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The effect of lesion size on cortical reorganization in the ipsi and contralesional hemispheres

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RÉSUMÉ

Bien que la plasticité ipsilesionnelle suite à un accident vasculo-cérébral (AVC) soit bien établie, la réorganisation du cortex contralésionnel et son effet sur la récupération fonctionnelle restent toujours non élucidés. Les études publiées présentent des points de vue contradictoires sur le rôle du cortex contralésionnel dans la récupération fonctionnelle. La taille de lésion pourrait être le facteur déterminant la réorganisation de ce dernier. Le but principal de cette étude fut donc d'évaluer l'effet des AVC de tailles différentes dans la région caudal forelimb area (CFA) du rat sur la réorganisation physiologique et la récupération comportementale de la main. Suite à une période de récupération spontanée pendant laquelle la performance motrice des deux membres antérieurs fut observée, les cartes motrices bilatérales du CFA et du rostral forelimb area (RFA) furent obtenues. Nous avons trouvé que le volume de lésion était en corrélation avec le niveau de récupération comportementale et l'étendue de la réorganisation des RFA bilatéraux. Aussi, les rats ayant de grandes lésions avaient des plus grandes représentations de la main dans le RFA de l'hémisphère ipsilésionnel et un déficit de fonctionnement plus persistant de la main parétique. Dans l'hémisphère contralésionnel nous avons trouvé que les rats avec des plus grandes représentations de la main dans le RFA avaient des lésions plus grandes et une récupération incomplète de la main parétique. Nos résultats confirment l'effet du volume de lésion sur la réorganisation du cortex contralésionnel et soulignent que le RFA est l'aire motrice la plus influencée dans le cortex contralésionnel.

Mots-clés : accident vasculo-cérébral, réorganisation contralésionnelle, microstimulation intracorticale, récupération fonctionnelle, taille de lésion.

ABSTRACT

While our understanding of ipsilesional plasticity and its role in recovery of hand function following ischemic stroke has increased dramatically, the reorganization of the contralesional motor cortex and its effect on recovery remain unclear. Currently published studies offer contradictory views on the role of contralesional motor cortex in recovery. Lesion extent has been suggested as the factor determining the type of reorganization of the contralesional motor cortex. The primary goal of this study was thus to evaluate the effect of unilateral strokes of different sizes in caudal forelimb area (CFA) of the rat on both physiological reorganization and behavioral recovery. At the end of a period of spontaneous recovery during which we monitored motor performance of both limbs, we obtained bilateral maps of the CFA and the putative premotor area of the rat – rostral forelimb area (RFA). We found that lesion volume in the CFA correlates with both the extent of behavioral recovery of the paretic hand and the extent of both ipsi and contralesional cortical reorganization. We found that rats with bigger lesions had larger hand representations in the ipsilesional hemisphere and more persistent deficits of the paretic hand. In the contralesional hemisphere we found that rats with larger hand representation in the RFA had bigger lesions and incomplete recovery of the paretic hand. Our results confirm the effect of lesion volume on the reorganization of the contralesional motor cortex and highlight contralesional RFA as the motor cortical area most influenced by lesion volume for future investigations.

Key words: cortical stroke, contralesional reorganization, intracortical microstimulation, functional recovery, lesion size.

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LIST OF ABBREVIATIONS

1a afferent fibers -	Primary afferent fiber
AP -	Anterior-posterior
CFA -	Caudal forelimb area
EMG -	Electromyographic
ET-1 -	Endothelin-1
GABA -	Gamma-Aminobutyric acid
H-reflex -	Hoffmann's reflex
Hz -	Hertz
ICMS -	Intracortical microstimulation
M1 -	Primary motor cortex
MAP2 -	Microtubule-associated protein 2
MCA -	Middle cerebral artery
MCAo -	Middle cerebral artery occlusion
ML -	Medial-lateral
NMDAR1 -	N-methyl-D-aspartate subunit 1
PMd -	Dorsal premotor cortex
PMv -	Ventral premotor cortex
RFA -	Rostral forelimb area
rTMS -	Repetitive transcranial magnetic stimulation
S1 -	Primary somatosensory cortex
SMA -	Supplementary motor area
TMS -	Transcranial magnetic stimulation

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Boris Touvykine

CONTRIBUTION OF AUTHORS

Chapter 2 contains a manuscript ready for submission to Neurorehabilitation and Neural Repair: Touvykine B., Mansoori B. K., Jean-Charles L., Deffeyes J., Quessy S., Dancause N. *The effect of lesion size on cortical reorganization in the ipsi and contralesional hemispheres*. For this article, the nature of my contribution is as follows: task familiarization of animals, lesion inductions, histological analysis, behavioral testing and preliminary data analysis. In addition I participated in all the terminal ICMS experiments, and was the lead surgeon for all of control group and half of experimental animals. I also wrote the first draft of the Methods and Results sections. Dr. Babak Khoshkrood Mansoori participated in most terminal experiments and was the lead surgeon in half of experimental animals. Loyda Jean-Charles assisted in all the control ICMS experiments as well as most terminal ICMS experiments. Dr. Joan Deffeyes created the software in MATLAB, which I used to analyze experimental data. Dr. Stephan Quessy provided guidance and oversight with the behavioral component of the experiment, as well as running additional statistical analysis on the data. My supervisor Dr. Numa Dancause provided guidance and instruction throughout the study, including data collection, data analysis and interpretation, and the preparation of the final manuscript.

Chapter 1

General introduction and literature review

1.1 General introduction

Stroke is a cardiovascular disease, which damages a part of the brain due to a disruption of normal functioning of the cardiovascular system. It is the leading cause of disability worldwide. In Canada alone each year there are approximately 50000 strokes (PHAC 2011). While many people survive stroke, they are left with multiple behavioral and cognitive deficits. Currently there are approximately 315,000 Canadians dealing with post-stroke complications (Hakim, Silver, and Hodgson 1998). To contribute to the design of more successful treatments for individuals with stroke-induced deficits, it is important that we gain a better understanding of the basic mechanisms of cortical reorganization that occur after stroke.

There are two types of strokes, hemorrhagic and ischemic. Hemorrhagic stroke is neuronal death due to a rupture of a blood vessel. This type of stroke accounts for approximately 13% of all stroke cases. The second type of stroke is ischemic, also known as cerebral infarction. Ischemic stroke is neuronal death due to a blockage of a blood vessel, most often by a blood clot. This either significantly slows down the blood flow or stops it completely, interrupting vital oxygen and nutrients supply to the brain. This type of stroke is much more common and accounts for approximately 87% of all stroke cases. There is also a phenomenon that has been identified as mini-strokes which are often asymptomatic. They are due to a very transient blockage of a minor blood vessel that does not last long enough to lead to significant neuronal damage. The major difference between mini-stroke (also

known as Transient Ischemic Attack) and ischemic stroke is the amount of damage done to the brain. Our study investigated cortical reorganization following the most prevalent type - ischemic stroke.

As much as 80% of ischemic stroke cases are due to blockage of the middle cerebral artery (MCA), which is the largest artery in the brain or one of its branches (Harrison 1994). MCA supplies multiple cortical (frontal, parietal and temporal lobes) and subcortical (basal ganglia and the internal capsule) regions of the brain. The extent of initial ischemic damage depends on whether the whole of the MCA or one of its multiple branches will be blocked. This leads to variability of lesion size and location, creating differences from patient to patient and complicating prognosis.

The overwhelming majority of strokes are unilateral and therefore result in a lesion in one hemisphere. Many stroke survivors are left with persistent deficits in motor control, from such extreme cases as hemiparalysis to milder cases such as difficulties in fine motor control. In a classic study in 1951 Twitchell observed that unilateral stroke affects the upper limb more than the lower limb, and recovery of the upper limb is worse.

While research into stroke recovery and rehabilitation has made great progress in the past decade, numerous stroke survivors with motor deficits of the upper limb are left with significantly lower quality of life and a large strain on the health care system. In particular, motor deficits of the hand following stroke are some of the most resilient motor impairments after stroke, meaning such survivors are unable to do even simple manipulations. As a consequence, better knowledge of how reorganization following stroke permits the recovery of hand is needed. To help us better understand the recovery process this study was designed to investigate motor recovery of the hand in the rat. Rats are able to grasp and manipulate small objects with their forelimbs. Vasoconstrictor endothelin-1 (ET-1) was used for lesion induction protocol. It is an endogenous molecule, which binds to receptors present on blood vessels and results in vasoconstriction (Black et al. 2003).

Vasoconstriction results in hypoxia, which in turn induces cortical lesions replicating the mechanism of ischemic stroke. One advantage of using a rat model is that there is incredibly high variability of lesion size and location in human patients, whereas inducing stroke in the rat circumvents this problem. The size of focal lesions we induce in the motor cortex of the rat can be controlled by injecting small amounts of ET-1 to limit its spread. This allows for examination of reorganization and recovery induced by a cortical lesion in the motor cortex.

1.1.1 Motor areas of the frontal cortex

In humans motor cortex is responsible for the planning and execution of voluntary movements. It is the region in the caudal part of the frontal lobe of the cerebral cortex. Currently the motor cortex is separated into a primary motor cortex (M1) and a variety of non-primary motor cortical regions (Fulton 1935; Penfield and Welch 1951). The execution of voluntary movements is through the corticospinal tract, the vast majority of which originates in M1 (Dum and Strick 1991). Most of the corticospinal tract consists of fibers originating from the large pyramidal neurons in Layer V of the motor cortex. The axons of these neurons form pyramids in the brainstem, and then most of those axons cross over to the side contra*lateral* to their hemisphere of origin (approximately 80% of pyramidal fibers) (Nathan and Smith 1973). In the spinal cord these axons form synapses with excitatory and inhibitory interneurons, which in turn synapse on motoneurons enervating the muscles. Humans, great apes, and some higher order non-human primates (e.g. Macaca) have corticomotoneuronal connections. In these cases, there is only one synapse between a cortical neuron and a motoneuron. This feature is limited to the hand and finger muscles of the forelimb and may support high manual dexterity of these species (Porter 1985).

In many primates, a series of non-primary motor areas are found rostral to M1. To date, at least six premotor areas have been described, which include the premotor ventral (PMv), premotor

dorsal (PMd), supplementary motor area (SMA) and three cingulate motor areas. Ablation studies in primate SMA have resulted in significant impairment of performance of bimanual tasks, suggesting its involvement in preparation and coordination of sophisticated bimanual movements (Brinkman 1984). PMv has been shown to be involved in the processing and transformation of visual information into internal set of coordinates which are consequently passed on to M1, which executes the motor command (Rizzolatti, Fogassi, and Gallese 2002; Davare et al. 2009). PMd is currently thought to process temporal demands of a task and prepare the necessary sequence for muscle activation (Davare et al. 2006). Cingulate motor areas have not been studied as well as other non-primary motor areas. Rostral cingulate motor area has been implicated in evaluating the reward benefit of the available motor repertoire and subsequent selection of the most rewarding movement (Shima and Tanji 1998). The authors were not able to distinguish between dorsal and ventral cingulate motor areas and grouped them into caudal cingulate motor area. The authors propose that it is involved in movement initiation and motor preparation. In summary planning and preparations of movement are understood to be performed by the higher order (non-primary) motor areas.

By comparison, rodents have a much simpler motor cortex. Currently, only two forelimb cortical regions have been identified. There is a larger caudal forelimb area (CFA), and a smaller rostral forelimb area (RFA). The connection patterns of CFA and RFA are different and suggest that these areas play different roles in the control of the forelimb. The first exhaustive examination of these two areas in the rat came from a study by Rouiller and colleagues (1993). This study examined and compared the pattern of connections to and from RFA and CFA. They found a significant difference in the pattern of incoming and outgoing connections between the two motor cortical areas. Among those was a segregation of both corticocortical and thalamocortical projections. RFA was interconnected with the insular cortex while the CFA was not, a pattern also seen for SMA and

the premotor cortex in primates (Matelli et al. 1986). In addition RFA and CFA were interconnected with different nuclei in the thalamus similar to segregation of thalamic input to the cortex between M1 and non-primary motor areas (SMA, premotor cortex) (Schell and Strick 1984). CFA is also the area from which the majority of the corticospinal neurons projecting to the cervical segment of the spinal cord originate (Starkey et al. 2012). The proportion of corticospinal projections from RFA is much smaller. This mirrors what has been found in primates, in which M1 is the area from which the most corticospinal neurons originate. The projections to the cervical enlargement from any single non primary motor area are significantly smaller (Dum and Strick 1991). These anatomical findings further support the proposed role of RFA as a non-primary motor area acting as either premotor cortex or SMA, with CFA acting as M1. Thus, based on these anatomical data, the RFA is likely to be homologue of a premotor motor area, while the CFA is likely to be a homologue of M1 (Rouiller 1993). However, to date the functional role of RFA is still is not clear, but lately with the advent of optogenetics different researchers have started to explore the functional significance of these anatomical differences in the pattern of connections. There is an increasing body of evidence that RFA acts as a higher-order motor cortical area comparable to non-primary motor areas in primates (Smith et al. 2010; Hira et al. 2013). Smith and colleagues (2010) found that inactivation of RFA leads to increased response time, but does not increase premature responding. Inactivation of the medial prefrontal cortex (mPFC) produced the opposite results. The response time did not change, but premature responding increased. Evaluating these results together with anatomical studies previously done on the interconnectivity of RFA, the authors propose that RFA acts as a premotor cortex and competes with mPFC for action selection. Hira and colleagues (2013) found that RFA and CFA have an asymmetrical pattern of reciprocal connections where the majority of corticocortical connections originating in layer 5b of RFA project towards Layer 5b of CFA. However the majority of corticocortical connections from CFA to RFA originate in layer 2/3 and projection towards layer 5b of RFA. Arguing

that there is laminar hierarchy in the motor cortex with neurons in layer 5b being the final outputs of corticospinal networks, the authors propose that the asymmetrical reciprocity of corticocortical connections between RFA and CFA suggests that RFA is a higher order motor area.

As of yet it is still unclear if RFA functions as a specific non-primary motor area or a fusion of two or more of them. Nonetheless the proposed hierarchical organization of the rat motor cortex makes the organization of the rat motor cortex significantly more relevant to primates than previously thought (Rouiller, Moret, and Liang 1993). All of these factors make the findings on cortical reorganization in the rat more clinically relevant.

1.1.2 Organization of primary motor cortex

Primary motor cortex is organized somatotopically for large regions of the body. The cortical area responsible for evoking movements for different segments of the body, such as upper limb, trunk, face and leg are always oriented the same way relative to one another. For example, the face representation is always found lateral to the forelimb representation. This type of organization was discovered by Penfield and Boldrey (1937) in the somatosensory and motor cortex. In 1957 Mountcastle described the organization of the somatosensory cortex by proposing the concept of the cortical column. According to this hypothesis, a cortical column is the basic processing unit of the somatosensory cortex. In a column, all the neurons have the same receptive fields and there is no overlap of receptive fields between cortical columns. In 1975, based on his previous work using intracortical microstimulation (ICMS) Asanuma proposed the cortical column as the basic functional unit in the motor cortex as well. In his view each cortical column in the primary motor cortex would project to a single muscle. This interpretation was based on his work with ICMS. This technique uses an insulated stimulation electrode to penetrate the cortex and to pass a train of pulses to evoke muscle contractions. By doing so, the volume of stimulated cortex is very small, potentially limited to

a single column. In his experiments using ICMS in primates Asanuma and Rosén (1972) observed that stimulation at threshold current typically induced contractions to a single muscle.

However a number of studies have cast doubts over the columnar organization of M1 corticospinal outputs. In 1980 Fetz and Cheney performed a study where the muscle activity of monkeys doing a simple manual task was correlated to single-neuron activity in M1. After averaging the EMG activity that followed the firing of cortical neurons, they found that several muscles can show facilitation after firing of a single neuron. They proposed that this effect is due the divergent connectivity of cortical tract neurons, which would synapse on different motoneuron pools, innervating different muscles. An anatomical study by Shinoda and colleagues (1981) supported this view by demonstrating that a single large pyramidal neuron originating in the motor cortex has collaterals at several levels of the spinal cord suggesting connections with multiple motoneurons.

The question remained as to how M1 manages to elicit specific muscle contractions that produce movements, considering that its projections are so divergent. The answer was provided by Schieber and Hibbard in 1993, when they recorded isolated neurons as the monkey moved its individual fingers. They found that neurons with activity related to the movements of the different fingers were intermingled and that there was no clear localization of neurons involved in the control of movements of one finger in relation to the others. Their conclusion was that the control of the digits is widely distributed through the hand area of M1, with no apparent clusters dedicated to single muscles. This divergent distribution of the origin of corticospinal projections in M1 and their destination in the spinal cord suggests that for a muscle contraction to take place there should be a temporal convergence of inputs onto appropriate motoneurons. This highly redundant organization of the corticospinal projections is thought to underlie the plasticity and rapid reorganization in the motor cortex, and is considered to be one of the underlying substrates that allow stroke recovery.

