DYNAMICS OF FUNCTIONAL CONNECTIVITY WITHIN CORTICAL MOTOR NETWORK DURING MOTOR LEARNING IN STROKE – CORRELATIONS WITH "TRUE" MOTOR RECOVERY

By

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ABSTRACT

Arm motor recovery after stroke is usually incomplete; six months after onset about two-thirds of patients suffer from arm motor impairment that significantly impacts the individual's activities of daily living. Thus, novel concepts beyond current strategies for arm motor rehabilitation after stroke are needed. An essential approach for this is to better understand whether motor learning-related neural changes in stroke are similar with those in healthy controls and how these neural changes relate to recovery of the pre-morbid movement pattern or "true" recovery. Abnormal task-related activation in primary and non-primary motor cortices has been a consistent finding in functional MRI studies of stroke. Disturbed functional network architecture, e.g., the influence that one motor area exerts over another, also impacts stroke recovery. The outcome measures chosen to evaluate recovery are also important for the interpretation of these brain changes.

Thus, the long-range goal of this work was to longitudinally investigate the changes in cortical motor function at two levels, regional (micro-circuitry, regional activation) and network (macro-circuitry, functional connectivity), following an arm-focused motor training in chronic stroke survivors and how these brain changes relate to recovery of the pre-morbid movement pattern or "true" recovery.

In the Chapter I, we reviewed the literature concerning the pathophysiology of stroke, neural substrates of motor control, and motor learning principles and neural substrates in healthy and pathological (stroke) brain.

In the Chapter II, we examined the relationships between task-related motor activation and clinical and kinematic metrics of arm motor impairment in survivors of subcortical stroke. We found evidence that primary motor activation was significantly correlated to kinematic metrics of arm motor impairment, but not with clinical metrics.

In the Chapter III, we longitudinally investigated the regional changes in motor-related activation (functional MRI) in primary and non-primary motor areas following an arm-focused motor training in stroke survivors and age-sex matched healthy controls. We demonstrated that similar changes in the motor areas contralateral to the trained arm were found with training in both stroke and healthy participants. We also demonstrated a significant increase in motor performance in both groups as well as a normalization of the correlations between bilateral motor activation and movement kinematics in participants with stroke.

In the Chapter IV, we also investigated the changes in functional connectivity between primary and non-primary motor areas following an arm-focused motor training and how these changes correlate with "true" motor recovery. We demonstrated significant enhanced functional connectivity in motor areas contralateral to the trained hand (or ipsilesional), although no "normalization" of the inter-hemispheric inhibition following training in our survivors. We also showed a "normalization" of the relationships between cortical motor functional connectivity and movement kinematics.

In the Chapter V, we concluded that the present dissertation work support the hypotheses that motor system is plastic at different levels, regional and network, even in the chronic stage of stroke and some of these changes are similar with those reported in healthy controls. Further, these changes provide a substrate for "true" recovery. These findings promote the use of

neuroimaging and kinematic metrics to improve our understanding of the neural substrates underlying reorganization in remaining intact brain structures after stroke. Such an approach may further enable monitoring recovery or compensation based on this reorganization and evaluating new treatment regimes that assist motor recovery.

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CHAPTER I - Introduction

LIST OF ABBREVIATIONS

CBF ..... Cerebral blood flow

ATP ..... Adenosine triphosphate

CST ..... Corticospinal tract

fMRI ..... Functional magnetic resonance imaging

CNS ..... Central nervous system

M1 ..... Primary motor cortex

BA 4 ..... Brodmann's area 4

BA 4a ..... Anterior division of Brodmann's area 4

BA 4p ..... Posterior division of Brodmann's area 4

LTP ..... Long-term potentiation

rTMS ..... Repetitive transcranial magnetic stimulation

PM ..... Premotor cortex

SMA ..... Supplementary motor area

PMd ..... Lateral/dorsal premotor cortex

PMv ..... Ventral premotor cortex

BOLD ..... Blood oxygen level dependent

PSC ..... Percent signal change

FC ..... Functional connectivity

PCA ..... Principle component analysis

KR ..... knowledge of results

KP ..... knowledge of performance

LTD ..... Long-term depression

CIMTs ..... Constraint-induced movement therapies

WMFT ..... Wolf Motor Function Test

FMUE ..... Fugl-Meyer Upper Extremity Assessment

EEX ..... Elbow Extension

PLIC ..... Posterior limb of the internal capsule

ROI ..... Regions of interest

PRE ..... Prior to

POST ..... Immediately after

CHAPTER I

INTRODUCTION

Introduction

The literature review covers the existing knowledge about arm motor impairment and recovery at different levels, behavioral and neural, following stroke. Specifically, it focuses on the principles of arm motor control and learning in healthy controls, the plastic changes in the motor system after stroke evidenced by functional brain mapping, and the motor system reorganization during arm-focused interventions after stroke. The gaps in the literature have been identified and hypotheses regarding motor impairment and recovery have been formulated.

Stroke physiopathology

Stroke is defined as "a condition characterized by rapidly developing symptoms and signs of a focal brain lesion, with symptoms lasting for more than 24 hours or leading to death, with no apparent cause other than that of vascular origin" (World Health Organization, 1989). In the United States, it is estimated that over 800,000 people suffer each year from stroke, from each about 600,000 are first attacks and 200,000 are recurrent attacks, with estimated 134,000 deaths nationwide. To date, stroke affects at least 6.4 million persons in the United States (Lloyd-Jones et al., 2010).

Mechanisms of stroke

The underlying mechanisms of stroke depend on the presence of preexisting conditions that are epidemiologically linked to stroke and possibly implicate the cause of stroke. Specifically, there are two mechanisms, ischemia and hemorrhage, that may cause brain damage.

The most common cause of ischemic stroke (about 80% of all strokes) is the sudden occlusion of a blood vessel by a thrombus or embolism, resulting in an almost immediate decrease or complete absence of circulating blood that deprives neurons of critical survival

substrates, i.e., oxygen and glucose. A common feature of the ischemic stroke is the narrowing of the arteries, particularly those in the neck or head, as results of atherosclerosis. Cardiovascular diseases, such as atrial fibrillation, myocardial infarction, endocarditis, cardiac tumors and valvular disorders, are among the most commonly recognized sources for embolism. Other causes may include certain drugs, traumatic brain injuries to the blood vessels of the neck, or blood clotting disorders. Therefore, a thrombotic stroke may occur when damaged cerebral arteries become blocked by the formation of a blood clot while an embolic stroke results from a detached intravascular mass clogging arterial capillary beds at a site, i.e., in the brain, or far from its origin, i.e., systemic.

Hemorrhagic stroke (less than 20% of all strokes) occurs when a weakened blood vessel bursts and bleeds into brain tissues, causing localized pressure injury and consequently irreversible neuronal injury and damage. Brain hemorrhages can be induced by some blood vessels disorders, including chronic unmanaged high blood pressure or cerebral aneurysms. Cerebral aneurysms are abnormally weak spots on blood vessels wall within the brain. As aneurysms usually don't cause any detectable problems until they break specifically with abnormally high blood pressure (Aguilar and Freeman, 2010). There are two recognized types of hemorrhagic strokes including subarachnoid and intracerebral hemorrhages. While in subarachnoid hemorrhages, bleeding is cause by burst of large arteries on or near the membrane surrounding the brain, i.e., meninges, in intracerebral hemorrhages, bleeding is cause by burst vessels within the brain itself. Hypertension is recognized as the primary cause of intracerebral hemorrhage (Woo et al., 2004). Other causes include abnormalities of the blood vessels, such as arteriovenous malformation, aneurysm amyloid angiopathy, and head injuries (Paolucci et al., 2003).

In this work we studied only survivors of an ischemic stroke. We restricted to this type of stroke to study a more defined patient subgroup (or homogeneous) because heterogeneity could be one reason for the failure of restorative therapies(Hermann and Chopp, 2012). In addition, because this type of stroke accounts for 80% of all strokes, we could reach our recruitment target.

In the adult human brain at rest, the average hemispheric cerebral blood flow (CBF) is maintained at a fairly constant level, near 50-55ml/100g/min. Following a stroke, regions with different degree of ischemia appears in the affected vascular territory. The brain region with a CBF lower than 10mL/100g/min, are referred as the ischemic core. In the ischemic core, irreversible cell damage appears within minutes after the stroke onset. The ischemic core is surrounded by a brain region with a CBF lower than 25mL/100g/min or oligemic region called the ischemic penumbra (Hakim, 1998; Heros, 1994). Brain tissue in the penumbra is a brain tissue at risk for several hours after the stroke onset and is referred as the "window of opportunity", since neurological impairment generated by ischemia can be partially or totally reversed by reperfusion of the ischemic yet viable brain tissue (Lo, 2008; Witte et al., 2000). However, if this window is not exploited, brain cells gradually become hypoxic and are depleted of cellular adenosine triphosphate (ATP), which results in a significant energy failure. Thus, brain cells switch to anaerobic metabolism to produce energy, resulting in accumulation of lactic acid. The depletion of cellular energy reserves triggers over-activation of certain neurotransmitters, particularly glutamate and aspartate. This process is called "excitotoxicity". Increased glutamate in the extracellular space opens the calcium channels associated with NMDA and AMPA receptors (Dirnagl et al., 1999; Heros, 1994; Rothman and Olney, 1986). Thus, glutamate overexcites the already deprived cells and causes influx of calcium, sodium, and

chloride ions and efflux of potassium ions. Increased in intracellular calcium stimulate a series of destructive enzymes that allows release of mediators, such as cytokines, resulting in the loss of cellular integrity (Dirnagl et al., 1999; Rothman and Olney, 1986; Yuan and Yankner, 2000). The neuronal death is accompanied by inflammatory response, resulting in vasodilatation, vasoconstriction, increased permeability, increased platelets aggregation, and immunoregulation.

For example, during a supratentorial acute ischemic stroke, about 1.2 billion neurons, 8.3 trillion synapses, and 7140 km myelinated fibers are lost (Saver, 2006). Therefore, a particular attention has been given to developing new acute care stroke interventions in order to reduce or limit the size of the brain acute lesion (Abou-Chebl, 2013). Unfortunately, for many patients this is not accomplished and stroke remains the leading cause of long-term disability in the United States (Sidney et al., 2013). Given the higher prevalence of stroke in the elderly, the burden of stroke is likely to increase as our population ages. Moreover, people at younger ages are increasingly suffering from this debilitating disease. Therefore, it is vital to understand the physiological mechanisms underlying functional recovery, particularly motor recovery, in these patients. This understanding might provide unexpected clues for approaching the rehabilitation process.

Clinical impairments after stroke

After stroke, impairments of motor, sensory and/or cognitive abilities have been described with different degrees of severity. While each can have debilitating effects independently, impairment in one area may affect performance in another.

Neurological recovery depends on the type (Bilic et al., 2009; Paolucci et al., 2003), size and location of the brain lesion (Chen et al., 2000; Luft et al., 2004b; Shelton and Reding, 2001). It is likely that patients with hemorrhagic stroke have better neurological recovery than those with

ischemic stroke (Bilic et al., 2009; Paolucci et al., 2003). Indeed, during hemorrhagic stroke, neurological deficit can be caused by brain compression, and as the hematoma resolves, neurological functions recover. However, these studies included the patients in the middle range of all hemorrhagic patients. Therefore, care must be taken in generalizing these findings. Studies investigating the effect of brain lesion location on neurological, particularly motor, recovery have shown worse arm motor recovery with lesion located in subcortical deeper brain structures, i.e., basal ganglia, internal capsule, compared to more superficial location, i.e., cerebral cortex (Chen et al., 2000; Luft et al., 2004b; Shelton and Reding, 2001). Specifically, patients with pure cortical stroke or those with mixed cortico-subcortical stroke have less motor deficits than those with pure subcortical stroke (Feys et al., 2000; Shelton and Reding, 2001). The corticospinal tract (CST) is the major pathway that mediates voluntary movements. Neurophysiological and anatomical studies have shown that the integrity of the CST fibers is critical for successful completion of the arm motor tasks. The degree of CST injury, for example after brain lesion at the subcortical level, is a significant predictor of motor outcome (Puig et al., 2011). However, recovery is possible in 10-15% of cases where CST damage seems complete and this could be possible by reorganization of spared brain regions and/or recruitment of new regions (see below).

Arm motor impairment after stroke

Arm paresis is one of the most common impairments after stroke (Carey et al., 1998; Yarosh et al., 2004). After six months, about two-thirds of patients suffer from arm sensorimotor impairment that impacts the individual's activities of daily living (Kolominsky-Rabas et al., 2001b). This impairment is most evident in the arm contralateral to the injured (ipsilesional) hemisphere (Mani et al., 2013) with a greater distal (wrist/hand) than proximal (elbow/shoulder)

impairment (Bourbonnais and Vanden Noven, 1989). Motor deficits consist of weakness of specific muscles (Bourbonnais and Vanden Noven, 1989), abnormal muscle tone (Burke, 1988; Lance, 1980; Wiesendanger, 1991), abnormal postural adjustments (Carr and Shepherd, 1987b), abnormal movement synergies (Bobath, 1990b), lack of mobility between structures at the shoulder girdle (Carr and Shepherd, 1987b), and incorrect timing of components within a movement pattern (Archambault et al., 1999a). Previous studies on arm movement kinematics (Cirstea and Levin, 2000b; Fisher et al., 2000; Platz et al., 1999) and/or kinetics (Mirbagheri et al., 2008) have shown decreased active range of elbow and shoulder movements and altered coordination between these joints (Cirstea et al., 2003b). When stroke survivors attempt to move and encounter such deficits, the natural reaction is to use alternative motor strategies (Cirstea and Levin, 2000b; Rehme et al., 2011). In other words, the stroke survivors retained the ability to recruit a new degree of freedom that is not typically used by healthy subjects. Such degree of freedom is represented by the use of compensatory movement strategies, i.e., trunk movement, that are related to the degree of motor impairment, i.e., greater compensation in severely to moderately impaired subjects while mildly impaired subjects tended to employ healthy movement patterns (Cirstea and Levin, 2000). Although the incorporation of motor compensations may result in better functional ability in the short-term, the task accomplished using atypical movement patterns might lead to long-term medical complications, e.g., reinforcement of distorted positions of the joints, excessive muscle shortening (Ada et al., 2000). One can argue that the goal of stroke rehabilitation is recovery of function whether achieved through “true” motor recovery, defined here as recovery of premorbid movement patterns, or behavioral compensation. It is generally agreed that for some patients with severe impairment and poor prognosis, compensatory movements should be encouraged to maximize arm and hand

functional ability. On the other hand, for those with good prognosis, several arguments support emphasizing motor recovery over compensation. First, recent research suggests that given appropriate training, motor improvements of the upper limb can continue into the chronic stage of stroke (Michaelsen and Levin, 2004). Second, permitting the use of motor compensations could lead to a pattern of learned non-use limiting the capacity for subsequent gains in motor function of the impaired arm (Allred et al., 2005; Cirstea et al., 2006; Taub et al., 1993). Third, this is also a real problem for functional brain imaging studies investigating recovery-related changes because such motor compensation might often lead to changes in brain activation even though they have nothing to do with the true recovery. Since the functional magnetic resonance imaging (MRI) is one of the main tool to investigate brain reorganization after stroke, the outcome measures have to be carefully chosen so that investigators know what they are attributing activation changes to. *This limited attention to movement quality is an important concern in stroke rehabilitation and we addressed this in the present research.*

Indeed, most arm motor recovery studies used clinical measures of impairment or function. However, many of these clinical measures suffer from serious limitations related to ceiling or floor effects and limited objectivity as well (Krakauer, 2005). As stated above, several research groups have proposed measures derived from kinematic data to quantify motor deficits after stroke (Cirstea and Levin, 2000a; Cirstea et al., 2003a; Dewald et al., 1995; Finley et al., 2005). In contrast to clinical measures, such measures provide high-resolution measurements and identify movement features that cannot be captured by clinical scales (Subramanian et al., 2010). For example, subtle movement deficits could be detected using kinematic analysis even in patients with mild impairment (Cirstea et al., 2003a; Hermsdorfer et al., 1999; Jeannerod, 1984). Despite of all these advantages, these metrics are largely ignored in functional brain imaging

studies.

Given that motor compensations are related to the deficits in the active range of the proximal joints, we used an objective measure of such deficit, i.e., the active range of elbow extension, to distinct between motor compensation and "true" recovery at the behavioral level and we studied the relationships of these measures and brain activation (see Chapter II).

Other stroke-related deficits

Although the focus of the present studies was on the arm motor deficits, these patients have also shown a widespread incidence (about 50% of cases) of somatosensory dysfunction (Kim and Choi-Kwon, 1996; Sullivan and Hedman, 2008). This dysfunction can range from involvement of just one type of sensation, for example light touch, to impairment of all somatosensory abilities. Since somato-sensation is fundamental for motor ability, any somatosensory impairment is linked to poor recovery of the arm motor capabilities (Sullivan and Hedman, 2008). Recognizing and monitoring sensory impairments is crucial to the patient's treatment and rehabilitation outcome. Therefore, these impairments have been taken in consideration in the present studies.

Since we studied capability of stroke patients to relearn a motor task, it is important to also mention the cognitive impairments in patients with stroke. In addition to physical impairments, there are often cognitive impairments, including problems with memory, perception, reasoning, language, and attention, which interfere with functional recovery of these patients (Makin et al., 2013). Such impairments may be associated with motor learning deficits (Doyon et al., 2009; Hanlon, 1996; Krakauer, 2006) and poor motor outcome (Tatemichi et al., 1994). However, a motor intervention may involve varying degrees of cognitive processing depending on its cognitive demands. For example, during a "simple" motor intervention, i.e., feedback about

precision, patients with moderate to mild cognitive deficits may simple improve their motor abilities by repetition since this intervention does not tap into intellectual or executive functioning processes (Cirstea et al., 2006; Platz et al., 2002). In contrast, during a "complex" motor training, i.e., feedback about movement pattern, clinical improvements are likely to be related to better memory, mental flexibility, and planning abilities (Cirstea et al., 2006). Presumably, these cognitive processes are more involved in making use of critical information about adapting motor behavior to improve efficiency. Thus, a battery of cognitive tests, including attention, apraxia, somato-agnosia, and receptive aphasia testing, has been delivered in our participants. Finally, depression is known to have a negative influence on functional recovery (Kanner and Barry, 2003) and patients with moderate to severe depression have been also excluded from our studies.

Arm motor control

Arm motor control in healthy controls

Proper central nervous system (CNS) contribution is required for efficient planning and execution of arm movements (Hogan, 1984; Soechting and Flanders, 1998). The CNS regions concerned with arm movement control are organized in a distributed fashion and interact at both cortical and subcortical levels to cooperate in the control of movement (see Fig. 1). For example, basal ganglia: putamen, globus pallidus, and portions of the thalamus (Alexander and Crutcher, 1990), along with the cerebellum (Allen and Tsukahara, 1974, Itō, 1984, Fine et al. , 2002) and substantia nigra, are critical in regulating movements. Specifically, basal ganglia exert their control on motor behavior through reciprocal connections to motor cortices, particularly premotor areas. This cortical-basal ganglia motor loop plays a major role in the control of the

force, speed, and amplitude of movement. Similar to this loop, the cortico-cerebellar loop (i.e, premotor areas via re-entrant neuronal circuitry) plays a major role in the control of kinematic parameters for skilled movements. The cerebellum also plays an important role in detection of movement error and in adjustment of the evolving movement to changing contextual requirements. Finally, these two subcortical structures, the basal ganglia and cerebellum are critically involved in different stages of motor learning (Alexander and Crutcher, 1990; Ashby et al., 2010; Doyon et al., 2009; Jueptner M Fau - Weiller and Weiller; Lehericy et al., 2005; Miyai et al., 1997).

