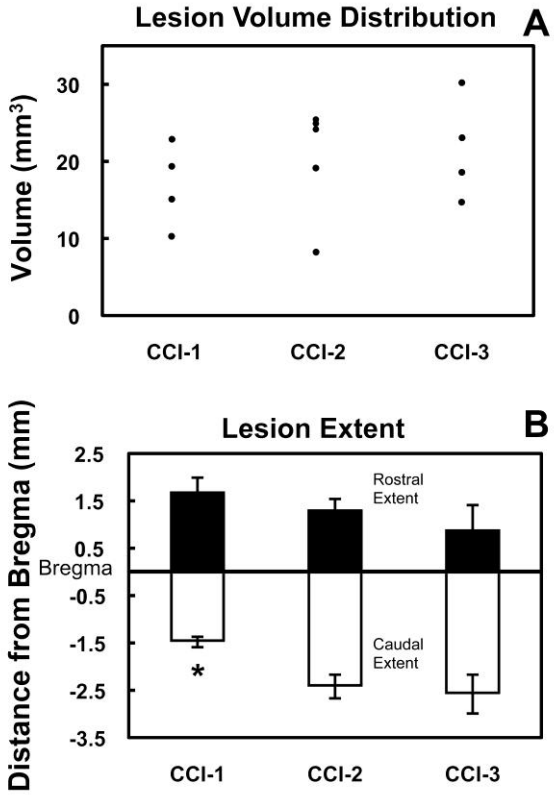
Lesion Volume Distribution
A
Volume (mm3)
30
20
10
0
CCI-1
CCI-2
CCI-3
Lesion Extent
B
Distance from Bregma (mm)
2.5
1.5
0.5
Bregma
-0.5
-1.5
-2.5
-3.5
Rostral Extent
Caudal Extent
*
CCI-1
CCI-2
CCI-3

Figure 2

Fig 2 Histological results. (A) Scatter plot showing distribution of lesion volume in CFA region in each of the three experimental groups. The three different sets of impact tip parameters produced no significant differences in lesion volume. Means are indicated by horizontal lines. (B) Bar graphs showing rostrocaudal extents (± SEM) measured from bregma. The three sets of impact tip parameters produced no difference in *rostral* lesion extent (upper portion of plot) but a significant difference in *caudal* extent (lower portion of plot). The caudal extent of CCI-1 was more anterior than that of CCI-2 and of CCI-3; *$p<0.05$.

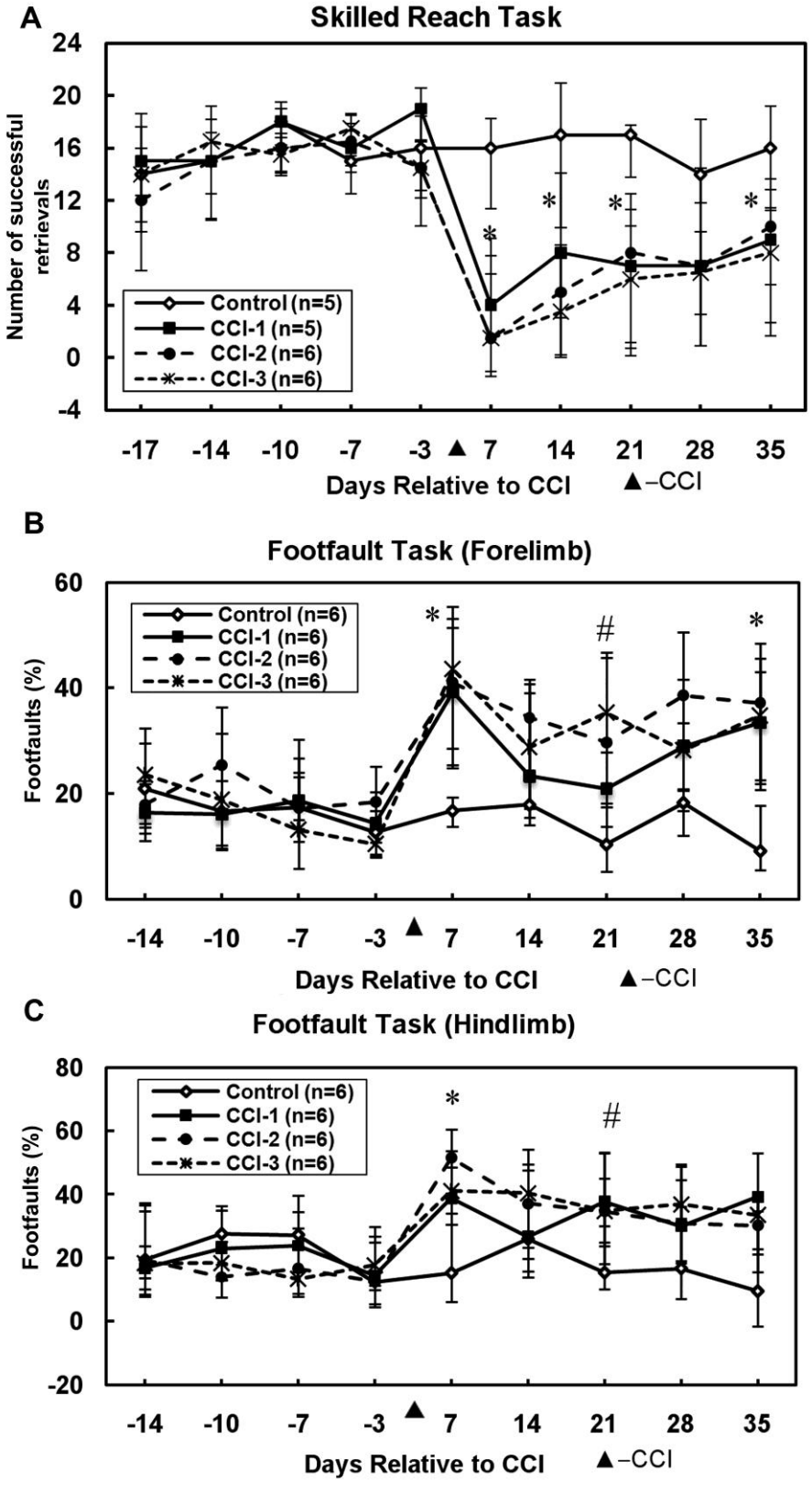
A
Skilled Reach Task
Number of successful retrievals
24
20
16
12
8
4
0
-4
Control (n=5)
CCI-1 (n=5)
CCI-2 (n=6)
CCI-3 (n=6)
-17 -14 -10 -7 -3 ▲ 7 14 21 28 35
Days Relative to CCI
▲–CCI
B
Footfault Task (Forelimb)
Footfaults (%)
60
40
20
0
Control (n=6)
CCI-1 (n=6)
CCI-2 (n=6)
CCI-3 (n=6)
-14 -10 -7 -3 ▲ 7 14 21 28 35
Days Relative to CCI
▲–CCI
C
Footfault Task (Hindlimb)
Footfaults (%)
80
60
40
20
0
-20
Control (n=6)
CCI-1 (n=6)
CCI-2 (n=6)
CCI-3 (n=6)
-14 -10 -7 -3 ▲ 7 14 21 28 35
Days Relative to CCI
▲–CCI

Figure 3

Fig 3 Behavioral results. (A) Median number of successful retrievals (+95% confidence intervals) on testing sessions before and after CCI. Deficits in skilled reach were observed during 4 of 5 post-lesion sessions. On post-lesion day 7, the pellet retrievals of the control and CCI groups correspond to a success rate of >70% and <20%, respectively. The number of successful retrievals of CCI groups on post-lesion day 35 corresponds to a success rate of 41-48%. *$p<0.05$ (each of the three CCIs compared to control group). (B) Median percent forelimb footfaults (+95% confidence intervals) on testing sessions before and after CCI. Increased errors in forelimb locomotion occurred in 3 of 5 post-lesion sessions. *$p<0.05$ (each of the three CCIs compared to control group); #$p<0.05$ (CCI-2 and CCI-3 compared to control group). (C) Median percent hindlimb footfaults (+95% confidence intervals) on testing sessions before and after CCI. Increased hindlimb footfault errors occurred on 2 of 5 post-lesion testing sessions. *$p<0.05$ (each of the three CCIs compared to control group); #$p<0.05$ (CCI-2 and CCI-3 compared to control group).

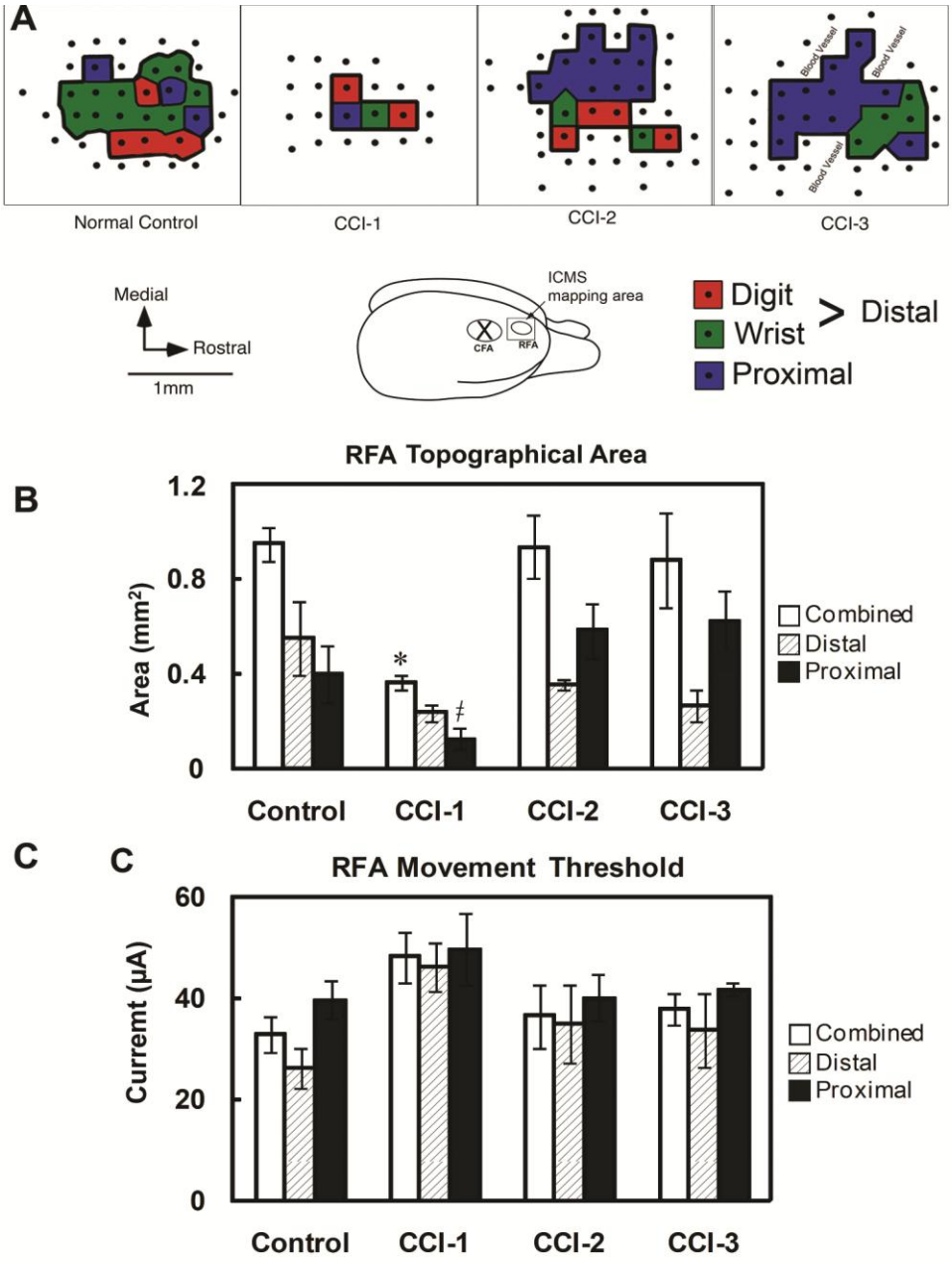
A
Normal Control
CCI-1
CCI-2
CCI-3
Blood Vessel
Blood Vessel
Blood Vessel
Medial
Rostral
1mm
ICMS
mapping area
CFA
RFA
Digit
Wrist
Proximal
> Distal
B
RFA Topographical Area
Area (mm²)
1.2
0.8
0.4
0
*
≠
Combined
Distal
Proximal
Control
CCI-1
CCI-2
CCI-3
C
C
RFA Movement Threshold
Curremt (μA)
60
40
20
0
Combined
Distal
Proximal
Control
CCI-1
CCI-2
CCI-3

Figure 4

Fig 4. Neurophysiological results. (A) Color-coded maps of movements evoked by ICMS in RFA 7 weeks after CCI. Inset shows ICMS mapping area illustrated on a dorsolateral view of the rat brain. Cases illustrated are representative of movement representations in each group. Each map was bordered by face, whisker or neck movements. Dots reflect the ICMS penetration sites. Note the obvious smaller RFA size in CCI-1. In this figure, distal forelimb movements are subdivided further into digit and wrist movements. Note that no digit movements were evoked from any of the CCI-3 animals. In these cases, distal forelimb movements consisted of wrist movement s exclusively (B) Movement representation areas (± SEM) in RFA. The combined RFA (distal + proximal representations) was smallest in CCI-1. *$p<0.05$ (CCI-1 compared to each of the other groups); # $p<0.05$ (CCI-1 compared to CCI-2 and CCI-3). (C) Threshold currents required to evoke movements in RFA (± SEM). Although there seem to be an overall tendency for higher currents in CCI-1, no significant difference was detected among groups.

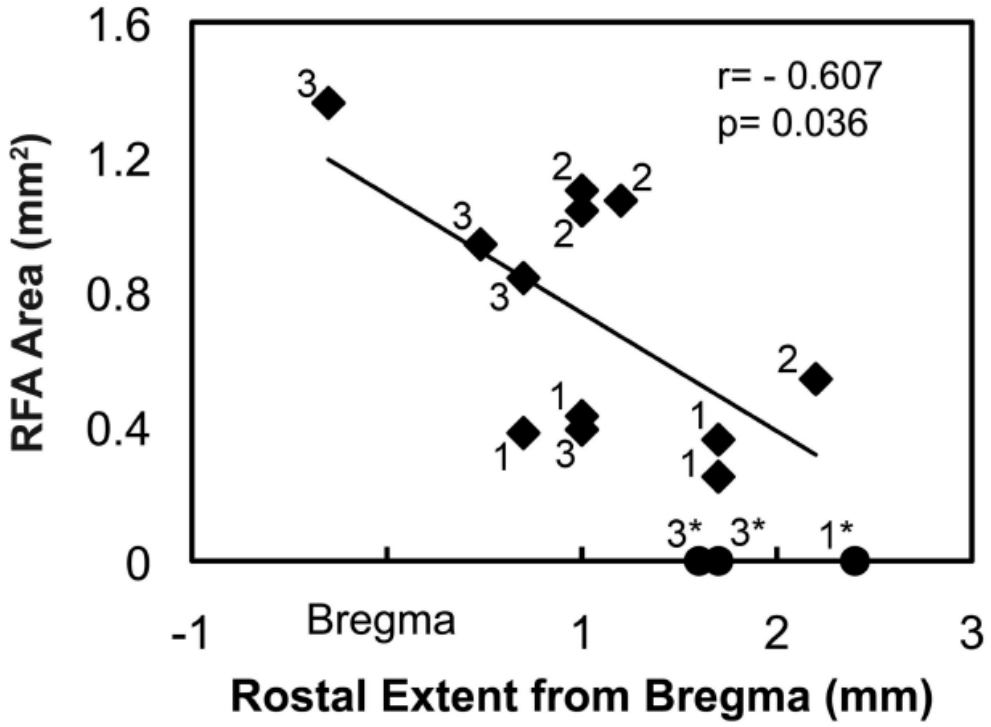

Fig 5 Neurophysiologically-based RFA area as a function of rostral lesion extent. The more rostral the lesion extended, that is, the closer the lesion was to RFA, the smaller the RFA area obtained during the post-CCI mapping procedure (diamond). Three cases in which we could not evoke movements (and therefore, excluded from the statistical analysis) are also illustrated (circle). Numbers indicate CCI group.

CHAPTER THREE

Use- and Time-dependent Reorganization of Motor Representations after Cortical Ischemic Injury

ABSTRACT

To better understand injury-behavior interaction on cortical plasticity in over time, the present study longitudinally examined the effect of rehabilitative training on map reorganization in the spared rostral forelimb area (RFA) associated with motor recovery after a restrictive ischemic lesion to caudal forelimb area (CFA). We tested the hypothesis that the rehabilitation-aided recovery may be supported by delayed, extensive cortical reorganization in the spared RFA after a focal lesion to CFA. We further tested the relative contribution of compensatory behavioral processes in recovery.

Adult rats were assigned to rehabilitative training at the tray and single pellet reach tasks or non-rehabilitation groups following endothelin-1 induced focal ischemic lesion in CFA. Half of the rats in each group underwent terminal cortical mapping procedure a day (post-lesion day, PLD 18) or 20 days after the completion of rehabilitative training (PLD 38), to be assessed for cortical reorganization RFA.