1.2 Plasticity in the ipsilesional hemisphere

1.2.1 Release of local inhibition can support rapid changes of motor outputs in M1

Fast acquisition of new motor skills is a huge evolutionary advantage. Motor cortex plasticity is thought to underlie mammalian capacity to quickly acquire new motor behavior. What permits this ability for rapid motor cortical plasticity? Reversal of cortical inhibition has been shown to play a very important role in the reorganization of the motor cortex. In a culmination of a series of experiments Jacobs and Donoghue (1991) assessed reorganization of motor cortex due to release of local GABAergic inhibition. In this study using ICMS the authors identified stimulation sites that evoked either only vibrissae or forelimb movements in the rat. They then applied a GABA antagonist (bicuculine) in the forelimb region to remove the effect of local inhibition on the motor outputs of that region. After the injection of the GABA antagonist, they stimulated sites from which vibrissae movements were evoked again. Along the border of the two representations, as early as 15 minutes after local application of GABA antagonist the stimulation of a vibrissae site started to also evoke forelimb movements. This time window is too short for synaptogenesis or any other anatomical changes to occur. Their results thus strongly suggest that there were already present, functional (but silenced) corticocortical connections between the vibrissae and the forelimb regions, which were supressed by tonic GABAergic inhibition. By removing the tonic inhibition, the previously silenced synapses become responsive to stimulation. This suggests that there is a significant amount of redundancy in the pattern of connections in the motor cortex. This mechanism is faster than establishing new synapses. By taking advantage of the high redundancy of both the descending projections from M1 and the local corticocortical connections within M1, the modulation of local inhibition would allow for fast cortical reorganization. The inherent plasticity of M1 is likely an important factor in the reorganization of the motor cortex after stroke that allows functional recovery of many patients.

1.2.2 Primary motor cortex plasticity and motor learning

Before looking at stroke-induced plasticity it is important to examine plasticity intrinsic to healthy individuals. Plasticity in the motor cortex is believed to support motor learning in adults. Indeed, several experiments have shown that motor learning is associated with cortical reorganization. In a study in squirrel monkeys, animals had to develop a new motor skill to perform precision pinch with an index and thumb to grasp food pellets in a small well (R. J. Nudo et al. 1996a). Following motor learning and an increase in performance, the digit representation in M1 of these animals expanded. Subsequently, the same animals were trained at a task that required the animals to engage in the skilled use of the forearm and not the digits. Cortical motor maps obtained after the training at the second task showed a decrease and return to baseline of the size of the digit representation in M1. Even though monkeys still had to use their fingers to perform the second task, the animals were performing an already acquired behavior and thus it did not require an increase in the size of the digit representation in M1. Thus cortical reorganization seems to be very dynamic and dependent on active learning of a new motor skill.

It has been previously demonstrated that there is an increase in excitability of the motor cortex at the initiation of motor skill learning (Rioult-Pedotti et al., 1998). This is further supported by an experiment in which hyperexcitation of M1 was achieved through application of high frequency repetitive transcortical magnetic stimulation (rTMS), and resulted in the improvement of sequential learning (Kim et al. 2003). What is the functional significance of this increased excitability of the motor cortex? As was previously discussed, there are plenty of potentially functional synapses in M1, which are suppressed by the inhibitory interneurons. The increased excitability of M1 could reflect that a certain number of previously “masked” synapses become functional. During the initial stage of motor skill learning there is an increase in muscle co-contraction (Osu et al. 2002). This increased co-

contraction is thought to increase task accuracy as it offers tighter control over limb dynamics and its placement in space and likely warrants larger corticospinal output.

Hikosaka and collaborators (2002) proposed that after initial learning, basal ganglion and cerebellum would come into play and mediate consolidation. These structures would reinforce the synapses in M1 that caused muscle contraction resulting in accurate performance of the task in a process not unlike “tuning”. As learning of the motor task proceeds, co-activation decreases without loss in accuracy, because limb dynamics have been optimized to the task. Eventually this process would result in a new set of functional synapses that are activated for the execution of this task. This can be seen as a consolidation, when synapses involved in the activation pattern necessary to produce muscle contractions to the right degree and at the right time, have been selectively reinforced.

Therefore, during motor learning, existing but silenced connections are activated. Those that best contribute to the new skill performance are selectively reinforced to be engaged in the particular motor skill. After the completion of motor learning, tonic inhibition in the motor cortex returns to normal. It is important to note that motor learning may not require axonal sprouting. It can simply take advantage of the redundant anatomic infrastructure already present and selectively reinforcing parts of it, while inhibiting other parts. This aforementioned redundant anatomical organization of M1 is thought to fast allow acquisition of new motor skills, and it is thought that it can also be used to support motor recovery after stroke.

1.2.3 Cortical reorganization after stroke in M1

Following injury, stroke patients recover at different speeds. After examining 46 stroke patients Fuji and Nakada (2003) separated the patients into three distinct groups. The first group demonstrated almost complete recovery a month after stroke, and was deemed the “fast” recovery

group. The rest of the patients demonstrated “slow” recovery. By three months post stroke some of these patients recovered to a level approaching that of the “fast” recovery group. They were thus classified as the “slow and good” recovery group. The remainder of patients did not recover much, even by the end of the three months period and were reclassified into “slow and bad” recovery group. The authors suggest that independent of the extent of recovery, the patients who recover slower do so through a different pattern of reorganization. Whereas the patients who recover quickly undergo one type of reorganization, the patients in both “slow” groups undergo a different type of reorganization that may or may not lead to good recovery of hand function.

1.2.4 Early changes in the ipsilesional hemisphere after stroke

We know that as early as one day after stroke there is widespread cortical disinhibition (Schiene et al. 1996). However the disinhibition appears to last longer than one day. Indeed, one week after injury, global down-regulation of GABA binding was reported (Qü et al. 1998). As discussed previously there are plenty of synapses in the cortex that are functional, but supressed by the tonic GABA inhibition (Jacobs and Donoghue 1991). Global disinhibition after stroke could allow for re-tuning of existing, but previously non-functional connections and selectively strengthen those which would result in return of function. This process can result in recovery if enough of M1 was spared by the lesion. In this case, at least part of the behavioural recovery would be sustained by physiological reorganization of the surviving M1 and would not require significant anatomical reorganization. This process would likely take advantage of the endogenous anatomical organization, and utilise the innate plasticity of the mammalian motor cortex which has evolved for fast acquisition of new motor skills. This could be the major route of reorganization of the “fast” recovery group described by Fujii and Nakada (2003).

1.2.5 Late changes in the ipsilesional hemisphere after stroke

However as Fujii and Nakada (2003) have demonstrated the majority of patients do not recover within a month. So what sort of processes might be involved in "slow, but good" recovery? Lashley (1938) proposed that it is the extent of the damage to the cortex that would drive subsequent reorganization. Thus if the damage to M1 is too extensive, where not enough of M1 remains, this would trigger significant reorganization of distant cortical areas. In particular non-primary cortical motor areas are the best candidates for where this reorganization takes place, as they are already heavily interconnected with M1 and form part of the corticospinal tract. This functional reorganization of distal areas was demonstrated by transiently inhibiting the premotor cortex in monkeys that recovered after stroke (Liu and Rouiller 1999). Following recovery from lesions in the sensorimotor cortex of macaque monkeys, inhibition of the premotor cortex in the ipsilesional side with muscimol, a GABA agonist, can re-instate behavioral deficits in the paretic hand. When the inhibition was done in the contralesional premotor cortex, there was no decrease in the task performance for the paretic hand. These results support the idea that during post stroke recovery the ipsilesional premotor cortex has taken on some of the function of M1.

Frost and colleagues (2003) looked at physiological reorganization of PMv following lesions in M1. They found that after large ischemic lesions in the hand area of M1, the hand area of PMv underwent expansion, presumably as part of compensatory functional reorganization. Building up on these results Dancause and colleagues (2005) conducted a study which looked into anatomical changes associated with stroke recovery and with the physiological reorganization of PMv. Following recovery, they injected the neuroanatomical tracer into PMv and compared the pattern of connections to the one found in intact animals. Injections of neuroanatomical tracer in PMv of control animals did not result in any significant labelling of either neuronal cell bodies or axonal terminals in

primary somatosensory cortex (S1). This indicates a lack of direct projections between S1 and PMv. Tracer injections in PMv of animals that recovered from the ischemic lesions resulted in a larger number of labelled axonal terminals and cell bodies in S1. Furthermore the orientation of labelled axons originating in PMv was towards S1 in experimental animals, but not in controls. As M1 is reciprocally connected to both PMv and S1, but PMv does not project directly to S1, the authors proposed that as part of compensatory reorganization, PMv needs to re-establish these connections with S1 to take on some of the function of M1. The expansion of the hand representation of PMv along with the long distance anatomical rewiring (which appear to try to reproduce the connectivity pattern of M1) strongly support that PMv is undergoing compensatory reorganization. This type of reorganization could explain the novel role of the premotor cortex following recovery from stroke and the return of deficits in the paretic hand following inactivation of ipsilesional PMv in recovered animals (Liu and Rouiller 1999). Furthermore, such mechanisms could be the major route of recovery of the “slow, but good” group of Fujii and Nakada (2003).

In summary, there are multiple processes taking place in the ipsilesional hemisphere following a lesion in M1 (R. Nudo 2006). Depending on the extent of damage, the motor cortex might reorganize relatively quickly, taking advantage of redundancy particular to the motor cortex. This would result in relatively fast recovery. However if the damage to M1 is too extensive, significant anatomical reorganization is required to achieve an adequate degree of functional recovery. The need to generate new axons and guide them to the right targets is significantly more demanding and takes longer. Therefore while recovery after relatively extensive damage to the motor cortex is possible, it takes significantly longer.

1.3 Plasticity in the contralesional hemisphere

1.3.1 Interhemispheric interactions in healthy adults

The majority of projections composing the corticospinal tract originate from neurons within the motor cortex to the forelimb. However the ipsi*lateral* motor cortex could also participate in the control of the forelimb by sending signals through corpus callosum, the largest bundle of nerve fibers in the mammalian brain, which connects the two hemispheres. One hypothesis is that the motor cortex of one hemisphere exerts inhibitory influence over its homologue in the other hemisphere to allow unimanual movements (Beaulé, Tremblay, and Théoret 2012). Supporting this hypothesis are studies demonstrating that stimulation of the motor cortex of one hemisphere with TMS produces suppression of EMG activity in the hand ipsi*lateral* to the stimulation (Ferbert et al. 1992; Harris-Love et al. 2007). In these experiments they examined the effect of a subthreshold conditioning pulse in M1 of one hemisphere on the electromyographic (EMG) output of a suprathreshold pulse in M1 of the other hemisphere. In both studies the authors observed that the conditioning stimulus resulted in a consistent suppression of muscles in the arm contra*lateral* to M1 stimulated with a suprathreshold pulse. To determine if the interhemispheric inhibition takes place at the spinal cord, the effect of the conditioning stimulus on the Hoffmann's reflex (H-reflex) was established. The H-reflex is EMG activity due to an electrical stimulus administered to 1a afferent fibers which are known to have a monosynaptic connection with alpha-motoneurons (Palmieri, Ingersoll, and Hoffman 2004). In other words, the H-reflex is analogous to an electrically evoked stretch reflex. In these two studies (Ferbert et al. 1992; Harris-Love et al. 2007), they used the H-reflex to examine changes in spinal cord motoneuron excitability. They found that conditioning stimulus to the ipsi*lateral* M1 did not modulate the H-reflex response, suggesting that interhemispheric inhibition takes place in the supraspinal structures.

In a 2009 study, Kobayashi and collaborators looked at the effect of low frequency subthreshold rTMS on motor learning. Subjects in all groups had to learn a unimanual sequential task after receiving the rTMS treatment. The first group received low frequency rTMS in M1 contra*lateral* to the hand performing the task, the second group in M1 ipsi*lateral* to the hand performing the task; the control group received rTMS treatment to the control scalp position (Cz). The subjects who received the rTMS treatment to the contra*lateral* M1 did not learn the task as effectively as the control subjects. This was expected as low frequency rTMS is thought to be inhibitory. However the subjects who received rTMS to the ipsi*lateral* M1 showed slight but significant improvements in motor skill learning compared to controls. Another study achieved similar results by exciting the contra*lateral* motor cortex (Kim et al. 2003). In this study high frequency rTMS, thought to cause cortical hyperexcitability, was applied to the M1 contra*lateral* to the hand performing the task and resulted in improvement of motor learning. It thus appears that either decreasing the activity of the M1 ipsi*lateral* to the hand involved in skilled motor learning or increasing the activity of the M1 contra*lateral* to the hand used improves motor skill learning. These studies further support the functional importance of interhemispheric inhibition for motor control.

There is a convergence of opinions that are singling out the corpus callosum as the important actor through which interhemispheric inhibition takes place (Ferbert et al. 1992; Harris-Love et al. 2007). Mayer and colleagues (1995) compared interhemispheric interactions of healthy subjects to patients with a complete or partial damage of corpus callosum. In both groups they found suppression in tonic muscle activity after ipsi*lateral* stimulation of M1. However in patients with callosal damage such as partial agenesis and hypoplasia, this suppression appeared later and was weaker than in healthy subjects. These findings are further corroborated by results from a study in cats in which Asanuma and Okamoto (1959) observed that in most recorded large pyramidal neurons

the stimulation of corpus callosum resulted in suppression. While these findings do not isolate the corpus callosum as the sole structure through which interhemispheric inhibition takes place, they do point to it as the major mediator.

The current assumption as to the role of the inhibitory interhemispheric activity is thought to be the lateralization of movement (Grefkes et al. 2008). This inhibitory network would allow us to perform unimanual tasks without simultaneous movements of the other arm. Whereas healthy adult humans can easily perform such unilateral movements, children up to the age of ten often show engagement of the other forelimb during performance of a unilateral task (Mayston, Harrison, and Stephens 1999). It is suggested that the difficulty encountered by children might come from an immature interhemispheric network. These unintentional and unwanted movements of the opposite hand during a tentative unimanual task are called mirror movements. As the child's brain matures they tend to disappear. Mirror movements are also observed in some stroke patients and something that has been proposed to be due to the disruption of the normal functioning of interhemispheric inhibition (Kim et al. 2003).

1.3.2 Early changes of interhemispheric interaction after stroke

When a region of the sensorimotor cortex is destroyed or silenced the input from that particular region to the contralesional hemisphere is lost. Even if it is a temporary lesion caused by transient inactivation there is a release of inhibition in the contralesional hemisphere. In monkeys, inactivating part of the motor cortex has resulted in expansion of receptor fields in the contra*lateral* somatosensory cortex immediately after inactivation (Clarey, Tweedale, and Calford 1996). In the rat, Maggiolini and colleagues (2008) documented acute changes in the contra*lateral* motor cortex. Immediately after lidocaine inactivation of the motor cortex in one hemisphere, they obtained an ICMS map of contralesional motor cortex. These motor maps were bigger than in sham animals that

did not receive cortical lidocaine injection. Thus, due to loss of input from the inhibited motor cortex, there is an expansion of motor representation in the opposite hemisphere. The short interval between the inactivation and the effect seen in the contralesional hemisphere suggests an unmasking of "dormant" connections. This acute disinhibition is most likely due to the loss of interhemispheric input that has been shown to be mostly inhibitory in healthy subjects. As part of the same study, Maggiolini and colleagues (2008) mapped the contralesional forelimb sensorimotor cortex 3 and 14 days after a chemical lesion in the forelimb motor cortex and found no difference from controls. These results suggest that the expansion of the motor map happens rapidly after the lesion and is transitory.

1.3.3 Late changes of interhemispheric interaction after stroke

As was discussed previously the ipsilesional motor cortex undergoes reorganization to recover functionality of the paretic limb. The contralesional motor cortex also undergoes reorganization to re-establish the interhemispheric balance disrupted by stroke (van Meer et al. 2012). While it might appear that disrupting the cortical reorganization might be detrimental to recovery, studies show that inhibiting the contralesional motor cortex with low frequency rTMS improves the recovery of the paretic hand (Takeuchi et al. 2005; Mansur et al. 2005). It is thought that the mechanism employed is through further disinhibition of the ipsilesional motor cortex which might act to speed up the reestablishment of a new interhemispheric balance. As discussed previously stroke recovery has been compared to learning a new motor skill by a healthy person. Just as motor skill acquisition improves after inhibition of the motor cortex ipsi*lateral* to the task in a healthy person, suggesting hyper-excitation of the contra*lateral* motor cortex, a similar mechanism is thought to be responsible for the beneficiary effect of contralesional inhibition in stroke patients. In fact hyper-exciting the ipsilesional cortex with 5 Hz rTMS resulted in improvement of functional

recovery of the paretic hand, similar to supressing the contralesional motor cortex with 1 Hz rTMS (Emara et al. 2010). All of these studies offer support for the detrimental effect of interhemispheric inhibition exerted by the contralesional motor cortex.