However, this review is primarily focused on the motor cortices, i.e., primary and non-primary motor areas (see Fig. 2), given strong evidences of their involvement in motor recovery and learning after stroke (Calautti et al., 2007; Johansen-Berg et al., 2002a; Ward et al., 2003b) (Carey et al., 2002; Fridman et al., 2004; Seitz et al., 1998) (Gerloff et al., 2006; Johansen-Berg et al., 2002b; Lotze et al., 2006).

Primary motor cortex (or M1)

M1, the Brodmann's area 4 (BA 4), is located on the anterior bank of the central sulcus with the caudal border lying in the depth of the central sulcus close to its fundus and anterior border abuts area 6, is considered the executive locus responsible for generating the motor outcome for simple and complex voluntary movements (Filimon et al., 2007). The M1 has a broad somatotopic representation of the different upper and lower body segments in an arrangement called "motor homunculus" (Fig. 3). The representation of body segments is unique in terms of location and size. First, the homunculus is arranged in an upside-down map of the contralateral body segments. The upper extremities and the facial body segments are closer to the lateral sulcus than lower extremities such as the leg and toes that are located more medially. The

amount of cerebral cortex devoted to a given body segment is proportional not its size but to how richly innervated that region is. In other words, the image of the human body segments in the brain are built in proportion to their motor significance, i.e., proportional to the complexity of the movements (and to sense nerves) executed by particular segments of the body. Therefore, the resulting body representation within M1 is a grotesquely disfigured body image with disproportionately large hands, lips, and face compared to the rest of the body (see Fig. 3). Similar to animal models, human M1 is subdivided into two distinct areas, including an anterior (BA 4a) and posterior (BA 4p) area based on their features relative to the performed task (Geyer et al., 2000; Rathelot and Strick, 2009; Terumitsu et al., 2009). BA 4a, the "old" motor cortex, is thought to be more "executive" in nature than the BA 4p since its output is conducted via corticospinal tract and spinal interneurons to produce movements (Rathelot and Strick, 2009). BA 4p, the "new" motor cortex, contains corticomotoneurons that synapse directly onto spinal motoneurons (Rathelot and Strick, 2009). Thus, BA 4p is likely involved in "non-executive" functions required by complex movements. Further, BA 4p is strongly recruited when sensory stimuli are present (Terumitsu et al., 2009). The latter is consistent with Geyer et all suggesting a differential specialization of BA 4a and BA 4p in roughness discrimination task, with BA 4p relatively not involved in generating the actual physical movement (Geyer et al.; Geyer et al., 2000; Rathelot and Strick, 2009). This issue is presently under investigation and is important for the recovery of function after stroke because these areas may participate differentially in recovery processes.

M1 also plays a major role in motor learning (Gerloff et al., 1998; Kawashima et al., 1994). M1 functional adaptation that accompanies motor skill learning depends on restructuring of M1 micro-circuitry including enlarged dendritic fields and increase in neuronal synapses (Greenough

et al., 1985; Withers and Greenough, 1989). Therefore, it is reasonable to conclude that M1 neurons may significantly contribute to learning-dependent network reorganization and improved synaptic efficiency and strength through mechanisms like long-term potentiation (LTP) (Hess and Donoghue, 1994; Racine et al., 1995). However, M1 is one of several brain areas that are involved in motor skills acquisition and learning (Ghilardi et al., 2000; Toni et al., 1998). Specifically, M1 is involved in early consolidation of the motor memory and makes an important contribution to procedural learning by stabilizing procedural memories (Muellbacher et al., 2002). Repetitive transcranial magnetic stimulation (rTMS) of M1 disrupted the retention of the behavioral improvement, suggesting that M1 is specifically engaged in the early stage of motor consolidation of newly learned tasks.

Non-primary motor areas (premotor cortex and supplementary motor area)

Non-primary (or secondary) motor areas (Brodmann's area 6, BA 6, Fig. 2) include premotor cortex or PM (on the lateral side of the central gyrus) and supplementary motor area or SMA (on the medial side). In comparison to M1, PM and SMA neurons are less related to the initiation of movements, but more related to the aspects of movement planning (Rushworth et al., 2003; Wu and Hatsopoulos, 2007).

PM participates in motor preparation (Churchland et al., 2006), action selection (Cisek and Kalaska, 2005), motor execution (Caminiti et al., 1991) and learning (Lee and van Donkelaar, 2006). PM, located on the anterior half of precentral gyrus and the anterior bank of the precentral sulcus, has two subdivisions, lateral dorsal and ventral areas (PMd and PMv respectively). PMd, located anterior to M1, has a separate yet similar representation of the body segments as that described in M1. PMd neurons are involved in the preparation phase and visual online control of movement (Kurata and Wise, 1988; Lee and van Donkelaar, 2006; Wise and Mauritz, 1985). In

other words, neuroimaging studies in human have shown that PMd is heavily involved in visual- and somato-motor control (Prado et al., 2005). Much like PMd, PMv is also thought to be involved in visually guided and somatosensory control of movement (Halsband et al., 1994; Hoshi and Tanji, 2004a; Mushiake et al., 1991). PMv has connections with many other brain areas (Dancause et al., 2006; Ghosh and Gattera, 1995; Stepniewska et al., 2006) and exerts prominent facilitatory inputs on M1 especially during the visually-guided movement of the hand and arm (Cerri et al., 2003; Shimazu et al., 2004). However, differences exist between PMv and PMd in terms of their projections into other brain areas. PMd is more connected to the visual and visio-motor areas on the medial parietal cortex where PMv is connected more laterally on the parietal cortex to the somatosensory areas (Dancause et al., 2006; Stepniewska et al., 2006). In summary, PMv guides limb movement by integrating internal somatosensory body representation (Kurata, 1994; Ochiai et al., 2005) while PMd is important for integrating visual information in the spatial guidance of limb movement (Kakei et al., 2001).

SMA is the medial aspect of PM (BA 6) and is located on the medial wall of the hemisphere from the top of the brain to the depth of the cingulate sulcus with the posterior boundary halfway between the extension of the central and precentral sulci onto the medial surface and the anterior boundary at the vertical line trough the anterior commissure. Evidence from animal models and humans suggests that SMA is involved in motor planning especially in high-order motor functions such as coordinating complex motor sequences of actions as in bimanual motor control (Hoshi and Tanji, 2004b; Lee and Quessy, 2003; Roland et al., 1980a; Roland et al., 1980b; Yazawa et al., 1998; Yazawa et al., 2000) as well as in motor learning (Perez et al., 2008). Although both PM and SMA are involved in motor planning, it has been shown that the SMA activation occurs prior to PM activation supporting the major role of SMA

in planning and programming voluntary movements (Tanji, 1996). Furthermore, unlike PM that controls actions externally guided by visual inputs, SMA involved in sequential actions that are internally guided by memory (Gaymard et al., 1990)

In conclusion, several anatomical properties make the non-primary motor areas optimal to assume some M1 functions in the event of stroke and justify the study of these areas in the present research:

1) they contain a somatotopic organization that parallels the M1 organization (He et al., 1993; Wise et al., 1996);

2) their efferents project directly to the spinal cord (Dum and Strick, 1991; Hutchins et al., 1988; Nachev et al., 2008);

3) they are heavily interconnected via the corpus callosum (Fang et al., 2008; Mochizuki et al., 2004); and

4) they are ipsilaterally interconnected with each other (Dum and Strick, 2005) as well as with M1 (Stepniewska et al., 1993).

Neuroimaging techniques to study motor control

The study of the motor system anatomy and function in human relies on a variety of non-invasive neuroimaging techniques, such as functional MRI (fMRI) and positron emission tomography (!!! INVALID CITATION !!!; Calautti et al., 2001b; Formisano et al., 2004; Friston et al., 1993; Kim et al., 2006; Nowak et al., 2008; Serrien et al., 2002; Takeuchi et al., 2005; Yozbatiran et al., 2009).

Functional magnetic resonance imaging (fMRI) is an MRI technique that measures brain activity by detecting the changes in cerebral blood oxygenation and flow (hemodynamic response) that occur in response to neural activity (i.e. when a brain area is in use, blood flow to

that specific area increases) (Huettel et al., 2009). Functional MRI uses the blood oxygenation level as a contrast mechanism known as blood oxygen level dependent (BOLD) contrast (Ogawa et al., 1993). The study of brain function using fMRI uses a repetitive scans the brain during presenting a stimulus (Martin and Potts, 2009) or carrying out a certain task (Cirstea et al., 2011a). There are two forms of experimental designs in fMRI studies, block and event-related designs. Block design includes two or more conditions (in our case, rest and move conditions) in an alternating pattern. The alternating conditions are separated into distinct blocks and each condition is presented for a pre-established period of time. In the event-related design, the stimulus presentation is random and the time between stimuli could vary. The commonly used methods to quantify BOLD signal are i) percent signal change (PSC) and ii) spatial extent of activation (Nielson et al., 2010). PSC is the most commonly used measures in fMRI studies and quantifies signal magnitude divided by the mean signal intensity across functional runs. PSC values are obtained within a certain brain region using the setting cluster spread range. It has been shown that PSC is a sensitive measurement of BOLD signal changes longitudinally (Lee et al., 2010). Spatial extent of activation is defined as the total number of activated voxels within a cluster of activity divided by the overall number of voxels within the target brain area.

The overall agreement in these studies is that complex movements produce activation of both motor and non-motor areas in left and right hemispheres. For motor areas, such activation has been seen bilaterally in PMd, PMv, and SMA (Prado et al., 2005; Ward et al., 2003a; Ward et al., 2003c; Ward et al., 2006; Wu and Hatsopoulos, 2007) and unilaterally in contralateral M1, somatosensory cortex, and cerebellum (Ward et al., 2003a; Ward et al., 2003c). Brain regions, such as M1 and PMd, ipsilateral to the moving hand/arm, have been identified to be age-dependent. Specifically, increased activation in these areas was found in aged compared to

young healthy controls executing the same motor task (Langan et al., 2010; Ward et al., 2003a). Yet, such age-dependent increased activation of the ipsilateral M1 has been shown to have a negative influence on motor output, i.e., increase in reaction times (Langan et al., 2010). However, it has been suggested that increased activation in these areas result from reduced inter-hemispheric connectivity or, in other words, decreased ability of the contralateral hemisphere to inhibit ipsilateral motor areas (Langan et al., 2010).

Functional connectivity within the motor system

In addition to quantification of activation in each area within the motor system, recent fMRI paradigms also quantify the **functional connectivity** (or FC), e.g., the influence that one area exerts over another. Functional and effective connectivity network is constructed from time series of brain dynamics on the anatomical network. In other words, a functional network represents patterns of cross-correlations between BOLD signals estimated from these dynamics. This section focuses particularly on the cortico-cortical connectivity between primary and non-primary motor areas.

For example, excitatory and/or inhibitory effects of one area on another were found between and within hemispheres. Between hemispheres, there is a common consensus that most connections between M1 are inhibitory in order to maximize the segregation of cortical-cortical activity, or inter-hemispheric inhibition (Daskalakis et al., 2002; Ferbert et al., 1992). Within hemisphere, different effects were found between SMA and M1 depending on the studies hemisphere. For example, within hemisphere contralateral to the studied **arm**, SMA facilitates M1, while in the ipsilateral hemisphere, SMA suppresses the activity of M1 (Grefkes et al., 2008a; Solodkin et al., 2001; Yu et al., 2011). Taken together, these data suggest a strong lateralization of motor activation during execution of arm movement.

As the individual participation of motor areas in movement execution, the FC between primary and non-primary motor areas is likely to be influenced by the age (Langan et al., 2010; Rowe et al., 2006). Specifically, a decrease in inter-hemispheric inhibition and consequently a decrease in lateralization have been found in aged population.

Motor learning also appears to change, e.g., enhance, FC within the motor system, by shaping both strength and timing of the cortical synchronization (Kilgard et al., 2007). Neuronal plasticity refers to structural as well as functional alterations of neuronal circuits in response to experience. Synaptic plasticity in adult neural circuits may involve the strengthening of existing synapses and forming of new synapses as well as structural plasticity (Fu and Zuo, 2011; Tropea et al., 2010).

Finally, this cortico-cortical FC is also altered in pathological condition, such as stroke (see below).

To study FC, various approaches have been proposed (Esposito et al., 2005; Formisano et al., 2004; Friston et al., 1993; Grefkes et al., 2008a; Mintzopoulos et al., 2009a; Rehme et al., 2011). Most of these approaches quantify the statistical dependencies (i.e., correlations) among remote neurophysiological events by using different statistical models and logarithms (Friston, 2011; Grefkes and Fink, 2011a; Westlake and Nagarajan, 2011). Thus, FC is mostly descriptive and is usually assessed with correlation coefficients (Friston, 2011). Other ways like eigenvectors and principle component analysis (PCA) are also considered correlation approaches, yet they do not provide a direct measure for the interregional connectivity. For example, PCA decompose or extract the observed connectivity patterns into few components and thereby ignore many of the original connectivity patterns within the investigated brain networks (Koch et al., 2010). Finally, inter-regional FC could be simply identified using signal time course correlation especially when

the main goal is exploratory (Eickhoff and Grefkes, 2011; Friston, 2011; Grefkes and Fink, 2011a; Koch et al., 2010; Westlake and Nagarajan, 2011).

In summary, there is still a huge debate about which approach is more sensitive and reliable to measure FC and there is no clear assumption in regard to the superiority of one approach over another (Grefkes and Fink, 2011a; Westlake and Nagarajan, 2011). As it is our goal to explore FC between primary and non-primary motor areas, we used signal time course correlation (i.e., correlation coefficients) (Eickhoff and Grefkes, 2011; Grefkes and Fink, 2011a; Westlake and Nagarajan, 2011).

Arm motor control reorganization after stroke

There is considerable evidence that the adult human (Chollet et al., 1991; Weiller et al., 1992; Weiller et al., 1993a) and non-human (Frost et al., 2003) motor system is capable of widespread functional and structural plasticity. If damage to a functional system is partial, recovery is more likely to occur through potentiation and extension of residual areas, whereas complete lesions require vicarious substitution by functionally related systems (Seitz and Freund, 1997). However, the degree to which brain regions could be recruited after an infarct is influenced by the location and the extent of the infarction itself as well as by "remote effect or diaschisis". Structural degeneration (Frost et al., 2003; Kobayashi et al., 2005) and functional disconnection of remote areas interconnected with the damaged area (Frost et al., 2003; Pantano et al., 1996; Seitz et al., 1999) have been described several weeks to months after subcortical stroke (Nelles et al., 1999). Although it has been established that a patient's neurological recovery parallels the resolution of neocortical diaschisis, the physiological aspects of diaschisis related to this resolution are unknown (Meyer et al., 1993; Seitz et al., 1999). In addition,

whether these two phenomena, vicarious function and diaschisis interact, i.e., if they have synergistic, independent or antagonist effects on recovery, is also not well known.

The effects of focal ischemia on surrounding motor areas have been examined in adult non-human primates after a focal infarct affecting 30% of the total M1 hand area by Nudo et al. (Nudo et al., 1996b). By the end of the first month, in animals undergoing spontaneous recovery, performance on a reach-and-retrieve task was nearly normal, with some residual deficits and compensation by the unaffected limb. After three months the deficits had essentially resolved. Examining cortical motor maps before and three months after the ischemic insult, widespread alterations are found in the cortical areas spared by the lesion. The digit area of M1 in the adjacent, undamaged tissue was reduced by about 50% possibly due to the disuse of the affected limb. To investigate whether the increased post-injury use of the affected limb might modulate this reduction in digit representations (maladaptive plasticity) constraint induced therapy was used to improve manual skills (Nudo et al., 1996b). In some cases, the hand representations expanded into regions formerly occupied by elbow/shoulder representations and this reorganization was accompanied by recovery of skilled hand function. These results suggest that after focal damage to the motor cortex, rehabilitative training can shape subsequent reorganization in the adjacent intact cortex, and that the undamaged motor cortex might play an important role in motor recovery. More recently, Nudo et al. have demonstrated alterations in the anatomy and physiology of more remote non-primary motor areas (PM, SMA) that appear to be related to functional recovery (Dancause et al., 2005; Eisner-Janowicz et al., 2008; Frost et al., 2003; Nudo et al., 1996b; Nudo, 1997; Nudo, 1999).

By using non-invasive neuroimaging (fMRI) and electrophysiological (transcranial magnetic stimulation or TMS, direct current stimulation or DCS) techniques, studies in humans have

demonstrated that similar mechanisms occur after stroke. Specifically, structures normally not involved in a specific task, such as sensory cortex and non-primary motor areas, are activated along with the displacement of primary motor peak activation both in subcortical (Calautti et al., 2001b; Eisner-Janowicz et al., 2008) and cortical infarcts (Cao et al., 1998; Luft et al., 2004b; Seitz et al., 1998). Since we studied the survivors of a subcortical stroke, this section is focused on the functional brain changes in this subpopulation. We selected this sample to allow us to have a homogeneous group of patients with comparable lesion size and location. In addition, subcortical location of the brain lesion is likely to induce bilateral activation of motor pathways and recruitment of additional motor structures not normally involved in motor function (Chollet et al., 1991; Cramer et al., 1997), potentially by leaving cortico-cortical circuitries intact (Ward et al., 2003c).

In cross-sectional studies, the activation pattern in patients was characterized by increased recruitment of the ipsilesional M1 (Byrnes et al., 2001; Murase et al., 2004; Ward et al., 2003c) and PM (Johansen-Berg et al., 2002a; Mima et al., 2001; Seitz et al., 1998; Weiller et al., 1992; Weiller et al., 1993a), ipsilesional cerebellum, and both ipsilesional and contralesional SMA, PM, and parietal cortices (Chollet et al., 1991; Cramer et al., 1997; Fridman et al., 2004; Nelles et al., 1999; Seitz et al., 1998; Weiller et al., 1992; Weiller et al., 1993a) compared to healthy controls (Fig. 4A).

Longitudinal studies show that successful recovery occurs in stroke survivors who exhibit relatively normal patterns of ipsilesional activation and less contralesional motor activation, whereas patients, who often show bilateral cortical activation, typically have less complete recovery (Calautti and Baron, 2003; Cramer et al., 2000; Marshall et al., 2000; Nelles et al., 1999) (Fig. 4B).