Rehabilitative training resulted in more rapid, greater functional recovery and more normalized movement quality. Typical bordered RFA could not be derived on PLD 18, but a few forelimb movement sites rostrally adjacent to the lesioned CFA were elicited in both rehab and non-rehab groups. Whereas on PLD 38, forelimb movements were bordered by face and trunk representations within the typical coordinates of RFA. The mapped RFA was larger in the rehabilitative group than non-rehab group; and the restored area of the rehabilitation group was indistinguishable from the size of intact RFA.

In summary, neural plasticity in RFA appeared to be dependent both on post-lesion motor experience and time. The neurophysiological examinations demonstrated that rehabilitation

restored the physiological integrity of the RFA, which otherwise, would have been partially lost to the lesion in CFA. Further, we showed that functional recovery without compensatory strategies is possible with adequate cortical engram through extensive reorganization. We speculate that RFA has vicariously taken over, at least partially, the original function that was mediated by the CFA.

INTRODUCTION

Cortical reorganizations after brain injuries have been consistently observed in animal models as well as in clinical studies (Castro-Alamancos and Borrel, 1995; Frost et al., 2003). For example, with post-injury rehabilitative intervention, the forelimb motor cortex adjacent to the lesioned area (peri-lesion area) undergoes extensive reorganization to preserve itself which otherwise would be lost to the focal ischemia (Nudo et al., 1996a). Most recently, we reported cortical reorganization in the rat rostral forelimb area during spontaneous recovery after inducing injury in the caudal forelimb area (CFA, analog to non-human primate M1) using cortical controlled contusion model (Nishibe et al., 2010). The rostral forelimb area (RFA) is thought to be an analog topographical structure to non-human primate premotor area and SMA (Neafsey and Sievert, 1982; Nudo and Frost, 2007) from which the cortico-spinal projections terminate in the motor nuclei of cervical level spinal cord, along with the CFA. These findings suggest that reciprocally connected areas to an area of a focal lesion, in particular, represent advantages in repairing the damaged function. Rehabilitative intervention was targeted on hand and arm use in this study. Because dexterity reflects represented area (Monfils et al., 2005), reinstating the distal forelimb areas post-injury should theoretically help restore some corresponding functional use.

It is important to note, however, neither the changes in motor representations nor the recovery of upper limb function appear to follow a linear progression as a function of time. Also, timing of interventions has been variable among clinical studies, producing ambiguous results (Barbay and Nudo, 2009). Various anatomical changes appear in the rat cortices during the 2nd to 5th post-stroke weeks, which often correspond to the period when most of the spontaneous recovery takes place (Jones and Schallert, 1992b; Stroemer et al., 1995; Jones and Greenough,

1996; Qu et al., 1998; Jolkkonen et al., 2003). Neurophysiological changes measured by cortical topography, in contrast, seem to occur later than the behavioral recovery, at least in non-human primates (Eisner-Janowicz et al., 2008). To better understand the experience-time interaction on post-injury cortical plasticity, the present study examined the effect of rehabilitative training on functional recovery at a skilled reach task and map reorganization in the spared rostral forelimb area after a restrictive ischemic lesion to caudal forelimb area, at two time points using intracortical microstimulation. The two neurophysiological assessment time points corresponded to a day after termination of rehabilitative training (post-lesion day PLD18) and 21 days after the termination (PLD 38)

Recent studies support the notion that functional recovery occurs through compensatory strategies (Alaverdashvili et al., 2008a; Alaverdashvili and Whishaw, 2010). Whishaw stated that "unless the lost innate cortical engram can be replaced, recovery occurs through compensation" (Whishaw, 2000). Thus, we secondarily tested whether functional recovery without compensation is possible with "adequate" cortical engram through reorganization.

The major findings were 1) rehabilitative training restored functional recovery quantitatively and movement quality to baseline pre-lesion levels at the skilled reach task, 2) improvements in movement quality occurred simultaneously with quantitative recovery and continued past quantitative recovery plateau, 3) on PLD 38, and not PLD 18, the rehab group displayed a larger representation area, indistinguishable size to the intact level, than the no rehab group, 4) RFA map expansion in the rehab group coincided with improvements in quality of the movements during a reach rather than with a increase in quantitative success, suggesting cortical areal expansion reflect not only the quantitative endpoint recovery but also the improvement in the behavior quality.

In summary, we showed that functional recovery without compensatory strategies is possible with adequate cortical engram through extensive reorganization. Further, the neurophysiological examinations demonstrated that rehabilitation restored the physiological integrity of the RFA, which otherwise, would have been partially lost to the lesion in CFA. Neural plasticity in RFA appeared to be dependent both on post-lesion motor experience and time. We speculate that RFA has vicariously taken over, at least partially, the original function that was mediated by the CFA.

MATERIAL AND METHODS

Subjects and Group Assignments

A total of 25 adult male Long-Evans hooded rats (Harlan, IN; 300-400g) were used. Rats were 4-5 months of age at the initiation of the study. All animal use was in accordance with National Institutes of Health regulations, and approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center. Rats were singly housed in a transparent cage with *ad libitum* food and water on a 12h:12h light:dark cycle, with ambient temperature maintained at 68-71^{o}F.

After the ischemic cortical injury procedure (see below), animals were randomly assigned to one of two post-lesion conditions: rehabilitative training (rehab; n=13) or no rehabilitative training (no rehab; n=12). Rats in the two conditions were randomly divided further into survival times of 18 days (n=6 from each condition; short-term survival) or 38 days (n=7 from rehab condition, n=6 from no rehab condition; long-term survival) from the time of injury induction, for neurophysiological assessment of cortical topography. Thus, there was a total of 4 groups: 1)

rehab/short-term survival (n=6), 2) rehab/long-term survival (n=7), 3) no rehab/short-term survival (n=6), 4) no rehab/long-term survival (n=6).

Behavioral Assessment

Pre-Lesion Training and Assessment on Skilled Reach Task. Pre-lesion training was conducted using a single-pellet reach and retrieval task (Fang et al., 2010). Each animal was placed in a Plexiglas reaching box (25cm in length * 25cm in width * 35cm in height) with a 1cm-wide reaching slot, designed to restrict the use of the non-dominant forelimb. An external shelf was positioned 3cm above the bottom plane of the box. A single banana-flavored food pellet (45mg, Bioserve) was placed on a shallow indentation on the external shelf 2cm from the front wall of the reaching box. Forelimb dominance was then determined during 20 pellet reaches on two consecutive days. Forelimb dominance was defined as the limb used on more than 50% of the reaches. Then, the rats underwent pre-lesion training for the subsequent 10 days (60 pellets per session per day) using the dominant forelimb. A pre-lesion training session typically lasted 10 to 20 minutes. Video recordings of each session were obtained for later analysis of motor performance and movement kinematics.

Pre-lesion motor scores were based on performance during an assessment session, consisting of 20 pellet trials, conducted after pre-lesion training was completed, and within 3 days prior to the lesion. As pre-lesion performance for each rat was consistent across days during the assessment week, only the last assessment day prior to the lesion was used to define the baseline performance scores. We tallied: a) total number of successful retrievals and b) total number of reaching attempts (limb advances).

a. *Total number of successful retrievals.* Each trial began with the presentation of a single pellet and ended with 1) a successful reach and retrieval, 2) a displacement of the pellet (unsuccessful attempt), or 3) 5 consecutive unsuccessful attempts accompanied by limb advance in which the pellet was not contacted. A successful retrieval required the rat to grasp the pellet and transport it to the mouth.

b. *Total number of reaching attempts (limb advances).* A reaching attempt was defined by a movement in which the forelimb passed through the reaching slot in an attempt to contact the pellet.

Post-lesion Assessment and Rehabilitative Training on Skilled Reach Task. Post-lesion sessions were conducted daily in the rehab groups starting on PLD 7. At the beginning of each session, 20 assessment trials (i.e., 20 pellets) were conducted. During rehabilitative training (PLD 8-17), assessment trials were followed by rehabilitative training trials (60 pellets). Rehabilitative training was conducted in two phases: During Phase I (PLD 8-12), training was conducted on a tray-reaching task (Whishaw et al., 1986; Maldonado et al., 2008). In this task, rats retrieved pellets from a pellet-filled tray (4cm in length * 3cm in width * 0.5cm in height) attached to the external shelf of the skilled reach box. During Phase II (PLD 13-17), training was conducted on the single-pellet retrieval task described above (60 pellets per session per day). Thus, throughout rehabilitative training, each rat received a total of 80 pellet trials per session (20 assessment trials + 60 training trials).

Rats assigned to the no rehab groups had a similar number of pellets available from the floor of the skilled-reach box. To minimize training effects, they were assessed using 20 pellets on PLD 7, 12 and 17. Data from corresponding days in the rehab groups [PLD 7 (pre-training), 12

(5^{th} training day) and 17 (10^{th} and last day of training)] were used for statistical comparisons to the no rehab groups.

On PLD 18, rats from the short-term survival groups underwent a terminal neurophysiological mapping procedure (see below). The remaining rats (long-term survival groups) were assessed every 5 days through PLD 37 (i.e., PLD 22, 27, 32 and 37). Rats in the long-term survival groups underwent the terminal neurophysiological mapping procedure on PLD 38.

Analysis of Movements During Skilled Reach Task. Kinematic analysis was performed using the Eshkol-Wachmann Movement Notation adapted by Whishaw and colleagues (Eshkol, 1958; Whishaw and Pellis, 1990). The kinematic assessments were performed on the long-term survival groups on PLD -1 (pre-lesion day), 7, 12, 17, and 37. For each session, three randomly selected trials that resulted in a successful retrieval were analyzed for the quality of forelimb movements. Specific movements consisted of pronation, grasp, supinate I, supinate II, and release (Whishaw et al., 1991; Alaverdashvili and Whishaw, 2010). For each movement, a score of 0 was assigned if the movement was normal, 0.5 if the movement was abnormal but present, or 1 if the movement was absent. Briefly, normal movements were defined as follows (Alaverdashvili and Whishaw, 2010)

1) Pronation: the elbow abducts with a movement of the upper arm pronating the paw over the target in an arpeggio movement.
2) Grasp: the arm remains still, while the digits close to grasp the food and then the paw is extended at the wrist and raised.

3) Supination I: the paw supinates by 90º by adducting the elbow as the paw holding the food is withdrawn through the slot.

4) Supination II: the paw is supinated as it approaches the mouth so that the palm faces the mouth.

5) Release: the food pellet is released into the mouth by opening the digits.

Footfault task. This task assessed forelimb performance during locomotion (Gilmour et al., 2005). The footfault task was conducted on PLD -1, (the pre-lesion day) 7, 12, 17 and in the long-term survival groups, every 5 days through PLD 37 (i.e., PLD 22, 27, 32 and 37). Each rat was placed onto an elevated grid (57cm × 44cm with 4cm × 4cm grid opening) and allowed to locomote freely for 3 minutes. The number of steps and the number of slips through the grid made with each forelimb were recorded. Performance was defined as the percentage of footfaults per step made with forelimb contralateral to the lesion.

Ischemic Cortical Lesion Procedure

First, rats were anesthetized with 3% isoflurane gas followed by ketamine (100mg/kg, IP) and xylazine (5mg/kg, IM). Additional doses of ketamine (20mg/kg/hr IM) were used when necessary to maintain an adequate anesthetic level. Following a scalp incision, exposing the skull over the frontal lobe contralateral to the dominant forelimb, six 0.7-mm diameter holes were drilled into the skull over the caudal forelimb area (CFA) at the following stereotaxic coordinates: anteroposterior +1.5, +0.5, and -0.5 mm and mediolateral +2.5 and +3.5 mm from bregma (Fang et al., 2010). To induce cortical ischemia, 0.33 μL of endothelin-1 (ET-1; Peninsula Laboratories, San Carlos, CA; 0.3mg dissolved in 1 mL saline) was injected into each

of the six holes at a rate of 3nL/s and at a depth of 1.5mm from the cortical surface, through a tapered micropipette (tip size 160μm outer diameter, barrel size 900μm outer diameter) attached to a Hamilton syringe using a microsyringe injector (UltraMicro Pump III,World Precision Instruments, Inc. Sarasota, FL). Following the completion of the ET-1 injections, the incision was closed. Then, an analgesic (Bupivicaine 0.5-1mL, topical) and triple antibiotic ointment (Neosporin, topical) were applied. Penicillin (0.15mL, SC) was administered once after surgery. Buprenorphine (0.05mg/kg, SC) and acetaminophen (20mg/kg, orally) were administered once after surgery and twice the following day.

Intracortical Microstimulation (ICMS) Procedure

As noted above, ICMS procedures were conducted on PLD 18 and PLD 38 in short-term and long-term survival groups, respectively. Standard ICMS techniques were used as described in previous publications (Nishibe et al., 2010). Rats were anesthetized with an initial dose of ketamine (100mg/kg IP) and xylazine (5mg/kg IM). Additional doses of ketamine (20mg/kg/hr IM) were used when necessary to maintain adequate anesthesia. Following a craniectomy over frontal cortex (1.5-4.5mm anterior, and 0.5-2.5mm lateral to bregma), the dura was removed. Then, a high-magnification digital image of the cortical surface vasculature was obtained and imported into a graphics program (CANVAS 3.5, Deneba Systems, Miami, FL). Grid lines ($250*250\mu m^2$) were overlaid on the digital photograph to reference the placement of the microelectrode. The stimulation microelectrode was a NaCl-filled glass micropipette (tapered to 15-20μm o.d. tip; impedence = 500-700kΩ). A platinum wire was inserted into the NaCl and attached to a constant-current stimulator (Model BSI-2, BAK Electronics, Mount Airy, MD). The electrode was positioned sequentially at each of the grid intersections and lowered

perpendicular to the cortical surface to reach the level of layer V (~1700µm) using a hydraulic microdrive (Model 650, David Kopf Instruments, Tujunga, CA). ICMS pulses consisted of a train of 13, 200µs monophasic cathodal pulses at a rate of 350 Hz delivered at the rate of 1/sec (Master-8; A.M.P.I., Jerusalem, Israel). Movements elicited by ICMS stimulation were inspected visually by two observers and recorded. The minimum current required to evoke a movement was also recorded. The maximum current used in the experiments was 80µA. Elicited movements were assigned a color code and recorded at the penetration site on the digital photograph using the graphics software. The rostral and caudal extents of the forelimb representation relative to bregma was determined using the superimposed ICMS grid in CANVAS and the areal size was measured from the reconstructed movement maps using NIH IMAGE vl.61 (Nudo et al., 1992). Digit and wrist movement representations comprised the "distal forelimb area", while elbow movement representations comprised the "proximal forelimb area". The combined distal and proximal movement representations comprised the "total forelimb area".

Histology

Immediately following the ICMS procedure, rats were euthanized by an overdose of Buthanasia (1.0cc) and perfused transcardially with 4% paraformaldehyde in 0.1M PBS, pH 7.35. Each brain was removed and postfixed in 20% glycerol for a minimum of two days, then sectioned coronally (30µm thickness) using a cryostat. A series of sections was stained with a Nissl stain for verification of the lesion location and volume (Donoghue and Wise, 1982). Lesion volume estimation was obtained by the difference of the cortical volume of the injured

hemisphere subtracted from that of the intact hemisphere (Nishibe et al., 2010), using the Cavalieri method in StereoInvestigator (Microbrightfield, Inc., Williston, VT).

Statistical Analysis

Statistical analyses were performed using a general linear model using SPSS 20 (SPSS IBM Inc., Chicago, IL). The three outcome measures (the number of successful retrievals, attempts and footfaults) were analyzed by two-way repeated measures ANOVAs (Group*Time) for each effect: a) lesion (2 groups, n=12 and 13, *2 assessment points), b) rehabilitative training (2 groups, n=12 and 13, *2 assessment points), and c) persistence (2 groups, n=6 and 7, *4 assessment points during the post-rehabilitation, no intervention period). Since the movement analysis score was based on an ordinal scale, a non-parametric test was used (Wilcoxon, two-tailed) to compare the normality score on post-lesion assessment days to baseline performance. Lesion volume, rostral and caudal extents of the forelimb topographical areas, ICMS topographical areas and stimulation thresholds were all analyzed using a one-way ANOVA. All post-hoc comparisons were performed with Tukey tests when appropriate. An alpha level of 0.05 was required for significance in all statistical tests.

RESULTS

Lesion Anatomy

Fig. 1A illustrates the Nissl-stained coronal section showing the lesion in the motor cortex from one representative rat at 1.2mm rostral from bregma, at the level of CFA. Histological inspection at the level of CFA showed that in all 24 cases all cortical layers were destroyed, with the corpus callosum intact. At the level of RFA, no cortical damage was evident under light

microscopy in any of the cases. There was no difference in lesion volume among the 4 groups ($F_{3, 23}$=0.206 p=0.891, Fig. 1B). Mean lesion volume was 11.84±1.22mm^2 and 12.04±1.52mm^2 for short-term and long-term survival groups, respectively. Although neurophysiological maps of CFA were not derived, in all cases, the lesion location corresponded to typical stereotactic coordinates for CFA (Neafsey et al., 1986; Nudo et al., 1990).