Nonetheless there is also some data that contradicts these conclusions on the adverse role of the contralesional hemisphere in recovery of the paretic limb. A patient who successfully recovered from a unilateral stroke and then suffered another one in the previously intact hemisphere had the functional deficits of the initial paretic hand reinstated (Song Y 2005). This suggests that the contralesional hemisphere can indeed contribute to control of the paretic hand. In fact there are studies showing that after stroke recovery, the contralesional hemisphere of patients exert a more facilitatory effect on the ipsilesional motor cortex, in contrast to healthy subjects (Bütefisch et al. 2003). It appears that with time after stroke, the contralesional motor cortex can assume a positive or a negative role in the recovery of the paretic hand. In the face of these contradictory results coming from multiple studies it becomes clear that we are most likely missing a key factor which would influence the kind of role the contralesional hemisphere would play in stroke recovery.

1.4 Effect of lesion size on contralesional reorganization

1.4.1 Effect of lesion size on physiological, anatomical and functional reorganization in the CL hemisphere

Why is there such conflicting data about the role of the contralesional motor cortex in stroke recovery? A potential explanation could be that the contralesional cortex participates in stroke recovery differently depending on the how much of the ipsilesional motor cortex remains intact following stroke. There is a body of evidence indicating that lesion size influences reorganization in the contralesional motor cortex. In a functional magnetic resonance imaging (fMRI) study in rats

Dijkhuizen and colleagues (2003) demonstrated that the extent of contralesional activity correlates positively with the lesion size. In this experiment after inducing a middle cerebral artery occlusion (MCAo) in rats, hemodynamic activity in both hemispheres in response to paw stimulation was evaluated with fMRI. The results show a strong correlation between the hemodynamic activity in the contralesional hemisphere and the lesion size. On the anatomical level we know that certain proteins, such as MAP2 and NMDAR1 are associated with cortical plasticity (Derksen et al. 2007; Carroll and Zukin 2002). These proteins were found to be expressed at different levels in the contralesional hemisphere after lesions of different size (Hsu and Jones 2006). MAP2 and NMDAR1 were expressed at higher levels in rats with larger lesions. This suggests that larger lesions in the ipsilesional cortex induce more extensive reorganization in the contralesional cortex.

As lesion size has already been shown to influence both physiological and neuroanatomical activity in the contralesional motor cortex, Biernaskie and colleagues (2005) looked into the interaction of these factors with behavior. After inducing stroke in the rat and letting the animals recover, the contralesional motor cortex was inhibited by lidocaine right before the test of the performance of the paretic hand. They found that the inhibition of the contralesional motor cortex in the rats with larger lesions resulted in significantly greater deficits of the paretic hand than in the rats with smaller lesions. These results suggest that the contralesional motor cortex contributes more to the functional recovery of the paretic limb after a large lesion, than after a small lesion.

In the ipsilesional hemisphere, the physiological reorganization of areas distant from the lesion has been found to be affected by the size of lesion. Using motor maps obtained with ICMS the authors found that lesions that destroyed less than 30% of the hand representation of M1 caused a contraction in the hand area of PMv. In contrast lesions almost completely destroying the hand area of M1 caused a 50% expansion of the hand area of PMv (Frost et al. 2003; Dancause et al. 2006;

Dancause et al. 2005) . While the monkeys with small lesions recovered within three weeks, the monkeys with large lesions still had mild behavioral deficits 5 months after stroke induction. The authors proposed that the capability of the hand area of M1 to reorganize was exhausted by large lesions. In these cases, PMv, a premotor area heavily interconnected with M1 and with its own corticospinal projections, underwent expansion of its hand area to support recovery.

Similarly if a lesion is large enough to eliminate the capacity of the ipsilesional cortex to reorganize, the contralesional motor cortex would then undergo adaptive reorganization to contribute to the recovery of the paretic hand. Summarising all the evidence presented above I propose that a lesion in the motor cortex will trigger a reorganization in the contralesional motor cortex. However the functional outcome of this reorganization and the observable physiological and anatomical changes will be influenced by the volume of the lesion.

1.4.2 Rationale for the set of experiments conducted in the present study

We wanted to investigate how lesions of different sizes in the motor cortex influence cortical reorganization in the contralesional motor cortex. Currently there are only two studies which examined the effect of lesion size on physiological reorganization in the contralesional hemisphere. The first one by Dijkhuizen and colleagues (2003) was discussed previously. Unfortunately the resolution of fMRI in rodents does not allow the separation of the rat motor cortex into rostral (RFA) and caudal forelimb regions (CFA), which are suspected to play different roles in motor control (Rouiller, Moret, and Liang 1993) and thus might play different roles in stroke recovery. Additionally an increase in hemodynamic activity does not actually reveal what sort of reorganization is taking place.

The only other study which looked at the effect of lesion size on contralesional reorganization was done by Gonzalez and colleagues (2004) using ICMS, a well-established technique that allows us

to examine cortical organization within each motor cortical region at high resolution. In this study unilateral stroke was induced in the sensorimotor cortex in rats with one of two methods: devascularisation of surface vessels or electrocoagulation of the middle cerebral artery (MCA). The strokes caused by MCAo were larger and more lateral when compared to strokes resulting from the devascularisation of the surface vessels. The authors did not find any effect of lesion size on the contralesional motor cortex. However, in this study not only size, but also lesion location varied between the two groups. Indeed, due to difference in rodent vascular anatomy, MCAo routinely leaves the motor cortex intact (Gharbawie et al. 2005). Thus, it is yet not clear what would be the effect of lesion of different sizes in M1 on the reorganization of the contralesional motor areas.

Our objective was to evaluate the effect of lesion size in the CFA of rats on cortical reorganization of both hemispheres and behavioral recovery of the paretic hand. We predict that lesions of different sizes should result in different reorganization patterns in the contralesional motor cortex. Our results will further the understanding of the physiological reorganization following ischemic stroke. In particular, as discussed in the sections above while the role of the ipsilesional motor cortex in functional recovery has been an area of active research, there is a current gap in understanding how the contralesional motor cortex contributes to recovery. Furthermore, there are clinical interventions that are currently being designed that rely on untested assumptions of how the ipsi and contralesional motor cortices interact. As a consequence, this study seeks to contribute to closing this gap and provides a better understanding of the processes that take place in the contralesional hemisphere following stroke and their relation to the functional recovery of the paretic limb.

Chapter 2

The effect of lesion size on cortical reorganization in the ipsi and contralesional hemispheres

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Introduction

Cortical lesions, such as may occur following stroke, trigger plasticity in diverse, distant regions of the brain that are spared from the injury. In humans, corticospinal tract disruption is a good predictor of motor impairments (Schaechter, et al., 2009, Stinear, et al., 2007, Ward, et al., 2006). In addition, patients with greater deficits show more activation in diverse areas of the ipsi and contralesional cortex during movement of the paretic limb (Cramer, et al., 1997, Ward, et al., 2007, Ward, et al., 2006).

In animal studies, comparable effects of lesion size have been reported. Following middle cerebral artery occlusions (MCAo) in rats, the reorganization of the pattern of hemodynamic activity (Dijkhuizen, et al., 2003) and of the functional and structural connectivity of the contralesional hemisphere (van Meer, et al., 2012) are more pronounced in animals with larger lesions. Many neuroanatomical changes are also known to occur in the contralesional hemisphere (Adkins, et al., 2004, Biernaskie and Corbett, 2001, Jones and

Schallert, 1992, Stroemer, et al., 1995) and are affected by the extent of injury (Hsu and Jones, 2006, Kim and Jones, 2010). Reorganization of cortical motor representations, or motor maps, in the ipsilesional hemisphere is also affected by the size of injury (Dancause, et al., 2006, Frost, et al., 2003). Altogether, these data support that the size of lesion has substantial effects on postlesion plasticity and recovery.

To date, the effect of lesion size on the reorganization of motor representations in the contralesional cortex have not been studied. Moreover, there has been no complete documentation of how the volume of the lesion affects the organization of cortical motor maps in the two hemispheres. In the present study, our objective was to evaluate the effect of cortical lesion size on the organization of motor areas of the ipsi and contralesional hemispheres. In a rat model, we induced cortical lesions of different size in the caudal forelimb area (CFA), the rodent equivalent of the primate primary motor cortex (M1) and the main source of corticospinal neurons in adult rats (Brosamle and Schwab, 1997, Miller, 1987). Following recovery, we used intracortical microstimulation techniques (ICMS) to study the organization of motor representations in both hemispheres.

Methods

18 Sprague-Dawley rats of approximately 3 months of age weighing from 250g to 300g were used for the study (Charles River Laboratories, Montreal, Québec, Canada). All animals were housed separately in a reversed day-night light cycle and were only handled in the dark, under red light. Animals were randomly assigned to one of three groups, controls (n= 5), a 'small' ($Group_{small}$; n=7) or a 'large' ($Group_{large}$; n=6) cortical lesion group. Animals in the $Group_{small}$ and $Group_{large}$ were familiarized with banana flavored food pellets in the Montoya Staircase task (Biernaskie and Corbett, 2001, Montoya, et al., 1991) for 10 work-days. Testing chamber was made out of Plexiglas (6-cm wide, 12-cm high and 30cm long) with a central platform (2.3-cm wide, 6-cm high and 19-cm long) which separates right and left forelimbs (Biernaskie and Corbett 2001; Montoya et al. 1991). Prior to lesion induction animals were familiarized with the task. Familiarization consisted of two sessions of Montoya staircase, one in the morning and one in the afternoon. In a session a rat had 4 three-minute trials with each hand (8 trials per day in totals). Number of pellets eaten per trial was established at the end of three minutes, and all 7 wells refiled for the next trial (one pellet per well). On the last two days of the familiarization period, the performance in terms of the number of eaten pellets was recorded and used to establish if the animal reached our inclusion criteria. To be included in the study, rats needed to eat 4 out of 7 pellets in 3 of the 4 trials on both days with both forepaws. Each forepaw was testing separately (i.e. 4 three-minute sessions with the right hand, then 4 three-minute sessions with the left hand and vice-versa). Prior to the lesion, grasping performance of both forelimbs in the Montoya Staircase task was collected

on the 9th and 10th days and averaged to establish a baseline performance. Following the lesion, behavior was reevaluated twice in the first week and then once per week for the three following weeks. At the end of this recovery period, motor mapping was conducted (Figure 1). In control animals, the mapping procedures were done after 5 weeks of being single housed in our facility. Controls did not undergo the familiarization period, as this was showed to have no effect on motor maps (Barbay, et al., 2013). The familiarization and behavioral data collection procedures have been described in detail previously (Mansoori, et al., in revision).

Behavioral recovery was calculated using the following formula:

$$Final\ recovery\ = Mean\ of\ pellets\ eaten\ using\ paretic\ hand\ on\ day\ 28 - Mean\ of\ baseline\ for\ paretic\ hand$$

Our experimental protocol followed the guidelines of the Canadian Council on Animal Care and was approved by the Comité de Déontologie de l'Expérimentation sur les Animaux of the Université de Montréal.

Lesion induction surgery

Lesion surgeries were done aseptically. Animals were fixed in a stereotaxic frame in a prone position. Anesthesia was induced with ketamine hydrochloride (80mg/kg; ip) and sustained with ~2% isoflorane and 100% oxygen. The temperature was monitored and maintained between 35.5°C and 36.0°C by a self-regulating heating mat (Harvard Apparatus, Holliston,

MA). The oxygen saturation was also monitored throughout the procedures (Nellcor Puritan Bennett, Model NPB-190, Mansfield, MA). In both $Group_{small}$ and $Group_{large}$, lesions targeted the CFA based on stereotaxic coordinates (Fang, et al., 2010, Mansoori, et al., in revision) (Figure 2). For $Group_{small}$, six 0.7mm diameter holes were drilled through the skull (+1.5, +0.5, -0.5mm anteroposterior, +2.5, +3.5mm mediolateral to bregma). In each hole, a Hamilton syringe (Hamilton Company, Reno, Nevada, United States) was lowered at a depth of -1.5mm in the cortex to inject 330nL of endothelin-1 (ET-1) (EMD chemicals, San Diego, CA, USA; 0.3µg/µL in saline) at a rate of 3nL/s with a microinjector (Harvard apparatus, Holliston, MA). For $Group_{large}$, ET-1 was injected in a similar manner in twelve holes (+2.0, +1.0, 0.0, -1.0mm anteroposterior, +2.0, +3.0, +4.0 mediolateral to bregma), doubling the area of targeted cortex in the CFA. Our lesion protocol was specifically designed to increase the area of the cortical gray matter damaged in $Group_{large}$, without damaging subcortical structures, which occurs following ET-1 injections of bigger volumes (Biernaskie, et al., 2005, Hsu and Jones, 2006, Kim and Jones, 2010). Upon completion of injections, the holes in the skull were sealed with bone wax and the skin sutured. After the surgery, animals received a regimen of pain, anti-inflammatory and antibiotics medication and their recovery was closely followed for 48 hours.

Electrophysiological mapping surgery

Five weeks after the lesion, in a terminal acute experiment, ICMS techniques were used to obtain cortical motor maps of forelimb movements in both hemispheres. A first craniotomy

and durectomy exposed the brain of the contralesional (CL) hemisphere under isoflurane anesthesia. Mineral oil was applied over the opening to protect the cortex. A digital photograph of the exposed brain was exported to Canvas 11 software (Seattle, Washington, USA). A grid with a resolution of 0.333mm was overlaid onto the photograph and was used to guide the electrode penetrations to generate the motor map (333µm interpenetration distance). As it is impossible to evoke any motor response with cortical stimulation under isoflurane, anesthesia was switched to ketamine hydrochloride (~10mg/kg/10 minutes; intraperitoneal) for the collection of electrophysiological data. A glass insulated tungsten microelectrode (~1.0 MΩ; FHC Bowdoin, ME USA) was lowered into the cortex to a depth of 1600 µm targeting cortical layer 5 using a microdrive (David Kopf Instruments Model 2662, Tujunga, CA). Each stimulation train consisted of 13 monophasic square pulses (0.2ms duration and 3.3ms interpulse interval) generated by a Master-8 stimulus generator (A.M.P.I. Jerusalem, Israel). ICMS trains were delivered at 1Hz with a constant current stimulus isolator (Bak Electronics, Model BSI-2, Sanford, FL, USA). At each stimulation site, the movement evoked at threshold current intensity, defined as the current at which movements were evoked by 50% of the stimulation trains, was used for subsequent analyses. If no movement was evoked at a maximum current intensity of 100 µA, the site was qualified as unresponsive. Evoked movements were divided in three categories: distal forelimb, proximal forelimb or other. Movements of digits, wrist and forearm were included in the distal forelimb and movements of the elbow and shoulder were included in the proximal forelimb representation (Dancause, et al., 2006, Kleim, et al., 1998, Nudo, et al., 1992). Movements of the neck, back, vibrissae, hindlimb or non-responsive sites defined the borders of the CFA

and rostral forelimb area (RFA; rodents putative equivalent of a primate premotor area (Rouiller, et al., 1993)). Following completion of the contralesional motor maps, the animal was put back on isoflorane anesthesia and a second craniotomy exposed the ipsilesional cortex. Similar ICMS mapping techniques were used to define motor areas in this hemisphere. In some cases, due to complications during the experiment, the motor mapping was limited to the contralesional hemisphere and was immediately followed by perfusion (see results).

During mapping procedures, a small circle with a color specific to the movement category was overlaid onto the image of the cortex in Canvas at each penetration site. At the end of data collection, the digital image with color circles was used for analysis of the surface area of each movement category. This analysis was performed with a custom-made program in Matlab (MathWorks, MA, USA). The algorithm used nearest neighbor interpolation between penetration points to assign each pixel to a movement category. Dimensions of pixels were scaled according to a ruler placed on the brain in the digital picture of the cortex. The total number of pixels with the same movement color was multiplied by the scaling factor to obtain the cortical surface area of distal and proximal forelimb representations. The distinction between pixels in the in the CFA and RFA was made using a k-means cluster analysis of the distal forelimb representations. Surface areas for distal and proximal forelimb representations in rats that recovered from small and large lesions were compared to each other and to control, naïve rats.