Primary motor cortex reorganization after stroke

Regarding **M1**, the best spontaneous recovery is associated with the greatest return of activity in ipsilesional M1 (Johansen-Berg et al., 2002a; Johansen-Berg et al., 2002b; Shumway-Cook and Woollacott, 2012; Ward et al., 2003b). For example, facilitation of ipsilesional M1 excitability is directly related to the improved function of the impaired hand (TMS (Traversa et al., 1998; Ward et al., 2003a); tDCS (Hummel and Cohen, 2005; Kim et al., 2006; Yozbatiran et al., 2009), while its inhibition resulted in altered behavior (Fregni et al., 2005). In addition, a shift of the M1 hand representation in the dorsal (Boggio et al., 2007), ventral (Jaillard et al., 2005; Weiller et al., 1993b; Werhahn et al., 2003b; Zemke et al., 2003) or posterior (Cramer and Crafton, 2006; Schaechter et al., 2008) direction has been related to the survival of distinct subsets of CST fibers.

However, the relationship between ipsilesional M1 activation and "true" motor recovery is unknown. Thus, we are the first to test the hypothesis that motor-related activation of the primary motor cortex is stronger correlated with the kinematic than clinical metrics of arm motor impairment after stroke (see Chapter II).

The functional significance of contralesional M1 involvement in motor recovery has been much debated. This debate is due, in part, to inconsistent results of TMS studies that found that inactivation of contralesional M1 improved (Calautti and Baron, 2003; Pineiro et al., 2001), altered (Mansur et al., 2005), or left unchanged (Takeuchi et al., 2005) the motor performance of the impaired arm. In the healthy brain, a TMS-induced M1 "virtual lesion" results in increased excitability of the "contralesional M1" without changes in hand motor function (Lotze et al., 2006). Therefore, it is conceivable that the increased contralesional M1 activation observed after stroke may reflect altered inter-hemispheric inhibition providing compensatory support for

impaired hand movements in the presence of ipsilesional CST damage. The mechanisms underlying altered inter-hemispheric inhibition between M1 after stroke is not well understood, e.g., direct involvement of the commissural fibers connecting M1s after a cortical stroke (Werhahn et al., 2003a) or indirectly by thalamic or cerebellar pathways in subcortical stroke (Schambra et al., 2003). Some argue that the contralesional M1 recruitment reflects the recruitment of un-crossed CST fibers (Nowak et al., 2008; Radlinska et al., 2012). However, contralesional M1 may reflect either a diffuse recruitment of the motor networks driven by higher orders areas during a task performance (Calautti and Baron, 2003), or a dendritic overgrowth due to overuse of the healthy hand and unmasked by lack of transcallosal inhibition from the ipsilesional M1. The recruitment of this area also depends on the location and extent of injury (Calautti and Baron, 2003; Nair and Lincoln, 2007; Nelles et al., 1998). Finally, increased activity in contralesional M1 is generally present in those with poor behavioral outcome (Gerloff et al., 2006; Ward et al., 2003a). Although the exact role of contralesional M1 in recovery remains elusive, its connection with ipsilesional M1 is critical for functional improvement (Cramer and Crafton, 2006; Cramer, 2008a; Ward et al., 2003a). Remodeling of contralesional motor tracts after stroke (Lindenberg et al., 2012; Perez and Cohen, 2009) might not necessarily provide the structural basis for further functional gains in chronic stage, but it might be necessarily in earlier stages of stroke.

Non-primary motor cortex reorganization after stroke

Regarding non-primary motor areas, increased ipsilesional (Benowitz and Carmichael, 2010; Fridman et al., 2004; Lindenberg et al., 2012) and contralesional (Carey et al., 2002; Johansen-Berg et al., 2002b; Seitz et al., 1998) **PM** activation, frequently associated with recovery, has been reported. Moreover, contralesional PM activation is more prominent in patients with

significant impairment (Gerloff et al., 2006; Lotze et al., 2006). However, one study failed to demonstrate a relationship between bilateral PM recruitment and recovery (Ward et al., 2006).

The relationship between **SMA** activation and post-stroke recovery is also inconsistent (Calautti et al., 2007; Johansen-Berg et al., 2002b; Loubinoux et al., 2003; Riecker et al., 2010). Finally, although functional connectivity between the ipsilesional non-primary areas was weaker in patients imagining and executing a motor task compared to controls (Calautti et al., 2007), altered connectivity during execution was not correlated with the motor impairment. Therefore, the exact function served by increased recruitment of non-primary areas after stroke remains to be clarified.

Reorganization of functional connectivity within motor system after stroke

After stroke, the motor system adapts not only in terms of what structures are engaged but also in how these structures communicate between them. Indeed, **disturbed functional connectivity within the motor system** also impacts stroke recovery (Sharma et al., 2009a; Zemke et al., 2003). Studies of FC may provide further insights into the mechanisms underlying the changes in activation patterns described above.

In subcortical stroke, a disrupted functional connectivity was found between cortical motor areas within and across hemispheres (Duque et al., 2005; Grefkes et al., 2008a; Grefkes et al., 2008b; Mintzopoulos et al., 2009a). Specifically, they reported an increased connectivity across primary and non-primary motor areas within each hemisphere (Grefkes et al., 2008b; Grefkes et al., 2010; Murase et al., 2004) and increased inter-hemispheric inhibition toward the ipsilesional hemisphere (van Meer et al., 2010a). However, the number of studies published so far on this topic is very limited and the relationship between FC alteration and behavioral recovery has been poorly interpreted and remains incompletely characterized. This lack of data interpretation

could be due to the use of different techniques assessing FC and/or the heterogeneity of studied population (van Meer et al., 2010b). Moreover, although cross-sectional designs are useful to determine the relationship between FC patterns and motor impairment, the longitudinal assessment truly defines the changes in connectivity associated with recovery.

Thus, in the present studies, we employed task-related fMRI, clinical and kinematic assessments of motor impairment, to investigate longitudinally the changes in functional connectivity between primary and non-primary motor cortices as well as their functional relevance (correlation with "true" recovery) after a subcortical stroke. Specifically, we have objectively monitored the changes in motor-related activation and functional connectivity between these areas and movement patterns employed to achieve the functional goal over an arm-focused motor learning paradigm. On the basis of this increased understanding and the availability of such objective measurements of recovery, novel rehabilitative approaches could be tested raising hope for the development of new treatments for post-stroke chronic disability.

Motor learning

Motor learning principles in healthy controls

In healthy controls, motor learning is defined as a "set of processes associated with practice or experience leading to relatively permanent changes in the capability for responding" (Grefkes et al., 2008b). Therefore, there are permanent changes in motor planning and execution resulting in a reduction in time and errors of execution of movement performed. Thus, "an internal model which represents the exact matching between perceived sensory and motor information" is formed (Wolpert et al., 1995). Four factors have been identified to influence motor learning: i) the stages of learning, ii) the type of task, iii) the type of feedback, and iv) the type of practice.

There are classically described two distinct stages of motor skill acquisition: i) early and fast learning stage, in which considerable improvement in performance could be seen within a single training session, and ii) late, slow learning stage, in which further gains can be observed during the course of practice (Grefkes and Fink, 2011a). Another definition of the learning stages has been suggested by Fitts and Posner (Fits and Posner, 1967), including cognitive, associative and autonomous stages (Schmidt and Lee, 1988). During the cognitive stage, there is a process of understanding the requirements of the motor task. This stage is characterized then by inconsistent performance, because of multiple different strategies that are explored and employed by the learner. During the associative stage, there is process of refining the motor skill with continuous practice and repetition. Consequently, the motor skill becomes more consistent and the numbers of errors are decreased. Moreover, during this stage, sensory feedback is a key element in correcting the movement, by developing error-detection mechanisms and increasing the ability to generalize to new motor tasks (Karni et al., 1998). Thus, motor skill progresses from explicit control in the early stages of learning to a more implicit or automatic control when well learned.

The task can be classified into closed (predicted) and opened (unpredicted) based on the environment in which they are being performed (Fitts and Posner, 1967). The key difference between the two tasks is whether or not the external environment controls the spatial and temporal features of the movement and the task demands (Kottke, 1980).

Although all four factors are considered essentials in designing treatment programs, greater emphasis is placed on two most potent learning variables, feedback and practice (Gentile, 1972). Thus, feedback is one of the two key motor learning elements of program prescription (Adams et al., 1972; Anderson et al., 2001; Badets and Blandin, 2012; Bilodeau and Bilodeau, 1958;

Cirstea et al., 2006; Cirstea and Levin, 2007; Gentile, 1972; Liu and Jensen, 2009; Salmoni et al., 1984; Schmidt and Lee, 1988; Sigrist et al., 2013). Feedback has been categorized into intrinsic or extrinsic. For intrinsic feedback, the feedback is given in the form of sensory information from body receptors such as those in muscles, joints, tendons, skin, and visual and auditory information. For extrinsic feedback, also called augmented feedback, the feedback is given in the form of information from an external source such as the therapist or biofeedback system (van Vliet and Wulf, 2006; Wulf et al., 1998). Extrinsic feedback can be provided in two forms: knowledge of results (KR) or knowledge of performance (KP) (Bilodeau and Bilodeau, 1958; Shumway-Cook and Woollacott, 2012; Sigrist et al., 2013). KR is the feedback about movement outcome and goal provided after the movement is completed, i.e., terminal feedback (Salmoni et al., 1984; Schmidt and Lee, 1988; Weeks and Kordus, 1998). KP is the feedback about the movement pattern used to achieve the goal that could be provided during or after movement execution, i.e., concurrent or terminal feedback. Although both types of extrinsic feedback facilitate and accelerate the learning process (Adams et al., 1972; Badets and Blandin, 2012; Cirstea et al., 2006; Cirstea and Levin, 2007; Liu and Jensen, 2009; Shumway-Cook and Woollacott, 2012; Sigrist et al., 2013; Winstein, 1991), KP is likely to be more commonly employed and has higher clinical applicability (van Vliet and Wulf, 2006; Wulf et al., 1998). As feedback is used in early learning to develop the previously mentioned error-detection mechanisms, the frequency of the feedback should be decreased (i.e. faded feedback) (Gentile, 1972) over the learning process to enhance more cognitive processes related to internal error detection and to prevent dependency on feedback (Shumway-Cook and Woollacott, 2012; Winstein, 1991).

As stated before, beside feedback, practice is considered the second most important variable

in motor learning. This is potentially due to the high degree of manipulation that can be made to structure practice sessions. More specific, practice sessions can be manipulated in regard to the rest periods (massed vs. distributed practice), the variability of the task across practice (variable vs. constant practice), the amount of components within a certain task (part vs. whole practice) and the order of the practiced tasks (blocked vs. random practice) (Shea et al., 1993; Shumway-Cook and Woollacott, 2012). The improvement in motor skill has been shown to increase as the amount of practice increases (Newell and Rosenbloom, 1980; Shumway-Cook and Woollacott, 2012). However, the initial stages of learning should focus on understanding the goal (i.e., cognitive stage) and closed, distributed, constant, part and blocked practice may be employed to increase performance. Then, through trial and error-detection, successful movement patterns will develop and in the later stages of learning, open, massed, variable, whole and random practices should be indicated (Carr, 2000; Davis, 1979; Gentile, 1972; Newell and Rosenbloom, 1980; Schmidt and Lee, 1988; Shumway-Cook and Woollacott, 2012).

Principles of motor learning are one of the most effective exercise paradigms for learning or re-learning of motor skills. The application of these principles depends on the stage of motor learning and the capabilities of the learners. The findings derived from research on motor learning in healthy controls have been used to develop treatment approaches in patients stroke (Davis, 1979; Doyon et al., 2009; Hanlon, 1996; Kawashima et al., 1994; Krakauer, 2006; Laforce and Doyon, 2002; Magill, 2004; Salmoni et al., 1984; Shumway-Cook and Woollacott, 2012; Sigrist et al., 2013).

Neural basis of motor learning in healthy

Motor learning depends on the plasticity of neurons and circuits within the motor system. As stated before, the motor system consists of cortical (primary and non-primary motor areas) and

extracortical areas (basal ganglia and cerebellum). Although the interaction between sensory and motor systems is a prerequisite for proper motor learning (van Vliet and Wulf, 2006), the next section is focused only in the motor system participation in motor learning.

At the neural level, a set of brain regions, including primary and non-primary motor areas, basal ganglia, and cerebellum, shows changes in their activation during different stages of motor learning (Asanuma and Pavlides, 1997; Ashby et al., 2010; Doyon et al., 2002; Doyon et al., 2003; Doyon and Benali, 2005a; Doyon, 2008; Doyon et al., 2009; Winstein, 1991). Specifically, the work by Doyon and colleagues revealed that cortico-cortical and cortico-subcortical networks contribute differently to different stages of motor learning (Laforce and Doyon, 2002; Lehericy et al., 2005). For example, the fast (early) stage of learning is associated with significant contribution of both cortico-striatal and cortico-cerebellar networks. Once the task is well learned, a shift to the cortico-striatal networks is observed. Therefore, the integrity of these networks is critical for efficient motor task acquisition.

Over the course of motor learning, as more movement strategies are employed and increased cognitive demands, increased activation in motor and non-motor areas has been observed (Alexander and Crutcher, 1990; Ashby et al., 2010; Doyon and Benali, 2005a; Doyon, 2008; Doyon et al., 2009; Lefebvre et al., 2012; Lehericy et al., 2005). Further, an increased functional connectivity between these areas has been also reported (Karni et al., 1995). In the early phases of motor learning, increased activation in prefrontal areas, particularly in the dorsolateral prefrontal cortex, was reported. This is not surprising since these areas are presumably related to explicit working memory and the establishment of a novel association between visual cues and motor commands (Platz et al., 2012). In addition, primary and non-primary motor areas of the right hemisphere (Sun et al., 2007) as well as cerebellum are particularly important in the early

stages of motor skill acquisition (Ashe et al., 2006; Grafton et al., 2002; Halsband and Lange, 2006a). For example, motor learning is disrupted when M1 function has been temporally altered (by using rTMS) immediately after the motor training (Karni et al., 1995; Karni et al., 1998). With practice, motor associate areas of the left-hemisphere show increased activity. This shift to the left hemisphere has been observed regardless of the hand used during training, indicating a left-hemispheric dominance in the storage of visuo-motor skills. Moreover, rTMS applied over M1, PM and SMA induces alterations in different aspects of motor skill learning (Karni et al., 1995).

Primary motor cortex in motor learning

It has been shown a learning-related increase of activation contralateral primary motor cortex over the course of motor learning (Baraduc et al., 2004; Karni et al., 1998) in M1, assemblies of neurons control specific movements of different joints and muscle groups (Halsband and Lange, 2006b; Seitz et al., 1990). Thus, an assembly projects to several pools of spinal motoneurons (Cheney et al., 2004; Nudo, 2008). To control a multi-joint movement, reach-to-grasp movement in our case, different assemblies are interconnected via horizontal intra-cortical projections (Cheney et al., 2004; Keller, 1993). Movements and arm parts are represented multiple times and are intermixed with representations of related movements of parts forming a complex mosaic pattern. This pattern, called motor map, reflects the output of M1. During motor training, these maps are reorganized, i.e., enlarged (Cheney et al., 2004; Keller, 1993; Kleim et al., 1998). This reorganization depends on restructuring of M1 microcircuitry. Structural modifications, as spine formation and synaptogenesis (Pascual-Leone et al., 1995), and modulation of synaptic weights or alterations of connectivity, i.e., (LTP) or long-term depression (LTD) (Nudo et al., 1996a), for the basis of such enlargement of the motor maps (Fig. 4). For example, pyramidal neurons in

layers II/III and V have enlarged dendritic fields (Kleim et al., 2004b; Rioult-Pedotti et al., 2000). This enlarged dendritic field is accompanied by an increase in the number of synapses per neuron in layer V, suggesting that learning may promote synaptogenesis (Withers and Greenough, 1989). The magnitude of spine formation during the early phase correlates with learning efficacy and they become stabilized after training. If the same task is over-trained no further spine turnover is induced, but training a new task did. Such structural changes do not occur randomly within the M1 circuitry but are confined to a subset of neurons directly recruited by the novel task (Greenough et al., 1985) and may represent a footstep of the motor memory trace. As mentioned above, electrophysiological alterations of M1 neurons may also contribute to learning-related reorganization of the network. LTP or LTD are correlates of synaptic plasticity induced by learning. For instance, acquisition of a reaching task induced LTP in the horizontal connections of layer II/III in the M1 forelimb representation contralateral to the trained paw. There were no changes of synaptic efficacy in the hindlimb representation (Kleim et al., 2004a). Thus, this training-related enlargement could be a reflection of the motor memory (Wang et al., 2011a).

However, changes in movement-related activation by using fMRI are fundamentally different than changes in evoked movements in response to cortical stimulation (used to describe learning-related map changes in the previous sections). In our case, movement-related activation reflects indirectly neuronal populations that control a movement. With training, it is likely that the constituents of these populations are changed and morphological changes and/or alterations of connectivity could form the basis of these changes. Thus, some newly generated synapses, that have functionally relevance for the learned movement, are formed, and synaptic transmission within horizontal connections are strengthened. These changes translate in a better connectivity

among neurons across M1 activating in concert the spinal motor neutron pools to enabling the performance of the trained movement. Since the results of stimulation mapping depend on the organization of cortical output and on cortical excitability, the movement-related activation is more physiologically and may be a better surrogate marker for the motor memory trace.

Non-primary motor cortex in motor learning

SMA showed a practice-related increase in activation (Monfils et al., 2005; Rioult-Pedotti et al., 1998), particularly in the left hemisphere, regardless of the hand trained (Grafton et al., 2002; Hazeltine et al., 1997b). This suggests that left SMA has a dominant role in the performance of sequential arm movement, and it is in agreement with the left-hemisphere dominance movement-planning hypothesis (Grafton et al., 2002).

A bilateral activation in PM was observed during the early stages of unilateral skill learning (Hazeltine et al., 1997b; Janssen et al., 2011). For this area, the right PM is likely to be involved in early stages of motor learning and such involvement suggests its role in spatial processing and high reliance on sensory feedback system which are critical during early motor learning (Deiber et al., 1997).

As in M1, same basic mechanisms of learning-induced plasticity could be applied in non-primary motor areas.

<u>Motor learning and rehabilitation after stroke</u>

A recent review of the stroke rehabilitation literature revealed 12 randomized controlled trials comparing specialized patient rehabilitation with conventional care in 2813 stroke survivors (Inao et al., 1998). Improved functional outcomes and reduced length of hospital stays were reported among patients receiving specialized rehabilitation (Halsband and Lange, 2006a).

Intensive and structured training is one key element of such rehabilitation programs and the improvement in the desired outcomes is likely to depend on two elements: the intensity of the training and the specificity of the task practiced (Foley et al., 2003a). Motor learning principles, i.e., intense and structured training, have been now included in two of the most used therapeutic approaches in this population, constraint-induced movement therapies (CIMTs) (Carr, 2000; Teasell et al., 2009) and motor relearning program developed by Carr and Shepherd (Carr, 2000). CIMTs have been shown to improve arm functionality even in the chronic stage of stroke (Carr, 2000; Taub, 2000; Taub and Morris, 2001) by inducing neuronal plasticity (Taub et al., 1999; Taub et al., 2013). Motor relearning program includes intensive task-oriented practice taking into account motor learning principles, such as task specificity, task repetition, type of practice, type of feedback, retention testing and generalizability of motor gains. There is increasing evidence that functional recovery occurs with this type of motor re-learning program even in the chronic phase of stroke (Butefisch et al., 1995; Cirstea et al., 2003c; Dean and Shepherd, 1997; Schaechter et al., 2002; Taub et al., 2003). Recently, electromyogram-triggered neuromuscular stimulation (Kwakkel et al., 2002), robot-assisted rehabilitation (Kwakkel et al., 1997), and virtual reality training are excellent examples of how concepts from research in motor control can be applied to generate new therapeutic approaches with regard to rehabilitation.