Effects of Rehabilitative Training on Behavior

As anticipated, there were no statistical differences in the retrieval, attempts and footfault data between the rehab/short-term survival and rehab/long-term survival groups through the end of rehabilitative training (PLD -1 through PLD 17; Group $F_{1, 11}$=0.012, p=0.916 for retrievals, Group $F_{1,11}$=1.067, p=0.324 for attempts, Group $F_{1, 11}$= 2.458, p=0.156 for footfaults). Therefore, the data for these groups were pooled for each outcome measure through PLD 17. Likewise, there were no statistical differences between the no rehab/short-term survival and no rehab/long-term survival groups during the same time period (Group $F_{1, 11}$=2.340, p=0.157 for retrievals, Group $F_{1, 11}$=0.487, p=0.500, footfault $F_{1, 11}$= 1.638, p=0.230). The data for these groups were also pooled through PLD 17. It should be noted that statistical significance in subsequent analyses was still evident without pooling.

Separate repeated measures ANOVAs then were conducted over three different time periods to determine the: 1) Lesion effect (PLD -1 and PLD 7), 2) Rehabilitative training effect (PLD 12 and PLD 17), and 3) Persistence effect (PLD 22 through PLD 37). The three time periods are illustrated in Fig. 2A.

Successful retrievals (Fig. 2B)

Lesion effect (PLD -1 and 7). The analysis revealed no significant Group ($F_{1,23}$=0.173, p=0.681) or Interaction effect ($F_{1,23}$=0.060, p=0.808) but a significant effect of Time ($F_{1,23}$=105.908, p<0.0001), demonstrating that the lesion resulted in a significant deficit in pellet retrieval ability at PLD 7 in both rehab and no rehab groups.

Rehabilitative training effect (PLD 12 and 17). There were significant Group (Group $F_{1, 23}$=8.465, p=0.008), Time ($F_{1, 23}$=20.828, p<0.0001) and Interaction effects ($F_{1, 23}$=9.428, p=0.005). Post-hoc Tukey tests showed that there was no difference between groups on PLD 12 (p>0.05), but the rehab group retrieved a significantly higher number of pellets than the non-rehab group on PLD 17 (p<0.01).

Persistence effect (PLD 22 through 37). There was a significant Group effect (Group $F_{1, 11}$=6.981, p=0.023) but no effect of Time ($F_{3,33}$=0.018, p=0.997) or Interaction effect ($F_{3, 33}$=0.165, p=0.919), demonstrating that the rehabilitative training effect was maintained during the follow-up period from PLD 22 to 37.

Reaching attempts (# forelimb advances) (Fig. 2C)

Lesion effect (PLD -1 and 7). Similar to the retrieval analysis, the ANOVA revealed neither Group ($F_{1,23}$=0.027, p=0.870) nor Interaction effects ($F_{1,23}$=0.004, p=0.949), but a significant effect of Time ($F_{1,23}$=31.064, p<0.0001), demonstrating that the lesion resulted in a significant increase in the number of reaching attempts at PLD 7 in both rehab and no rehab groups.

Rehabilitative training effect (PLD 12 and 17). The ANOVA revealed no Time ($F_{1,23}$=1.168, p=0.291) nor Interaction effect ($F_{1,23}$=1.974, p=0.173) but a significant Group effect ($F_{1,23}$=5.922, p=0.023), indicating that the rehab groups made fewer reaching attempts to retrieve pellets compared with the no rehab groups.

Persistence effect (PLD 22 through 37). A significant Group effect ($F_{1,11}$=27.353, p<0.0001) was detected while there was no Time ($F_{3,33}$=0.613, p=0.611) nor Interaction effect ($F_{3,33}$=0.786, p=0.510), suggesting that the rehabilitative training effect was maintained during the follow-up period from PLD 22 to PLD 37.

Footfault task (Fig. 2D)

Lesion effect (PLD -1 and 7). The analysis showed no Group ($F_{1,23}$=0.102, p=0.752) nor Interaction effect ($F_{1,23}$=0.055, p=0.816), but a significant Time effect ($F_{1,23}$=43.201, p<0.0001). Thus, both groups displayed a similar increase in the percent footfaults after the lesion.

Rehabilitative training effect (PLD 12 and 17). None of the effects were significant during the rehabilitative period (Group $F_{1,23}$=1.500, p=0.233; Time $F_{1,23}$=1.466, p=0.238; Interaction $F_{1,20}$=0.118, p=0.734).

Persistence effect (PLD 22 through 37). The analysis revealed that there was a Time effect ($F=_{3,\ 33}$=3.953, p=0.016) but no Group ($F_{1,\ 11}$=0.924, p=0.357) nor Interaction ($F_{3,\ 33}$=0.392, p=0.760) effect, suggesting that the percent footfaults decreased in both groups at a similar rate after PLD 22.

Analysis of movements during skilled reach task (Fig. 3)

No significant difference was detected in supination I, pronation or grasp in either group at any of the time points (all Z<-1.4, p>0.1). Therefore, the following description will focus only on supination II and release.

Lesion effect (PLD -1 and 7). On PLD 7 both groups displayed a significant change from baseline performance in supination II (Fig. 3A) (rehab group Z=-2.214, p=0.027; no rehab group

Z=-2.032, p=0.042) and release (Fig. 3B) (rehab group Z=-2.207, p=0.027; no rehab group Z=-2.060 p=0.039).

Rehabilitative training effect (PLD 12 and 17). On PLD 12, both groups displayed a significant deviation from baseline performance in supination II (Fig. 3A) (rehab Z=-2.214, p=0.027; no rehab Z=-2.207, p=0.027) and release (Fig. 3B) (rehab Z=-2.207, p=0.027; no rehab Z=-2.032, p=0.042). Further, compared with baseline scores, both groups still showed a significant difference on PLD 17 in supination II (rehab Z=-2.023, p=0.043; no rehab Z=-2.207, p=0.027) and release (rehab Z==2.023, p=0.043; no rehab Z=-2.032, p=0.04).

Persistence effect (PLD 22 through 37). On PLD 37, the no rehab group showed a significant difference compared with the baseline score in supination II (Z=-1.992, p=0.046), but not release (Z=-0.949, p=0.343). In contrast, the rehab group no longer demonstrated a significant difference in supination II (Z=-1.826, p=0.068) or release (Z=-0.577, p=0.564) compared with baseline.

Effects of Rehabilitative Training on Cortical Physiology

RFA Topography

Previously, we reported in intact rats the topographical area of RFA (Nishibe et al., 2010), a separate forelimb representation found rostral to CFA, similar to other reports (Neafsey and Sievert, 1982; Sanderson et al., 1984). The face and neck representations typically divide RFA and CFA. Normal RFA is surrounded caudally by areas where neck and face movements are evoked (jaw and tongue), medially by vibrissae movements, laterally by face movements and rostrally by non-responsive sites. Based on our previous ICMS data using identical procedures in intact rats (n=5), we determined the typical location of RFA with respect to bregma. The expected caudal limit of RFA was defined as the mean caudal limit of the normal, intact group

minus 2 standard deviations. Thus, the expected caudal limit of RFA was 2.39mm rostral to bregma. Likewise, the expected rostral limit of RFA was defined as the mean rostral limit of the normal, intact group plus 2 standard deviations. Thus, the rostral limit of RFA was 4.58mm rostral to bregma (see Fig. 5).

Post-lesion maps on PLD 18 and PLD 38 were derived by exploration of a wide swath of cortical territory rostral to the lesion, extending well beyond the typical location of RFA. On PLD 18, forelimb movement representations were bordered caudally by the lesion and rostrally by neck and face movement representations and non-responsive sites. On PLD 38, forelimb topography was contiguously surrounded caudally by areas where neck and face movements were evoked (jaw and tongue), medially by vibrissae movements, and laterally by face movements and rostrally by non-responsive sites. On both PLD 18 and 38, forelimb movements were evoked largely from a cortical territory within two standard deviations of the rostral and caudal limits of typical RFA. However, on PLD 18, a small portion of RFA extended caudal to these limits (see Fig 4C and D, Fig. 5).

Area and threshold results were successfully obtained in 21 of 25 rats (6/6 in the rehab/short-term survival group, 6/6 in the no rehab/short-term survival group, 5/7 in the rehab/long-term survival group and 4/6 in the no rehab/long-term survival group). In the remaining 4 rats (two rat from rehab/long-term survival group and two rats from no rehab/long-term survival group), no evoked movements were observed from ICMS stimulation in RFA. Such outcomes are not uncommon in ICMS experiments, and are typically attributable to improper anesthetic depth that cannot be corrected during the course of the procedure. The same outcomes were observed during our previous mapping experiments (Nishibe et al., 2010). A Pearson chi square test show

that the map experimental outcome was independent of the post-lesion experience (chi-square=0.034, 2 tailed p=0.853).

RFA topographical area (Fig 4A)

ANOVA demonstrated a significant group difference in the total forelimb area, inclusive of the distal and proximal forelimb area ($F_{3, 20}$=20.837, p<0.0001). Post-hoc tests indicate that RFA of the rehab/long-term survival group was larger than any of the other three groups (ps≤0.008). Also, the RFA of *no rehab*/long-term survival group was larger than the *rehab*/short-term survival group (p=0.01). Thus, while some increase in RFA area was observed in both groups, the rehab group experienced a substantial increase during the period following rehabilitation.

Further analysis to differentiate distal vs. proximal representations revealed that most of the change in total forelimb area was due to changes in distal area ($F_{3, 20}$=14.595, p<0.0001). Post-hoc tests indicate that the distal forelimb area of the rehab/long-term survival group was larger than any of the other three groups (ps≤0.006). No group difference was detected in the proximal forelimb areal representations ($F_{3, 20}$=1.086, p=0.382). In Fig. 4A, RFA area in normal, intact rats is shown for comparison.

RFA Threshold Currents (Fig 4B). ANOVA showed no significant difference in the threshold currents required to evoke total forelimb movements ($F_{3,20}$=0.685,p=0.574), distal forelimb movements ($F_{3,20}$=0.130,p=0.941), or proximal forelimb movements ($F_{3,20}$=0.073,p=0.974). In Fig. 4B, RFA threshold currents in normal, intact rats is shown for comparison.

Rostral and caudal extent of forelimb representations (Fig. 5)

An ANOVA demonstrated a significant group difference in the rostral extent of RFA ($F_{3, 20}$=5.424, p=0.008). The map extended more rostrally in rehab and no rehab/long-term survival groups compared with rehab/short-term survival group (ps<0.05, Tukey post-hoc). Thus, the map extended more rostrally in both rehab and no rehab groups. No other paired-comparisons were significant (p<0.05). There was no significant difference among groups in the caudal extent ($F_{3,20}$=2.952, p=0.062).

DISCUSSION

Our aim was to determine longitudinal changes in the spared cortical physiology adjacent to the lesion during both spontaneous and rehabilitation-aided motor recovery following a restrictive ischemic lesion to CFA. Also, we aimed to determine whether motor recovery without compensation was possible with adaptive cortical plasticity through topographical reorganization. In summary, we demonstrated 1) rehabilitative training restored functional recovery quantitatively and qualitatively to baseline pre-lesion levels, 2) quantitative behavioral plateau occurred earlier in rehabilitated animals than in non-rehabilitated ones, 3) qualitative recovery occurred simultaneously with quantitative recovery and continued past quantitative recovery plateau, 4) continued practice was not necessary to retain the recovery level and to induce expansion in forelimb representations, 5) rehabilitative training group showed an expansion in the rostral edge of the forelimb area while maintaining the caudal edge over time whereas no rehabilitation group show no map shift in either edges, 6) Maps derived on PLD 18 were bordered caudally by lesion, rostrally by face and neck representations, 7) on PLD 38, forelimb

representations were isolated by trunk and neck representations, 8) on PLD 38 but not on PLD 18 the rehab group displayed a larger representation area, indistinguishable size to the intact level, than the no rehab group, 9) RFA map expansion in the rehab group coincided with improvements in performing supination during a reach rather than with a increase in quantitative success, suggesting cortical areal expansion mediate the improvement in behavior quality (Fig 6).

Together, restoration of the RFA appears to be dependent both on post-lesion motor use and time. Quantitative behavioral recovery may be contingent on and may precede the expansion in the spared cortex. Further, improvements in the movement quality may be supported by expansion of the forelimb representations.

Qualitative Behavioral Recovery may still occur past quantitative recovery plateaus.

CFA exhibits more pertinent neural adaptation in acquiring the motor skills to reach and retrieve pellets than RFA (Rample et al., 2001; Kleim 2003; Gharbawie et al 2007), the relation similar to the primary motor cortex in non-human primates (Friel et al., 2005). We induced a focal damage in CFA, the area from which most cortical motor fibers descend, and produced significant impairments in forelimb motor function. Our lesion model produced significant impairments in forelimb motor function detected in all behavioral outcome measures.

While the rehabilitated group reached a recovery plateau in the number of successful pellet retrieval by PLD 17 (the last day of training for the rehab group), no rehabilitated group reached its plateau, later on PLD 22. The result suggests rehabilitated subjects may recover more rapidly. The plateau often occurs within a few weeks after similar cortical lesions (Whishaw, 2000). The rehabilitated group kept a superior performance to the no rehab group at the pre-lesion level through the rest of the experiment. The difference in the two groups in the number of attempts also persisted, suggesting that the motor training helped reach to be better aimed. The sustained

increase in reaching attempts in non-rehabilitated rats indicates that they may have developed "learned-bad use" (Alaverdashvili et al., 2008a).

The continued practice was not necessary to retain the behavioral recovery and to induce cortical topographical expansion in rehabilitation group. Some forms of neural plasticity in intact rat is said to require the continued performance of the skill to induce topographical changes (Kleim et al., 1998a). Though the present finding offers an example of how post-lesion cortical excitability alters the learning (Schubring-Giese et al., 2007; Clarkson and Carmichael, 2009), in intact rats behavioral changes preceded the map changes (Kleim et al., 2004). Thus, it further supports the notion that functional improvement following brain injury is a relearning process that similarly requires the placing of new neurons and synapses in the right locations to reproduce lost functions (Kleim, 2011). Together, the current results may have implications in the design of effective rehabilitative training (Kleim and Jones, 2008).

Rats and non-human primates often adopt effective compensatory movements to perform the function lost to brain damage (Whishaw, 2000; Knieling et al., 2009; Moore et al., 2011). Our lesioned animals exhibited abnormality in performing supination/release rather than in pronation/grasp during a successful reach. The lost movements and developed compensation seem to be unique to the method of lesion induction (Alaverdashvili et al., 2008b), the extent and location of the brain damage or the age of the subject. For example, other stroke models such as a small photothrombotic lesion in the sensorimotor cortex causes persisting impairments in pronation and supination II (Moon et al., 2009) while aged subjects rely heavily on compensating aim and supination I after a large damage to the motor areas (Alaverdashvili and Whishaw, 2010).

During spontaneous recovery, overall qualitative compensation appears to continue at least until the assessment time on PLD 14 (Gharbawie et al., 2005a) or on PLD 19 (Moon et al., 2009), and, in the present study, on PLD 37. For example, as we also observed, rats with poor recovery dropped the forelimb which the mouth followed in order to consume the pellet, instead of the forelimb bringing it to the mouth, or frequently supinated immediately against the base of the reaching slot which the mouth approached, which both interfered with supination (Whishaw et al., 1991; Gharbawie et al., 2005a; Metz et al., 2005).

Recent studies suggest rehabilitation certainly reduces the movement compensation; nevertheless, such compensation underlies the mechanisms of not only spontaneous but also rehabilitation-aided forelimb functional recovery (Gharbawie et al., 2005a; Alaverdashvili and Whishaw, 2010). Compensatory movements played a significant role in functional recovery in our study; yet, functional recovery without compensation may also be possible with adequate spared corticospinal projections. That is, Alaverdashivili and Whishaw damaged both CFA and RFA (Whishaw, 2000; Alaverdashvili and Whishaw, 2010) while we spared RFA. Indeed, Whishaw states that "unless the lost innate cortical engram can be replaced, recovery occurs through compensation" (Whishaw, 2000). Neural recovery in the spared RFA may explain the lesser degree of endpoint behavioral compensation in our rehabilitated rats in comparison with their results.

To our knowledge, the present study is the first to show that some qualitative improvements are possible even past most of the quantitative recovery plateaus in rehabilitated animals. The rehabilitated rats with the re-instated RFA, reflected by the expansion of the distal forelimb area, showed improvement in supination/release. Thus, the distal forelimb areal expansion may reflect re-building of spatio-temporal coordinated muscle synergy of supination/release. Limited

restoration of the distal forelimb area was exhibited in the non-rehabilitated subjects which showed some residual abnormality in supination. It is not certain, however, whether the lack of complete map restoration caused maladaptive compensatory strategies, thus resulting in lower quantitative functional recovery, or whether it caused a lower quantitative recovery and thus necessitated the compensation in order to fulfill the function. Our lesion model, which produced relatively mild deficits, showed that with rehabilitative training the presumed motor recovery seems possible with a vicarious RFA substitutive functioning for the damaged CFA. The degree to which the cortical reorganization supported the compensation or true recovery was not definitive.