Histology

Upon completion of the electrophysiological data collection the animal was given a lethal dose of sodium pentobarbital. It was transcardially perfused with heparinized saline solution (1% NaCl in H_20; 0.2% heparine; total volume = 500ml), followed with a 4% paraformaldehyde in 0.1M phosphate buffer saline (PBS) (total volume = 500ml). The brain was extracted and cryoprotected with a 20% sucrose, 4% paraformaldehyde 0.1M PBS solution overnight. It was then transferred to 20% sucrose, 2% dimethyl sulfoxide 0.1M PBS for 2 hours and then in 20% sucrose 0.1M PBS for 48 hours. The brains were frozen and cut coronally with a cryostat (40um thickness). One out of six sections were Nissl stained and reconstructed using Neurolucida (MicroBrightField, Colchester, VT, USA). Reconstructed sections were used to calculate the lesion extent with Neuroexplorer (MicroBrightField, Colchester, VT, USA). Lesion volume was obtained by subtracting the volume of the ipsilesional cortex to the volume of the contralesional cortex. The volume was then transformed to percentage using the contralesional hemisphere according to the following formula (Mansoori et al 2013):

$$Lesion\ extent\ = \frac{volume\ of\ contralesional\ cortex - volume\ of\ ipsilesional\ cortex}{volume\ of\ contralesional\ hemisphere}\ X\ 100$$

Statistical Analysis

Statistical analyses of behavioral data were carried out with SigmaPlot Version 11 (Systat Software, San Jose, CA). Repeated measure ANOVA was conducted using lesion size group,

time and lesion size x time as factors. Post-hoc multiple comparisons were done using Holm-Šídák test (Holm, 1979). The volumes of the lesions between the two groups of animals were compared with a one-way ANOVA.

Statistical analyses of motor maps data were carried out using custom scripts in Matlab (MathWorks, Nantick, MA, USA). Because of the large number of conditions, we performed multiple t-test using Holm-Šídák methods to correct for multiple comparisons.

Pearson's correlation coefficient and their significance were calculated using custom scripts in Matlab (MathWorks, Nantick, MA, USA).

Results:

Effective volume of cortical lesions

In animals with small and large lesions, the ischemic injury destroyed all cortical layers of the sensorimotor cortex (Fang, et al., 2010, Mansoori, et al., in revision). Two rats from $Group_{large}$ had subcortical lesions and were excluded from the study. In $Group_{small}$, the 6 ET-1 injections induced lesions of 5.18±1.25mm^3 (mean ± standard deviation). In $Group_{large}$ The 12 ET-1 injections in induced lesions of 16.26±5.58mm^3, which were significantly larger than (t= -8.64*; P<0.001*). These lesion volumes corresponded to 4.1±0.96% and 11.4±2.0% of the hemisphere for $Group_{small}$ and $Group_{large}$ respectively (Figure 3).

Effect of lesion volume on behavioral recovery

There was no difference of behavioral performance on the Montoya staircase task between experimental groups prior to the lesions. In contrast, the paretic forelimb function was affected by lesion volume (Figure 4). For $Group_{small}$, there was a significant decrease of grasping performance in the Montoya staircase task during the first week ($t=3.74$; $p<0.01$) that returned to baseline by end of the week ($t=2.1$; $p>0.05$). The $Group_{large}$ had a poorer performance than $Group_{small}$ throughout the postlesion recovery period ($t=4.1$; $p<0.01$). Although grasping performance showed some recovery with time, animals in $Group_{large}$ never reached back pre-lesion performance ($t=5.0$, $p<0.001$). Finally, there was a strong negative correlation between the final recovery score at day 28 and the lesion volume ($r=-0.64$) and

the slope was significantly different from zero (t=2.74; p=0.02). Thus, animals with larger lesions had poorer performance on the Montoya task.

Effect of lesion volume on motor representations of the ipsi and contralesional hemispheres

In naïve control animals, ICMS techniques revealed that the CFA was significantly larger than the RFA (5.75±0.82mm^2 and 1.23±0.19mm^2 respectively; t=11.98; p<0.0001) (Figure 5). In the CFA, movements of the wrist and digits (distal forelimb representation) represented 68±12% of the total surface area and were typically surrounded by movements of the elbow and shoulder (proximal forelimb representation). The RFA comprised 19±3% of distal representation and was separated from the CFA by cortex from which movements of the trunk and vibrissae were elicited (Kleim, et al., 1998, Mansoori, et al., in revision, Rouiller, et al., 1993).

In 5 rats with small lesions and 5 rats with large lesions, we were able to conduct ICMS mapping in the ipsilesional cortex (example ICMS maps shown in Figure 6). In the ipsilesional CFA (Figure 7), the proximal representation of animals in $Group_{large}$ was smaller than controls (p<0.01). The proximal representation of animals in $Group_{small}$ was not different from controls or from $Group_{large}$. For the distal representation in the ipsilesional CFA, $Group_{small}$ (t=4.57, p<0.01) and $Group_{large}$ (t=6.12, p<0.001) were smaller than controls. However, there was no difference between the two experimental groups. Thus, rats with large lesions had smaller proximal forelimb representations in the ipsilesional CFA, but the

effect of lesion size on this representation was not clear. In the ipsilesional RFA (Figure 8), the size of the proximal representation was similar in all groups but the distal forelimb representation was smaller in $Group_{small}$ than $Group_{large}$ ($t= -3.45$, $p>0.01$) but not different from controls.

We documented the motor cortex organization in the contralesional of 7 rats with small and 6 rats with large CFA lesions. In the contralesional hemisphere, we found no difference for the size of proximal or distal representations in the CFA (Figure 9). In RFA, there was also no difference for the size of the proximal representation. As in the ipsilesional hemisphere, we found that the distal forelimb representation in the contralesional RFA of $Group_{small}$ was smaller than in $Group_{large}$ ($p<0.05$; Figure 10). In summary, this first analysis revealed that the volume of lesion affected the organization the distal forelimb representations in the ipsi and contralesional RFAs.

To establish more clearly the relationship between the volume of lesion and the organization of the motor cortex following spontaneous recovery, we conducted regressions between the effective lesion volume, determined histologically, and the forelimb representations for which we found differences among our groups of animals (Figure 11). The negative correlation between the size of the proximal representation in the ipsilesional CFA the volume of lesion ($r=-0.66$) was not significantly different from zero ($p=0.55$). In contrast, distal forelimb representation of both the ipsi and contralesional RFAs were positively correlated with the volume of lesion ($r=0.73$; $p=0.02$ and $r=0.68$; $p=0.001$

respectively). Thus, rats that recovered from larger lesions had larger distal forelimb area in RFAs of both hemispheres.

Interaction between cortical reorganization and final recovery

Finally, we looked at the interaction between the size of motor areas affected by the volume of lesion and final recovery. We conducted regressions between cortical surface areas of the ipsi and contralesional distal forelimb area in the RFAs and the final recovery score on post-lesion day 28 for each rat (Figure 12). Whereas the size of the distal forelimb area in the ipsilesional RFA was not significantly correlated with recovery ($r=-0.36$, $p=0.3$), there was an inverse correlation between recovery and the size of the distal forelimb representation in the contralesional RFA ($r=-0.62$, $p=0.02$).

Discussion:

Our objective was to study the effect of lesion volume on the organization of ipsi and contralesional motor areas. In two groups of rats, we induced lesions at similar location in the sensorimotor cortex that destroyed all cortical layers but that affected different proportions of the CFA. Following 30 days of spontaneous recovery, we studied the organization of cortical motor representations, or motor maps, in the ipsi and contralesional hemispheres with ICMS techniques. This model allowed us to isolate the effect of the volume of cortical damage in CFA, the equivalent of M1 in rats, on physiological plasticity and behavioral recovery. Large lesions induced greater and more sustained functional deficits of the paretic forelimb (Figure 13). Animals that recovered from larger lesions had bigger distal forelimb representations in both ipsi and the contralesional RFAs. Moreover, the size of the distal representation in the contralesional RFA was inversely correlated to recovery. Animals with poorer recovery had larger distal representation in the contralesional RFA.

The effect of lesion size on motor recovery

Larger lesions of the CFA resulted in greater and more sustained behavioral deficits of the paretic forelimb. Lesion size was negatively correlated with behavioral performance of this forelimb. Similar results have been reported following strokes induced with MCAo in rats (Biernaskie, et al., 2005). Rats with larger MCAo lesions have a greater number of unsuccessful grasps and inaccurate reaches. As our lesions specifically targeted the CFA, the

primary origin of corticospinal projections in rats (Brosamle and Schwab, 1997, Miller, 1987), these results are also consistent with the human literature supporting that disruption of the corticospinal tract correlates with motor impairments (Schaechter, et al., 2009, Stinear, et al., 2007, Ward, et al., 2006).

The effect of lesion size on the reorganization of ipsilesional motor maps

We found that the size of the distal forelimb area in the ipsilesional RFA was smaller in $Group_{small}$ than controls and $Group_{large}$ and there was a significant linear relation between the size of lesion and the distal forelimb area in the ipsilesional RFA. These results are reminiscent of the ones reported in New World monkeys. In a series of experiments, it was shown that small lesions in the hand representation of M1 result in a decrease of the size of the hand representation in the ipsilesional ventral premotor cortex (PMv). In contrast, larger M1 lesions are associated with an increase of PMv hand representation (Dancause, et al., 2006, Frost, et al., 2003). Thus, has we found for the ipsilesional RFA of rats, in monkeys there is a linear relationship between the size of M1 lesion and reorganization of PMv. In monkeys, the relationship between lesion size and motor map reorganization in distant cortex has also been shown for the supplementary motor area (SMA) (Eisner-Janowicz, et al., 2008), suggesting that all ipsilesional premotor areas of primates are affected by lesion size in a comparable fashion. Our study extends these principles to rodents and supports that the RFA underdoes changes that are comparable to ones found in premotor areas of the primate following cortical lesion.

The effect of lesion size on the reorganization of contralesional motor maps

We did not find any difference between motor representations in the contralesional CFA across our different groups of animals. For the contralesional RFA, neither $Group_{small}$ or $Group_{large}$ were significantly different from controls but, the distal forelimb representation in $Group_{small}$ was smaller than in $Group_{large}$. Moreover, we found a significant relationship between the volume of lesion and the size of the distal forelimb representation in the contralesional RFA. It is possible that the relatively small size of RFA, allowing only for a limited number of stimulation sites and the inter-animal variability inherent to motor maps (Nudo and Milliken, 1996) hinders the identification of differences from controls in this cortical area. In the present set of experiments, the use of two groups of animals with lesions of different sizes highlighted the relation between lesion volume and the distal forelimb area in the contralesional RFA.

To date, the few studies that have looked at cortical motor maps in the contralesional hemisphere have not found differences between recovered animals and controls (Barbay, et al., 2013, Gonzalez, et al., 2004, Maggiolini, et al., 2008). Thus, so far, the absence of changes in the contralesional CFA appears to be common to all studies in rodents. The failure to identify changes in the contralesional RFA in other studies may be explained by the restricted range of lesion sizes used or by differences in lesion location. For example, a recent study using methods similar to ours, conducted motor mapping in the contralesional following recovery from lesions induced with 8 microinjections of ET-1 (Barbay, et al., 2013). ICMS

revealed no difference between the RFA of recovered animals and controls. The relationship we found between lesion size and the distal forelimb area in the contralesional RFA predicts this result (see figure 11). Lesions induced with 8 microinjections should fall between our $Group_{small}$ and $Group_{large}$ and produce little, if any changes in contralesional RFA. One study has conducted motor mapping in the contralesional hemisphere following recovery from devascularisation lesions of the sensorimotor cortex destroying approximately 8% and MCAo lesions destroying 18% of the ipsilesional hemisphere (Gonzalez, et al., 2004). Whereas the lesions resulting from MCAo were likely larger than the ones in our $Group_{large}$, MCAo lesions in rodents typically spare the motor cortex (Gharbawie, et al., 2005). Thus, the difference of lesion location in animals with MCAo could explain the absence of reorganization of motor areas of the contralesional hemisphere.

The reorganization of the contralesional RFA in rats is reminiscent of the atypical activation of the contralesional premotor cortex following stroke reported in numerous human imaging studies (Gerloff, et al., 2006, Lotze, et al., 2012, Seitz, et al., 1998). Abnormal contralesional premotor activity after stroke appears to correlate with decreased of corticospinal tract integrity, suggesting that patients with more affected corticospinal outputs are more likely to recruit the contralesional premotor cortex to perform more demanding tasks (Lotze, et al., 2012). In rats, MCAo lesions produce an increase of contralesional hemodynamic activity and a decrease of ipsilesional activity. This shift of activation between the two hemispheres is greater following larger lesions (Dijkhuizen, et al., 2003). In light of our results, it appears that the topographic organization of RFA, the

tentative equivalent of premotor cortex in rats, is more sensitive to lesions in the opposite hemisphere than the CFA. It is tempting to propose that this area is more likely to be involved in recovery, positively or negatively, following strokes in the sensorimotor cortex. Perhaps motor map changes in the contralesional CFA are only present following recovery from even larger sensorimotor cortex lesions than the ones that were induced in the present study. Regardless, it is interesting to note that there appears to be a dissociation between the numerous anatomical changes in the contralesional hemisphere affected by lesion size and motor map reorganization in this hemisphere.

The relation between motor map reorganization and recovery

In monkeys, reversible inactivation of ipsilesional premotor areas after recovery reinstates the initial motor deficits caused by the lesion, thus supporting that they can contribute to the recovery of the paretic limb (Liu and Rouiller, 1999). In humans, many studies have shown atypical activation of the ispilesional premotor cortex after stroke (Carey, et al., 2006, Jaillard, et al., 2005, Loubinoux, et al., 2003, Seitz, et al., 1998) and transcranial magnetic stimulation studies have provided evidences that this area can play a novel role in the control of the paretic hand (Fridman, et al., 2004, Johansen-Berg, et al., 2002). In the present study, we did not find a significant relationship between the size of the ispilesional RFA and behavioral recovery.

The relation between reorganization in the contralesional hemisphere and recovery has been and still is a topic of debate. There are evidences in the literature that reorganization of the contralesional hemisphere can interfere with recovery of the paretic limb, support its recovery or favor motor learning with the non-paretic limb (Dancause, 2006, Jones and Jefferson, 2011, Nowak, et al., 2009, Schallert, et al., 2003). In the present study, rats that with poorer recovery had a larger distal forelimb representation in the contralesional RFA. Similarly, in humans, atypical activity in the contralesional premotor areas is more frequent in patients with poor recovery (Calautti, et al., 2007, Ward, et al., 2003). Such data led to the hypothesis that atypically high activity in the contralesional hemisphere interferes with the paretic limb function. Studies in humans showing that inhibition of this hemisphere after stroke can favor recovery of the paretic limb support that at least part of the contralesional activity does has a negative effect on recovery (Fregni, et al., 2005, Nowak, et al., 2008, Takeuchi, et al., 2005). In rats, we found that pharmacological inhibition of the contralesional CFA with a GABA agonist can improve recovery of the paretic arm following cortical lesions (Mansoori, et al., in revision). It is however interesting to point that none of the inhibition studies to date have specifically targeted premotor areas and thus, do not support conclusions on the role of these areas on the function of the paretic limb.

In rats that recovered from large MCAo lesions, reversible inhibition of the contralesional cortex induces greater deficits in the paretic limb than in control rats or animals that recovered from small lesions (Biernaskie, et al., 2005). These data suggest that

the contralesional cortex can contribute to the recovery of the paretic limb following large lesions. In humans, inhibition of the contralesional hemisphere can also have different outcomes depending on the degree of impairment and the size of lesion (Bradnam, et al., 2011). Contralesional inhibition improved the control of the paretic limb for mildly impaired patients. However, the same treatment for patients with more ipsilesional white matter damage and severe impairments worsened the paretic arm function. These studies emphasize that the inverse relationship between the size of contralesional RFA and the final recovery score we found must be interpreted with caution. Following larger lesions that cause greater motor deficits, the ipsilesional network may be insufficient to support recovery and require the contribution of the contralesional RFA.

Rats that suffered a lesion are better at learning novel task with the non-paretic limb than control animals (Bury and Jones, 2002). However, when lesions of greater sizes are produced, rats rely more on their non-paretic limb but they are not as efficient at learning novel tasks with this limb. The lower learning capacity following larger lesions is associated with a decrease of anatomical plasticity in the contralesional cortex (Hsu and Jones, 2006, Kim and Jones, 2010). It is possible that the changes in the contralesional RFA we found following large lesions support learning of compensatory behavior of the non-paretic forelimb. However, if the reorganization of contralesional motor maps we found was only due to motor learning and use of the non-paretic limb, reorganization would have been expected to occur in the CFA, not the RFA. In intact rats, motor training on a precision reaching or lever-pushing task affect the organization of the CFA, but not RFA (Kleim, et al.,

1998). Thus, if changes in motor maps of the contralesional hemisphere strictly support motor learning with the non-paretic forelimb, our result suggest that after cortical lesions, this learning is achieved through a very different mechanisms that preferentially involves RFA over CFA.