However, one concern in stroke rehabilitation is whether identification of movement component(s) that are missing or that interfere with motor performance can lead to specific impairment-oriented training strategies to maximize recovery. It has been emphasized that the best training approach must specifically address the individual's impairments and should incorporate strategies to enhance the transfer of performance gains from the training situation to everyday life (Cauraugh et al., 2000). Such training programs include repetitive practice,

incorporate optimal feedback, and need to be sufficiently difficult to challenge the motor system and to induce changes in motor cortex topology likely relevant for motor recovery (Volpe et al., 2000). Previous studies suggested that during an intensive repeated practice, if an altered movement component is addressed by training, the gains in motor behavior reflect recovery compared to the condition when one movement outcome was targeted by training (Platz et al., 2005b).

Since our goal of the second manuscript was not to train patients in a novel task but to investigate longitudinal the neural changes at different levels, individual activation and functional connectivity within cortical motor network, occurring as a function of "true" motor recovery following a specific motor training, we used an intensive training of an altered movement component (i.e., elbow extension) to measure changes in movement quality and whether these changes are related to changes within the motor system. Thus, this study takes the first step in a systematic approach to this problem within such a theoretical context (see Chapter II).

Measures of motor recovery

However, the criteria for assessing and defining functional recovery have been ambiguous. Most studies have used clinical indicators of impairment (i.e., Fugl-Meyer scale), function (i.e., Barthel Score), and/or kinematic outcomes (i.e., movement speed) to measure intervention effectiveness without consideration of *how these gains were attained* (i.e., movement quality). Thus, there is a lack of distinction between "true" recovery and behavioral compensation. Indeed, many outcomes used in stroke rehabilitation have limited objective ability to characterize movement strategies (Platz et al., 2005b). For example, the Wolf Motor Function Test (WMFT), a reliable and a common "timed" task-based, is a standard outcome measure in stroke research

involving arm rehabilitation [175][175]. WMFT assesses gross- and fine-motor components during a set of functional tasks. All tasks are timed and rated based on the functional ability. However, several concerns are present in regard to using WMFT in stroke rehabilitation field. One limitation related to the validity of using this outcome in severely impaired patients who cannot complete many of the tasks considering the time limit of 120 seconds for each task. Therefore, this test has limited ability to quantify overall changes in performance in moderate to severely impaired patients (Cirstea and Levin, 2007). Another common tool used in stroke rehabilitation is Fugl-Meyer Upper Extremity Assessment (FMUE). **FMUE**, composed of scales for sensation, proprioception, joint pain, range of motion (shoulder, elbow, wrist and fingers), reflex activity, and joint co-ordination and having an excellent intra-rater and inter-rater reliability (Platz et al., 2005a; Platz et al., 2005c), is one of the most comprehensive quantitative measures of motor impairment after stroke (Michaelsen et al., 2004). However, the FMUE components neither assess purposeful reaching tasks nor quantify the functional impairments due to spasticity or weakness (Taub et al., 1999). In addition, ceiling effect, particularly for the patients with mild impairment, and the presence of some components (such as reflexes) that do not make a significant contribution to the assessment of impairment (Morris et al., 2001) have been identified as further limitations of FMUE. Furthermore, FMUE scores can be obtained by using combined measures of the trunk and shoulder flexion movements during a reach-to-grasp task (Hodics et al., 2012). Therefore, it may be reasonable to exclude some components, i.e., reflexes, and to decompose FMUE score in sub-scores accordingly to proximal and distal segments. In summary, the WMFT and FMUE assessments provide valuable information regarding motor performance and motor impairment after stroke, yet they do not provide precise quantitative data

on movement strategies and thereby lack the sufficient sensitivity to characterize changes in movement strategies especially longitudinally over time.

Kinematic motion analysis is an effective quantitative tool to capture movement strategies during movements with the impaired arm (Duncan et al., 1992; Gladstone et al., 2002; Woodbury et al., 2008). Indeed, movement kinematics can be used to distinguish between recovery and compensation. Many studies have documented an indirect relationship between the use of behavioral compensation and the impaired reaching ability characterized by decreased active range of elbow/shoulder movements (Cirstea and Levin, 2000b; Cirstea et al., 2003b; Gladstone et al., 2002; Levin et al., 2002; Subramanian et al., 2010; Woodbury et al., 2007). Thus, it is reasonable to conclude that an increase in active elbow extension is a feature of "true" recovery, being accompanied by a decrease in compensation. In support of this, Roby-Brami and her team used the increase in active range of elbow extension as a main outcome measure to quantify intervention-related arm motor recovery after an intervention (Michaelsen et al., 2004). Finally, the assessment of the elbow extension during a reaching task predicts the performance on both WFMT and FMUE (Levin et al., 2002; Roby-Brami et al., 2003a). Thus, we also used the clinical and kinematic metrics to quantify the training-related changes in behavior (see Chapter III).

Neural substrates of motor learning after stroke

Despite an enormous research on the neural mechanisms underlying motor recovery in humans, these mechanisms are still largely unknown. In animals, acquisition of motor skill appears to be a prerequisite for driving plasticity in the motor cortex (Cirstea and Levin, 2000b). In humans, limited number of stroke studies examined the relationship between motor improvements and brain activation pattern following different therapeutic approaches (Cirstea et

al., 2003b; Levin et al., 2002; Massie et al., 2011; Michaelsen et al., 2006). Despite methodological and sample differences, three findings were consistently found: i) before training, cortical activation is predominantly bilateral; ii) after training, the cortical activation is shifted from the contra- to the ipsilesional hemisphere, at least in those patients with return of motor function; and iii) training-induced plasticity is possible in chronic phases of stroke. As stated before, bilateral activation of primary and non-primary motor areas and recruitment of additional sites have been reported in the early stages after a stroke and persist to the chronic stages especially in those with more severe impairments (J. R. Bloedel et al., 1996). A trend toward more normalized activation patterns has been seen specifically in patients with moderate to mild impairments (Carey et al., 2002). However, these findings suggest that central nervous system retain the ability to reorganize toward a more physiological (more efficient) activation pattern even in the chronic stage of stroke. Furthermore, the main mechanism underlying recovery of motor abilities involves enhanced and predominant activity in preexisting networks within the affected-side.

Further investigations are needed not only to confirm these findings in a larger sample as well as to assess whether these neural changes are related to "true" recovery or compensation. This is indeed a real problem for fMRI studies investigating brain changes related to an intervention. As mentioned before, motor compensation could lead to changes in brain activation even though they have nothing to do with the "true" recovery. Thus, our aims are the following: i) to study the relationships between task-related motor activation and clinical and kinematic metrics of arm motor impairment in the chronic stage of ischemic subcortical stroke (see Chapter II), and ii) to longitudinally investigate the changes in cortical motor function at two levels, regional (micro-circuitry, regional activation) and network (macro-circuitry, functional connectivity), following

an arm-focused motor training in a subgroup of survivors studied in the Chapter II and how these brain changes relate to recovery of the pre-morbid movement pattern or "true" recovery (see Chapters III and IV). Finally, we study whether training-related brain and behavioral changes are similar in stroke survivors with those in age-sex matched controls. The results of these studies will provide further support for the ability of the central nervous system to reorganize at different levels, regional and network, even in the chronic stage of stroke and these changes may provide a substrate for "true" recovery. Such understanding would be a significant addition to the current literature and fulfills several gaps that have not been addressed for years.

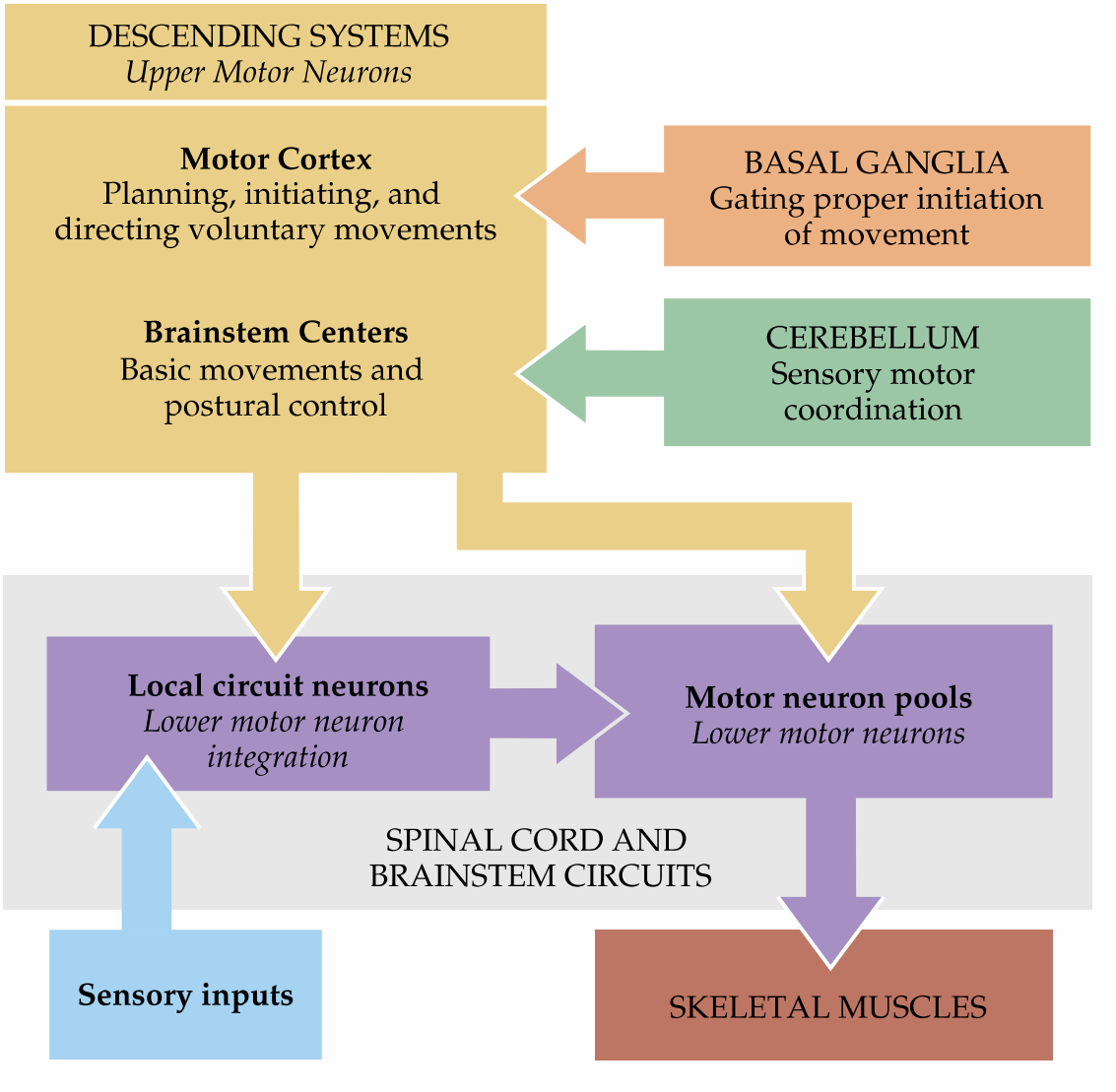


Fig. 1 Cortical and subcortical structures involved in control of movements. There are 4 systems: local spinal and brainstem circuits, descending modulatory pathways, cerebellum, basal ganglia, make major and distinct contributions to motor control. From Fig. 15.1, page 372 Nueroscience (third edition) Eds. Purves D et al., 2004

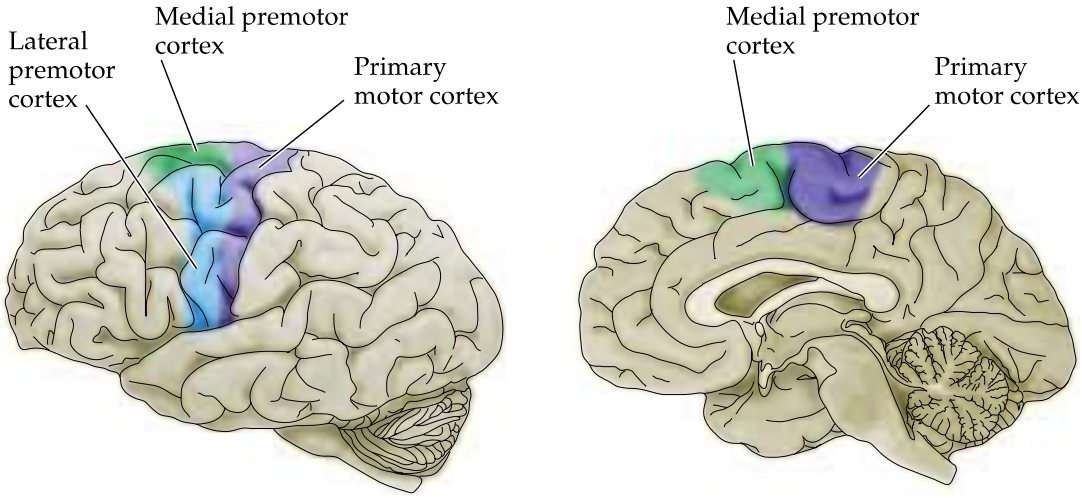


Fig. 2 Primary and non-primary (lateral or premotor; medial or supplementary motor) motor cortices seen in lateral (left panel) and medial (right panel) views. Primary motor cortex is located in the precentral gyrus. Non-primary motor areas are located more rostral. From Fig. 16.7, page 402 Nueroscience (third edition) Eds. Purves D et al., 2004

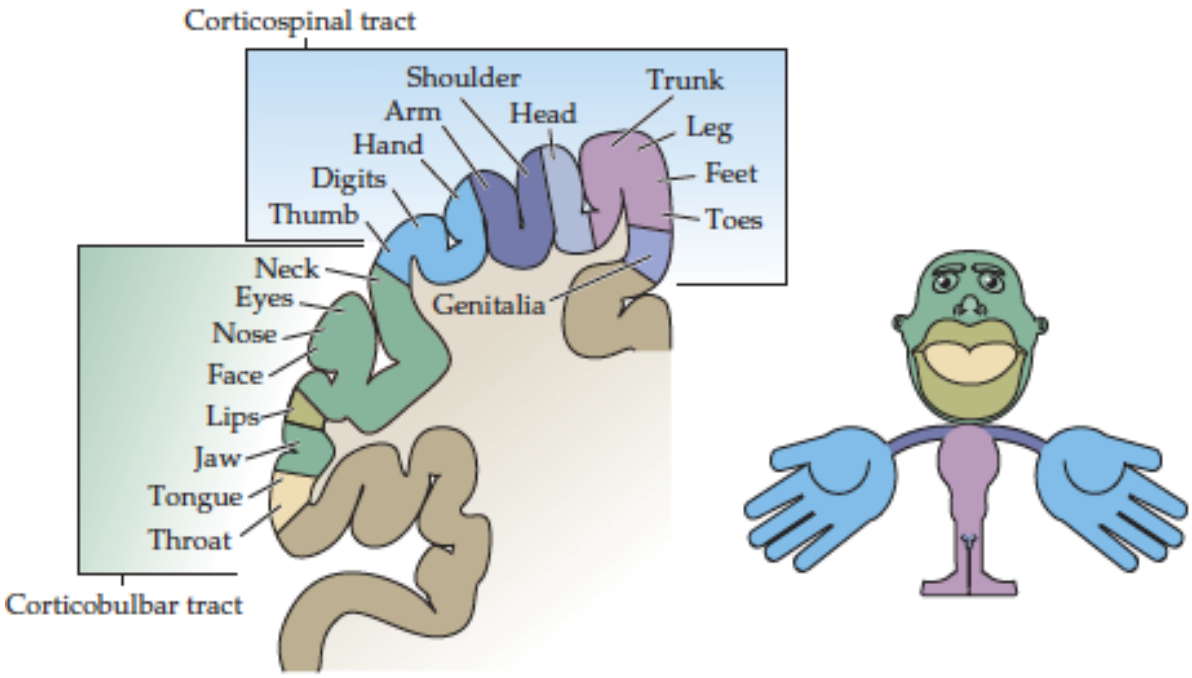
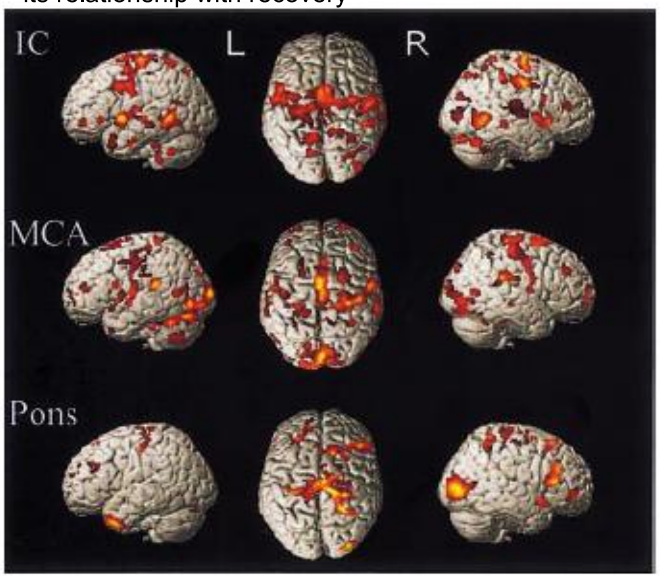


Fig. 3 Topographic representation of the body muscles in the primary motor cortex. Left: section along the precentral gyrus: the most lateral protions of the primary motor cortex control muscles in the face and arm while the most medial portions control muscles in the trunk and legs. Right: Disproportional representation of the body segemenst with larger representations for the hands and face (who exihibit fine motor control capabilities) compared to trunk and legs (who exhibit less precisse control). From Fig. 16.9, page 406 Nueroscience (third edition) Eds. Purves D et al., 2004

A. Cross-sectional differences in brain activation and its relationship with recovery



B. Longitudinal difference in brain activation its relationship with recovery

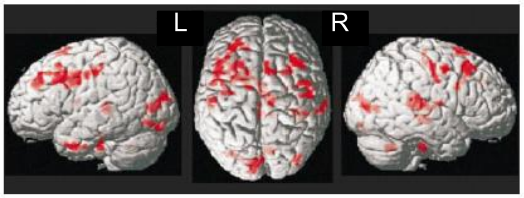


Fig. 4 A. Brain significant voxels ($p<0.05$) in which there was a negative correlation between recovery and task-related activation in patients with different lesion locations: internal capsule (IC), top line; middle cerebral artery (MCA), middle; pons, bottom. L, left, R, right. From Fig. 3, page 1441, Ward et al. Correlates of outcome after stroke: a cross-sectional fMRI study. Brain 126: 1430-1448, 2003a. B. Brain significant voxels ($p<0.05$) in which there was a decrease in task-related activation across longitudinally as a function of recovery. This represents effect group analysis for a group of patients with subcortical stroke. L, left, R, right. From Fig. 4, page 2484, Ward et al. Neural correlates of motor recovery after stroke: a longitudinal fMRI study. Brain 126: 1430-1448, 2003b.