Early diaschisis-like effect on cortical map

The results indicate that the remote RFA undergoes a diaschisis-like effect and exhibits diminished functional integrity during early stages of recovery. Literature suggests that early behavioral recovery may occur due to the attenuation of diaschsis rather than to reinstated neural connectivity and functional integrity (Witte, 1998; Nudo and Friel, 1999). The theory of diaschisis attenuation as a recovery mechanism states that the peri-infarct area suffers a temporary loss of function but regains its original excitability during the course of recovery (Von Monakow, 1914; Glassman, 1971). Present-day research expands the concept of diaschisis to a wide range of neurophysiological phenomena, any remote effects initiated by a focal lesion and caused either through neural projections or systemically (e.g cortical spreading depression or edema). In a recent rat study, for example, hypometabolism was detected in areas S1, S2 and CFA after a focal ischemic lesion induced in the barrel cortex (Carmichael et al., 2004). In our study, the depressed activity of the RFA, the remote and functionally relevant structure to CFA,

is most likely the effect of diaschisis. Such remote consequences of the lesion, however, occur simultaneously, and so it is difficult to isolate the causal and beneficial links to behavioral manifestation (Witte et al., 2000).

The remote effects are not necessarily depressive. The excitability of the projection areas remote from the lesion increases (Reinecke et al., 1999), mainly due to downregulated $GABA_A$ inhibitions (Schiene et al., 1996; Qu et al., 1998; Que et al., 1999; Reinecke et al., 1999) and NMDA receptor upreglation (Humm et al., 1999). Hagemann et al found a propensity for long-term potentiation in the ipsilesional cortex that was unique to post-lesioned animals (Hagemann et al., 1998). Such hyperexcitability is speculated to increase spontaneous cellular activities and thus may facilitate cortical plasticity (Witte et al., 1997). For example, following a focal lesion in the rat hindlimb S1 area, the vibrissa S1 (B3) area, when stimulated, shows enhanced intercolumnar transmission compared with the control animals (Schiene et al., 1999).

Altogether, the balance of early cortical changes impacted our physiological results. It was to our surprise that rehabilitation induced non-responsiveness of the forelimb areas; however, in a primate model, forelimb representations were suppressed prior to their substantial expansions (Eisner-Janowicz et al., 2008). The cortical map represents the intracortical horizontal connectivity. Thus, the silenced area may reflect the extensive area that rehabilitative training engages with neural repair following the early disruption of intracortical synaptic connections due to diaschisis.

Rehabilitative training restored RFA to the intact level.

20 days after the behavioral practice discontinuation, the earlier detected responsive coordinates reorganized to form face and trunk representations which surrounded the formerly

silenced but now reorganized forelimb motor representations that came to exist more rostrally. In the cases which RFA bordered with whiskers/nose and trunk/neck representations, the areal measure was significantly larger in the rehab than the no rehab group. That is, only with motor training was areal organization normalized and spontaneous restoration of RFA limited in its extent. Corticospinal neurons from adjacent representations that numerously interconnect with intracortical afferents are speculated to have over time altered the descending projections to distal forelimb muscles, re-circuiting the sensorimotor physiological function. As a result, non-response sites were taken over by forelimb motor representations.

Previously, the effect of rehabilitative training has been demonstrated in the peri-infarct area after incomplete M1 lesions (Castro-Alamancos and Borrel, 1995; Nudo and Milliken, 1996; Kleim et al., 2003b), and in spared rat RFA (Conner et al., 2005). This study provides the first evidence of how rehabilitative training affects once-diminished cortical areas remote from the lesion. In other words, it completely restores the RFA after ischemic lesion to CFA.

Despite the behavioral improvement and behavioral intervention in our study, the lack of RFA maps in some rats suggest either that in those animals neural substrates are located elsewhere (e.g. contralateral motor cortices) or that ICMS fails to detect the map in certain conditions that rats underwent (e.g. anesthesia level). In our previous study, which used a controlled cortical impact lesion model in CFA, we reported 3 cases out of 24 where we could not evoke RFA at post-lesion week 7 (Nishibe et al., 2010). It seems that RFA can become undetectable during recovery due to certain lesion effects to the sensorimotor cortex.

Temporal mismatch of behavioral recovery and map expansion

Our results show qualitative recovery (i.e. improvement in movement quality), rather than quantitative behavioral recovery, temporarily correlated with the cortical map expansion (Fig 6). It may be possible that the map expansion supports improvement in movement quality. A temporal mismatch between quantitative behavioral improvement and cortical changes was previously reported from our laboratory in a study on non-human primates during spontaneous recovery after destroying both M1 and PMv. According to their results obtained in spontaneously recovered monkeys mapped at the post-lesion weeks 3rd and 13th, the longitudinal profile of map expansion occurred after behavioral recovery plateau. It suggested that quantitative behavioral recovery may precede cortical map expansions in supplementary motor area SMA. (Eisner-Janowicz et al., 2008). While the present results also support such notion, there are subtle differences speculated from the difference in species, and extensiveness and methods of lesion inductions. First, our spontaneously recovered group did not show any areal expansions over time, displaying only an incomplete areal restoration. Behavioral recovery occurred later than the rehab group. It is, however, the monkeys did not plautatued at the level of pre-lesion performance while our rats reached the basline performance level and platatued thereafter. Second, in their study, the restored area of the supplementary motor cortex exceeded the pre-lesion area substantially after damage both to M1 and premotor cortex. In the present study, with rehabilitation, the RFA was restored at least to the intact level; presumably, the remaining afferents restored the formerly connected representations but did not reconnect with areas beyond their typical borders. It may be possible that cortical plasticity in rats is more limited than in non-human primates (Jain et al., 1995).

As discussed, the early behavioral recovery may be due to the attenuation of diaschsis rather than to reinstated neural connectivity and functional integrity (Witte, 1998; Nudo and Friel,

1999). Remote hyperexcitability is thought to subsequently contribute for cortical plasticity (Witte et al., 1997). Various molecular and anatomical changes in the cortices coincide with most of the spontaneous recovery that takes place as early as the 2nd post-stroke week (Jones and Schallert, 1992b; Schallert and Jones, 1993; Stroemer et al., 1995; Jones and Greenough, 1996; Jones et al., 1996; Qu et al., 1998; Jolkkonen et al., 2003). Physiological changes measured by cortical topography thus seem to occur later than the behavioral improvements.

One rehabilitated rat, mapped during the 4th week after lesion (on PLD 25), exhibited the complete bordered RFA (data not shown), therefore it can be speculated that the RFA map restoration in our model may have occurred as early as the 4th post-lesion week.

The overall study results were congruent with previous findings in that rehabilitative intervention promotes cortical reorganization which, in turn, plays a role in supporting functional recovery after a focal lesion to primary motor cortex (Nudo et al., 1996b; Ward et al., 2003; Conner et al., 2005). Importantly, other studies have shown that lesioning of reorganized areas after behavioral recovery re-instates the motor impairment formerly induced by various types of CFA lesions (Castro-Alamancos and Borrel, 1995; Liu and Rouiller, 1999; Gharbawie et al., 2007). We herein showed how early diaschisis-like effects are recruited by rehabilitative intervention for subsequent cortical plasticity. The neural strategies for motor improvement observed in this study were to restore once abolished RFA function which vicariously substituted for the lost CFA function of producing movements necessary for single-pellet reach and retrieval.

ACNKOWLEDEMENTS

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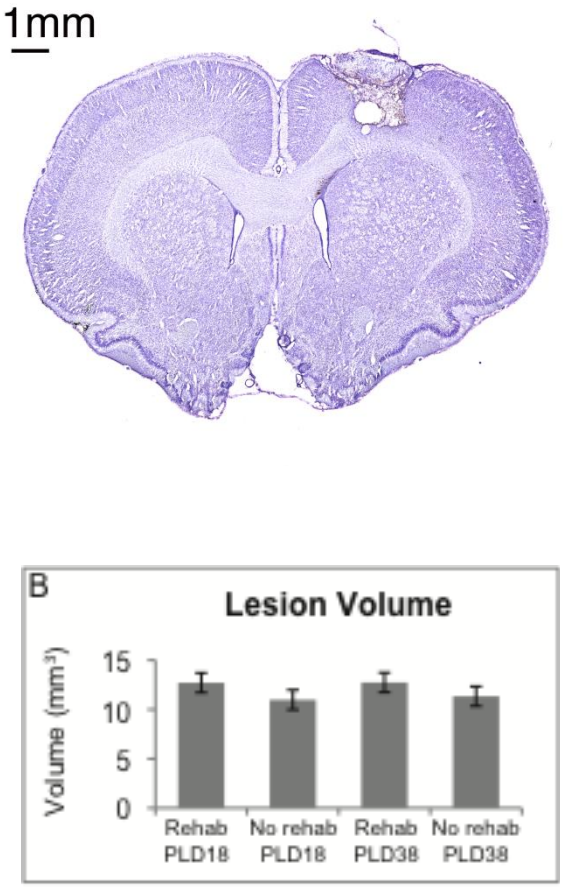
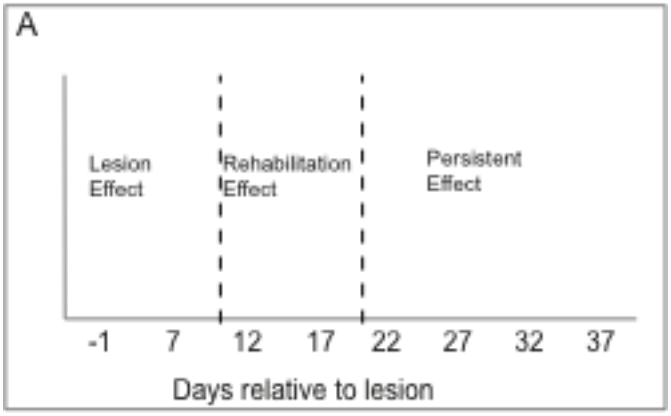
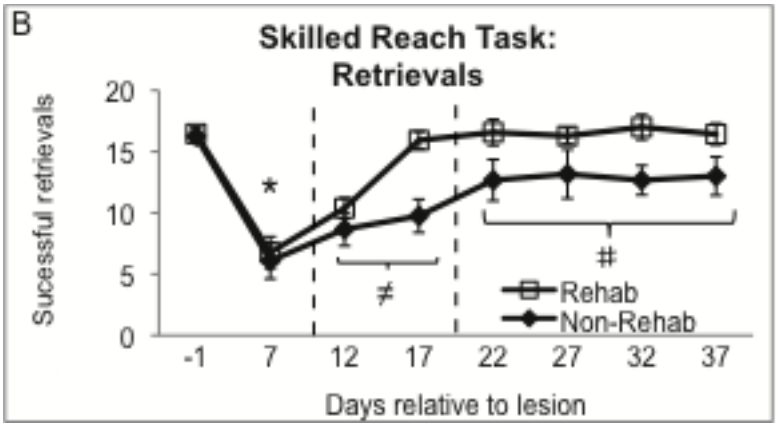
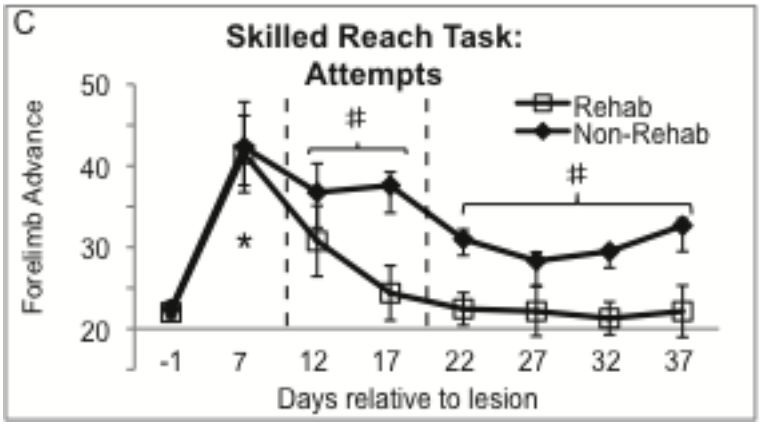


Fig 1 (A) illustrates Nissl-stained coronal section showing the lesion in the motor cortex from one representative rat at 1.2mm rostral from bregma (Scale bar =1.0mm). (B) Lesion volume. There was no difference in the lesion volume between the four groups ($p>0.1$).


A
Lesion Effect
Rehabilitation Effect
Persistent Effect
-1
7
12
17
22
27
32
37
Days relative to lesion


B
Skilled Reach Task:
Retrievals
Sucessful retrievals
20
15
10
5
0
*
≠
#
Rehab
Non-Rehab
-1
7
12
17
22
27
32
37
Days relative to lesion


C
Skilled Reach Task:
Attempts
Forelimb Advance
50
40
30
20
#
*
#
Rehab
Non-Rehab
-1
7
12
17
22
27
32
37
Days relative to lesion

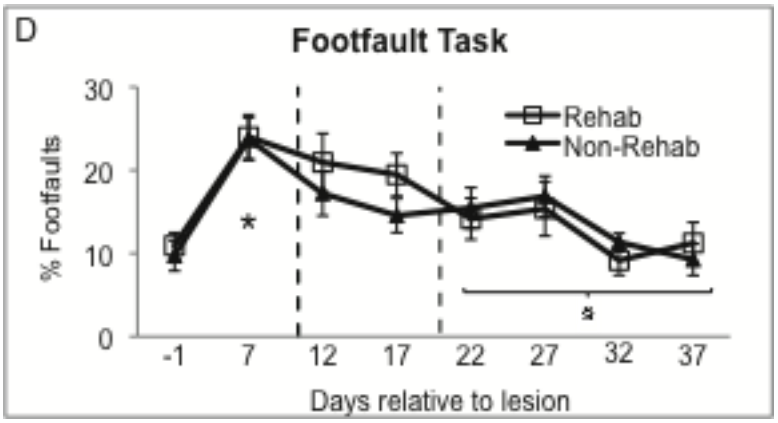


Fig 2 Behavioral results. (A) Three time periods analyzed by separate repeated measures ANOVAs (B) Mean number of successful retrievals (±SEM). *p<0.01 Time effect on PLD-1 and 7. The lesion caused a significant deficit in pellet retrieval ability at PLD 7 in both rehab and no rehab groups. ≠p<0.01 Group effect on PLD 12 and 17. Tukey test shows the rehab group successfully retrieved a statistically higher number of pellets than the non-rehab group on PLD 17. #<0.05 Group effect from PLD 22 through 37. Rehabilitative training effect was maintained during the follow-up period. (C) Mean number of reaching attempts (forelimb advances) (±SEM). *p<0.01 Time effect on PLD-1 and 7. The lesion caused a significant increase in the limb advances during skilled reach task at PLD 7 in both groups. #<0.05 Group effect on PLD 12 and 17. The rehab group made fewer reaching attempts to retrieve pellets compared with the no rehab group. #<0.01 Group effect from PLD 22 through 37. The rehab effect persisted. (D) Mean percentage of footfaults (±SEM). *p<0.01 Time effect on PLD-1 and 7. The lesion caused a significant increase in percent footfaults. §p<0.05 Time effect from PLD 22 through 37. The percent footfaults decreased in both groups at a similar rate after the discontinuation of rehabilitation.