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Figure 1. Experimental design. Timeline of experimental procedures for each animal which underwent lesion induction. ICMS mapping at day 35 was terminal and animals were perfused at the end of the experiment.

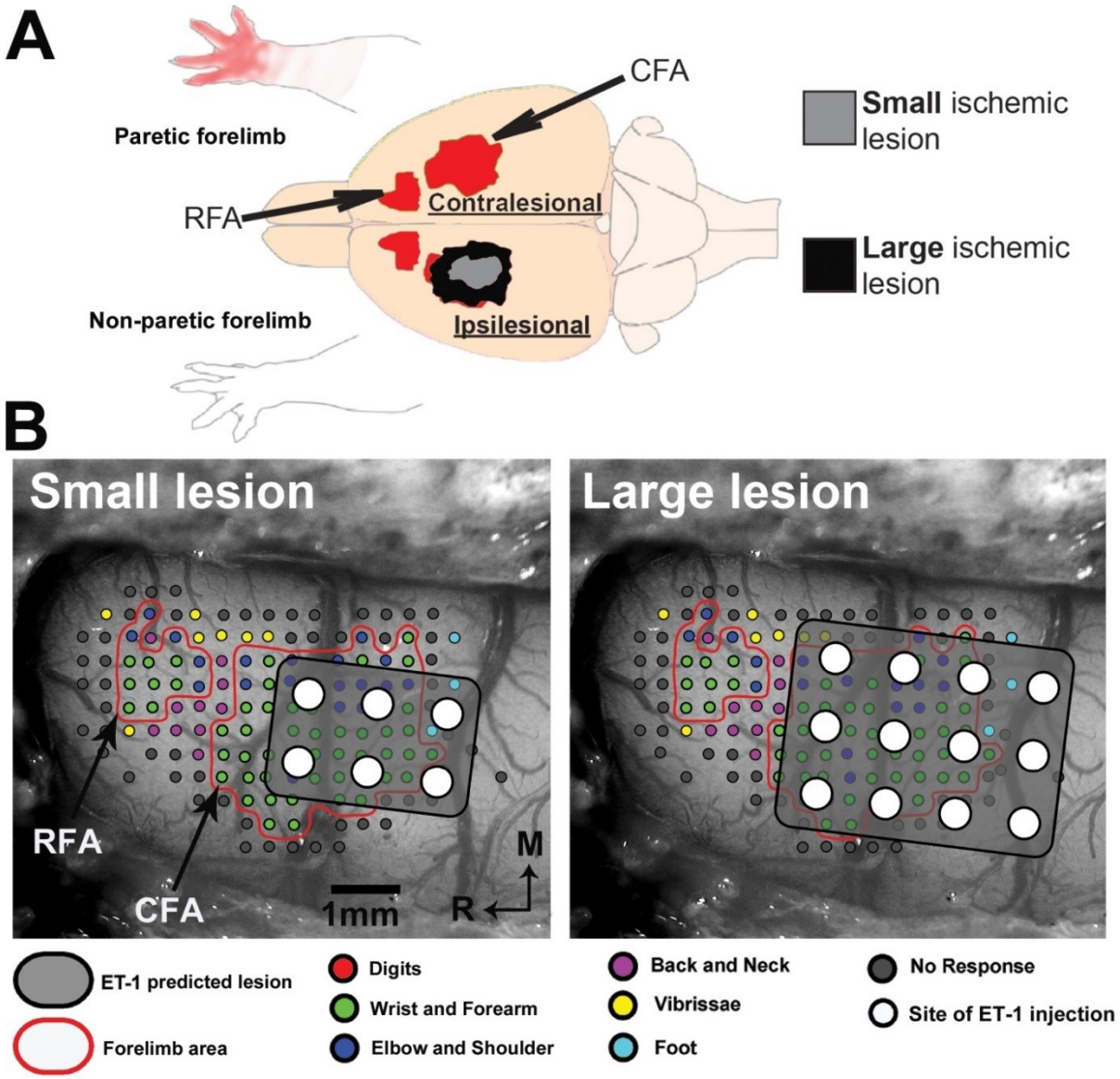

Figure 2. Experimental design. A) Cartoon of the experimental design. Lesions targeted the caudal forelimb area (CFA) but were of different size in two experimental groups (gray and black area). Following a recovery period of 28 days, intracortical microstimulation techniques (ICMS) were used to study the motor cortex organization in the CFA and rostral forelimb area (RFA) of the ipsi and contralesional hemispheres (red area). B) Typical ICMS map showing the CFA (large red contour) and RFA (small red contour). Each small dot is a penetration site where microstimulations were delivered. Evoked movements are color-coded. Based on stereotaxic coordinates, the locations of the endothelin-1 (ET-1) injections are overlaid onto the motor map in CFA for the small (left) and large (right) lesions. The expected spread of the lesion is drawn around the sites of ET-1 injections (gray area). Although small lesions ($Group_{small}$) should spare the RFA and a portion of the CFA, large lesions ($Group_{large}$) should spare RFA and completely destroy CFA. M: Medial, R: Rostral.

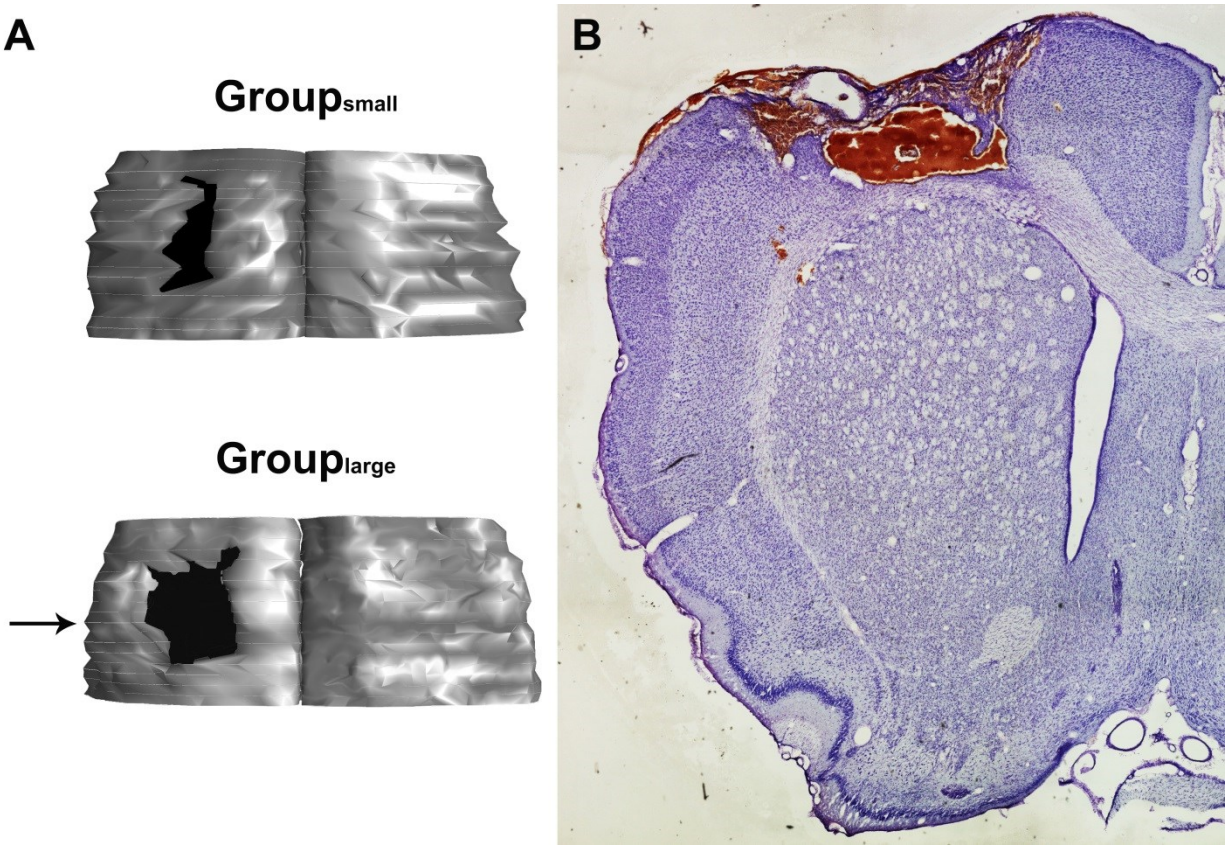

Figure 3. Histological reconstruction of lesions. For each animal, one out of six sections were reconstructed using Neurolucida software (Microbrightfield, inc.) to calculate the effective lesion volume. A) Example of anatomical of a 3D reconstruction of the lesion extent of an animal in $Group_{small}$ (top) and an animal in $Group_{large}$ (bottom). For $Group_{large}$, an arrow shows the location of the section shown in B). B) Cresyl stained section from the animal in $Group_{large}$. The lesion is wide but damage is mostly restricted to the gray matter.

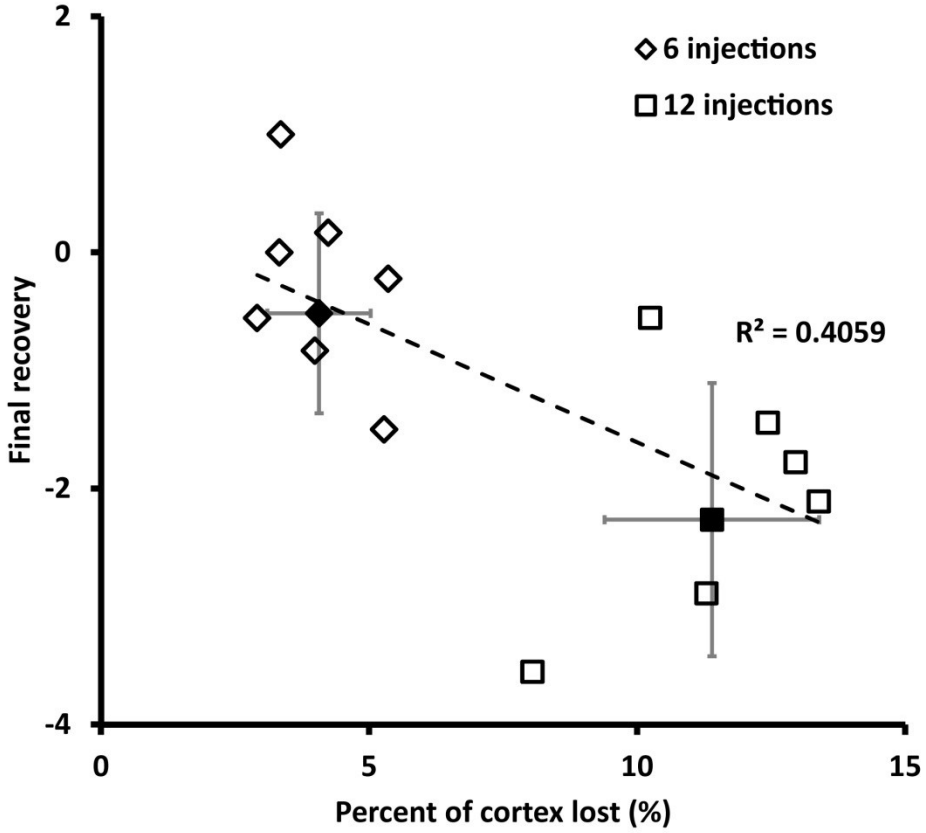

Figure 4. Effect of lesion size on the final recovery of the paretic hand. Although there was some variability of effective lesion size for each group, lesion induction protocols resulted in two distinct populations of lesion sizes for $Group_{small}$ and $Group_{large}$. There was a significant negative correlation between the size of lesion and the final recovery. Lesion size is given as a percentage of IL cortex lost. Recovery is the difference in the number of pellets eaten on the last behavioral test and baseline for that hand obtained before lesion induction. Negative values illustrate incomplete recovery.

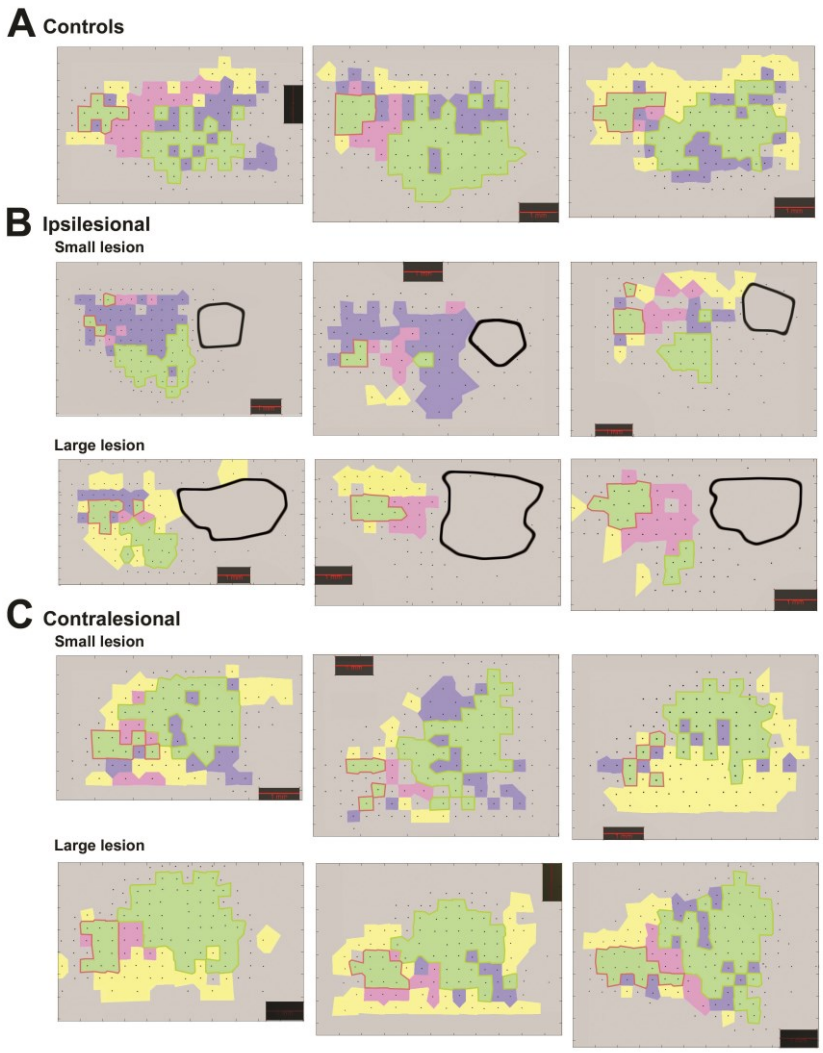

Figure 5. Examples of analysed motor maps. Figure showing examples of motor maps derived from the different experimental groups. A) Three ICMS maps of control rats. Each dot indicates a stimulation site for which the evoked movement was identified and color-coded (Green = digit/wrist/forearm; blue = elbow/shoulder; magenta = neck/trunk; yellow = vibrissae; no response = gray). The distal forelimb representation of the CFA is outlined in green and of the RFA in red. Black rectangle is scaled to 1mm. B) Three examples of motor maps in the ipsilesional hemisphere of rats that recovered from small (upper row) and from large (lower row) lesions ($Group_{small}$ and $Group_{large}$ respectively). The distal forelimb representation in the ipsilesional RFA was generally smaller in animals of $Group_{small}$ than $Group_{large}$. The lesion location identified visually from the digital photograph acquired during the mapping procedure is outlined by a black contour. Note that tissue distortion occurs at the site of injury so that the actual size of the lesion cannot be accurately extrapolated from this picture and relied on histological reconstructions. Color codes as in A. C) Three examples of motor maps in the contralesional hemisphere of rats from $Group_{small}$ and $Group_{large}$. In this hemisphere as well, the distal forelimb representation in RFA appears larger in $Group_{large}$ than in $Group_{small}$. Color codes as in A.