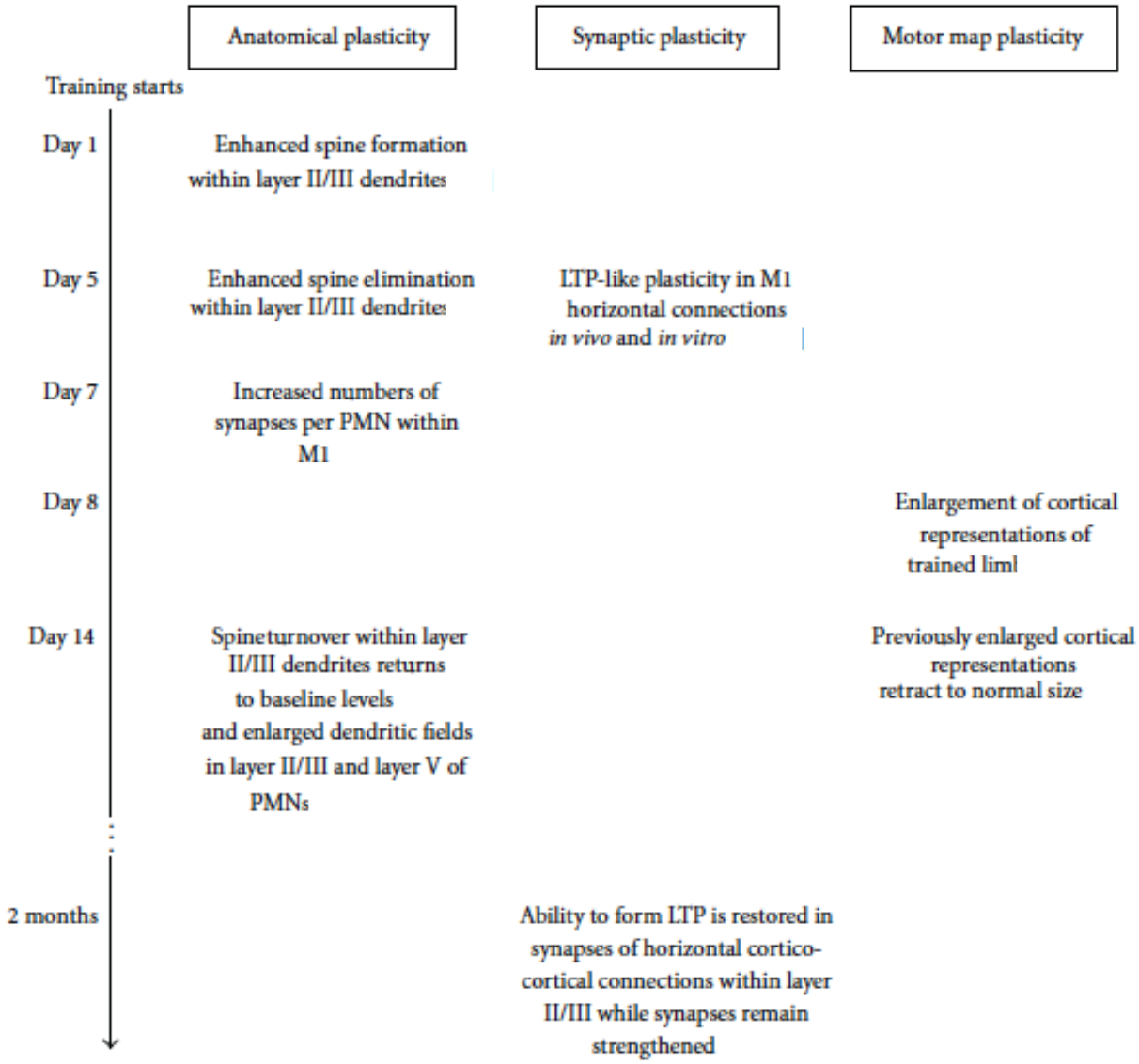


Fig. 5 Time course of the microscopic and macroscopic changes in primary motor cortex of rodents during learning of a reaching task. PMN, pyramidal motor neuron, LPT, long-term plasticity. From Fig. 1, page 4, Hosp and Luft. Cortical plasticity during motor elarning and recovery after ischemic stroke. Neural Plasticity volume 2011, article ID 871296, 9 pages, 2011.

CHAPTER II

KINEMATIC VERSUS CLINICAL METRICS OF ARM MOTOR IMPAIRMENT AND MOTOR-RELATED PRIMARY MOTOR CORTEX ACTIVATION IN CHRONIC STROKE

(in preparation for submission to Experimental Brain Research)

Abstract

Background: Reaching is one of the arm major functions and has poor recovery after stroke. Although kinematic metrics of reaching reflect "true" impairment after stroke, these metrics are largely ignored in functional MRI studies. In this study, we examined the relationship between motor–related activation of the primary motor cortex (or M1) and clinical and kinematic measures of arm motor impairment in chronic stage of stroke. We hypothesized that participants with stroke would show increased handgrip-related M1 activation, particularly for those with poor outcome, and decreased active range of elbow extension during a reach-to-grasp task compared to healthy controls. We also expected that M1 activation would be negatively related to clinical/kinematic metrics of arm motor impairment. Finally, we hypothesized that M1 activation would have a stronger correlation with kinematic than clinical metrics.

Methods: Nineteen survivors of an ischemic subcortical stroke (confirmed on T2-weighted images) at more than six months post-onset and twelve age-sex matched healthy controls participated. All participants underwent functional MRI assessment (TR=2000ms; TE=50ms; FOV=240mm; matrix=64x64; slice thickness=5mm; 0 skip; resolution=5x5mm^2; 100 time points) during the impaired hand (dominant hand in controls) grip. Kinematic metrics of elbow extension during a reach-to-gasp task (Vicon system) were also assessed in all participants. In stroke survivors, arm motor impairment was evaluated using Fugl-Meyer Upper Extremity (FMUE) scale. Percentage change of blood oxygen level-dependent response (or activation) was measured in M1 contralateral to the tested hand (ipsilesional in patients). We focused on M1 given the strong previous evidences for its involvement in motor recovery after stroke and which should not be confused with the location of the subcortical lesion. Relationships between M1 activation and clinical and kinematic measures were determined.

Results: Compared to controls, significant increase in activation of M1 contralateral to the tested hand ($p=0.006$) along with decrease in elbow extension ($p<0.001$) was observed in stroke survivors, particularly in those with poor outcome. In patients, M1 activation was negatively and significantly correlated with elbow extension ($p=0.02$) and tended to be negatively correlated with FMUE ($p=0.06$).

Conclusions: Our preliminary results demonstrated that kinematic metrics of reaching play a complementary role to the current clinical assessments and might increase our understanding of the neural mechanisms underlying altered reaching ability after stroke. Although kinematic analysis is time-consuming, we suggest that when an intervention appears to enhance recovery, the use of a combined approach, including fMRI and kinematic measures, will facilitate the distinction between recovery and compensation at both levels, neural and behavioral. This approach will be also extremely helpful to control for the effect of the possible variations in the task execution on the resulting cortical maps.

Introduction

Stroke remains the leading cause of long-term motor disability among adults in the United States (Lloyd-Jones et al. 2010). Given the higher prevalence of stroke in the elderly, the burden of stroke is likely to increase as our population ages. Moreover, people at younger ages are increasingly suffering from this debilitating disease. This is an issue of considerable impact, since it affects at least 6.4 million persons in the United States (Lloyd-Jones *et al.* , 2010).

Arm paresis, one of the most common impairments after stroke, is most evident in the arm contralateral to the injured (ipsilesional) hemisphere. After six months, about two-thirds of patients suffer from arm motor impairment that impacts the individual's activities of daily living (Kolominsky-Rabas *et al.* , 2001). This impairment consists in muscle weakness (Bourbonnais and Vanden Noven, 1989), abnormal muscle tone (Lance, 1980), abnormal movement synergies (Bobath, 1990), decreased arm joint mobility (Carr and Shepherd, 1987), and incorrect timing of components within a movement pattern (Archambault *et al.* , 1999, Levin, 1996, Cirstea *et al.* , 2003). Such impairments recover dramatically in the first month after stroke in patients with less severe paresis, and continue to recover up to three months in those with more severe paresis (Duncan *et al.* , 1994, Wade *et al.* , 1983). However, despite intensive and prolonged rehabilitation a subset of patients does not recover (Prabhakaran *et al.* , 2008) and their natural reaction is to compensate with alternate motor strategies. We define motor compensation as the use of alternative muscles groups to accomplish a task compared to healthy aged-matched controls. For example, during reaching, these patients engage excessive trunk displacement and rotation to compensate decreased active ranges of elbow/shoulder motion (Cirstea and Levin, 2000, Roby-Brami *et al.* , 2003). Although the incorporation of such compensatory behavior might result in better short-term functional ability, this behavior might lead to long-term medical

problems, i.e., pain, discomfort and joint contractures (Ada *et al.* , 2000). Moreover, this behavior limits the capacity to for subsequent gains in motor function of the impaired arm (Levin *et al.* , 2009, Taub *et al.* , 2006). This is also a real problem for functional brain imaging studies investigating recovery-related changes because such motor compensation might often lead to changes in brain activation even though they have nothing to do with the true recovery. Since the functional magnetic resonance imaging (fMRI) is one of the main tools to investigate brain reorganization after stroke, there is a need to carefully choose the outcome measures to better understand at what the activation changes are attributed.

Most functional MRI studies have used measures of motor impairment based on the assumption that impairment reflects most accurately true biological repair mechanisms. The primary clinical measure of impairment used in these studies is the Fugl-Meyer Upper Extremity (FMUE) scale. FMUE, composed of scales for sensation, proprioception, joint pain, range of motion (shoulder, elbow, wrist and fingers), reflex activity, and joint co-ordination and having an excellent intra-rater and inter-rater reliability (Woodbury *et al.* , 2008, Duncan *et al.* , 1992), is one of the most comprehensive quantitative measures of motor impairment after stroke (Gladstone *et al.* , 2002). However, the FMUE components neither assess purposeful reaching tasks nor quantify the functional impairments due to spasticity or weakness (Gladstone *et al.* , 2002). In addition, ceiling effect, particularly for the patients with mild impairment, and the presence of some components (such as reflexes) that do not make a significant contribution to the assessment of impairment (Woodbury *et al.* , 2007) have been identified as further limitations of FMUE. Recently, several research groups have proposed measures derived from kinematic data to quantify motor deficits after stroke (Cirstea and Levin, 2000, Dewald *et al.* , 1995, Cirstea *et al.* , 2003, Finley *et al.* , 2005). In contrast to FMUE, such measures provide high-

resolution measurements and identify movement features that cannot be captured by clinical scales (Subramanian *et al.* , 2010). For example, subtle movement deficits could be detected using kinematic analysis even in patients with mild impairment (Cirstea *et al.* , 2003, Hermsdorfer *et al.* , 1999, Jeannerod, 1984). Despite of all these advantages, these metrics are largely ignored in functional MRI studies.

In this study, we examined the relationship between task–related activation of the primary motor cortex (or M1) and clinical and kinematic measures of arm motor impairment in chronic stage of an ischemic subcortical stroke. We focused on M1 reorganization given the strong previous evidences for its involvement in motor recovery after stroke (Johansen-Berg *et al.* , 2002, Calautti *et al.* , 2007, Ward *et al.* , 2003) and which should not be confused with the location of the lesion at the subcortical level. We used as functional paradigm the handgrip task that has been shown to robustly activate M1 contralateral to the tested arm (Ward *et al.* , 2003, Cirstea *et al.* , 2011). For clinical assessment, we used the FMUE scale. We also assessed the active range of elbow extension during a reach-to-grasp task executed with the impaired arm to measure "true" impairment. Based on previous work, we hypothesized that stroke survivors would show i) increased handgrip-related M1 activation, particularly for those with poor outcome, and ii) decreased active range of elbow extension compared to age-sex matched healthy controls, and iii) an inverse relationship between M1 activation and clinical/kinematic metrics of arm motor impairment. We also hypothesized that M1 activation would have a stronger correlation with elbow extension than FMUE score. Such relationships would help us to better understand the functional relevance of plastic brain changes following stroke.

Materials and Methods

Study participants

A total of 19 patients with chronic stroke and 12 age-matched healthy controls participated.

Patients met the following inclusion criteria: 1) ischemic subcortical stroke at least six months previously, 2) primary motor areas intact based on T2-weightd MR imaging, 3) pre-stroke right-handed (Edinburgh handedness inventory, (Oldfield, 1971), 4) able to reach with the paretic arm (≥ 10 on FMUE total score), 5) able to understand consenting and study-related instructions (Token test), 6) no visual attention deficits (Cancellation test), 7) no apraxia (clinical observation of the use of scissors to cut paper and making coffee); For criteria *5-7*, neuropsychological evaluation was supervised by coauthor (Dr. Savage) and clinical impairment on all tests was defined as performance less than 2 SD below the mean. Other tasks, e.g., the use of scissors, making coffee, were based on clinical observation; 8) no other neurological disorders (medical chart review), and 9) no MRI contraindications. Most patients were on anti-hypertensive (n=18) and cholesterol-lowering (n=12) therapy, and some were on anti-diabetic (n=7) and/or antiplatelet therapy (n=8).

Right-hand dominant healthy controls, without any neurological and psychiatric disorder or MRI contraindications, and with normal T1- and T2-weighted images, were enrolled. All participants provided written informed consent in accordance with the Human Subjects Committee of the Kansas University Medical Center.

Study design

Kinematic (Vicon system, Landon Center on Aging) and magnetic resonance imaging (3T Allegra MR system, Siemens Medical Solutions, Erlangen, Germany, Hoglund Brain Imaging

Center) assessments were performed in each participant in two different sessions. Clinical assessment was performed in stroke patients.

Structural and functional MRI

An axial T1-weighted, a proton density (PD)/T2-weighted, and one run of gradient echo blood oxygen level dependent (BOLD) were administrated in each participant. Full details of the MRI protocol appear elsewhere (Cirstea *et al.* , 2011, Cirstea *et al.* , 2012)

The parameters were TR 2300ms, TE 3ms, FOV 240mm, matrix size 256 x 256, axial slice thickness of 1mm, and resolution of 1x1x1 mm^3 for T1-weighted series, and TR 4800ms, TE1/TE2 18/106ms, FOV 240mm, matrix 256 x 256, and slice thickness of 5mm with no gap for PD/T2-weighted series. These structural series were collected parallel to the anterior commissure - posterior commissure plane. The T2-weigted images were used to: (1) confirm the presence of a single ischemic subcortical lesion that did not involve the primary motor areas, and (2) exclude other pathological conditions.

For BOLD scan, the parameters were TR 2000ms, TE 50ms, FOV 240mm, matrix size 64 x 64, axial slice thickness of 5mm, 0 skip, in-plane resolution 5 x $5mm^2$, 100 time points, and 25 slices coincident with the PD/T2 series. One BOLD scan was performed for the impaired (dominant) hand and consisted of two alternating conditions: movement (20s) and rest (20s) conditions repeated five times (total time=3min 28s). A MRI-compatible custom designed device, that maintains constant pressure, was used throughout movement and rest conditions, i.e., active grip followed by passive opening of the hand. In the movement condition, the participant performed a single brief handgrip task, repeated at every 4s. To ensure similar performance across the participants, a target pressure (25% of handgrip maximal voluntary contraction, MVC) was

displayed graphically, and participant performed the handgrip until the target pressure was attainted, at which point the grip was released. All participants were able to attaint the target pressure. If we detected mirror movements by visual inspection, we stopped the acquisition and instructed the subject accordingly that this movement is prohibited. If on a repeat scan there was continued mirror-movement, the data collection was aborted. In the rest condition, participants were instructed to lie motionless (Fig. 1).

BOLD data was analyzed using Brain Voyager software (Brain Innovation B.V., Maastricht, Netherlands). Motion correction, estimating three translation and three rotation parameters, was performed using a rigid body transformation to match each functional volume to the reference volume (the third volume, since the first two are discarded to avoid T1 saturation effects). These parameters were inspected to estimate head movements. None of the participants moved their head more than 2 mm in any direction. We then used 3D spatial smoothing with a 4-mm Gaussian filter to validate statistical inference according to Gaussian random field theory. The time series in each voxel was then filtered at 0.01Hz to remove low frequency confounds. Movement and rest periods were modeled by a boxcar function with hemodynamic response modification (predictor movement). The general linear model was used to extract percentage signal change (PSC, cluster threshold=100 contiguous voxels; $p_{Bonferroni}$=0.01) and create a hand representation mask in left (ipsilesional) M1 (Fig. 1).

Kinematics: arm reach-to-grasp task

Since the ability to reach is critical for virtually all activities of daily living, we assessed the active range of elbow extension movement during a self-paced reach-to-grasp task (Fig. 2A). At the initial position, seated participants had the tested (paretic arm) arm rested on an ipsilaterally

located, height-adjustable table (elbow flexed at 90°) and the contralateral arm rested alongside the body. The seat height was normalized by placing it at a height equivalent to each participant's lower leg length (Chari and Kirby, 1986, Dean and Shepherd, 1997). The hip and knee joints were flexed at 90° and the feet made full contact with the floor. A 4 cm diameter cylinder was fixed on a height-adjustable platform located in front of the participant on the midline of the trunk, within a comfortable range for grasping (90% of passively extended arm's length (Mark *et al.* , 1997). The height of the cylinder was determined according to the participant's shoulder height.

Kinematic data were recorded using a motion analysis system (VICON, Oxford Metrics). Four reflective balls were positioned on arm (1-ulnar process; 2-lateral epicondyle; 3-acromion process of the ipsilateral shoulder; 4-acromion process of the contralateral shoulder). Participants were instructed to reach and grasp the cylinder in response to an auditory tone and hold the hand in the final position until a second tone signaled the end of trial. Prior to recording, participants practiced the movement five times. Then, 20 movements performed with full vision were recorded in a single experimental session. No verbal feedback about movement execution has been administrated before or during recording. Thus, the stroke survivors who could not successfully reach the target were able to use trunk movement to compensate. Each movement was recorded for 3-6 sec at a sampling rate of 100 Hz. The vectors joining the appropriate reflective balls were used to compute, using vector algebra, the elbow angles (flexion/extension).

Clinical outcome measure

All clinical assessments were performed by one trained physical therapist. Arm motor impairment was assessed using the FMUE scale, including 22 items in four sections: arm, wrist,

hand, and coordination for a maximum score of 66.

Statistical analysis

The analysis focused on one variable (PSC in M1 contralateral to the tested hand) and two outcome measures (FMUE, elbow extension). Descriptive statistics such as means and standard deviations were computed for each variable/outcome.

Between-group differences in demographic data, M1 variable, and elbow extension outcome were used parametric (t-test) statistics (SPSS16.0, Chicago, Il, USA).