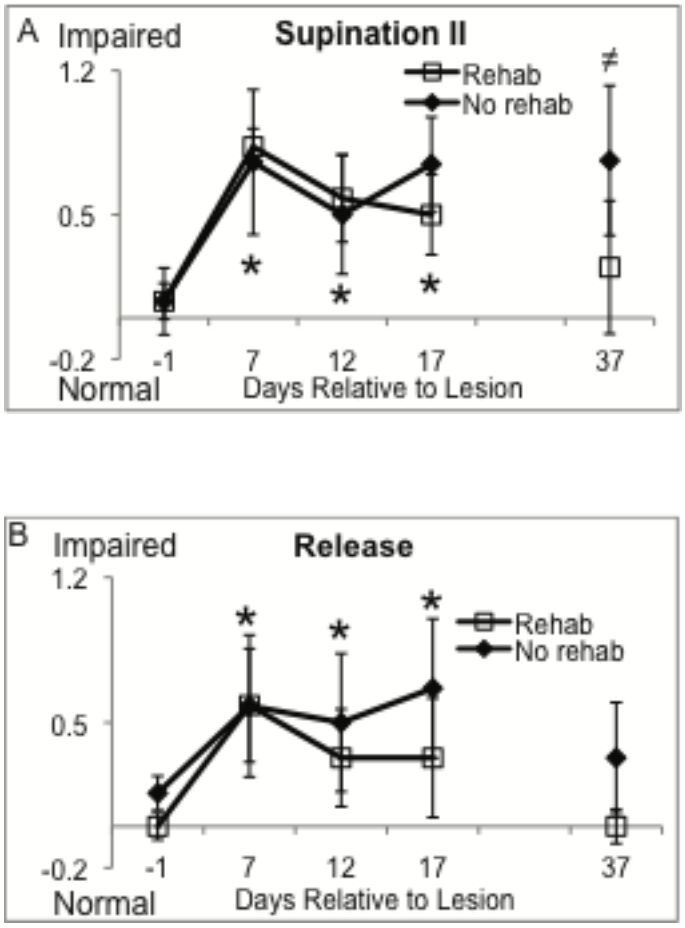
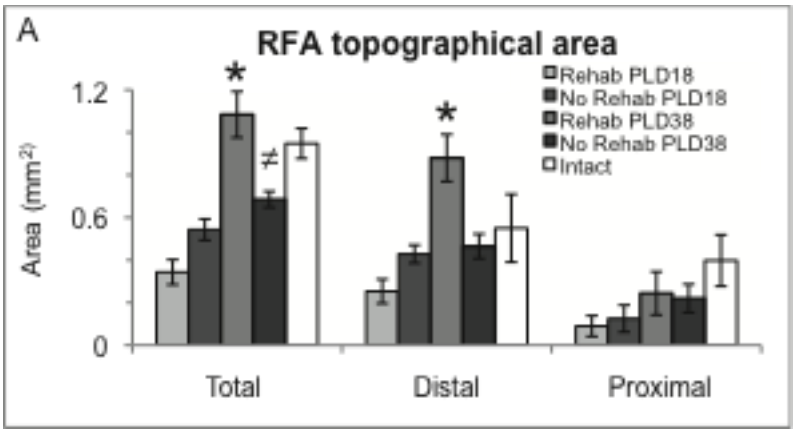
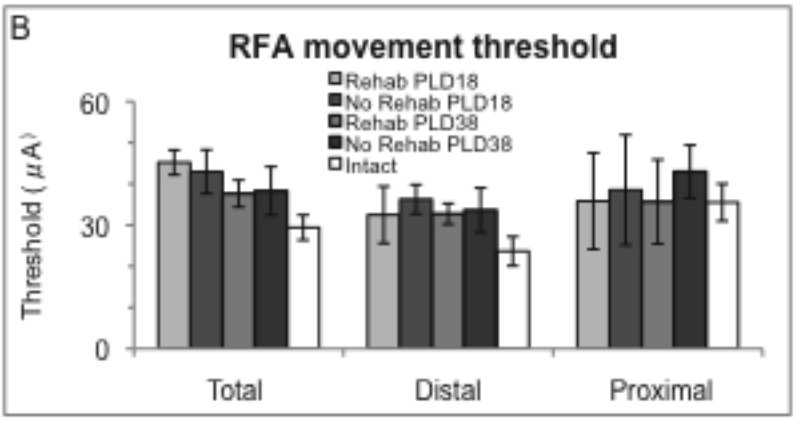
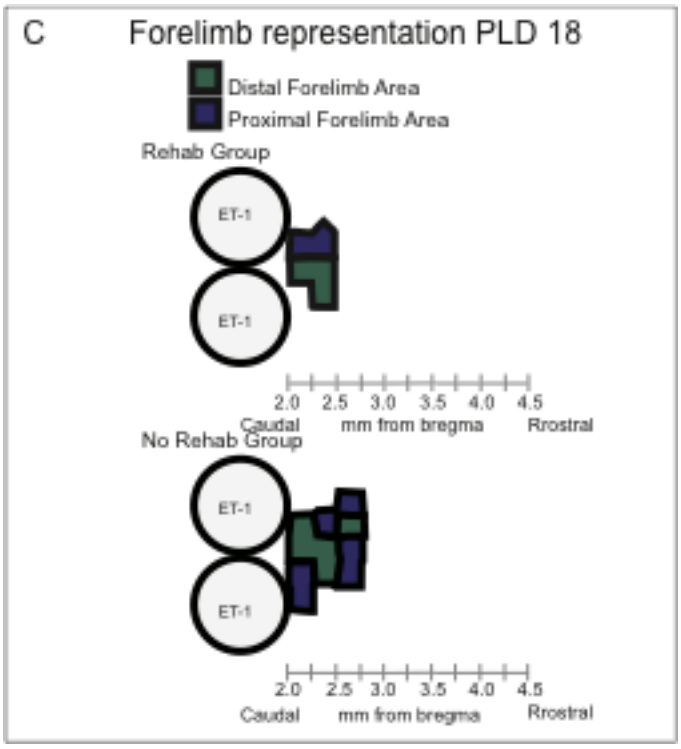


Fig 3 Movement Analysis (A) Supination II. On PLD 7, 12 and 17, a significant deviation was detected from the baseline performance in both groups (*$p<0.05$ in both groups from their baseline). On PLD 37, only no rehab group demonstrated the deviation from the baseline (≠ $p<0.05$ in no rehab group from its baseline). (B) Release. On PLD 7, 12 and 17, a significant deviation was detected in both groups (*$p<0.05$ in both groups from their baseline). On PLD 37, however, both groups recovered in how release is performed during the skilled reach task.


A
RFA topographical area
Rehab PLD18
No Rehab PLD18
Rehab PLD38
No Rehab PLD38
Intact
Area (mm²)
1.2
0.6
0
*
*
≠
Total
Distal
Proximal


B
RFA movement threshold
Rehab PLD18
No Rehab PLD18
Rehab PLD38
No Rehab PLD38
Intact
Threshold (μA)
60
30
0
Total
Distal
Proximal


C
Forelimb representation PLD 18
Distal Forelimb Area
Proximal Forelimb Area
Rehab Group
ET-1
ET-1
2.0 2.5 3.0 3.5 4.0 4.5
Caudal
mm from bregma
Rrostral
No Rehab Group
ET-1
ET-1
2.0 2.5 3.0 3.5 4.0 4.5
Caudal
mm from bregma
Rrostral

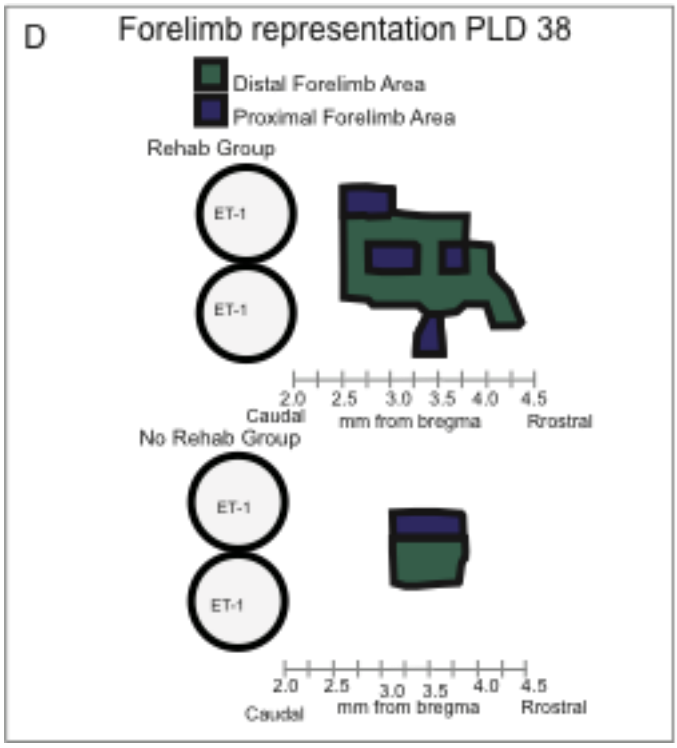

D
Forelimb representation PLD 38
Distal Forelimb Area
Proximal Forelimb Area
Rehab Group
ET-1
ET-1
2.0 2.5 3.0 3.5 4.0 4.5
Caudal
mm from bregma
Rrostral
No Rehab Group
ET-1
ET-1
2.0 2.5 3.0 3.5 4.0 4.5
Caudal
mm from bregma
Rrostral

Fig 4 (A) Mean RFA topographical areas (mm^2) (±SEM). Tukey test indicates that RFA of the rehab/long-term survival group was larger than any of the other three groups (*ps≤0.008). Also, the RFA of *no rehab*/long-term survival group was larger than the *rehab*/short-term survival group (≠p=0.01). Thus, the forelimb area expanded in the rehab groups longitudinally but not in the no rehab groups. (B) Mean RFA movement threshold (±SEM μA). There was no difference in current threshold required to evoke forelimb movements between groups on either PLD 18 or PLD 38. (C) Representative maps of forelimb movements evoked by ICMS on PLD 18 and (D) on PLD 38. Distal forelimb areas are shown in green and proximal forelimb area in blue.

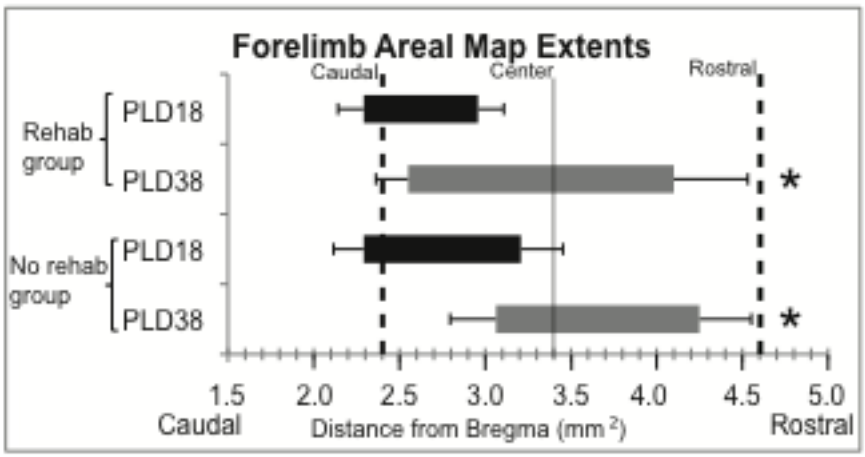
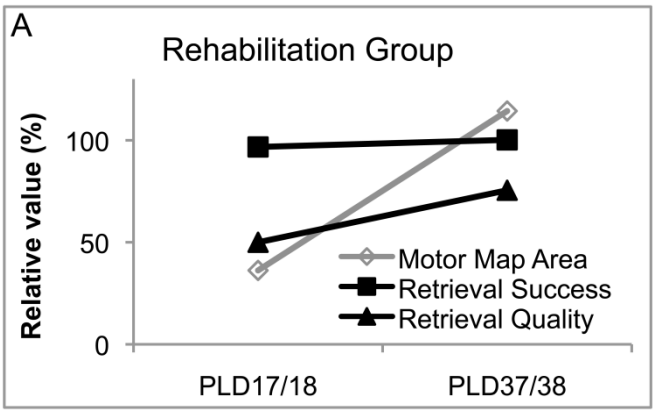


Fig 5 Mean distance (mm) of the rostral and caudal edge of the forelimb areal map from the bregma (±SEM). The map extended more rostrally in the rehab/long-term survival and no rehab/long-term survival groups in comparison to rehab/short-term group (*$p<0.05$ Tukey paired-test). No significant difference was detected in the caudal map extent. On both PLD 18 and 38, forelimb movements were evoked from a cortical territory within the typical RFA stereotaxic coordinates. The vertical dash lines represent the expected caudal and rostral limits of typical RFA of the normal, intact group (see text). The middle vertical line represents the mean center of the typical RFA representation.


A
Rehabilitation Group
Relative value (%)
100
50
0
Motor Map Area
Retrieval Success
Retrieval Quality
PLD17/18
PLD37/38

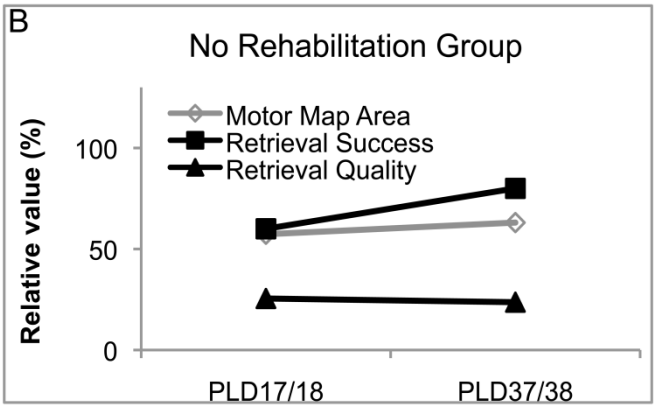

B
No Rehabilitation Group
Relative value (%)
100
50
0
Motor Map Area
Retrieval Success
Retrieval Quality
PLD17/18
PLD37/38

Fig 6 Temporal mismatch and match during post-rehabilitation period. Data was derived from the present study, as the PLD-1 baseline behavioral measures and intact RFA areal data set at 100%. Retrieval quality reflects the exclusive outcome on supintation II results since its group effect persisted until PLD 38. (A) Rehabilitation group. Map expanded from 36% on PLD 18 to 114% on PLD 38 (intact RFA=100%), and performance in supination II during the skilled reach task improved from 50% to 75% during the post-rehabilitation period. On the other hand, the number of successful retrievals at the skilled reach task had already reached baseline performance level on PLD 18 (96%) and maintained the plateau thereafter (100% on PLD 38). (B) No rehabilitation group. Neither substantial areal expansion (57% to 63%) nor improvement in supination II performance (25% to 23%) was observed during post-rehabilitation period. The number of successful retrieval slightly improved from PLD 18 (60%) to PLD 38 (80%). Map areal changes appear to temporally match more with retrieval quality and mismatch with retrieval success in both groups.

CHAPTER FOUR

Consequences of Contusion- and Ischemic-Type Lesion to Motor Forelimb Areas on the Behavioral Function and Integrity of Remote Forelimb Representations

ABSTRACT

The method of injury induction is important in the subsequent morphological and physiological reorganization as well as in the rate of recovery and the effectiveness of therapeutic intervention. Increasing attention is being paid to neural plasticity because it is speculated to underlie the mechanism for rehabilitation-dependent functional reorganization and for improving functional recovery after CNS injuries. Our recent studies on a TBI and ischemia rat model demonstrated that the ipsilesional rostral forelimb area (RFA) may provide a substrate for recovery of affected motor function after a focal damage to the caudal forelimb area (CFA). Therefore, we herein compare the consequences of the contusion-type with ischemia-type lesion in motor behavior and in ipsilateral RFA physiology, during spontaneous recovery. The comparison was made using a group from the TBI study (*controlled cortical impact injury*, the lesion diameter 3mm, epicenter at bregma and round shape, n=5) and a group from the ischemia study (*endothelin-1* induced ischemic injury, anteroposterior lesion extent 3mm, n=6). The behavioral measures were collected within one week before and for 5 weeks after lesion at the single-pellet reach and retrieval task. To quantify the area of RFA representations, the intracortical microstimulations (ICMS) procedures were performed in the ischemia group at the 6^{th} and in the contusion group at the 7^{th} post-lesion week. We demonstrated that 1) focal lesions to CFA by contusion and ischemic methods result in similar initial deficits, 2) the overall recovery was better in rats subjected to cortical ischemia than those with contusion, 3) both CFA lesions diminished the remote cortical integrity of RFA, 4) while the histological lesion volume was statistically not different, the RFA physiological integrity was more compromised by the contusion.

The method of injury induction is important in the subsequent morphological (Voorhies and Jones, 2002; Gonzalez and Kolb, 2003) and physiological (Glees, 1949; Nudo et al., 1996b) reorganization of spared cortical areas as well as in the rate of recovery (Alaverdashvili et al., 2008b) and the effectiveness of therapeutic intervention (Zeng et al., 2000). For example, a study suggested that host-to-graft intervention led to greater benefits when fetal cortical tissue was implanted in an ischemia-damaged cavity than in an aspirated cavity (Zeng et al., 2000).

Nevertheless, rehabilitative therapy for non-progressive diseases such as stroke and TBI should offer strategies to amend specific motor impairments, rather than for treating the disease itself. Increasing attention is now paid to the principle of neural repair which provides the capacity for behavioral adaptation. Previous studies showed that the vicarious reorganization of the spared, intact motor regions of the ipsilesional hemisphere may provide a substrate for affected motor function and be a therapeutic focus. Within our scope of studying reorganization in the spared premotor cortex after a focal damage to M1, we recently reported studies on a TBI rat model using controlled cortical impact (CCI), and an ischemic lesion model using intracortical injections of endothelin-1 (ET-1). We herein compare the consequences of the contusion-type with the ischemia-type lesion to CFA in motor behavior and in ipsilateral RFA physiology.

The comparison was made using a group from the TBI study (*CCI*, the lesion diameter 3mm, epicenter at bregma and round shape, n=5) (Nishibe et al., 2010) and a group from the ischemia study (*ET-1* induced ischemic injury, anteroposterior lesion extent 3mm, n=6). The two lesion groups were spontaneously recovered after the lesion designed to destroy the entire CFA. The behavioral measures were collected within one week before and for 5 weeks after lesion at the single-pellet reach and retrieval task. To quantify the area of RFA representations, the

intracortical microstimulations (ICMS) procedures were performed in the ischemia group at the 6^{th} and in the contusion group at the 7^{th} post-lesion week.

Statistical analyses were done using a general linear model on SPSS 20 (SPSS IBM Inc., Chicago, IL). The lesion volume was compared between groups through one-way ANOVA. The behavioral measure was analyzed by two-way repeated measures ANOVAs (Group*Time) for a lesion effect and a recovery effect. ICMS data was analyzed by one-way ANOVA. Probability value of 0.05 was required for significance.

The result showed that the lesion volume was not statistically significant between CCI- and ET-1 induced ($F_{1,9}$=2.637, p=0.143). The repeated measure of baseline (pre-lesion) and the 1^{st} post lesion week showed a time effect ($F_{1,9}$=43.488, p=0.0001; Fig 1) but no group ($F_{1,9}$=0.612, p=0.454) or interaction effect ($F_{1,9}$=0.763, p=0.405), suggesting that both lesions introduced severe motor deficits at a similar level. The repeated measures from the first post-lesion assessment to the fifth showed there was no interaction effect (Interaction $F_{4,36}$=0.796, p=0.536) but there was a Time ($F_{4,36}$=7.398,p=0.0001) and Group effect ($F_{1,9}$=5.100, p=0.05), suggesting that the recovery occurred spontaneously but the recovery level was, in fact, different following contusion- and ischemia-type injuries. It appears that both persisting motor deficits were milder with ischemia and more severe with contusion when compared to each other.