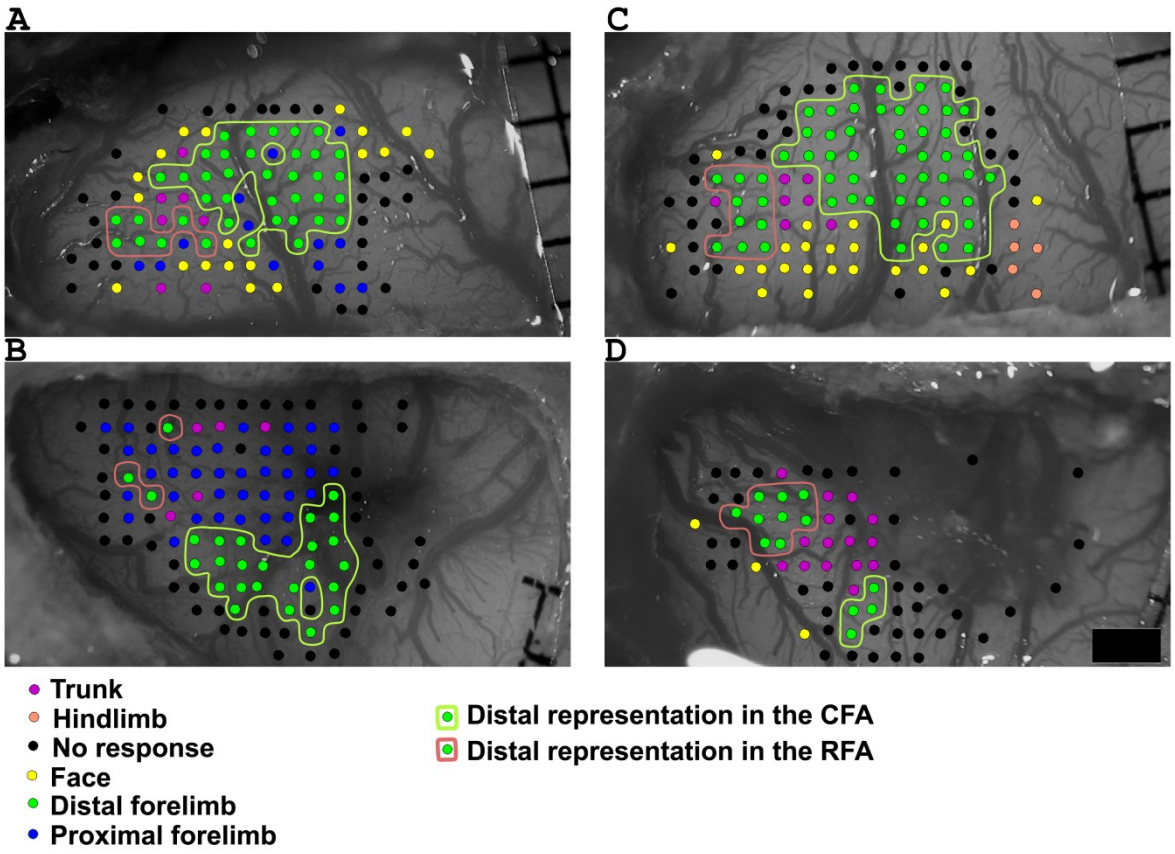

Figure 6. Examples of motor maps of lesioned animals. Distal forelimb sites in RFA and CFA were outlined. A and B are ICMS maps of rat with small lesion. C and D are ICMS maps of rat with large lesion. A and C are contralesional maps. B and D are ipsilesional maps. Black rectangle in the corner is 1mm in length.

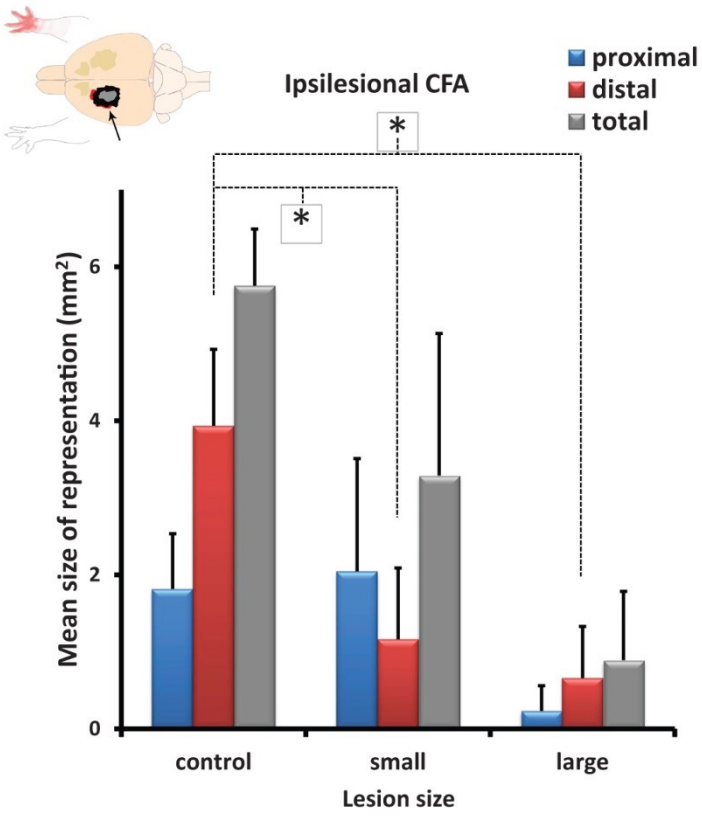

Figure 7. Motor representations in the ipsilesional CFA. The total CFA area (proximal + distal representations) and the distal forelimb representation were smaller in $Group_{small}$ and $Group_{large}$ than controls (*). For the proximal representation, there was no difference between $Group_{small}$ and controls. But the proximal representation of $Group_{large}$ was smaller than $Group_{small}$ and controls. Thus, animals with large lesions had smaller ipsilesional CFA than animals with small lesions. This difference between the two lesion groups was mainly accounted by the proximal representation. Cortical areas are reported in mm^2.

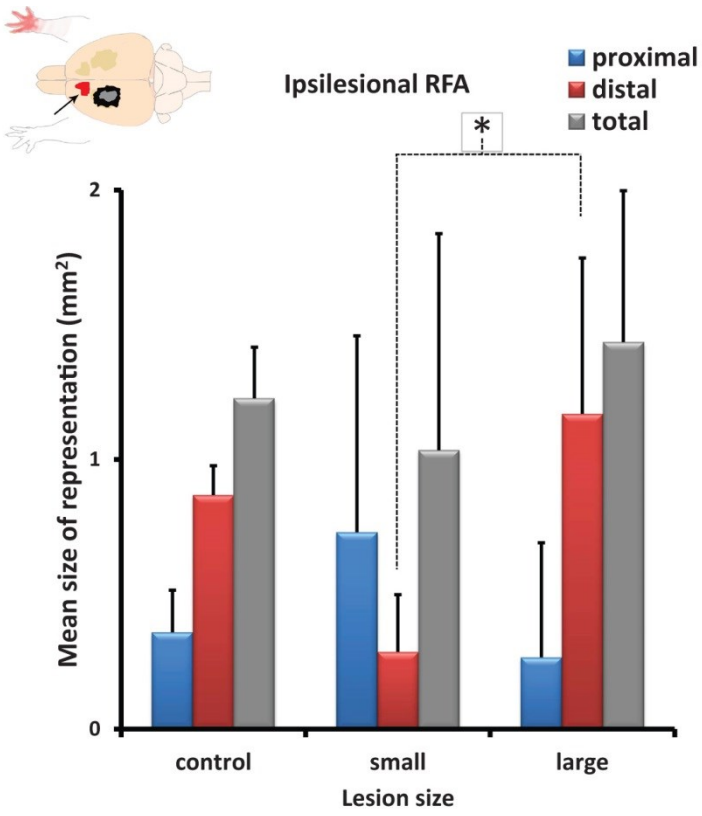

Figure 8. Motor representations in the ipsilesional RFA. Following small lesions or large lesions, there was no significant changes of the area from which proximal movements could be evoked. Whereas there was no significant difference from controls, the distal representation in rats that recovered from small lesions tended to be smaller than controls and larger than controls in rats that recovered from large lesions. In fact, the distal forelimb representation in the ipsilesional RFA of animals in $Group_{small}$ was significantly smaller than in $Group_{large}$. Cortical areas are reported in mm^2.

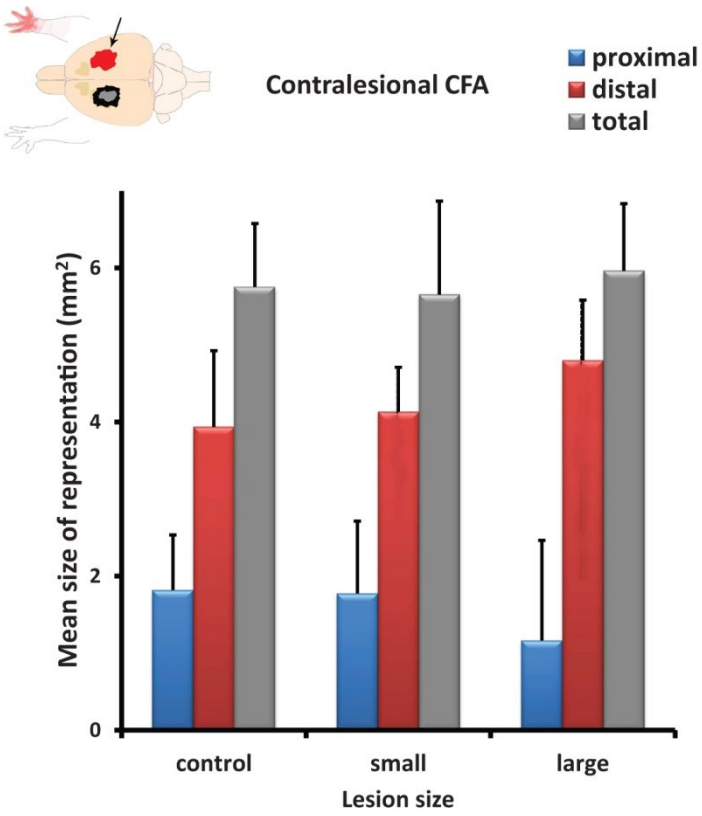

Figure 9. Motor representations in the contralesional CFA. The distal and the proximal representations in the contralesional CFA were of comparable size in all three groups. Our lesions did not affect the organization of this cortical area. Cortical areas are reported in mm^2.

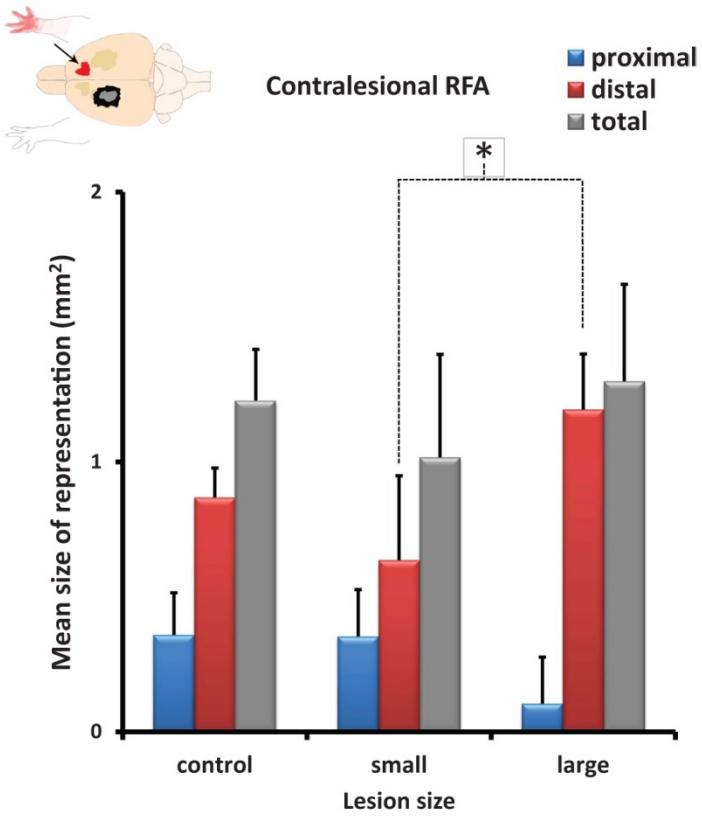

Figure 10. Motor representations in the contralesional RFA. In the contralesional RFA, the area of proximal representation was similar in all groups. However, the distal forelimb representation of $Group_{large}$ was significantly larger than $Group_{small}$. Thus, animals that recovered from large lesions had larger distal forelimb representation in the contralesional RFA than animals that recovered from small lesions. Cortical areas are reported in mm^2.

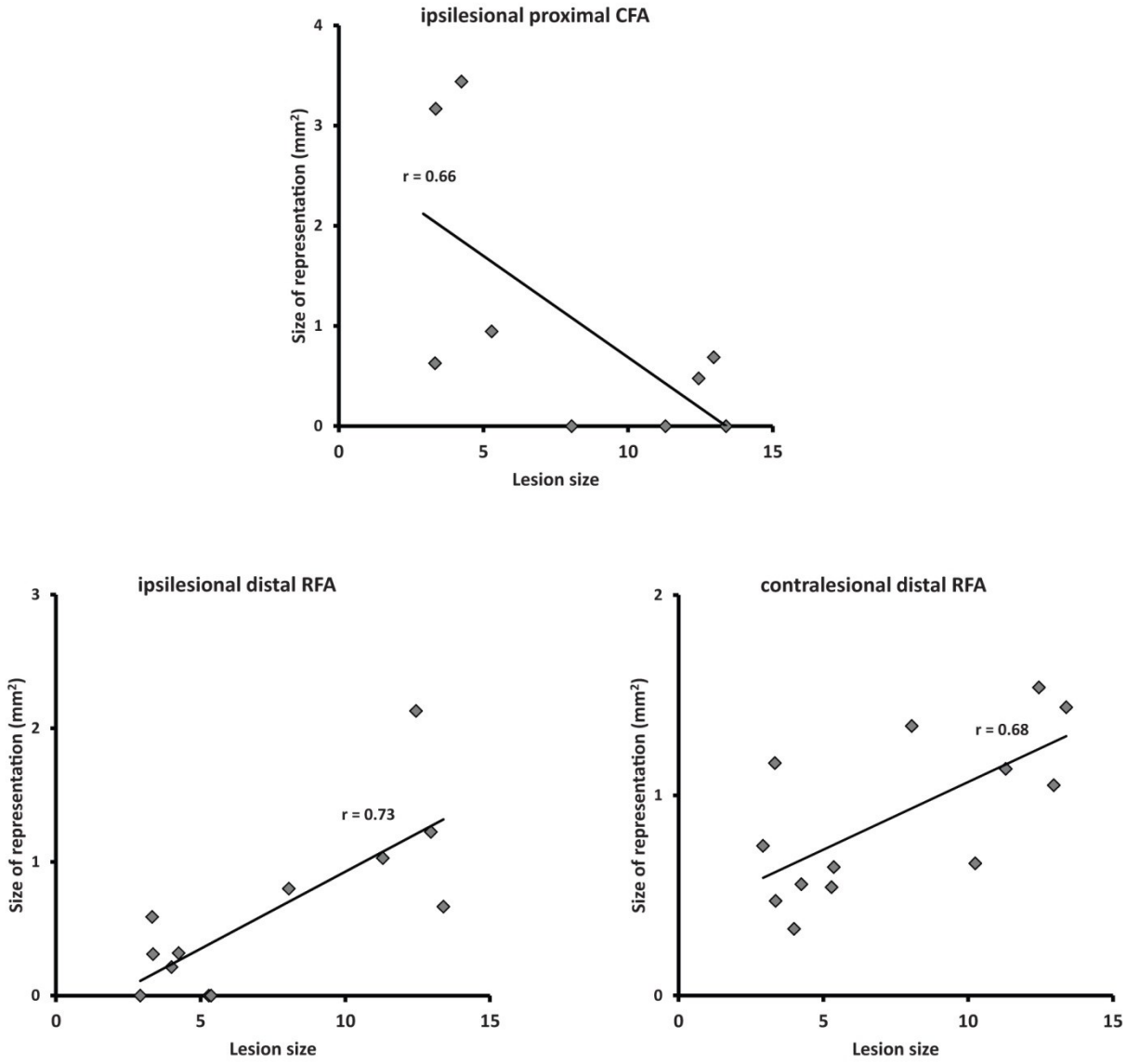

Figure 11. The effect of lesion size on motor representations. Regressions between the effective lesion size and the motor representations for which we found significant differences across groups were conducted. The interaction between lesion size and proximal representation of the ipsilesional CFA was not significant. However, there was a significant interaction between lesion size and the distal forelimb representations of the ipsi and contralesional RFA. Animals with larger lesions had larger distal forelimb representations in the RFA of the ispi and contralesional hemispheres.