Pearson correlation analysis was used to quantify the relationships between i) M1 PSC and outcome measures, and ii) between outcome measures. Since we run power analysis, we did not assess the differences in correlations coefficients because to reach a power of 0.80, we actually needed a sample size of 38 survivors. The significance level was set at $p<0.05$.

Results

Participants

Patients: Table 1 summarizes patient demographics and stroke location. A total of 19 patients (13 men) of mean age 55.8 ± 8.2yrs (range from 43 to 68yrs) and mean years of education 13.4 ± 2.3yrs (range 10-16) participated in the study. All patients had sustained a single infarction in middle cerebral (n=16), posterior cerebral (n=1), and basilar (n=2) artery territory 41.6 ± 38.4 mo previously (range from 6 mo to 144 mo). The site of cerebral infarction was determined from the T2-weigted images. The infarcts were located in the basal ganglia (n=14), with extension to posterior limb of the internal capsule (PLIC) in six patients, to anterior limb in two patients, to both posterior and anterior limbs of the internal capsule in two patients, and to corona radiata in

five patients. One patient had an infarct in the PLIC with extension to thalamus, one had an anterior limb infarction, one survivor had cerebral peduncles infarction, and two had an infarct in pons. No damage was detectable in M1. Thirteen stroke participants had left-sided infarcts.

Patients vs. Controls: There were no significant differences between patients and controls in age (stroke, 55.8 ± 8.2yrs vs. controls, 56.8 ± 5.4yrs, *NS*), male/female distribution (male 46% vs. 50%, *NS*), or years of education (13.4 ± 2.3 vs. 14.0 ± 2.5yrs, *NS*).

M1 activation during handgrip task

Controls: A robust contralateral BOLD response was seen in all controls while using the right (dominant) hand (left M1, 0.68 ± 0.16%, Fig. 1B for range of PSC).

Patients vs. controls: Images for patients with right-sided lesions (n=6) were flipped about the mid-sagittal line, so that all patients were assumed to have a lesion on the left side, with right arm weakness. Thus, we compared the ipsilesional hemisphere with the left hemisphere from controls.

As group, patients showed a significant increase in activation intensity in the ipsilesional M1 compared to controls while using the impaired arm (1.03 ± 0.48% vs. 0.68 ± 0.16% in controls, p=0.007). At the individual level, the patients with more severe impairment (9 out of 19 patients) activated more ipsilesional M1 compared to the range of our control group (Fig. 1B).

Kinematic measure of arm motor impairment - Elbow extension during reach-to-grasp task

Controls: Healthy controls executed the reach-to-grasp movement by initially raising their arm (shoulder flexion), pulling it across the body (shoulder horizontal adduction), and extending their elbow (57.9 ± 5.2 deg) to move the hand forward to grasp the cylinder (see Fig. 2A).

Patients vs. controls: In contrast with this homogenous pattern across healthy participants, patients used different patterns to reach the target. While most stroke participants were able to initially flex the shoulder, many (n=12) did not extend their elbow and moved the trunk substantially to bring the hand to the target. As group, patients used significantly less elbow extension to reach the target than controls (36. 6 ± 20.9 deg, $p < 0.001$, Fig. 2B).

Clinical measure of arm motor impairment

Patients were moderately affected with a mean total FMUE score of 37.9 ± 19.5 (range from 66 to 10).

Relationship kinematic-clinical measure of arm motor impairment

Significant positive correlation was found between the active range of elbow extension and FMUE ($r=0.97$, $p<0.001$).

Relationships M1 activation – clinical and kinematic measure of arm motor impairment

There was no significant correlation between ipsilesional M1 activation and total FMUE score ($r=-0.44$, $p=0.06$). Significant correlations were found between ipsilesional M1 activation and elbow extension ($r=-0.52$, $p=0.02$) (Fig. 3). However, since these correlations seem to be driven by two individuals (Participants 15 and 17 in the Table 1), we excluded them from our analysis. Interesting, we found a similar trend in the correlations between M1 activation and FMUE ($r=-0.32$, $p=0.21$) and elbow extension ($r=-0.43$, $p=0.08$) even in the subgroup of 17 patients.

Discussion

Summary of findings

To the best of our knowledge, this is the first study to investigate the relationships between motor-related M1 activation and clinical and kinematic metrics of arm motor impairment in chronic survivors of a subcortical stroke. We found significantly greater ipsilesional M1 activation in patients, particularly in those with poor outcome, compared to healthy controls performing the same handgrip task. In the same patients, we found significantly lower active range of elbow extension during a reach-to-grasp task than in controls. We also found negative correlation between M1 activation and clinical (FMUE) measure of arm motor impairment in our patients, although without reaching statistical significance. Finally, we found evidence that M1 activation was significantly correlated to kinematic metrics of arm motor impairment (elbow extension).

Handgrip-related activation in primary motor cortex after stroke

In agreement with previous studies in chronic stroke survivors (Johansen-Berg *et al.* , 2002, Ward *et al.* , 2003, Calautti and Baron, 2003), the activation pattern associated with impaired hand movement consistently included contralateral (or ipsilesional) M1. The mean activation was significantly different from that seen in uninjured individuals, particularly in patients with poorer motor outcome, i.e., impaired selective movements of the fingers (Fig. 1B). Despite intense research on this topic, the specific mechanism by which ipsilesional M1 activation is increased in some patients remains the topic of some conjecture. Since the nervous system retains the ability to exploit the redundancy within the somatotopy of M1 to generate an output via the intact portion of corticospinal tract (Sanes and Donoghue, 2000, Newton *et al.* , 2006), an

excessive recruitment of this area could be found in an attempt to perform the task despite cortico-spinal tract (CST) damage. Changes in somatotopic representation of the hand also occur in other areas, i.e., premotor areas (Johansen-Berg *et al.* , 2002, Newton *et al.* , 2006), and might alter the anatomical connections between premotor areas and M1, potentially enlarging the motor output zone. This enlargement could also result from a simple disinhibition that has no relationship to actual motor performance (Ghosh and Porter, 1988, Feydy *et al.* , 2002). In patients with poorer motor outcome, a wider sensori-motor recruitment, including deep central sulcus (or Brodmann area 4p) (Johansen-Berg and Matthews, 2002) may be also explained by the change in the patient' perception of the task. Precisely, these patients perceive a simple task as a complex task. Although the effort levels of the handgrip tasks were matched at 25% of their individual MVC, we did not control some aspects of cognitive performance, such as attention. Thus, the possibility that attention differences contributed to larger BOLD activations in ipsilesional M1 cannot be ruled out.

We selected the handgrip task based on the following reasons: i) since handgrip returns earlier than fractioned finger movements (Heller *et al.* , 1987), we studied patients with the degree of impairment ranged from severe to none, and thus they are representative of a wide range of performance after stroke, ii) this task compares well with other clinical measures of arm function (Heller *et al.* , 1987, Sunderland *et al.* , 1989), and iii) this task allows us to minimize movement artifact during scanner; none of our patients had moved their head more than 2 mm during the scanner. In addition, recent work on the somatotopic organization of M1 argues against divisions of modules controlling hand, elbow, or shoulder (Devanne *et al.* , 2002). Indeed, the cortical mechanisms controlling the hand are integrated with those of the elbow (and shoulder), as part of the system underlying reaching, prehension, and object manipulation

(Capaday, 2004). Moreover, the handgrip strength is closely associated with the strength of elbow flexors/extensors (Bohannon *et al.* , 1991) as well as with arm motor performance (Sunderland *et al.* , 1989, Boissy *et al.* , 1999, Mercier and Bourbonnais, 2004) Thus, we were able to study the correlations between brain activation during a handgrip task, elbow kinematics during a reach-to-grasp task, and clinical test of arm motor impairment.

FMUE and kinematic metrics of arm motor impairment

We chose the FMUE as one of our measures of arm motor impairment because this scale is one of the most widely used measures after stroke (Gladstone *et al.* , 2002). However, as stated above, this measure suffers from serious shortcoming: ceiling effort, no info about movement quality, observer ratings. The latter is a great threat of bias, particularly in trials in which a double-blind protocol is not possible (Krakauer, 2005).

We argue that using kinematic measures of motor deficit during a reach task would minimize these shortcomings. Although reach requires coordination of multiple joints, this task could be considered as an easy task because it does not need to be learned. We focus on the elbow movement based on significant correlation between elbow kinematic measures and FMUE scores (Levin, 1996, Cirstea and Levin, 2000). It is important to emphasize that we use this kinematic measure to identify "true" motor deficit. As expected, since our patients had moderate hemiparesis, we found significantly decreased active range of elbow extension compared to controls. This may reflect a deficit in motor control. Indeed, since reaching trajectory involving more than one joint consistently have invariant characteristics, such as straight paths and bell-shaped velocity profiles (Morasso, 1981), it is likely that reaching trajectory are planned in advance. Indeed, the left hemisphere is likely to play a special role in motor programming; one

focuses upon its dominance for movements which are independent of sensory feedback and the other emphasizes its specialization for processing rapid temporal information (Haaland and Harrington, 1989, Haaland and Harrington, 1994, Haaland *et al.* , 2004). Most of our patients had the lesion located in the left hemisphere, and this could explain decrease the active joint range. Finally, limitations in elbow extension may have been caused by agonist muscle weakness (Bourbonnais and Vanden Noven, 1989) and/or antagonist muscle spasticity (Bobath, 1990). However, all our patients have full passive range of elbow motion. It is thus unlikely that elbow limitation due to contracture could be responsible for the decreased elbow motion that we observed.

Correlations between handgrip-related primary motor activation and clinical and kinematic metrics of arm motor impairment

In agreement with our hypothesis, we found that M1 activation have a significant correlation with elbow extension than with FMUE. It is interesting to note that we found this even though both measures quantify impairment and more importantly, are significantly correlated one with each other. As previously shown (Calautti *et al.* , 2007), we found a non-significant correlation between ipsilesional M1 and clinical measure of motor impairment in our study. In contrast, a significant correlation has been observed between the activated clusters in ipsilesional M1 and "true" arm motor impairment. Although the present study is underpowered, our data suggest that kinematic measures not only objectively identify the motor deficits but may enhance the reliability of the fMRI data interpretation as well. Specifically, kinematic measures may shed light on the relationship between brain activation and motor strategies used during a goal-directed reach.

Limitations

There are some weaknesses to the study. First, our focus on subcortical infarcts provides statistical power by minimizing patient variance. In addition, studying subcortical stroke limits our ability to explore the effects of infarct location on the relationships brain function-kinematics. Future studies are needed to explore the relationships between motor-related motor activation and kinematics in participants with cortical or cortico-subcortical stroke. Our sample included thirteen left and six right hemispheric strokes. Due to small sample size, the potential differences between brain activation-kinematics of left-sided versus right-sided stroke were not addressed in this study. Finally, we focused our analysis on ipsilesional M1; accordingly, we cannot comment on the relationships between other brain regions that are critical to stroke recovery and movement kinematics.

Conclusions

Kinematic metrics of reaching play a complementary role to the current clinical assessments and might increase our understanding of the neural mechanisms underlying altered reaching ability after stroke. Although kinematic analysis is time-consuming, we suggest that when an intervention appears to enhance recovery, the use of a combined approach, including fMRI and kinematic measures, will facilitate the distinction between recovery and compensation at both levels, neural and behavioral. This approach will be also extremely helpful to control for the effect of the possible variations in the task execution on the resulting cortical maps.

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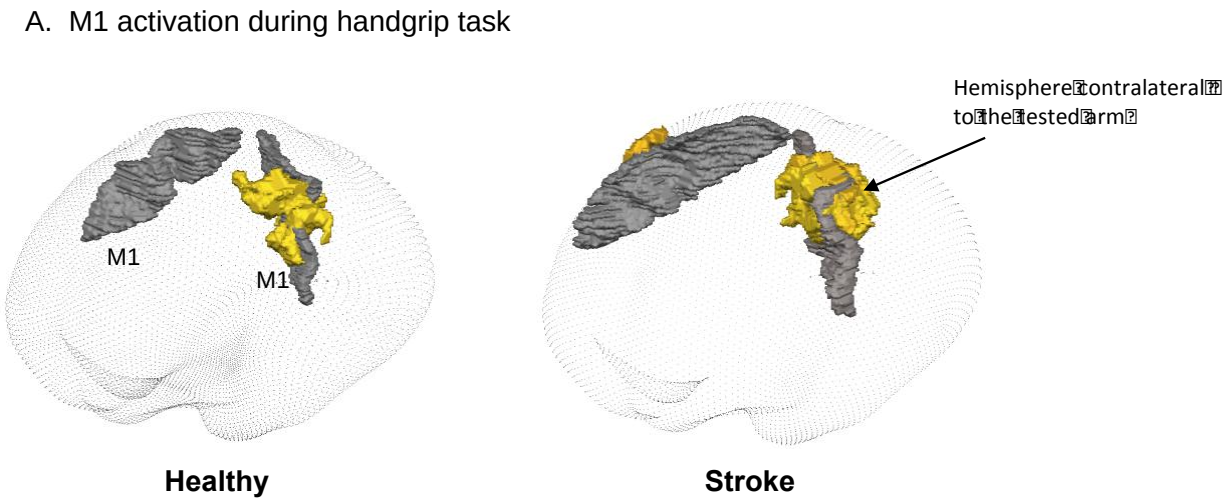
Table 1. Demographic and clinical data in participants with stroke.

Age	Sex	Time post-stroke	Sit of Stroke	FMUE
58	F	87	L/Basal ganglia, internal capsule	66
46	M	43	L/Basal ganglia, internal capsule	63
68	M	27	L/Basal ganglia, internal capsule	63
44	F	25	L/Basal ganglia, internal capsule	63
48	M	11	L/MCA, striato-capsular distribution	61
61	F	98	L/Corona radiata, basal ganglia	57
57	M	41	R/Basal ganglia, internal capsule	50
65	M	106	L/MCA, striato-capsular distribution	43
45	M	39	R/MCA, striato-capsular distribution	36
61	M	52	L/Basal ganglia, internal capsule	31
61	M	27	L/MCA, striato-capsular distribution	29
43	F	27	L/MCA, striato-capsular distribution	26
63	F	63	L/Basal ganglia, internal capsule	25

67	M	24	R/Basal ganglia, internal capsule	25
57	M	48	L/Pons	25
57	M	29	L/Basal ganglia, internal capsule	24
45	F	15	L/Pons	13
54	M	36	L/MCA, striato-capsular distribution	11
61	M	26	L/MCA, striato-capsular distribution	10
Mean (SD) 55.8 (8.2)	13M /6F	41.6 (38.4)		37.9 (19.5)



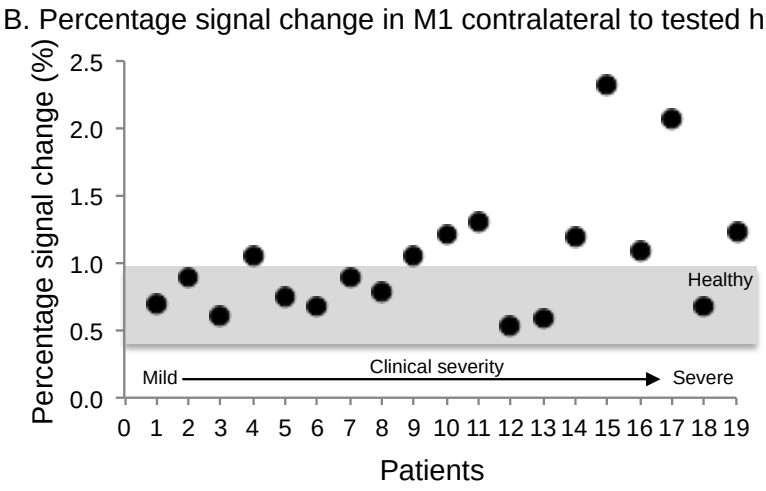


Fig. 1 A. Primary motor cortex (grey shadow) activation (yellow shadow) during a handgrip task in one healthy control (M, 61 years old) and one patient (M, 61 years old, infarct located in left basal ganglia and internal capsule, FMUE=31). **B** Percentage signal change in M1 contralateral to the tested arm in patients and control group (grey rectangle). Patients are ordered from no impairment (#1) (FMUE=66, first Patient in the Table 1) to severe impaired (FMUE=10, last Patient in the Table 1).

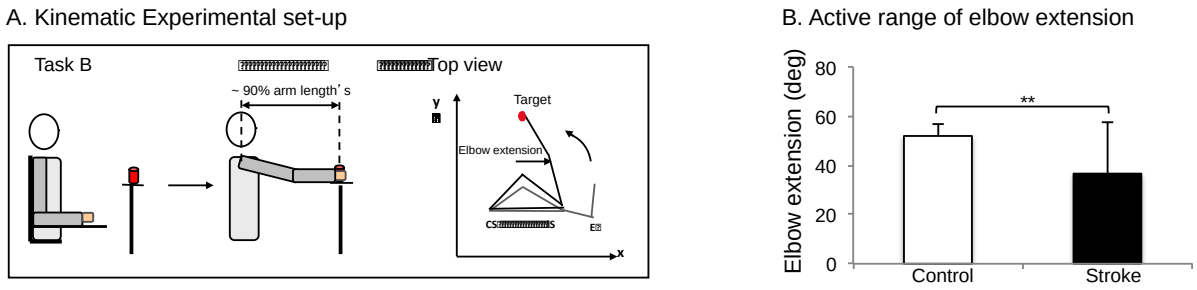


Fig. 2. A. Experimental design for kinematic measure of elbow extension during reach-to-grasp task. CS, contralateral shoulder, IS, ipsilateral shoulder, E, elbow. **B**. Mean (SD) of active range of elbow extension (deg) in control (white bar) and stroke (black bar) groups. **, $p<0.01$

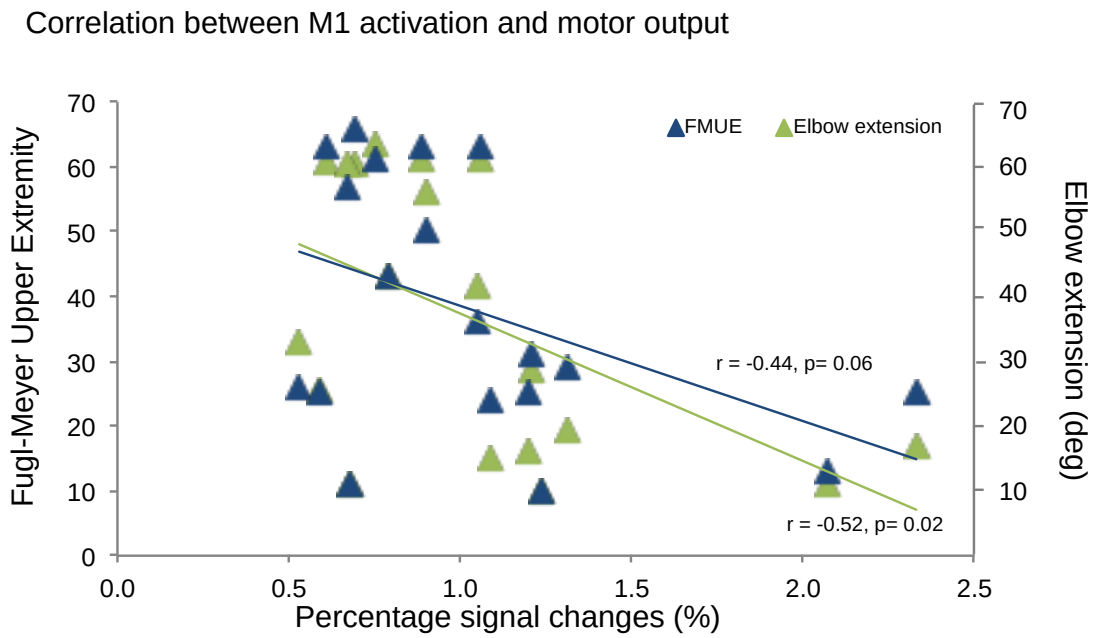


Fig. 3 Correlations between activation of the primary motor cortex (M1) contralateral to the tested arm and FMUE scores (blue triangles) and elbow extension (green triangles) in patients. Significant correlation was found between M1 activation and elbow extension (p=0.02).