The analysis on topographical areal measure showed that RFA size was different after the two lesions ($F_{1,8}$=46.811, p<0.0001; Fig 2), but the sizes of distal and proximal forelimb area did not reach significance (distal forelimb $F_{1,8}$=-4.333, p=0.076, proximal $F_{1,8}$=0.771, p=0.409). The results suggest both lesion groups underwent and retained the remote, lesion effect at the chronic stage. The effect was larger in the group that received contusion than in the one with ischemic damage.

We demonstrated that 1) focal lesions to CFA by contusion and ischemic methods result in similar initial deficits, 2) the overall recovery was better in rats subjected to cortical ischemia than those with contusion, 3) both CFA lesions diminished the remote cortical integrity of RFA, 4) while the histological lesion volume was statistically not different, the RFA physiological integrity was more compromised by the contusion. Our comparisons provide a ground of neural reorganization in the remote area associated with spontaneous recovery in rat models during chronic stages. The results may suggest that secondary degeneration, unique to contusion, in remote areas cause such differences.

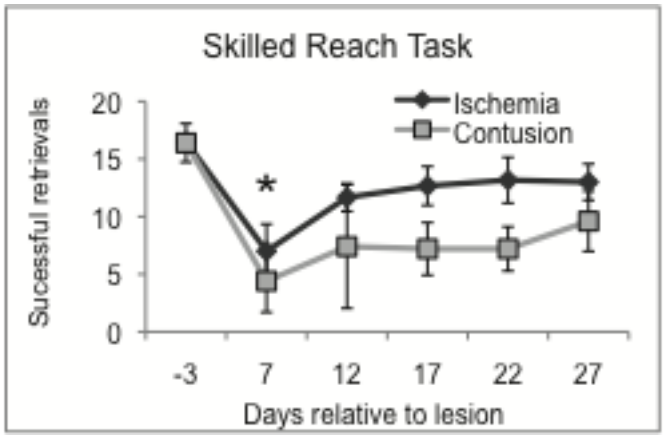


Fig 1 The repeated measure of baseline (pre-lesion) and the 1st post lesion week showed a time effect (*p=0.0001), suggesting that both lesions introduced severe motor deficits at a similar level. The repeated measures from the first post-lesion assessment to the fifth showed there was no interaction effect (Interaction $F_{4,36}$=0.796, p=0.536) but there was a Time ($F_{4,36}$=7.398, ≠p=0.0001) and Group effect ($F_{1,9}$=5.100, ≠p=0.05), suggesting that the recovery occurred spontaneously but the recovery level was, in fact, different following contusion- and ischemia-type injuries.

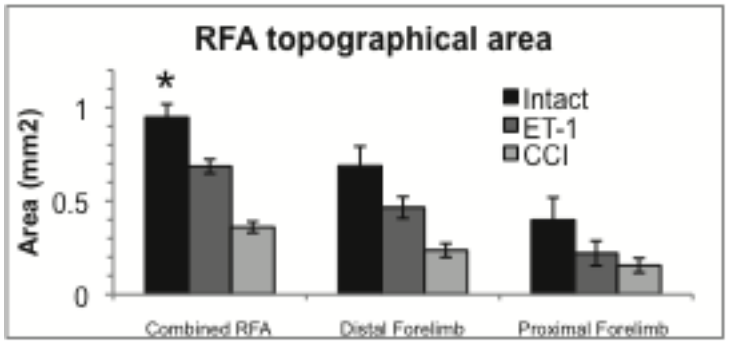


Fig 2 The analysis on topographical areal measure showed that RFA size was different after the two lesions (*$p<0.0001$), but the sizes of distal and proximal forelimb area did not reach significance (distal forelimb $p=0.145$, proximal $p=0.409$). The results suggest both lesion groups underwent and retained the remote, lesion effect at the chronic stage. The effect was larger in the group that received contusion than in the one with ischemic damage.

CHAPTER FIVE

Conclusions

Cerebral Cortex, the "rind"

> *"To Gall and Spurzheim (1810-1819) the cortex housed the faculties listed on their phrenological maps, e.g., mirthfulness, acquisitiveness, amativeness, etc. To Flourens (1824) the cortex was the interface with the mind and so must have mental attributes such as intelligence and will. To Loeb (1900) and Pavlov (1927) the cortex was the organ of associative learning. To Goltz (1892) the cortex was the seat of understanding, reasoning power, and intelligence. C. Judson Herrick (1926) felt much the same way, but stated his position if not as clearly at least more prosaically. To Lashley (1950) the cortex was the organ of learning and memory. More recently Oakley (1981) has made the cortex the organ of representational and abstract memory. To MacLean (1982) the cortex was the organ of emotion and expression. Phillips and colleagues (1984) speculate that the cortex is the Sherlock Holmes of the brain, sensitive to suspicious coincidence. The feature of these theories is that they do little more than substitute the neocortex for a facsimile of Rene Descartes's (1664) mind". (p239). (Whishaw, 1990).*

Historical context of the present studies

The first prominent work of the systematic examination on cerebral cortex was performed by Pierre Paul Broca, and it ushered in the localizationists. His identification of the language area is the most celebrated example of ascribing a behavioral function, i.e. language output (Broca,

1861). The motor cortex was first identified in an unanesthetized dog in 1870's by Fritsch and Hitzig (Fritsch and Hitzig, 1870). J. Hughlings Jackson then proposed in *West Riding Lunatic Asylum Medical Reports*, the first journal devoted to brain research, the localization of the hierarchically organized anatomical and physiological substrates of brain functions (Jackson, 1873). In 1875, D. Ferrier, a localizationist, reported that the motor functional maps in monkeys (Fig 1), using low intensity stimulation of the cortex, which had lost function due to lesion (Ferrier, 1873; Ferrier, 1875). Faced with skepticism, Beevor, Horsley, Sherrington and others began punctuating cortical mapping using the minimum current to elicit the smallest discernible movement (Horsley and A, 1883; Beevor and Horsley, 1887; Leyton and Sherrington, 1917). Skeptics suggested that the evidence for the localized motor function in the cortex was artifactual due to the "spread of current" to the striatum—the structure considered then to be the highest motor center.

Localizationists have focused on the punctuation of precise mapping to such an extent that by 1980's, a century after the first mapping, scientists could trigger and record small changes in the electromyographic activity of muscles in response to single pulses of cortical stimulation, a technique called the spike-triggered averaging, which identified neurons from the primary motor cortex that connect directly to motor neurons—the corticomotoneuronal cells (Fetz et al., 1976; Cheney and Fetz, 1985). Another technique was developed to assess motor representations by identifying the muscle contraction joint groups to which the descending tracts activate when electricity is provided to the vicinity of the soma (Asanuma and Rosen, 1972). Intracortical microstimulation became essential to electrophysiological studies in a variety of fields, including this dissertation work.

Neural plasticity was first described by Hebb in 1940's. Hebb tested the effects of the environment on rats by raising them in his home (Hebb, 1974). In comparison with lab-raised rats, the ones raised by Hebb, his students and later by others showed more flexibility in the choice of cues for solving a task. In 1962, the "enriched condition" was standardized, as grouped housing with a changing set of objects and toys in a large cage by Rosenzweig and associates, who showed that cortices of rats raised in an enriched condition were thicker and weighed more than those of rats raised in an impoverished condition (Rosenzweig et al., 1962). Diamond found a larger neuron size in the enriched environment group (Diamond et al., 1976).

During the mid 1980's, pioneering studies on cortical plasticity in somatosensory system were conducted. Researchers demonstrated that inactivating a subset of the peripheral inputs (i.e. peripheral nerve injury, removal of a digit, sectioning of the dorsal roots of peripheral nerves) causes reorganization of somatotopic representations such as the expansion of previously existing representations, the development of novel representations, and responsive inputs from adjoining representations (Wall and Egger, 1971; Kaas et al., 1983; Merzenich et al., 1983; Jenkins and Merzenich, 1987; Pons et al., 1991). Suturing two fingers abolished the sharp boundaries of sensory representations of each digit, normally evident in intact monkeys, by increasing the activity correlation of the skin afferents from the two fingers, (Clark et al., 1988). Moreover, Jenkins found that repetitive sensory inputs of the middle finger expand the cortical areas devoted to the tip of the middle digit at the expense of the adjacent proximal digits, which did not receive repetitive inputs (Jenkins et al., 1990).

Motor cortical reorganization was first noted in the 1950s by Glee and Cole who found reappearance of the representations in the peri-lesion area after damage to thumb representations (Glees, 1949; Glees, 1950). Studies on motor system plasticity were continued in the 1990s by

Nudo, Castro-Alamancos and Xerri. Through the use of ICMS, the peri-lesion area was discovered to be a potential substrate for functional recovery after focal cortical damage, particularly in the presence of rehabilitative training (Castro-Alamancos and Borrel, 1995; Nudo et al., 1996b; Xerri et al., 1996).

First described by Hicks and D'Amato as a rostral patch of corticospinal neurons near the frontal pole (Hicks and D'amato, 1977), a second motor representation was then confirmed by Wise and Neafsey in separate electrophysiological studies of rat frontal cortex (Wise and Jones, 1977; Neafsey and Sievert, 1982). They delineated two forelimb motor areas, one just rostral and lateral to bregma, corresponding to a formerly described forelimb area (Woolsey, 1958; Hall and Lindholm, 1974), and another located further rostrally in which a complete body representation existed.

Extensive evidence on the brain-behavior relation particularly in rats is attributed to the work of I.Q. Whishaw. Specifically, Whishaw and colleagues used the model of decorticated rats and demonstrated the roles of cortex in behaviors including but not limited to posture, grooming skilled movements, swimming, social behavior and maternal behavior. Further, Whishaw and Kolb developed many behavioral testing measures, such as skilled reach task, grid walk, ladder-rung walk and maze (Whishaw et al., 1983).

Those pioneering works have advanced rehabilitative medicine for nervous system injury. Under the supervision of Randolph Nudo, this dissertation work from 2008-2012 tackled the phenomenon of neural reorganization in the remote cortex after cortical injury in rat models.

The research goal in the field of neurorehabilitation after CNS injuries is to elucidate the behavioral and neural mechanisms that drive neural plasticity which allows maximization of functional recovery, patient-specific or population-specific. The application of neural plasticity

in clinical treatments for both progressive and non-progressive neurodegenerative diseases has received substantial attention in recent years. There is still some disparity between basic science research and clinical practice, however. This dissertation work was translational and it addressed such disparity by analyzing the behavioral and neurophysiological characteristics after clinically relevant CNS injuries.

Summary and Conclusions

In summary, we demonstrated in Chapter 1) the evidence of reorganization in the ipsilesional RFA after a focal traumatic injury to CFA during spontaneous recovery; in Chapter 2) the evidence of extensive reorganization in RFA unique to the rehabilitated animals after a focal ischemic injury to CFA; in Chapter 3) the evidence of a larger consequence of the traumatic- than the ischemic-injury on the RFA integrity and motor behavior.

In the present discussion, the major findings of the dissertation will be first summarized. Second, the limitations of rat models of CNS injuries will be addressed. Third, the dissertation will end with a general discussion on remote, intact cortical areas as neural substrates for functional recovery after brain damage.

Ch 2; the evidence of reorganization in the ipsilesional rostral forelimb representations after a focal traumatic injury to the caudal forelimb area during spontaneous recovery

The study tested the hypothesis that spared motor regions in the same hemisphere are still intact, and may functionally reorganize after contusion injuries. The cortical injury created by the

CCI device using three lesion parameters (CCI-1, 2 and 3) consistently reproduced a tissue cavity in CFA and adjacent cortical areas, though some variability was observed in cortical depth and white matter involvement. The study confirmed that 1) the cortical damage produced chronic motor deficits at the skilled reach task and footfault tests though there was some spontaneous recovery. 2) The restricted damage to CFA spared neurons in RFA and 3) RFA was functionally intact, and significant reorganizations in RFA movement representations were detected. 4) The resulting size of the RFA representation area varied according to lesion proximity to RFA.

The finding on RFA reorganization in a rat contusion-injury model was novel and was in congruent with earlier non-human primate studies demonstrating that PMv reorganized after M1 lesion (Frost et al., 2003). Group CCI-1 showed a reduction in total forelimb area of more than 60% (69% proximal forelimb and 57% distal forelimb areal reduction). In CCI-2 and -3 groups, a redistribution of movement representations from distal to proximal forelimb areas was observed while the groups maintained the total forelimb area. Interestingly, intact motor skill training induces an expansion of distal forelimb area at the expense of proximal area in CFA as well as in monkey primary motor cortex (Kleim et al., 1998; Nudo et al., 1996a). Although CFA and RFA play a different role in intact movement control (Barth et al., 1990), after CCI in CFA, it is reasonable to speculate that spontaneous behavioral recovery on the skilled reach task was related to functional changes in RFA. If RFA reorganization formed the basis for motor recovery, and if motor skill acquisition drives the changes in cortical representations, we can speculate that observed plasticity in RFA probably have supported the functional recovery through compensatory movements rather than recovery of the original movement patterns.

Ch 3; the evidence of differential effect of rehabilitative training over time on reorganization in RFA associated with functional recovery after a focal ischemic injury to CFA

We sought to determine longitudinal changes in the spared cortical physiology adjacent to the lesion during both spontaneous and rehabilitation-aided motor recovery following a restrictive ischemic lesion to CFA. Our major findings were 1) rehabilitative training restored functional recovery quantitatively and qualitatively to baseline pre-lesion levels, 2) improvement in movement quality occurred simultaneously with quantitative recovery and continued past the plateau in quantitative recovery, 3) on post-lesion day (PLD) 38 the rehab group displayed a larger representation area, indistinguishable size to the intact level, than the no rehab group, 4) RFA map expansion in the rehab group coincided with improvements in quality of the movements during a reach rather than with an increase in quantitative success, suggesting cortical areal expansion reflects not only the quantitative endpoint recovery but also the improvement in the behavioral quality. Together, restoration of the RFA appears to be dependent both on post-lesion motor use and time. Quantitative behavioral recovery may be contingent on and may precede the expansion in the spared cortex. Further, improvements in the movement quality may be supported by expansion of the forelimb representations.

Previously, the effect of rehabilitative training has been demonstrated in the peri-infarct area after incomplete M1 lesions (Castro-Alamancos and Borrel, 1995; Nudo and Milliken, 1996; Kleim et al., 2003b), and in spared rat RFA (Conner et al., 2005). This study provides the first evidence of how rehabilitative training affects once-diminished cortical areas remote from the lesion; it restored the RFA after ischemic lesion to CFA.

Our results show qualitative recovery temporarily correlated with the cortical map expansion. It may be possible that the map expansion supports improvement in movement quality. A temporal mismatch between quantitative behavioral improvement and map expansion was previously reported from our laboratory in a study on non-human primates during spontaneous recovery after destroying both M1 and PMv. According to their results obtained in spontaneously recovered monkeys mapped at the post-lesion weeks 3rd and 13th, the longitudinal profile of map expansion occurred after behavioral recovery plateau. It suggested that quantitative behavioral recovery may precede cortical map expansions in supplementary motor area (SMA). (Eisner-Janowicz et al., 2008). While the present results also support such notion, there are subtle differences speculated from the difference in species, and extensiveness and methods of lesion inductions. First, our spontaneously recovered group did not show any areal expansions over time, displaying only an incomplete areal restoration. The behavioral recovery occurred later than the rehab group. The behavioral plateau in monkeys was not at the level of pre-lesion performance while our rehabilitated rats reached the baseline performance level and plateaued thereafter. Second, in the previous study, the restored area of the supplementary motor cortex exceeded the pre-lesion area substantially after damage both to M1 and premotor cortex. In the present study, with rehabilitation, the RFA was restored at least to the intact level; presumably, the remaining fibers restored the formerly connected representations but did not reconnect with areas beyond their typical borders. It may be possible that cortical plasticity in rats is more limited than in non-human primates (Jain et al., 1995).

The early behavioral recovery may be due to the attenuation of diaschisis rather than to reinstated neural connectivity and functional integrity (Witte, 1998; Nudo and Friel, 1999). Remote hyperexcitability is thought to subsequently contribute for cortical plasticity (Witte et al.,

1997). Various molecular and anatomical changes in the cortices coincide with most of the spontaneous recovery that takes place as early as the 2nd post-stroke week (Jones and Schallert, 1992b; Schallert and Jones, 1993; Stroemer et al., 1995; Jones and Greenough, 1996; Jones et al., 1996; Qu et al., 1998; Jolkkonen et al., 2003). Physiological changes measured by cortical topography thus seem to occur later than the behavioral improvements.