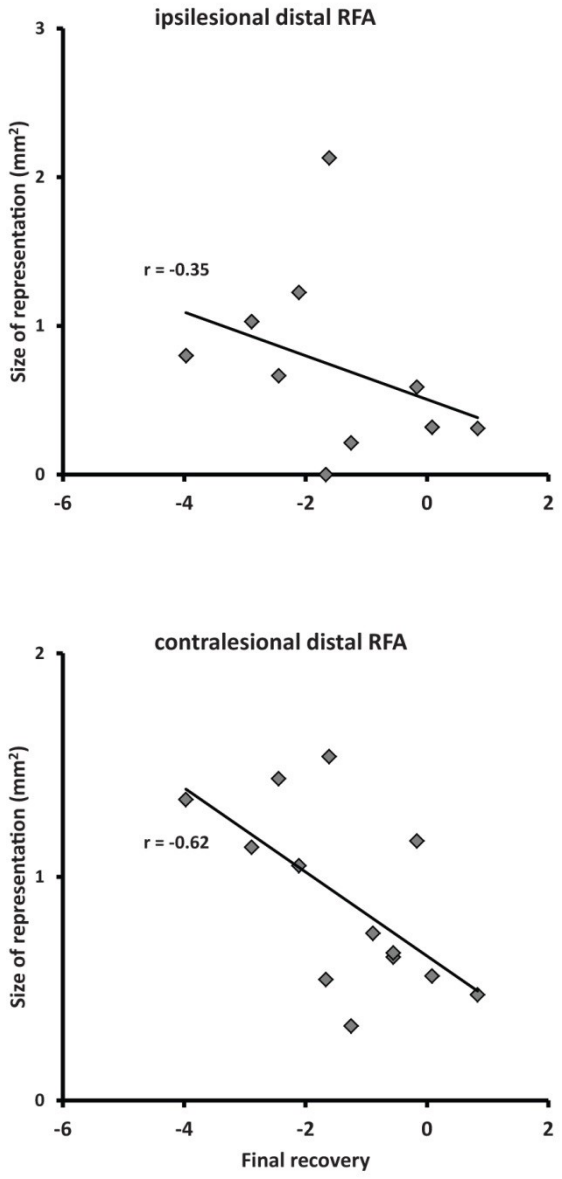

Figure 12. The relation between motor representations and final recovery. Final recovery of each rat was calculated by subtracting its baseline performance from its performance on postlesion day 28. Thus, negative values represent a persistent decrease of performance. The interaction between the distal forelimb representation of the ipsilesional RFA and final recovery was not significant. However, the one between the distal forelimb representation of the contralesional RFA and final recovery was. Animals with bigger lesions had larger distal forelimb representation in the contralesional hemisphere.

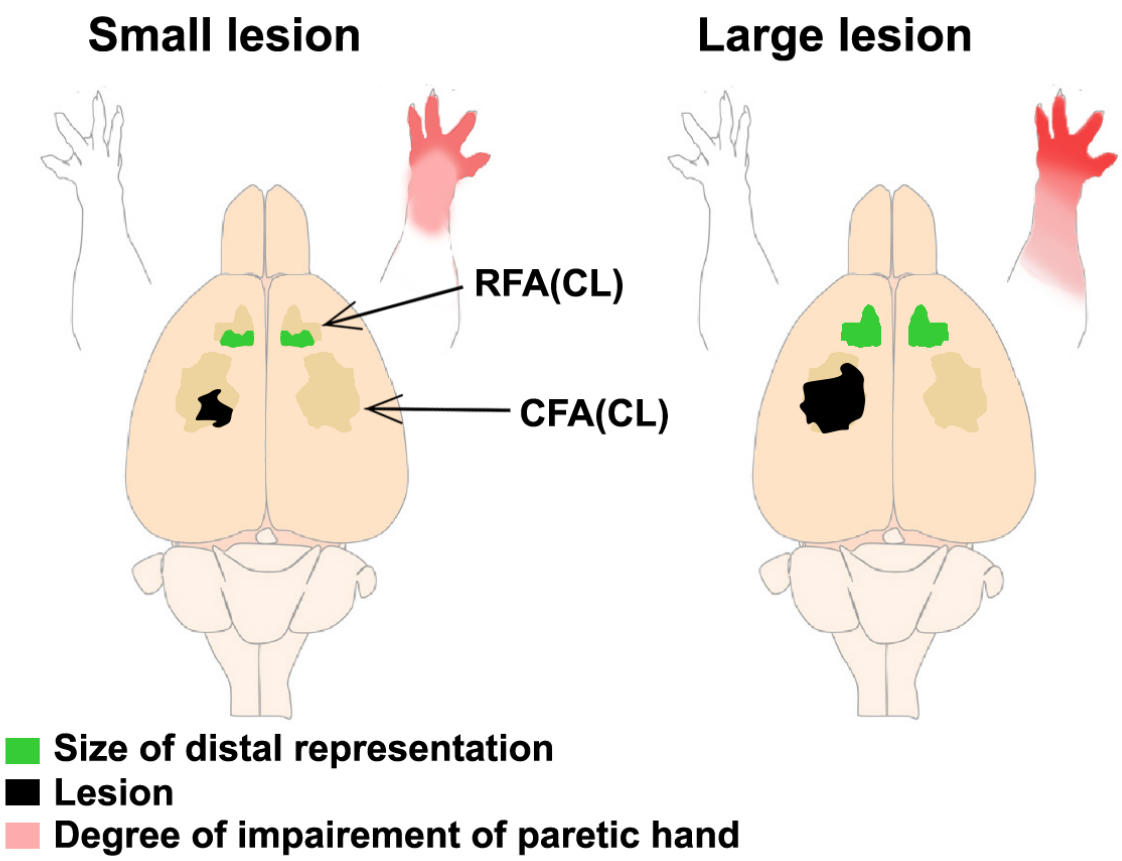

Figure 13. Schematic summary of results. Summary of results following smaller and larger lesions. Paretic forelimb in rats with small lesions was not as impaired as in rats with large lesions. Rats with smaller lesions had smaller distal forelimb representation in both IL and CL RFAs than rats with large lesions.

Chapter 3

General summary and discussion

3.1 General summary

As discussed in Chapter 2, in the present work we have confirmed that larger lesions in the CFA cause more persistent behavioral deficits of the paretic hand. We have confirmed the finding about the lack of change in cortical maps in the contralesional CFA (Maggiolini, Viaro, and Franchi 2008; Barbay et al. 2012). We have also established that cortical reorganization in the ipsi and contralesional RFA correlates inversely with lesion size. In addition we found that the size of hand representation in the contralesional RFA correlates inversely with the final recovery score.

Recent discovery of direct reticulomotor projections has increased interest in the role of reticulospinal tract and how it could contribute to motor recovery (Riddle, Edgley, and Baker 2009). Upon re-examination of older studies indications can be found further increasing interest in the reticulospinal tract. After Lawrence and Kuypers (1968a and b) severed the pyramidal and the rubrospinal tracts of macaques, they found the animals unable to effectively grasp food due to inability to efficiently control both distal and proximal muscles of the forelimb. While this highlighted the importance of these tracts in voluntary movements, there was also important information about the reticulospinal tract hidden within. The animals were able to move around the cage and were actually able to hang off the cage by grasping it with their hands with enough force to support their

weight. After lesions of rubrospinal and corticospinal tracts, of the three remaining tracts (reticulospinal, tectospinal and vestibulospinal) only reticulospinal projects to the distal muscles of the forelimb (Baker 2011). This could suggest that the reticulospinal pathway could be a venue for functional recovery after stroke. The major functional role of the reticulospinal pathway has been thought to be the initiation and control of locomotion (Kiyoji Matsuyama et al. 2004). Anatomical studies support this by demonstrating a wide patter of arborisation of single reticulospinal neuron in both the lumbar and the cervical enlargement (K Matsuyama and Drew 1997; K Matsuyama et al. 1999). This suggests a motor network designed for co-activation of large muscle groups. This point of view is supported by most studies, which examined the functional role of the reticulospinal tract, implicating it in initiation and control of locomotion (Kiyoji Matsuyama et al. 2004). These studies suggest that there should be further investigation of the reticulospinal pathway to establish its involvement in the recovery of locomotion after large cortical strokes. However the wide ranging arborisation of reticulospinal neurons in the spinal cord, along with reticulospinal pathway's role in locomotion suggests that it is unlikely to be the first priority target for investigation of recovery of voluntary reaching movements. Therefore in the following sections, this general discussion will be focused on the reorganization in the contralesional RFA and the potential mechanisms that can explain the relationship between changes in the contralesional RFA, lesion size and the behavioral recovery of the paretic hand. I also suggest potential future experiments that could verify my hypotheses.

In regard to the recovery of the paretic hand, the reorganization of the contralesional RFA could be either detrimental or adaptive. If the reorganization in the contralesional RFA is detrimental then it could be through either a) interhemispheric inhibition or b) learned non-use. In contrast, if the reorganization in the contralesional RFA is adaptive then it would be so through c) plasticity resulting

in increased corticospinal influence from the contralesional RFA onto muscles of the paretic limb. Alternatively, adaptive reorganization in the contralesional RFA could be through d) the contribution of contralesional RFA to the function of the ipsilesional RFA, mediated through interhemispheric connections. I address each of these possibilities in the following sections.

3.2 Relation between the reorganization of the contralesional RFA and behavioral recovery

3.2.1 Detrimental plasticity

A) Detrimental effect of contralesional RFA on behavioral recovery

We found a negative correlation between the size of hand forelimb representation of the contralesional RFA and final recovery score of the paretic hand. Previously, we discussed that there are clinical studies that found that suppression of the activity of the contralesional motor cortex resulted in improved recovery of paretic hand (Emara et al. 2010). The mechanism thought to be responsible for this phenomenon is a change of interhemispheric inhibition following stroke. After the lesion, increased interhemispheric inhibition from the contralesional hemisphere onto the ipsilesional hemisphere could interfere with adaptive plasticity in the ipsilesional hemisphere. According to this hypothesis, the most immediate conclusion after examining our results would be that the larger hand representation in the contralesional RFA is detrimental to the recovery of the paretic forelimb. However some caution is necessary when interpreting a correlational result. In the present set of experiments, great care was taken to make sure there is as little variability as possible between experimental animals. Rats were the same gender and age, and descendant from the same line (Sprague Dawley), thus assuring very limited genetic variability between animals. In addition, the experimental procedures, (i.e. task familiarization, behavioral recovery testing, and terminal bilateral

mapping) were the same for all animals. The only variable that was different between the two groups was the size of the ischemic lesion we induced in the CFA. Consequently, the negative correlation between lesion size and final recovery score is likely to be due to the only variable we introduced – lesion size. It is reasonable to assume that there is a causal relationship between the size of the lesion in the CFA and the recovery of the paretic hand. These results merely confirm what has already been established in primates. Similarly to our findings, lesions of progressively larger size in M1 of squirrel monkeys induce greater and more sustained behavioral deficits (Frost et al. 2003; Dancause et al. 2006; Dancause et al. 2005).

The hand representation in the contralesional RFA correlates positively with the only variable we introduced - lesion size. It is highly unlikely that the rats with larger lesions had larger hand representation in the contralesional RFA due to random chance. Assuming this is the case, we can conclude that prior to lesion induction rats which ended up with larger lesions did not have a larger hand representation in the contra and ipsilesional RFA. Therefore it is safe to assume that the correlation between lesion size and size of hand representation in the contralesional RFA is likely to be causal relationship with lesion size. If these assumptions are correct we can state two things. First, all other variables being equal, lesion size influences the extent of recovery and the size of hand representation in the contralesional RFA and is unlikely to be the cause of incomplete recovery of paretic hand in rats with larger lesions. As per our assumptions, both final recovery score of the paretic hand and the hand representation in the contralesional RFA have a causal relationship to lesion size. Therefore the relationship between the size of hand representation in the contralesional RFA and final recovery score is not causal. Thus larger hand representation in the contralesional RFA should not result in worse recovery of paretic hand. A simple way to test this hypothesis is to conduct an additional experiment.

In this experiment, the task, familiarization and behavioral testing schedule would be the same as the experiments that I have presented in the current work. Solely large lesions will be induced, as only rats with larger lesions tended to have bigger hand representation in the contralesional RFA. 24 hours after the behavioral test on day 28 a small surgery will be performed where the experimental groups will receive one injection of ET-1 in the contralesional hemisphere. The injection will be made based on stereotaxic coordinates, and will target contralesional RFA. Based on preliminary ICMS maps from five of our control animals an injection made at +3.6mm anterioposterior (AP) +2.2mm mediolateral (ML) relative to bregma is likely to create a lesion in the contralesional RFA (Figure 14). Sham animals will receive a saline injection of the same volume. 24 hours after such surgery our animals will have an additional behavioral testing session (day 30). The decrease in performance of the non-paretic hand between days 28 and 30 will indicate if the additional lesion was successful. If the lesion was successful then difference in performance with paretic hand between days 28 and 30 would be analyzed. By comparing the decrease in performance of paretic hand between sham and experimental groups, we would be able to say if a lesion in the contralesional RFA would cause reinstatement of deficits in the experimental group but not in sham. If the drop in performance after the additional lesion is greater in the experimental groups this would indicate that the contralesional RFA was contributing to recovery. If such results would be obtained, then this would demonstrate that larger hand representation in the contralesional RFA is not responsible for worse recovery of the paretic hand.

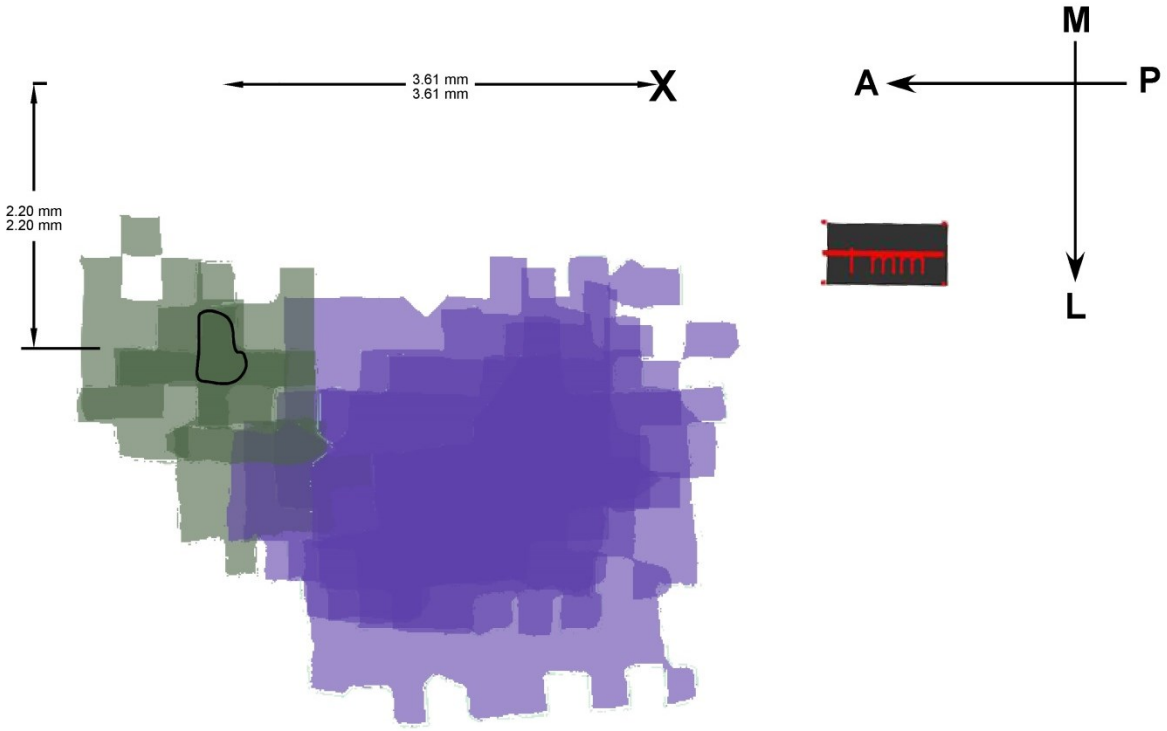

Figure 14. Stereotaxic coordinates for RFA lesion. Combined surface plot of ICMS maps of 5 control rats. ICMS map extracts have been aligned to bregma (X). Green represents RFA and blue represents CFA. Darker colour indicates areas where these respective maps overlapped between different animals. Black circle in the RFA represents the location, where all the control rats had forelimb response in the RFA. The arrows represent the location of this area in relation to bregma +3.6mm AP +2.2mm ML.

B) Expansion of RFA due to learned non-use

A possible explanation for the aforementioned correlations between the final recovery score, lesion size and the size of hand representation in the contralesional RFA is learned non-use of the paretic forelimb. Animals with greater impairments of the paretic limb likely relied more on the non-paretic limb. It is possible that to compensate for the greater loss of function of the paretic limb, the animals used the non-paretic hand more frequently and thus acquired new motor skills. These compensatory behaviors may have in turn led to the increase in size of hand representation of the RFA in the contralesional hemisphere. However, new motor skill acquisition in intact rats is associated

with a reorganization of the CFA, but not RFA (Kleim, Barbay, and Nudo 1998). As we did not see any changes in the contralesional CFA but did in RFA, if the cortical reorganization of the contralesional hemisphere is caused by acquisition of new motor skills with the non-paretic hand, the reorganization pattern is different after the lesion than in control animals.