CHAPTER III

MOTOR RELEARNING AFTER STROKE: MOTOR CORTICAL REORGANIZATION AND TRUE RECOVERY

(in preparation for Neurologic Physical Therapy –special issue: Motor learning after stroke)

Abstract

Background: Although strong experimental neurobehavioral evidences suggest that intensive motor training improves recovery after stroke, we still have only limited knowledge whether similar neural mechanisms underlie both relearning during rehabilitation and *de novo* motor learning. It is also unclear whether improvement results from adaptive reorganization (recovery of pre-morbid movement pattern) or behavioral compensation. In this study, we aimed to investigate the reorganization within motor networks (functional MRI) during an arm-focused motor training in patients with subcortical ischemic infarction and age-sex matched healthy controls. We also studied whether learning-related motor network changes are related to recovery of premorbid movement pattern (kinematics).

Methods: Eleven survivors of a first ischemic stroke located outside cortical motor areas (confirmed by T2-weighted MRI) and twelve age-sex matched healthy controls were recruited. Participants underwent functional MRI, kinematic, and clinical (in patients) testing prior to and immediately after a motor training period. Participants were scanned performing a dynamic isometric handgrip task with the dominant (impaired) hand (TR=2000ms; TE=50ms; FOV=240mm; matrix=64x64; slice thickness=5mm; 0 skip; resolution=5x5mm^2; 100 time points). The percentage signal changes of blood oxygen level-dependent (BOLD) signal were determined in hand representation area in our regions of interest (ROI): primary motor (M1), dorsal premotor (PMd), and supplementary motor (SMA) areas. Kinematic assessment of elbow extension during a reach-to-grasp task and clinical measures of arm motor impairment (Fugl-Meyer Upper Extremity, FMUE) were also administrated. Intervention consisted of a repetitive variable practice of a reach-to-grasp task with the impaired (dominant) arm during a four-week acquisition phase (12 training days, 90 repetitions per day). Independent sample t-test (2-tails)

was used to evaluate between-sessions and between-groups BOLD and motor outcome differences. Pearson correlation was used to analyze the relationships between BOLD and motor outcome.

Results: After training, in healthy controls, we found a significant increase in movement-related activation in the ROIs (an overall increase of 17%) contralateral to the trained arm along with a significant increase in elbow extension (by 6%). In patients, contralateral activation was also increased (an overall increase of 25%) but did not reach statistical significance. We also found a decrease in ipsilateral activation, reaching statistical significance only in ipsilateral M1. Patients significantly increased the elbow extension (by 19%) and improved clinical scores (by 11%). Correlations between ROIs activation and elbow extension were similar with those in controls after training. Significant negative correlation was found between ipsilateral M1 activation and FMUE after training.

Conclusions: Our preliminary results suggest that learning-related changes in activation in the motor areas contralateral to the trained arm in stroke were similar with those in healthy controls. Similar changes were also detected in behavior and its relationship with motor-related brain activation. Further studies investigating training-induced brain changes on the cellular (see our magnetic resonance spectroscopy studies) and network level (see the next Chapter) as well as on the time course of these changes may help us to better clarify whether principles of motor relearning in an injured brain are similar with those of motor learning in an uninjured brain for a better transfer to optimize neurorehabilitation.

Introduction

Although strong experimental neurobehavioral evidences suggest that intensive training improves motor recovery after stroke, we still have only limited knowledge why some patients recover more completely and others do not. It is also unclear whether improvement results from adaptive reorganization (recovery of pre-morbid movement pattern) or behavioral compensation.

The principles from the motor learning or motor memory literature have been only recently applied to stroke rehabilitation. Only 12 randomized controlled trials comparing specialized rehabilitation with conventional care have been reported (Haaland and Harrington, 1989). Improved outcome was generally present among patients receiving specialized rehabilitation in the majority of studies. Intensive and structured training is one key element of specialized rehabilitation and the improved outcomes depend on two elements: training intensity and task-specificity (Haaland and Harrington, 1994). Examples of how concepts from research in motor control have been applied to generate new therapeutic approaches with regard to rehabilitation are the constraint-induced movement therapy (Bobath, 1990a; Bourbonnais and Vanden Noven, 1989; Haaland et al., 2004), motor relearning program (Calautti et al., 2007), electromyogram-triggered neuromuscular stimulation (Foley et al., 2003a), and robot-assisted rehabilitation (Foley et al., 2003b). Overall, despite that there is increasing evidence that functional recovery can occur with these approaches (Butefisch et al., 1995; Carr and Shepherd, 1987b; Cauraugh et al., 2000; Cirstea et al., 2006; Cirstea et al., 2003c; Dean and Shepherd, 1997; Dromerick et al., 2000; Kunkel et al., 1999; Taub et al., 1993; van der Lee et al., 1999; Volpe et al., 2000), it still remains unclear whether similar plastic mechanisms underlie both relearning during rehabilitation and *de novo* motor learning. Indeed, no studies have compared functional imaging changes that occur with learning with those that occur with motor recovery. Further, what of type

of recovery is actually occurring during relearning is still unclear (Hanlon, 1996). In addition, the criteria for assessing and defining motor recovery have been ambiguous. Consequently, there is a misrepresentation in the literature that improvements in endpoint measures of performance represent true recovery. It can be argued that two distinct mechanisms account for post-injury improvement. First, true recovery leads to a return of pre-lesion performance and subsequent restoration of the original function (Nudo, 1999; Whishaw and Kolb, 1988). Second, compensation is based on the development of a new behavioral strategy that differs from the original performance. Nevertheless, the adaptive changes of compensation lead to improved end point measures (Whishaw, 2000; Whishaw et al., 1991). The differentiation between recovery and compensation requires a through behavioral analysis that can be accomplished by the analysis of skilled movement performance (Metz and Whishaw, 2002; Whishaw et al., 1993). The distinct feature of skilled movement tasks is that they have been developed to assist in simultaneous assessment of quantitative end point measures and qualitative analysis. The former reflects the ability to perform a particular motor act, while the latter characterizes movement patterns to differentiate original from alternative movement strategies (Metz and Whishaw, 2000). It is likely that recovery and compensation are mediated by different kinds of structural rearrangements. Moreover, compensation might often lead to changes in brain activation even though they have nothing to do with the recovery. Thus, distinction between these two mechanisms based on behavioral means is a valuable tool for inductive interpretation of post-rehabilitation neural changes.

Motor skill acquisition is paralleled by neural changes on two levels, regional (microcircuitry) and network (macrocircuitry). In the present study, we focus on the regional changes within the motor system. In animal models, motor skill acquisition appears to be a

prerequisite for driving plasticity in the motor cortex (Platz et al., 1994). In humans, a limited number of studies examined the relationship between motor improvements and altered brain activation pattern (Krakauer, 2006; Kwakkel et al., 1997; Kwakkel et al., 1999; Kwakkel et al., 2002; Whitall et al., 2000) after motor rehabilitation. Despite methodological differences, three important and consistent findings emerged: (i) before training, cortical activation is predominantly bilateral including radiologically normal-appearing (or spared) motor and premotor areas in both injured (ipsilesional) and un-injured (contralesional) hemispheres; (ii) after training, the cortical activation is shifted from the contralesional to the ipsilesional hemisphere, at least in those patients with return of motor function Some studies have shown however that a shift from bilateral to predominantly ipsilesional activation may not be universal (Carey et al., 2002; Nudo, 1997); and (iii) training-induced plasticity is possible in chronic phases of stroke. However, no studies in stroke examined the relationships between training-related neural changes and movement kinematics. Such relationship would help to interpret whether brain changes result from adaptive reorganization or behavioral compensation.

In this study, we aimed to investigate the reorganization within motor networks (functional MRI, fMRI; motor-related brain activation) following an arm-focused motor training based on motor learning theories in patients with subcortical ischemic infarction and age-sex matched healthy controls. Since this study builds upon previous findings on rehabilitation-related motor recovery, we expected a “normalization” or “focus” of the motor-related activation within motor networks, i.e., increased ipsilesional activation along with decreased contralesional activation. This would reflect the potential of the primary/non-primary motor areas to undergo learning-related reorganization although these areas already underwent a lesion-induced reorganization. Moreover, this would help us to identify whether the recovery-related changes in these networks

are the same or different from those in learning-related networks. Finally, to test further whether learning-related motor network changes are related to recovery and not to compensation, we assessed movement kinematics longitudinally. Since we consider that kinematic measure of the active range of elbow extension is a marker of "true" recovery (Johansen-Berg et al., 2002a; Nelles et al., 2001), we also expected that the correlations between motor activation and elbow extension after training would "normalize". This would allow interpreting the neural changes as adaptive mechanisms.

Materials and Methods

Participants

Eleven stroke survivors and twelve healthy controls (all right-handed and without MRI contraindications) signed informed consent in accordance with the University of Kansas Medical Center Human Subjects Committee (Institutional Review Board) prior to their recruitment in the study.

Patients were included if they had i) a single ischemic subcortical stroke more than six months prior to study recruitment, ii) radiologically-normal appearing motor and premotor areas based on T2-weighted magnetic resonance imaging (MRI), iii) ability to understand consenting (Token test), iv) no visual attention deficits (Cancellation test), apraxia (clinical observation of the use of scissors to cut paper and making coffee), or other chronic or degenerative neurological disease (medical chart review). Patients were on anti-hypertensive (95%), cholesterol-lowering (45%) and/or antiplatelet (45%) therapy, but were not receiving inpatient or outpatient treatment.

Age-, sex-, and education-matched healthy controls, with normal T2-weighted images and free of any medication, with no known vascular risk factors and no history of neurological or

psychiatric disease, head trauma, or alcohol or substance abuse, were recruited.

Study protocol

Participants underwent neuroimaging, kinematic, and clinical (in patients) testing on two separate occasions: prior to (PRE), and immediately after (POST) a motor training period. MRI studies were conducted on a 3 Tesla MR system (Siemens Medical Solutions, Erlangen, Germany) at the Hoglund Brain Imaging Center. Our experimental protocol has been detailed previously (Jang et al., 2003). Kinematic studies and motor training sessions were conducted at the Human Performance Lab, Landon Center on Aging.

Motor learning paradigm – variable practice of a reach-to-grasp task (Task A)

Participants participated in 12 daily practice sessions (one hour per session) for 4 weeks consecutively. The number of repetitions per sessions (n=90) was considered "intensive" practice according to our previous results (Cirstea et al. 2003; Cirstea et al. 2006). The intervention, supervised by an experienced physical therapist, consisted of repetition of a reach-to-grasp task (Task A) with the dominant (impaired) arm.

Specifically, participants were seated in front of an adjustable height table so that the initial position is located approximately half of distance between the low-sternum and the umbilicus. As stated before, the seat height and extent of thigh and foot support can affect reaching distance (Kopp et al., 1999; Levy et al., 2001), thus, the experimental seat height is normalized by placing it at a height equivalent to each patient's lower leg length. The hip and knee joints are flexed at 90° and the feet make full contact with the floor. At the initial position, the dominant (impaired) arm rested on the table at approximately 10cm in front of the body (Fig. 1). The contralateral arm

rested alongside the body. A cylindrical object was placed within a comfortable range for grasping on the platform located in front of the participant. The platform height corresponded to the shoulder height determined for each participant, and the platform distance was determined according to the length of the arm with the elbow in full extension (180°) and the hand comfortably grasps the object (about 90% arm's length (Schaechter et al., 2002)). The size (4, 6, 8 cm diameter, 10cm height) and weight (50, 100, 150 grams) of nine cylindrical objects were presented in a randomized order (90 trials). In response to an auditory signal, the participant was instructed to reach and grasp the object with the whole hand, and to move it back to the initial position at a comfortable self-selected speed. During training, subjects received feedback about elbow extension angle (knowledge of performance, KP) by using an electrogoniometer (Exos, Inc., Woburn, MA, USA) located on their lateral epicondyle. Video images of elbow movement were presented on a computer screen after the end of the task (terminal KP). To minimize dependency on feedback, the KOP was administrated with a decreasing frequency (faded KP) throughout the practice session (for the first 30 trials, KP will be given every trial, for the second 30 trials, every 2nd trial and for the last 30 trials, every 5 trials). This task was selected because it is not entirely novel, and it should be re-acquired during recovery from stroke. In addition, this task involves the coordination of arm and hand movements. So our aim was not to train patients in a novel task but to identify whether intensive training of an altered movement component (i.e., elbow extension) improved movement quality and efficiency and whether this improvement is underlined by functional changes within the motor system. It has to be noted that in this study, we focused only on the reaching component of the task.

Assessments (PRE-, POST-training)

Kinematic recording of elbow extension during a reach-to-grasp task (Task B): We assessed the active range of elbow extension movement during a self-paced reach-to-grasp task (Task B, Fig. 2A). At the initial position, seated participants had the tested (paretic arm) arm rested on an ipsilaterally located, height-adjustable table (elbow flexed at 90°) and the contralateral arm rested alongside the body. The seat height was normalized by placing it at a height equivalent to each participant's lower leg length (Cirstea and Levin, 2000a; Levin et al., 2009). The hip and knee joints were flexed at 90° and the feet made full contact with the floor. A 4 cm diameter cylinder was fixed on a height-adjustable platform located in front of the participant on the midline of the trunk, within a comfortable range for grasping (90% of extended arm's length (Cirstea et al., 2011b). The height of the cylinder was determined according to the participant's shoulder height.

A motion analysis system (VICON, Oxford Metrics) was used to record kinematic data. Four reflective balls were positioned on arm (1, ulnar process; 2, lateral epicondyle; 3, acromion process of the ipsilateral shoulder; 4, acromion process of the contralateral shoulder). We instructed participants to reach and grasp the cylinder in response to an auditory tone and hold the hand in the final position until a second tone signaled the end of trial. Prior to recording, participants practiced the movement five times. Then, during a single experimental session, 20 movements were performed with full vision. We did not provide any feedback, verbal or visual, about movement execution during recording.

Functional MRI acquisition during a handgrip task (Task C): In short, an axial proton density/T2-weighted MRI (TR = 4800ms; TE1/TE2 = 18/106ms; FOV = 240mm; matrix =

256x256; slice thickness = 5mm, no gap) and a whole-brain 3D T1-weighted MRI (TR = 2300ms; TE = 3ms; FOV = 240mm; matrix = 256x256; resolution = 1x1x1mm^3) were acquired to confirm the location of a lesion that did not involve the motor and premotor cortices and to exclude undiagnosed pathologies.

A gradient echo blood oxygen level-dependent (BOLD) scan (TR = 2000ms; TE = 50ms; FOV = 240mm; matrix = 64x64; slice thickness = 5mm; 0 skip; resolution = 5x5mm^2; 100 time points) was acquired for the impaired hand (dominant in controls) to identify the hand representation within our regions of interest (ROI), M1, PMd and SMA, in each hemisphere. For BOLD scans, two alternating conditions were repeated (3min 28s): movement condition (20s), where participants performed a handgrip (Task C) until a target pressure (25% of handgrip maximal voluntary contraction) was attained; and rest condition (20s), where participants were resting motionless.

Clinical assessment of arm motor impairment: All clinical assessments were performed by one trained physical therapist. Arm motor impairment was assessed using the FMUE scale, including 22 items in four sections: arm, wrist, hand, and coordination for a maximum score of 66.

Data analysis (PRE-, POST-training)

Elbow extension quatification: Each movement was recorded for 3-6 sec at a sampling rate of 100 Hz. The vectors joining the appropriate reflective balls were used to compute, using vector algebra, the elbow angles (flexion/extension) (Fig. 2B).

Functional MRI processing: Analysis methods for BOLD data have been detailed previously (Chari and Kirby, 1986). Briefly, BOLD data were analyzed using Brain Voyager software (Brain Innovation B.V., Maastricht, Netherlands). Thus, motion correction was performed by a

rigid body transformation, estimating six parameters, three translational and three rotational. These parameters were inspected for head movement. None of the participants moved their head more than 2mm in any direction. Then, 3D spatial smoothing with a 4mm Gaussian filter was used to permit valid statistical inference according to the Gaussian random field theory. The time series in each voxel was high pass filtered at 0.01Hz to remove low frequency confounds. Movement and rest periods were modeled by a boxcar function with hemodynamic response modification (predictor movement) and the general linear model was used to extract foci of activation and create a hand representation mask in our ROIs (cluster threshold=100 voxels and $p_{Bonferroni}$=0.01).

Regions of interest: Specifically, the voxels in M1 were selected in the omega shape or hand knob on the anterior bank of the central sulcus, PMd voxels on the anterior half of precentral gyrus and the anterior bank of the precentral sulcus, while SMA voxels on the medial wall of the hemisphere from the top of the brain to the depth of the cingulate sulcus, between a posterior boundary, halfway between the extension of the central and precentral sulci onto the medial surface, and an anterior boundary, a vertical line through the anterior commisure (Dean and Shepherd, 1997). The ROIs are illustrated in Fig. 3.

Statistical analysis

Means and standard deviations were computed for each variable (PSC in each ROI) and outcome (EEX, FM only for stroke group) in each session (PRE, POST) for each group. In addition, for each variable and outcome, between-session differences in mean concentrations were expressed as percent change of the control group (percent change (%) = (mean $measure_{control}$ – mean $measure_{stroke}$) x 100/mean $measure_{control}$, see Fig. 4)

Within session, between-group differences in variable/outcome were assessed using independent sample t-test (2-tails).

Within group, independent sample t-test (2-tails) was used to evaluate between-session differences in variable/outcome. Pearson correlation was used to analyze the relationships between PSC and EEX or FMUE. The significance level was set at $p<0.05$ (SAS version 9.3).

Results

Healthy Controls

Participants' characteristics

Healthy controls were aged between 46 and 62 years (58.8 ± 5.4 years), comprised 7 male and 5 female participants, and had 12.9 ± 2.8 years of education years.

Elbow extension during Task B

PRE: Healthy controls reached to the target by first flexing the elbow slightly (~15º) and then extending the elbow (57.9 ± 5.2deg).

POST: With training, controls significantly increased the elbow extension from 57.9 ± 5.2deg to 61.3 ± 5.7deg ($p = 0.005$). Specifically, the active range of elbow extension was increased by 5.6% from PRE to POST.

Brain activation during Task C

We were interested primarily in the activation of the primary and non-primary motor areas for the handgrip task described above.