The overall study results were congruent with previous findings in that rehabilitative intervention promotes cortical reorganization which, in turn, plays a role in supporting functional recovery after a focal lesion to primary motor cortex (Nudo et al., 1996b; Ward et al., 2003; Conner et al., 2005). Importantly, other studies have shown that lesioning of reorganized areas after behavioral recovery re-instates the motor impairment formerly induced by various types of CFA lesions (Castro-Alamancos and Borrel, 1995; Liu and Rouiller, 1999; Gharbawie et al., 2007). We showed how early diaschisis-like effects are recruited by rehabilitative intervention for subsequent cortical plasticity. The neural strategies for motor improvement observed in this study were to restore once abolished RFA function which vicariously substituted for the lost CFA function of producing movements necessary for single-pellet reach and retrieval.

Ch 4; the evidence of a larger consequence of the traumatic- than the ischemic-injury on the RFA integrity and motor behavior

In Chapter 4, We demonstrated that 1) focal lesions to CFA by contusion and ischemic methods result in similar initial deficits, 2) the overall recovery was better in rats subjected to

cortical ischemia than those with contusion, 3) both CFA lesions diminished the remote cortical integrity of RFA, 4) while the histological lesion volume was statistically not different, the RFA physiological integrity was more compromised by the contusion. Our comparisons provide a ground of neural reorganization in the remote area associated with spontaneous recovery in rat models during chronic stages. The results suggest that secondary degeneration, unique to contusion, in remote areas may cause such differences.

Limitations of the Rat Model of CNS Injuries

It is not surprising that treatments developed in young, healthy lab-raised animals that were subjected to CNS injuries, approximating clinical injuries, often fail clinical trials. It is no exaggeration to say that the effectiveness of a specific treatment can be limited to the lesion induction methods. Current experimental lesions do not simulate clinical CNS injuries (Nudo 2007). Rather they allow us to investigate the mechanisms of neural death, neural repair as well as functional deficits and recovery. Clinical strokes, on the other hand, are caused by predisposed conditions including cardiovascular diseases, obesity, diabetes, high blood pressure, age and genetic variance. Clinical TBIs often cause multi-traumas not limited to CNS and many complications necessarily follow, let alone not limited to one area of CNS. To take advantage of

neural plasticity effectively in human patients, it is expected to require the next decades of translational research.

For the studies in Ch. 2 and 3, one technical limitation was that we compared RFA maps in animals with CFA lesions to those in normal animals. Repeated mapping in the same animal, a common practice in our non-human primate studies and an alternative approach for the proposed studies, is much more difficult in rodents, and it compromises the cortical tissue due to reopening the craniectomy. We also relied on stereotaxic coordinates for the placement of the cortical contusion and the ET-1 injections in CFA. Previous studies indicate that relying on the stereotaxic coordinates is a reliable approach for the CFA damage induction.

Particular to the study in Chapter 2, though the cortical injury created by the CCI device consistently reproduced a tissue cavity in CFA and adjacent cortical areas, some variability was observed in cortical depth and white matter involvement. The lesion volume, however, was not statistically different among the CCI groups.

Also, the control group did not undergo any sham surgery. For logistical reasons (i.e. location of the surgery suit), we kept the necessary animal transportation to a minimum. Without craniectomy of one kind or another, this control served as intact control for both contusion and ischemia groups.

For the study in Chapter 3, a longitudinal assessment of cortical plasticity was performed in different groups of animals rather than in the same group repeatedly. As stated, we faced a

technical difficulty in performing repeated mapping procedure in rats. Though it could have, individual variability did not confine our neurophysiological results over time.

The limitation for Chapter 4 was that we compared the physiological results assessed during post-lesion week 6 and 7, both of which are within the chronic stages of injury. Though we do not anticipate substantial error in comparing these time points, it is possible that reorganization still occurs within post-lesion weeks 6 and 7.

Vicarious Function of RFA after CFA Injury as a Mechanism of Recovery

Our experiments systematically demonstrated spontaneous recovery and rehabilitation-aided recovery from cortical injuries in rats kept under the restricted, controlled environment. We showed that functional recovery without compensatory strategies is possible with adequate cortical spared substrates through extensive reorganization. Further, the neurophysiological examinations demonstrated that rehabilitation restored the physiological integrity of the RFA, which otherwise, would have been partially lost to the lesion in CFA. We speculate that RFA has vicariously taken over, at least partially, the original function that was mediated by the CFA. The following sections first summarize the anatomical and physiological implications by ICMS maps and discuss how vicarious substitution of cortical function may be possible.

<u>What does ICMS map reveal?</u>

Cortical maps derived by ICMS elucidate the integrative physiological function of the nervous system in controlling target muscles. The current delivered at layer V of the motor cortex recruits pyramidal neurons and evokes visible movement at joint(s) or recordable electromyographic (EMG) activity. ICMS activates soma or fibers in three ways; 1) by direct stimulation of local soma via current spread; 2) direct stimulation of intracortical collaterals of pyramidal tract axons whose soma may be remote (Asanuma et al., 1976); and 3) indirect transynaptic simulation of neurons receiving sufficient convergence of excitatory input from the directly activated cells (Jankowska et al., 1975). Thus, anatomical substrates determine the somatotopic organization of motor cortices derived by ICMS. I herein briefly review the important aspects of the anatomical framework.

First, outputs from motor cortical territories converge on the spinal motoneuron pool of any given muscle. Also, the motor territories from which outputs converge on upper extremity muscles overlap extensively. Thus, any instance of eliciting a muscle (or muscle group) contraction by ICMS means that the evoked output from the motor cortex to the muscle was greater than the output to other muscles, not that output was selective to the muscle. That is, the output to other muscles was insufficient to result in discharge to produce a detectable movement given that the ability to evoke movement requires a sufficient level of synaptic connectivity at the cortical level. Different topographical maps of forelimb (Andersen et al., 1975) and hindlimb (Jankowska et al., 1975), therefore, can be derived depending on the stimulation strength. Those studies, in turn, provide evidence of the extensive convergence and overlaps of the corticospinal tracts (Fig 2).

Second, muscle contractions elicited by ICMS also depend on the divergent arrangement of the corticospinal projections. The evidence of divergence from wide motor territories onto spinal motoneuron pools of more than one muscle was obtained from anatomical and physiological studies. Anatomically, collateral branches of a single corticospinal axon have been shown to ramify over several spinal segments providing terminal arbors in the motoneuron pools of up to four muscles Fig 3 (Shinoda et al., 1981). Physiologically, many single M1 neurons have been shown to produce postspike effects of relatively direct connections of different muscles in the forearm Fig 4 (Fetz and Cheney, 1980) or in the hand (Lemon et al., 1986) of macaques.

Third, long-range horizontal interconnections within motor areas also provide substrates for ICMS mapping. For example, neurons within the M1 cortical area which evokes digits may extend its terminal arbors throughout the upper extremity regions, including the areas that evoke movements of the shoulder, elbow and wrist. Reciprocally, axon collaterals from widespread neural somata of the upper extremity representation terminate in the digit region Fig. 5 (Huntley and Jones, 1991). Those horizontal axon collaterals particularly originate from pyramidal neurons in layers III and V, and often exert excitatory, glutamatergic effects (Aroniadou and Keller, 1993; Keller and Asanuma, 1993).

Corticospinal control is heavily mediations by the reticulospinal tract or by segmental interneurons in rats. Nevertheless, cortical maps elucidate the functionality of the cortical control to target muscles. Movements with a greater degree of control are more easily evoked to ICMS and occupy a larger proportion of the topography. Thus, representations corresponding to trained movements occupy a larger area of the topography. The increase in the map area supports enhanced control of the acquired movements.

Normal functions of RFA and CFA in skilled reach

Whishaw and Pellis have demonstrated that skilled reaching requires rats to first engage the paw advancement with shoulder and forelimb movements. Then, coordinated wrist and digit movements must be learned to grasp and retrieve a presented object (Whishaw et al., 1991). Acquiring a motor skill in intact rats is accompanied by LTP-like increased synaptic strength (Rioult-Pedotti et al., 1998) and morphological changes of pyramidal cells **within the contralateral CFA to the trained limb,** including extensive dendritic arborizations (Greenough et al., 1985) and synaptogenesis (Kleim et al., 2002). Physiologically, the wrist and digit cortical areas expand within the contralateral CFA at the expense of shoulder movement representations (Kleim et al., 1998a). On the contrary, no morphological or physiological changes in RFA are associated with skilled motor acquisition. Kleim et al suggest that those two areas exhibit different patterns of plasticity because they have different roles in motor control. In intact rats, extensive reorganization in RFA appears to be non-essential to supporting the acquired behavior. When neurons in CFA become dysfunctional, e.g. due to lesion, then reorganization in RFA may become essential to supporting or reestablishing the behavior.

Rouiller et al suggest that RFA can be considered "hierarchically superior to CFA". Shown for the visual (Coogan and Burkhalter, 1990) and auditory (Rouiller et al., 1991b) cortical areas, feedforward projections (ascending) typically originate from neurons in upper cortical layers, and feedback projections (descending) originate from neurons in deeper layers. The relations of laminar distribution between CFA and RFA connectivity appear to indicate that CFA to RFA is ascending and RFA to CFA is descending. Similarly, their connectivity to SI and SII also supports the notion that RFA may play a role of feedforward. Supportive of the feedforward

theory by RFA, an early study indicated that neurons in CFA discharge in conjunction with forelimb movements on an isometric bar pressing task while neurons in RFA discharge prior to and during forelimb force changes (Donoghue, 1985). A more recent study also showed that cortical field potential, the presumed "readiness potential", can be detected approximately within 1.2s from RFA and 1.0 s from CFA prior to the onset of the self-initiated forelimb movements (Seki et al., 2005). Taken together, neurons in RFA fire slightly earlier than neurons in CFA during the motor control, suggesting RFA may mediate motor planning and initiation.

Though the motor cortical areas in rats appear to specialize for different aspects of motor control, some connectivity features are shared. **It is for the anatomical substrates that RFA may vicariously take over the function of CFA when the latter is damaged.** RFA sends direct projections to CFA and to the cervical spinal cord of the hand and proximal limb musculature, meaning the cortical pathway from **RFA can control movements separate from the CFA**. According to Laing et al, latencies to elicit muscle contraction of the wrist and digit by single ICMS of relatively low intensity (<35μA) were similar. Also, both areas evoked comparable *groups* of muscles by stimulation, presumably owing it to the prominent divergent corticospinal axons (Liang et al., 1993). The findings suggest that the corticospinal connectivity from the two regions is anatomically similar; and hence, the relative strength of connectivity to motoneurons is similar in neurons of RFA and CFA.

Neural substrates of movements shown in lesion studies

Inducing a focal damage in CFA but not in RFA produces significant impairments that can readily be detected in the performance at the single-pellet reach and retrieval task (Gharbawie et al., 2007) and impairs individual digit movement (Erickson et al., 2007; Alaverdashvili et al.,

2008a). A lesion in CFA and not RFA also produces deficits in forelimb motor function at the footfault task (Barth et al., 1990). According to the authors, the animals exhibited difficulty in inhibiting the inaccurate placing response. Thus, the studies suggest CFA makes the main contribution to skilled motor performance, and that there is a lesser contribution by RFA.

Though CFA mostly mediates skilled motor learning in intact rats (Kleim et al., 1998a; Gharbawie et al., 2007) and overlaps with cutaneous receptive fields, it is important to note, as described above, that RFA also maintains connections with SI and SII in a feedforward manner (Chapin et al., 1987; Rouiller et al., 1993). Indeed, an RFA focal lesion produces more enduring deficits in a bilateral stimulation test with heavier neglect in the contralateral forelimb than in the case of a CFA lesion (Barth et al., 1990).

When both CFA and RFA are destroyed, the subsequent motor deficits are readily detected in vibrissae-forelimb placing, forelimb-forelimb placing, footfaults, bilateral asymmetry sensory test, and they persist over 6 months, and hence the obtained recovery is limited (De Ryck et al., 1992; Hoane et al., 2000).

RFA plasticity

RFA can exhibit more plasticity than CFA. For example, hemidecortication at the neonatal stage leaves relatively normal forelimb movements on the contralesional side through reorganized corticospinal fiber termination from the undamaged hemisphere, more densely from RFA than CFA (Umeda and Isa, 2011). In this study, contra- and ipsi- corticospinal fibers were widely distributed equally in the undamaged RFA whereas ipsi-corticospinal fibers were found just at the periphery of the cluster of contra-corticospinal fibers in CFA.

After electrolytic lesions in CFA, RFA plasticity has been demonstrated with a form of ICMS that uses long-duration trains of stimulation to elicit complex movements (Ramanathan et al., 2006). In rats that were not rehabilitated, the RFA map of complex movements was similar to that of intact rats. Meanwhile, in rats that received rehabilitative training on a pellet retrieval task, the RFA map expanded.

Conner showed the basal forebrain cholinergic system plays an essential role in enabling RFA reorganization after a focal CFA injury. Further, secondary lesion selective to RFA (but not secondary lesion to hindlimb area) after behavioral recovery plateau after a focal CFA lesion disrupted skilled reaching performance (Conner et al., 2005).

Is RFA normal function compromised by vicarious reorganization?

As discussed, damage in RFA particularly manifested motor dysfunction in the sensory tactile test while damage in CFA produced deficits in footfaults task. Thus, in normal, intact rats CFA and RFA appear to have different roles in motor control. In our results, functional recovery was associated with RFA physiological restoration. To determine whether the normal function of RFA would be compromised by its role in compensating for the CFA function, we could assess the rats on the bilateral stimulation test after recovery reaches a plateau on the skilled reach task. If we observed motor deficits in the sensory tactile test, it may suggest that RFA function was indeed compromised.

Structural bases that support RFA expansion

Proposed mechanisms for cortical motor map reorganization include: a) the triggering of pre-existing excitatory connections by disinhibition (Jacobs and Donoghue, 1991), which provides

an opportunity for b) long-term potentiation (LTP) of synaptic transmission in local horizontal cortical connections (Hess and Donoghue, 1994), c) synaptogenesis, (Kleim et al., 1996), and d) collateral sprouting (Carmichael, 2003). Together, these mechanisms suggest that the cortical map topography should change depending on the synaptic strengths among neurons.

There are, indeed, silent structures in many areas of the brain that may be functionally inactive because of the competition among the neural pathways. Recruitment of such previously silent synapses occurs after injury. Rehabilitative training or pharmacological intervention is thought to aid in unmasking synaptic connections (Goldstein, 1990, 2003). Long-term potentiation, accompanied by protein synthesis, is maintained through an increase in presynaptic transmitter release and new synaptic release sites (Kendal 2000).

Collateral sprouting is of interest in vicarious substitution because it may change the termination on the same motor nuclei through different interneurons. Axonal sprouting in corticocorical connectivity has been shown in peri-lesional area (Carmichael et al., 2001). It is reasonable to assume axonal sprouting changes the convergent and divergent connectivity of cortico-spinal, cortico-reticulo-spinal, and cortico-cortical projections from RFA. The projections from RFA may increase to the ventrolateral nucleus of the thalamus, or to the gray matter of the cervical spinal cord where motor neurons for intrinsic hand muscles are located, particularly after rehabilitative training to produce coordinated synergistic movements of digits and hand. Hence, efferent neurons from adjacent representations that interconnect with intracortical afferents are speculated to have over time altered the descending projections to distal forelimb muscles, re-circuiting the sensorimotor physiological function.

Extensive Reorganization from Supplemental Motor Area in Non-Human Primates.

Extensive reorganization from the ipsilesional supplemental motor area (SMA) has been documented after lesion to M1 and premotor cortex in non-human primates during spontaneous recovery (Aizawa et al., 1991; Eisner-Janowicz et al., 2008). Wrist and forearm representation enlarged significantly between the assessment time of 3 and 13 weeks post-lesion, attaining a substantially larger size than the baseline area (Fig 6). The study further found that the better the motor recovery, the larger the absolute size of the SMA distal forelimb area became (Eisner-Janowicz et al., 2008). Paralleling this study result, McNeal et al showed that cortical projections from SMA reorganized specifically to terminate in the contralateral, and not ipsilateral, gray matter of the cervical spinal cord (Mcneal et al., 2010). The contralateral plasticity was detected in lamina VII and dorsally within lamina IX, suggesting selective intraspinal sprouting transpired in the regions containing interneurons, flexor-related motor neurons, and motor neurons supplying intrinsic hand muscles (Fig 7). Thus, selective reorganization in the corticospinal connectivity played important roles in mediating reaching and digit movements.

Conclusion Statement

In neuroscience, definitive causal relations are challenging to prove, for the brain is an intricate, interactive system. It is likely that functional recovery involves the M1, premotor cortex, supplementary cortex, cerebellum, somatosensory cortex, subcortical areas, as well as peripheral inputs. As Diamond stated, “every area of the cortex can be viewed as a motor area, or layer V itself could be termed the motor cortex” (Diamond, 1979).

In conclusion, this present dissertation work supports the hypothesis that cortical plasticity within the spared RFA after restrictive damage to CFA mediates use-dependent physiological reorganization, which provides a substrate for sustaining rehabilitation-aided motor functional recovery. The research results offer a step toward the development of effective, evidence-based rehabilitative strategies for people who experienced brain damage.