To clearly establish if the negative correlation between the size of hand representation in the contralesional RFA and final recovery score is due to increased dexterity of the non-paretic hand, further experiments are needed. A relatively straight forward way to verify this hypothesis could be by constraining the non-paretic limb during recovery. The experimental design would be almost the same as the one we performed. The animals would first be familiarized with the Montoya staircase task. They would then undergo large lesion induction and during the 35 days of recovery their non-paretic hand will be constrained to prevent it from being utilized. Constrain induced therapy has been demonstrated to enhance behavioral recovery in stroke survivors (Wolf SL et al. 2006). Behavioral testing schedule will be the same as in our study and allowed to recover for the rest of the time. Five weeks after lesion induction bilateral ICMS mapping would be performed. The motor maps of rats with large lesions and restrained non-paretic hand will be compared to the motor maps of rats with large lesions that recovered spontaneously in the course of the current study presented in this work. As discussed previously studies in both squirrel monkeys and rodents have demonstrated that motor skill acquisition causes an increase in the size of the specific representation of the motor cortex (R. J. Nudo et al. 1996b; Kleim, Barbay, and Nudo 1998). Therefore restricting the non-paretic hand should prevent the excessive reliance on it following the lesion. More precisely, if larger hand representation in the contralesional RFA is due to motor skill acquisition of the non-paretic hand, then restricting the use of this limb and motor skill acquisition with it should prevent contralesional RFA from having a larger hand representation. If we see a significantly smaller hand representation in the contralesional

RFA of the group of rats with restrained non-paretic hand compared to unrestrained non-paretic hand, this would support the hypothesis that these changes are associated with the increased use of the paretic limb. However, in contrast I predict that the motor maps obtained from the two groups will not show significant differences in the contralesional RFA. If predicated results will be obtained this would suggest that the correlation between the size of hand representation in the contralesional RFA and behavioral recovery is not due to solely the acquisition of new motor skills by the non-paretic hand in the rats with large lesions.

3.2.2 Compensatory plasticity

C) Increased importance of contra and ipsilateral corticospinal projections from contralesional RFA

An alternative possibility is that the reorganization of the contralesional RFA is an example of adaptive plasticity. We know that the axons originating in the large pyramidal neurons in the motor cortex form most of the corticospinal tract. In rats about 5% of the corticospinal fibers do not crossover and descend down the spinal cord on the ipsi*lateral* side (Vahlsing and Feringa 1980). Due to bigger impairment of the paretic hand in the rats with large lesions perhaps the contralesional RFA underwent strengthening and arborisation of the ipsi*lateral* corticospinal projections to the paretic hand (Figure 15). While the percentage of corticospinal ipsi*lateral* projections is very low it is possible that further arborisation of these connections at the spinal cord would increase the importance of ipsi*lateral* projections from the contralesional RFA (Hypothesis 1). This contribution to paretic limb through ipsi*lateral* corticospinal projections would explain larger hand representation in the contralesional RFA. Alternatively it is possible that large lesions could trigger extensive reorganization in the spinal cord. As a result of this process it is possible that the major (decussated) part of the corticospinal tract originating in the contralesional hemisphere, would be able to contribute to the motor control of the paretic hand. This might take place through the arborisation and strengthening

of connections with commissural interneurons in the cervical enlargement. Commissural interneurons project across the midline of the spinal cord and synapse with neurons on the other side. Thus the reorganization in the spinal cord, which would allow the decussated part of the corticospinal tract originating in the contralesional hemisphere to contribute to recovery of paretic limb, could be responsible for larger hand representation in the contralesional RFA (Hypothesis 2). However, both of these hypotheses seem unlikely considering that during ICMS mapping we did not evoke any movements of the paretic hand in the contralesional RFA. However it is possible that the input from the corticospinal tract originating in the contralesional hemisphere was not large enough to evoke consistent muscle twitches. As we did not collect EMG data during our surgeries, we can exclude this possibility with absolute certainty. Thus further experiments would be needed to verify the contribution of the corticospinal tract originating in the contralesional hemisphere to the recovery of the paretic hand.

To verify that the corticospinal pathway originating from the contralesional RFA could be responsible for larger hand representation in the contralesional RFA, the following experiment could be performed. The terminal procedure would be divided into two stages. The first one will answer if the input to the muscles of the paretic hand from the corticospinal pathway originating from the contralesional RFA is greater after the large lesion. The second stage will tell us whether this contribution takes place through ipsi*lateral* (Hypothesis 1) or contra*lateral* (decussated) (Hypothesis 2) part of the corticospinal tract. As only rats with larger lesions tended to have bigger hand representation in the contralesional RFA, only large lesions will be induced in experimental rats. Control rats will be single caged for five weeks before undergoing the same terminal experiment as experimental rats. Task, task familiarization, behavioral testing schedule and terminal surgery timing will be the same as in experiments I have presented in the current work. Five weeks after lesion

induction animals would undergo a terminal experiment. In the first part of the terminal experiment EMG electrodes will be implanted bilaterally in both proximal and distal forelimb muscles. After craniotomies, the contralesional motor cortex and the pyramids would be exposed. Ipsilesional pyramidotomy would be performed rostral to pyramidal decussation (Figure 15). This should destroy all corticospinal input from the ipsilesional motor cortex to the paretic side. A regular ICMS electrode will then be lowered into the contralesional RFA and large single shocks will be delivered with this electrode. The intensity of the shock will be adjusted so that it evokes a large EMG response in the non-paretic arm and some EMG response in the paretic arm. If EMG response in the paretic arm can be observed, the amplitude is established and should be kept constant for the duration of the terminal surgery. EMG response of the paretic limb ($EMG_{pyramidotomy}$) should be normalized as percentage of EMG activity of the non-paretic limb ($EMG_{paretic/non\text{-}paretic}$). This will simplify comparison between animals and groups. This would conclude the first part of the experiment. If the $EMG_{paretic/non\text{-}paretic}$ obtained from animals that recovered from large lesions are significantly larger than $EMG_{paretic/non\text{-}paretic}$ values obtained from controls, then the input to the muscles of paretic hand from the corticospinal tract originating in the contralesional motor cortex is greater after recovery from large lesion. This reorganization could arguably be responsible for larger hand representation in the contralesional RFA. Absence of difference between the rats with large lesions and controls would indicate that it is not the corticospinal tract from the contralesional motor cortex, which is responsible for the reorganization in the contralesional RFA.

If there is an increase in the contribution of the corticospinal tract originating in the contralesional hemisphere, the second part of this experiment would answer whether it is taking place through the ipsi*lateral* (Hypothesis 1) or contra*lateral* (decussated) (Hypothesis 2) projections. An acute hemisection of the spinal cord above the cervical enlargement (rostral to third cervical

vertebrae) would be performed on the ipsilesional side (Figure 15). Its purpose is to exclude any contribution of decussated corticospinal tract originating from the contralesional hemisphere from contributing to EMG activity of paretic hand through commissural interneurons. Another set of data of EMG activity time locked to the stimulus in the contralesional motor cortex would also be collected from the paretic arm ($EMG_{hemisection}$). We will need to examine the difference between $EMG_{pyramidotomy}$, obtained before the hemisection and $EMG_{hemisection}$, obtained after. This is the only way to normalize this value between different groups and reduce variability. If the difference between $EMG_{pyramidotomy}$ and $EMG_{hemisection}$ in experimental rats is greater than in controls, this would suggest that contra*lateral* (decussated) corticospinal tract originating from the contralesional motor cortex contributes to the recovery of the paretic hand (Hypothesis 2). If the difference between $EMG_{pyramidotomy}$ and $EMG_{hemisection}$ in rats with large lesions is not different from controls, this would suggest that it was the strengthening of the ipsi*lateral* corticospinal tract originating from the contralesional motor cortex that contributed to the recovery of the paretic hand (Hypothesis 1). This experiment would help answer if the expansion of the hand representation in the contralesional RFA might be due to increased contribution of the contralesional motor cortex descending projections coinciding with significant reorganization in the spinal cord.

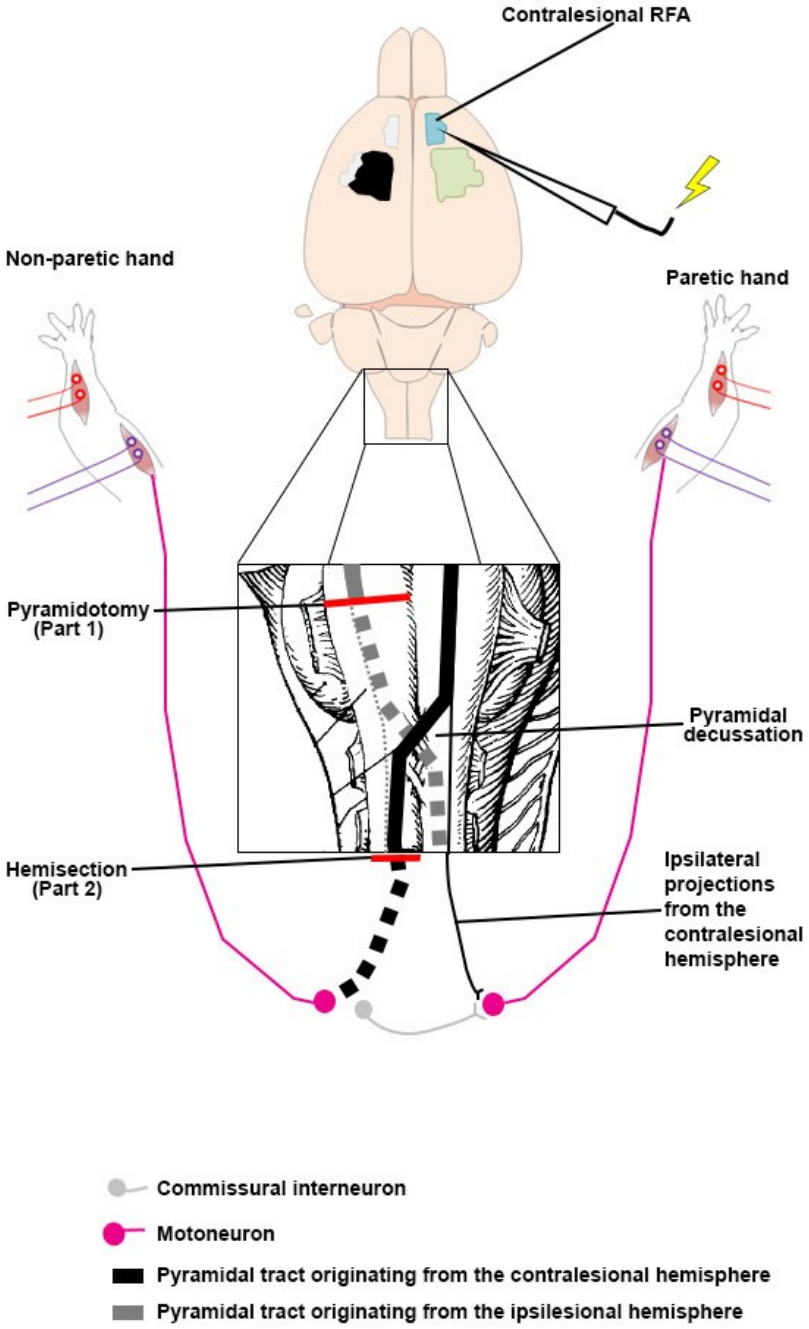

Figure 15. Proposed experiment setup. Large CFA lesion is the blacked out area of the cortex. Stimulation will be conducted through the electrode in the contralesional RFA. EMG activity during stimulation will be recorded. Blow up panel in the center shows a schematic representation of the pyramids and their decussation. The experiment will progress through two stages. At the first stage EMG data will be collected during stimulation after ipsilesional pyramid section (pyramidotomy). During the second stage EMG data will be collected during stimulation after ipsilesional hemisection of the spinal cord rostral to cervical enlargement.

D) Contralesional RFA contributing to the function of the ipsilesional RFA

Recovery from large lesions would require reorganization in the remote, interconnected regions of the brain. In healthy animals RFA is the cortical region most heavily interconnected with the CFA in the same hemisphere (Rouiller, Moret, and Liang 1993). RFA also has the highest number of corticospinal projections to the cervical enlargement after CFA (Starkey et al. 2012) and is the only other cortical region from which muscle twitches in the contra*lateral* forelimb can be evoked with ICMS. Therefore after excessive damage to the CFA, ipsilesional RFA is the primary candidate to assume CFA's function as this would require less reorganization than for any other cortical region. As the result of this compensatory plasticity ipsilesional RFA would function as a hybrid, assuming some of the function previously controlled by the ipsilesional CFA. The ipsilesional RFA might not be able to meet all of these additional processing demands. There is another possible recovery mechanism which might explain why rats with larger lesions and bigger impairment of paretic hand had larger hand representation in the contralesional hemisphere. In healthy animals the RFA is interconnected with contra*lateral* CFA. However it is most heavily interconnected to with the contra*lateral* RFA. (Rouiller, Moret, and Liang 1993). Therefore it is not unreasonable to presume that if in the course of recovery from large lesion the ipsilesional RFA cannot cope with additional processing demands, they might get "outsourced" to the contralesional RFA. Thus the correlation between the size of hand representation in the contralesional RFA and lesion size could be due to contralesional RFA, assuming some of the processing demands of the ipsilesional RFA. This sort of reorganization would contribute to functional recovery and would be most pronounced in the rats with the largest lesions and biggest deficits.

As the result of this reorganization interhemispheric balance is likely to change between the contra and ipsilesional RFA. To contribute to functional recovery of paretic limb it is likely that

modulation exerted by the contralesional RFA will be more facilitatory in the rats with large lesions compared to rats with small lesions or controls. The simplest way to verify the effect of this reorganization on interhemispheric balance would be to conduct an experiment that uses paired pulse stimulations. This experiment will examine the effect of subthreshold conditioning pulse in the contralesional RFA on the EMG output of paretic hand due to suprathreshold test stimulus in the ipsilesional RFA. Just as in other experiments proposed only the terminal experiment will be different from the study presented in the work. Task, task familiarization, behavioral testing schedule and age will be kept identical. There will also be three groups: rats with large lesions, rats with small lesions, and controls with no lesion. Terminal surgery will be conducted five weeks after lesion induction. During the terminal surgery EMG electrodes would be implanted into the forelimb muscles of the animal. After bilateral craniotomies, contra and ipsilesional RFA would be identified and stimulation electrodes placed in these two areas of interest. The effect of conditioning subthreshold stimulus to the contralesional RFA on the EMG output of suprathreshold pulse to the ipsilesional RFA would be quantified in recorded EMG. By comparing data between the controls and rats with large and small lesions, we would be able to establish how the contralesional RFA conditioning modulates the output of ipsilesional RFA after the lesion. If contralesional RFA modulates muscle activity evoked by ipsilesional RFA in rats with large lesion significantly stronger than in rats with small lesion and control animals, it would suggest a change in interhemispheric balance which occurs only after the large lesion. This would in turn support the hypothesis that following recovery from large lesion contralesional RFA undergoes reorganization to take up some processing demands from the ipsilesional RFA.

3.3 General conclusion

At present I foresee the hypothesis described in the previous section as the most likely explanation for the larger hand representation in the contralesional RFA. That is, more persistent deficits of the paretic hand in rats with larger hand representation in the RFA are less likely to be caused by the reorganization in the RFA and more likely to be due to larger lesions in those animals. Neither is learned non-use likely to explain larger hand representation in the RFA. It has been conclusively shown that the new skill acquisition causes reorganization in the CFA (Kleim, Barbay, and Nudo 1998). Therefore it is unlikely that the larger hand representation in the contralesional RFA is due to the animals' excessive use of the non-paretic hand. The fact that no muscle twitches were observed in the paretic hand during ICMS in the contralesional RFA makes it unlikely that reorganization of the corticospinal tract originating in the contralesional RFA is responsible for larger hand representation in the contralesional RFA. Thus it seems to me that the hypothesis that following recovery from large lesion, contralesional RFA undergoes reorganization to take up some processing demands from the ipsilesional RFA is the most likely one.

While we lack the data to conclusively explain the mechanisms underlying the physiological reorganization we observed, we feel that we have identified a crucial phenomenon in contralesional motor cortex. Correlation between the size of hand representation in the contralesional RFA with both lesion size and final recovery score singles out contralesional RFA as the area of interest. To the best of our knowledge no one has yet examined the role if this motor cortical area in stroke recovery. As such it singles out contralesional RFA for further investigation to the role that this area serves prior to and post stroke induced reorganization. In addition our result paves the road for more in-depth investigation of non-primary motor cortical areas in both primates and human stroke patients and might contribute to developing improved post stroke rehabilitation treatments.

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