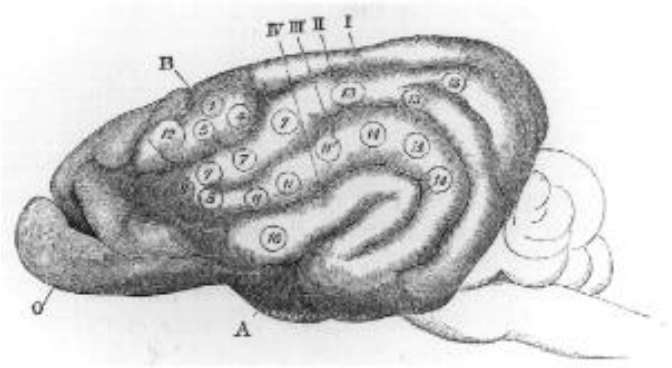


Fig 1 Illustration of motor map (Ferrier, 1873) *West Riding Lunatic Asylum Medical Reports.*

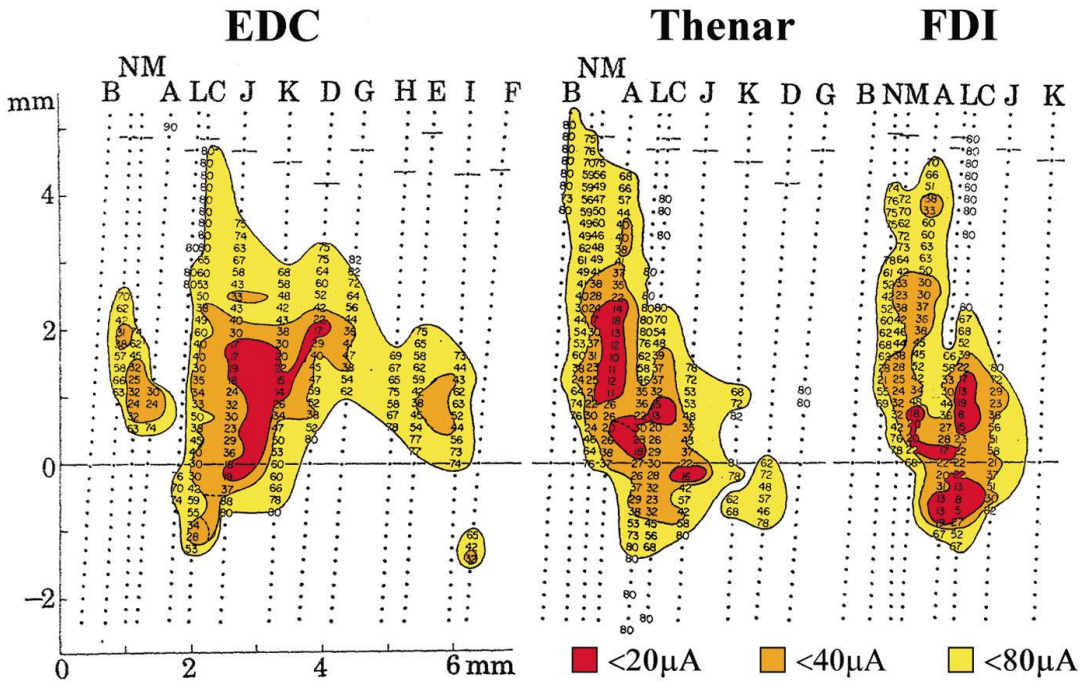


Fig 2 Convergence and overlap demonstrated by ICMS from Fig 5, p2132 *J of Neurophysiology* (Schieber, 2001) ICMS results in 12 electrode penetrations (A through N) down the anterior wall of a baboon's central sulcus while recording 3 single motor units: one in extensor digitorum communis (EDC, which extends all 4 fingers, radial nerve innervations); second in the thenar muscles (Thenar, which act only on the thumb, median nerve innervations); and a third in the 1st dorsal interosseous (FDI, which acts on the index finguer, ulnar nerve innervations). With currents up to 20 μA, multiple small zones from which each motor unit could be discharged are shown in red. Although the zones for the different motor units were largely interdigitated, on close inspection these small zones also overlapped to some degree. At higher currents up to 40, and then 80 μA, the small zones for each motor unit expanded into large cortical territories, increasing their mutual overlap. Current spread cannot solely count for this degree of convergence and overlap in the cortical territories of the 3 motor units, which each were served by different peripheral nerves and each acted on different digits.

Fig 3 Anatomical evidence of divergence in single corticospinal neuronal projection from Fig 2, p10 *Neusoscience letters* (Shinoda et al., 1981) A horseradish peroxidase (HRP)-filled corticospinal axon has been reconstructed in the transeverse plane of the ventral horn of the monkey spinal cord. (Left) The cord midline and central canal (Right) The lateral column The filled corticospinal axon enters the spinal gray matter from the lateral column and branches repeatedly, giving off terminal ramifications in the outlined motoneuron pools of 4 different muscles.

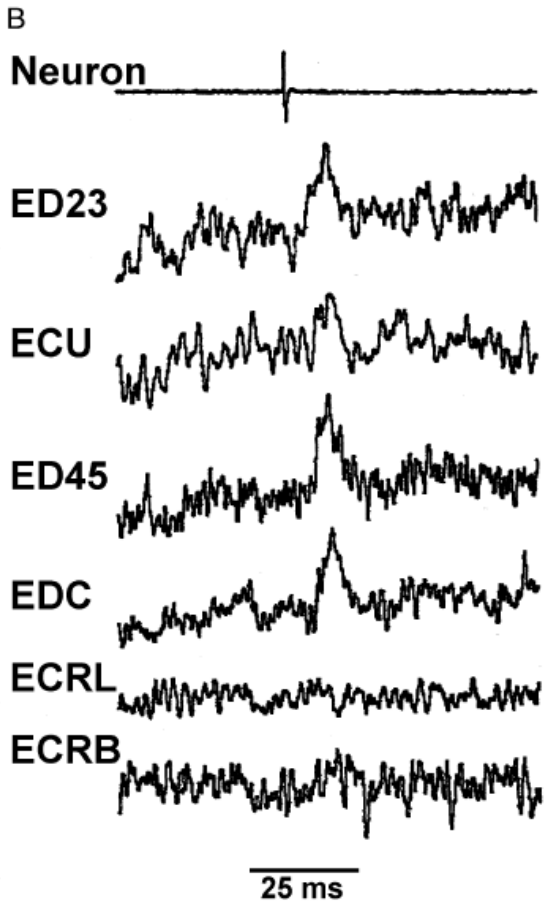


Fig 4 Physiological evidence of divergence of single corticospinal projection from Fig 6, p 759 *J of Neurophysiology* (Fetz and Cheney, 1980) Averages from rectified electromyographic activity are shown from 6 muscles (extensor digitorum secundi et ertii (ED23), extensor carpi ulnaris (ECU), extensor digitorum quarti et quinti (ED45), extensor digitorum communis (EDC), extensor carpi radialis longus (ECRL), and extensor carpi radialis brevis (ECRB) that act on the wrist and/or fingers in macaque monkeys. The brief peaks that appear in each of the 1st 4 EMG traces shortly after the neuron spike indicate that motoneurons innervating these 4 muscles received synaptic excitation at a short and fixed latency from the discharge of action potentials by the single M1 neuron.

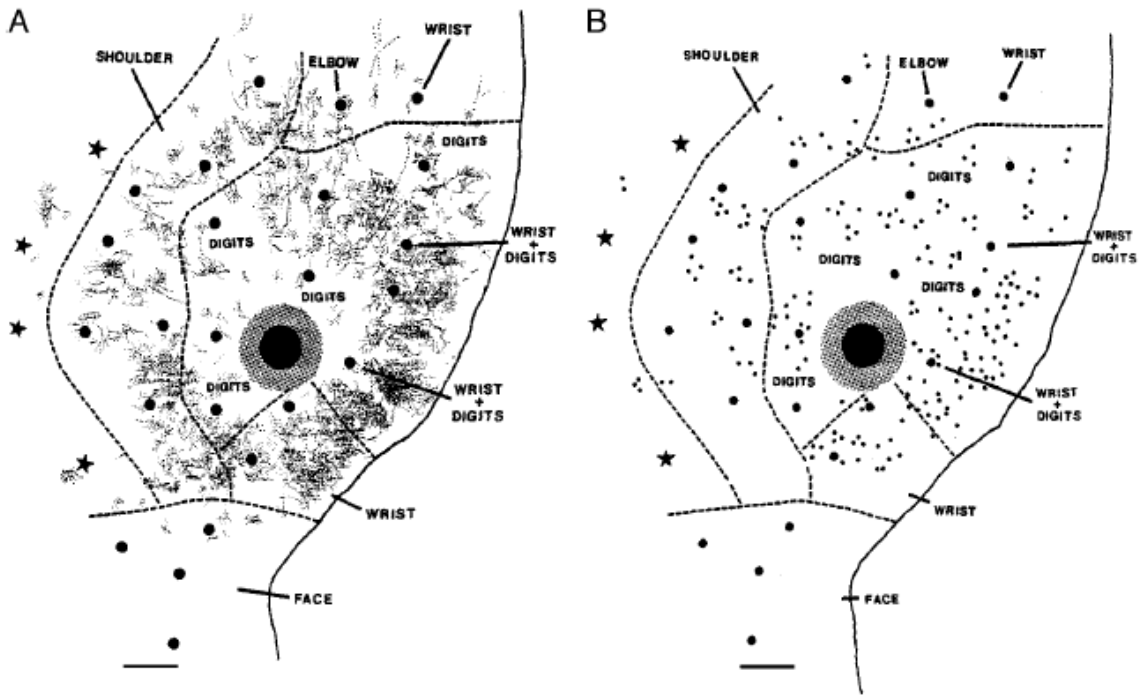


Fig 5 Horizontal interconnection in the M1 upper extremity representation from (Huntley and Jones, 1991) A horseradish peroxidase (HRP) was injected in the low-threshold digit representation (large, filled black circle) which resulted in widespread terminal labeling (fine stippling in A) and retrograde filling of neuronal somata (small black dots in B). Dash lines divide the representations of evoked body parts. Starts indicate points where stimulation at 40μA failed to evoke observable movements. Scale bar=1mm

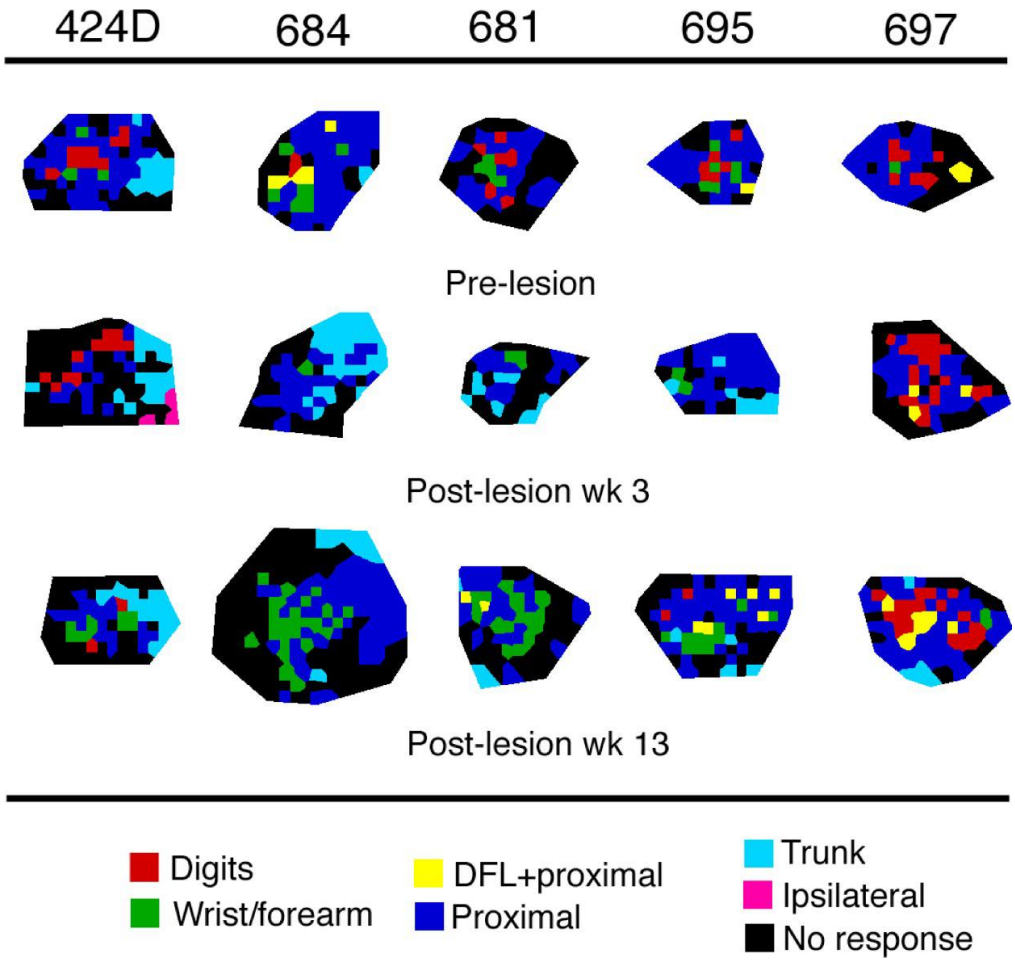


Fig 6 Color coded ICMS maps of SMA distal forelimb area (DFL) before and after cortical lesion from Fig 7, p1506 *J of Neurophysiology* (Eisner-Janowicz et al., 2008), derived by electrical stimulation of 60μA. The top numbers are indicative of animal case number. Spontaneously recovered squirrel monkeys were mapped at post-lesion 3 and 13. The total SMA DFL representation averaged 0.83±0.05mm^2 at baseline, 0.56±0.3mm^2 on post-lesion week 3, and 1.41 ±0.23mm^2 on post-lesion week 13.

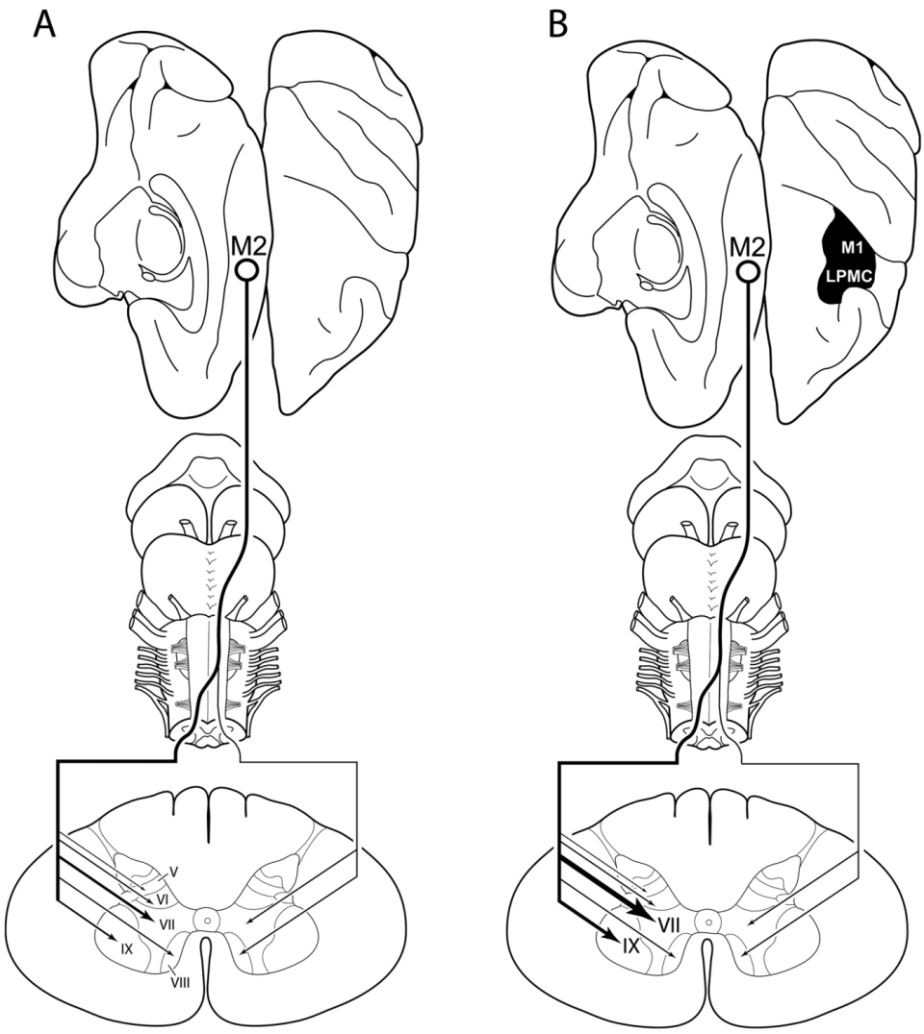


Fig 7 Illustration of corticospinal projections from supplementary motor area from Fig 13, p 612 *J of Comparative Neurology* (Mcneal et al., 2010) Most descending fibers cross at the midline at inferior brainstem levels and terminate in the spinal cord. The relative intensity of the projection to the spinal cord laminae is indicated by line thickness and arrow size. A) Projections of an intact animal. B) Corticospinal projections of animals subjected to lesion in M1 and premotor cortex (blackened area). Extensive enhancement of the contralateral projection to lamina VII and IX occurred following the lateral motor cortical injury but no in other contralateral, ipsilesional laminae.

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