PRE: The neural correlates of this handgrip task in controls included the M1 contralateral to the

tested hand (0.68 ± 0.16 %) and bilateral non-primary motor areas (contralateral: PMd, 0.69 ± 0.22, SMA, 0.52 ± 0.15%; ipsilateral, PMd, 0.46 ± 0.30, SMA, 0.52 ±0.08%) (Fig. 3B).

POST: In the group analysis, significant increase in task-related activation was found in contralateral areas, i.e., by 15.5% in M1 ($p = 0.001$), 7.5% in PMd ($p = 0.005$), and 18.6% in SMA ($p < 0.001$; Figs. 3b and 4). Training has not significantly changed ipsilateral activation.

Correlations between brain activation and elbow extension

PRE: There were no significant correlations between task-related activation and early performance levels (left, M1, r=-0.17, p=0.60, PMd, r= -0.53, p=0.08, SMA, r = 0.52, p=0.08; right, M1, r=-0.52, p=0.08, PMd, r=-0.24, p=0.46, SMA, r=0-0.06, p=0.86).

POST: In the post-training session, no significant changes were found in the correlations between brain activation and elbow extension.

Stroke Patients

Participants' characteristics

Patients' characteristics are listed in Table 1. Stroke survivors (between 43 and 71 years, 56.6 ± 8.7, years, 13 male/9 female) having a single subcortical infarction were recruited at 5 to 144 months (44.2 ± 36.7 months) post-injury. We used T1-weighted MRI to determine the site of cerebral infarction (Mark et al., 1997) and we found 19 patients had experienced infarcts involving the striato-capsular area. Of these, six were found to have anterior limb of internal capsule involvement, nine posterior limb of internal capsule, and three striato-capsular infarcts with extension to the corona radiata. In addition, one patient had suffered from pontine infarctions (pons). Thirteen patients had experienced right hemiparesis and six left hemiparesis.

The degree of arm motor impairment at the time of participation was variable, ranging from 10 (severe impairment) to 65 (mild impairment) on Fugl-Meyer Upper Extremity test (35.9 ± 17.9). *PRE:* Stroke and control groups did not differ statistically with respect to age (*p>0.05*), sex (65% vs. 63% male), or education (13.6 ± 2.0 vs. 12.9 ± 2.8, p>0.05*)*

Elbow extension during Task C

PRE: Patients used less elbow extension (33.7 ± 19.9deg, $p < .001$, Fig. 2B) than controls to reach the target (by using compensatory trunk movement).

POST: With training, patients significantly increased the elbow extension (from 33.7 ± 19.9deg to 40.3 ± 17.2deg, $p < 0.001$; Fig. 2B). Specifically, the active range of elbow extension has been increased from PRE to POST by 5.6% in control group and by 16.2% in patients. At the individual level, all patients showed an increase in elbow extension ranging from 0.6 to 13.4 deg (Fig. 2C).

Brain activation during Task B

Brain regions are described as either ipsilesional (i.e. contralateral to the moving hand) or contralesional. All patients were able to execute the handgrip task at 25% of their MVC. When performing the motor paradigm outside the scanner, there was no evidence of mirror movements by visual inspection.

PRE: Compared to controls, patients generally showed increased activation in these areas in both hemispheres (ipsilesional: M1, 1.03 ±0.51, p=0.048, PMd, 1.07 ± 0.45, p = 0.01, SMA, 0.75 ± 0.20, p=0.007; contralesional, M1, 1.11 ± 0.67, p<0.001, PMd, 1.36 ± 0.63, p = 0.001, SMA, 0.96 ± 0.49, p=0.01) when performing the same task (Fig. 3B).

POST: As in controls, the patients showed increased activation in the ipsilesional areas, although without reaching statistical significance (Fig. 3b and 4). In contrast, a decrease in activation was generally found in the contralesional areas, i.e., by 50% in M1 ($p = 0.02$), 18.1% in PMd ($p = 0.4$), and 17.2% in SMA ($p = 0.4$).

Correlations between task-related motor activation and elbow extension

PRE: In our patients, the correlations between motor activation and elbow extension reached statistical significance for contralesional M1 ($r=-0.81$, $p=0.003$) and both ipsilesional ($r=-0.68$, $p=0.02$) and contralesional ($r=-0.77$, $p=0.005$) PMd.

POST: The PRE significant correlations became non-significant in POST (contralesional M1, $r=-0.59$, $p=0.06$; ipsilesional PMd, $r=-0.26$, $p=0.44$; contralesional PMd, $r=0.01$, $p=0.97$ respectively).

Correlations between task-related motor activation and clinical motor impairment

POST: All patients decreased their arm motor impairment with training, as evidenced by an increase in FMUE scores (from 35.0 ± 17.9 to 38.9 ± 17.0, $p = 0.001$, $\Delta_{POST-PRE} = 4.1 \pm 2.6$; Fig. 5A). Significant negative correlation was found between contralesional activation of M1 and FMUE score after training ($r=-0.65$, $p=0.03$, Fig. 5B).

Discussions

Although it is well known that motor-related activation in primary and non-primary motor areas are heavily altered after a hemispheric stroke (Chari and Kirby, 1986; Cirstea et al., 2011b; Constable et al., 1998; Dean and Shepherd, 1997; Mark et al., 1997) and this could “normalize”

during rehabilitation (see review (Mai et al., 2008), none of these studies have addressed the question whether the recovery-related changes within motor networks are the same or different from learning-related changes within these networks and how these changes are related to true recovery or compensation.

By using a combined approach, functional imaging and movement kinematics, we have demonstrated for the first time that: (i) similar training-related changes within contralateral motor network to the trained hand in patients and controls, (ii) "normalization" of contralesional activation; and (iii) "normalization" of the correlations between bilateral motor activation and movement kinematics in patients.

Training-related brain changes in healthy controls

We found increased movement-related activation in primary and non-primary motor areas in the hemisphere contralateral to the tested (trained) arm after training. These results are consistent and complement other work derived from learning studies in healthy controls and animal models (Calautti and Baron, 2003; Cramer, 2008b; Foley et al., 2003a; Gerloff et al., 2006; Grafton et al., 2002; Halsband and Lange, 2006a; Hazeltine et al., 1997a; Loubinoux et al., 2003; Ward et al., 2003a). As expected, the training-related increased activation was found only in the cortical areas controlling the trained arm/hand (Seitz and Freund, 1997). Indeed, ipsilsilateral primary and non-primary motor contributions showed slight increases of activation in activation. This result is also consistent with previous studies indicating that dominant cortices influences learning of both the dominant and non-dominant hand (Van Mier et al., 1999).

Specifically, in M1, assemblies of neurons control specific movements of different joints and muscle groups (Karni et al., 1995; Shadmehr and Holcomb, 1997). Thus, an assembly projects to

several pools of spinal motoneurons (Doyon and Benali, 2005b; Floyer-Lea and Matthews, 2005). To control a multi-joint movement, reach-to-grasp movement in our case, different assemblies are interconnected via horizontal intra-cortical projections (Hund-Georgiadis and von Cramon, 1999; Xerri, 2011). Movements and arm parts are represented multiple times and are intermixed with representations of related movements of parts forming a complex mosaic pattern. This pattern, called motor map, reflects the output of M1. During motor training, these maps are reorganized, i.e., enlarged (Cheney et al., 2004; Keller, 1993; Nudo, 2008), increased signal strength (Cheney et al., 2004), increased spiking reliability (Keller, 1993). Such reorganization depends on restructuring of M1 microcircuitry. Structural modifications, as spine formation and synaptogenesis (Cheney et al., 2004), and modulation of synaptic weights, i.e. long term potentiation (Kleim et al., 1998), form the basis of such changes in motor maps. For example, changes in local GABA concentrations induce unmasking of existing horizontal connections within the cortex, which allows rapid changes in motor representations (Nudo et al., 1996a; Pascual-Leone et al., 1995). All these training-related changes support the notion of M1 as a locus of the long-tem acquired representation of specific motor skills, i.e., encoding novel (learned) mappings between limb motion and required muscle forces (Kargo and Nitz, 2004; Schieber, 2002).

However, changes in movement-related activation described here are fundamentally different than changes in evoked movements in response to cortical stimulation used to described learning-related map changes. In our case, increase in amplitude of movement-related activation indirectly reflects an increase in neuronal populations that control a movement. With training, the constituents of these populations could also be changed and morphological changes and/or alterations of connectivity are the neural substrates subserving the plastic changes in M1. Thus,

some newly generated synapses, that have functionally relevance for the learned movement, are formed, and synaptic transmission within horizontal connections are strengthened. Thus, the information from one region of M1 would be spread more effectively to other regions (Kleim et al., 2004b). These changes translate in a better connectivity among neurons across M1 resulting in activation in concert of the spinal motoneuron pools to enabling the performance of the trained movement. Some studies have suggested that, as learning proceeded, blood flow in M1 increased (Huntley, 1997; Rioult-Pedotti et al., 2000). However, no significant changes were found in M1 activation when the rates of movements in the trained and untrained conditions were controlled (Floyer-Lea et al., 2006; Monfils et al., 2005).

It is likely that these changes are not specific only to M1. Synaptic plasticity in the horizontal oriented axon collaterals may operate thought many areas within the motor network to restructurate representation patterns. Thus, the neuronal plasticity in the primary neurons directly involved in projection pathways and horizontal connections is important contributor learning-related processes in the non-primary motor areas, such as PMd and SMA. A consistent increase in activation in SMA was found to occur with practice (Hatakenaka et al., 2007; Kawashima et al., 1994; Rioult-Pedotti et al., 1998; Schlaug et al., 1994). Important to note is that such increase was reported particularly in the left hemisphere (Friston et al., 1992; Jenkins et al., 1994), which is in agreement with our results. Although some studies have suggested that, as learning preceded, activation increase in both left and right PM (Grafton et al., 2002; Hazeltine et al., 1997b), we found increased activation only in the left PMd. Since bilateral PM activation was described in early stages of motor learning, when spatial processing and high reliance on sensory feedback are critical for learning (Grafton, 1992), we did not expected to find bilateral increased in PMd recruitment since data presented here have been acquired in late stages of motor learning.

However, a major input to the PMd is from the posterior parietal cortex (Hund-Georgiadis and von Cramon, 1999) and these cortico-cortical connections has been proposed to code reaching movements as the result of a combination of visual and somatic information. Learning-related changes in this network (Grafton et al., 2002) may also explain the changes in PMd described here.

Thus, the final outcome of the trained task is a more extensive representation in primary and non-primary motor areas in the hemisphere contralateral to the trained arm, reflecting the dynamic nature of the neural representation of motor function.

Training-related brain changes in stroke patients

A widespread recruitment of primary and non-primary motor areas during motor performance was followed by a contralesional reduction and an ipsilesional increase in this task-related recruitment over training. Only the reduction in the contralesional M1 recruitment (by 50.2%) was significantly for the group. Although half of our patients recruited significantly less contralesional non-primary motor areas, there but there were no consistent effects across the group. In contrast, ipsilesional activation tends to increase as a function of training in half of our patients. Such `focusing' of brain activation has been reported before (Marshall et al., 2000; Calautti et al., 2001a; Feydy et al., 2002(Deiber et al., 1997; Halsband and Lange, 2006a; Hazeltine et al., 1997b; Inao et al., 1998), but has never been described in relation to both the true recovery and the brain changes in a control group who underwent a similar motor training.

If contralesional motor recruitment is the consequence of impairment to ipsilesional corticomotoneuronal pathways, then the explanation for the reduction of this recruitment (by 17-50% from PRE- to POST-training) is that recovery of motor function is a direct result of

restitution of ipsilesional anatomical link. Indeed, we generally found an increase in ipsilesional recruitment of both primary and non-primary motor areas, similar with learning-related cerebral reorganization observed in our healthy controls. A number of mechanisms may be involved in driving this reorganization.

Similar to controls, we found an increase (18%) in activation of M1 contralateral to the trained (impaired) arm in our patients. This is an intriguing finding since M1 output, in our patients is damaged and it is likely that this area already underwent a lesion-induced reorganization. Moreover, given that M1 is a key structure for the storage of motor engrams, relearning of a motor task may be hindered by "residual" elements of previously stored memory traces (Tanne et al., 1995) or dysfunctional spontaneous reorganization (Shadmehr and Holcomb, 1997). We can interpret ipsilesional M1 increase as a reestablishment of previously damaged, but not destroyed output of this area. Alternatively, this enlargement is an attempt to access to undamaged fast cortico-motoneuronal pathways since the nervous system, although injured, retains the ability to exploit the considerable redundancy within the somatotopy of M1 during learning, to generate an output via the intact portion of corticospinal tract (Ward et al., 2003b; Ward, 2006). Finally, neuronal plasticity is expressed not only in the primary neurons directly involved in projection pathways but also in the horizontal connections. (Liepert et al., 1998; Luft et al., 2004a). Overall, this result is in accord with a previous study showing that decreased hand, precisely the abductor digit minimi muscle, representation in M1 significantly increased after a period of 8-10 weeks of rehabilitation after stroke (Schubring-Giese et al., 2007). However, we did not find a decrease in hand representation before training in our group of patients. This could be explained by the differences in methodology, i.e., movement-related activation used in our study compared to evoked-movements in response to transcranial magnetic stimulation in the

other study (see above), patient sample, chronic subcortical versus subacute cortical and subcortical stroke.

With regard to the recruitment of contralateral non-primary motor areas, similar changes, i.e., increase, albeit greater, was exhibited in our patients (PMd, 8.1% in controls vs. 15.0% in patients, SMA, 22.9% vs. 41.5%, see Fig. 4). Since experimental data suggest that these areas operate in a parallel rather than a hierarchical fashion with primary motor area, they are able to functionally substitute for each other (Krishnan, 2006; Sanes and Donoghue, 2000). Therefore, the over-increased recruitment of these areas could be explained by a recruitment of more ischaemia-resistant small diameter myelinated corticospinal fibers of these cortices to compensate loss of large diameter fibers from M1. Alternatively, changes in M1 topography might alter the anatomical connections between premotor areas and M1, potentially enlarging the motor output zone (Grossman et al., 2002; Newton et al., 2006). Indeed, anatomic connections between M1 and SMA or PMd have been well-characterized in non-human primates (Fries et al., 1993; Johansen-Berg et al., 2002a; Rioult-Pedotti et al., 1998; Strick, 1988; Traversa et al., 1997). These non-primary motor areas also contain neurons whose activity is related to execution of relatively simple movements similar to M1 neurons (Dum and Strick, 2002; Dum and Strick, 2005; Newton et al., 2006). In addition, corticospinal projections from each non-primary area display a high degree of topographic organization (Boussaoud et al., 2005; Marconi et al., 2003; Mochizuki et al., 2004). Although we have very little direct information about anatomic connections in the human brain (Hoover and Strick, 1993), these findings indicate that function of any of these areas can only be understood in the context of a distributed network, which acts in concert to generate motor commands. In summary, these two premotor areas, PMd and SMA, are likely to remodel with training and thereby compensate for the injury of the M1 output.

Relationships training-related brain and motor outcome

We did not find significant correlations between learning-related brain changes and task performance in healthy controls. These findings are not consistent with prior transcranial magnetic stimulation studies in controls (Cadoret and Smith, 1997), which found correlations between increased size of representational maps in primary sensorimotor cortex and the performance of the trained task. However, as mentioned before, there is an enormous difference between movement-related activation described here and evoked movements in response to cortical stimulation. In accord with Shadmehr and Holcomb (1997), we interpret that brain reorganization of the representation of a motor skill increases stability of the representation of this motor skill rather a direct correlation with task performance.

After training, in patients, no significant correlations were found between activation of both ipsilesional and contralesional motor cortex and elbow extension performance. Moreover, the PRE significant correlations between elbow extension and contralesional M1 and bilateral PMd become non-significant with training. In other words, it is likely a "normalization" or a return to "normal" pattern of these relationships. However, these patients also showed a significant decrease in motor impairment, clinically evaluated. Moreover, this improvement was significantly correlated with contralesional activation in M1. In other words, a decrease in contralesional M1 activation was related with clinical improvement. This correlation suggests a potential compensatory role of contralesional M1 activation in these patients. However, further studies are required to elucidate the role of this area in recovery in chronic stage of stroke.

Limitations

A limitation of this study was certainly that not all areas involved in motor learning were fully covered. Thus, our results represent only a part of a puzzle of neuronal changes underlying motor learning. Especially, the hemodynamics of the cerebellum and the basal ganglia (Boudreau et al., 2001; Dum and Strick, 1991) were not included in the present study. Thus, enhanced activation in our regions of interest might reflect enhanced activation in these loops. Further drawback of the study may be the lack of evaluation of the generalizability and persistence of the skill performance. Since this is an important topic in post-stroke rehabilitation, we will specifically address it in a further manuscript. Performance during the session was controlled via video monitoring, but not continuously recorded an evaluated on-line by EMG, due to technical limitation in the scanner. Thus, it is possible that changes in muscle groups participating in performing the task rather than motor learning *per se* are responsible for changes in brain activation patterns. However, it is more likely that same muscles, but with increasing efficacy, are used to perform a task (He et al., 1993). In other words, the strategies used to perform a task are basically constant across the repetitions, particularly for the handgrip task (He et al., 1995).

Conclusions

A number of conclusions can be drawn on the basis of our observations in patients: (i) in chronic stage of stroke, the motor cortex has still the ability to undergo a remarkable reorganization even after 1180 movement repetitions, (ii) such reorganization is mainly reflected by increase of ipsilesional activation of primary and non-primary motor areas and decrease of contralesional activation, (iii) the correlations between brain activation and task performance normalized with training. Thus, we may conclude that learning-related brain and behavioral changes in stroke were somehow similar with those in healthy controls. Further studies

investigating training-induced brain changes on the cellular and network (i.e., functional connectivity) level as well as on the time course of these changes may help us to better clarify whether principles of motor relearning in an injured brain are similar with those of motor learning in an uninjured brain for a better transfer to optimize neurorehabilitation.

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Table 1. Demographic data, radiological status and clinical scores for stroke patients

Age/Sex	Time post-Onset	Sit of Stroke	FMUE
58/F	87	L/Basal ganglia, internal capsule	65
48/M	11	L/MCA territory, striato-capsular distribution	61
61/F	98	L/Corona radiata, basal ganglia	57
45/M	39	R/MCA territory, striato-capsular distribution	36
61/M	52	L/Basal ganglia, internal capsule	31
43/F	27	L/MCA territory, striato-capsular distribution	26
63/F	63	L/Basal ganglia, internal capsule	25
67/M	24	R/Basal ganglia, internal capsule	25
57/M	48	L/Pons	25
57/M	29	L/Basal ganglia, internal capsule	24
61/M	26	L/MCA territory, striato-capsular distribution	10

M = male, F = female; mo = months; L = left, R = right; MCA = middle cerebral artery; FMUE = Fugl-Meyer (maximum score = 66).

Table 2. Mean (SD) values of BOLD signal change (%) in primary motor cortex (M1), dorsal premotor cortex (PMd), supplementary motor area (SMA), measured bilaterally, in PRE- and POST-training in both control and stroke groups.

	M1		PMd		SMA	
PRE-training – Comparison stroke versus healthy						
	Left/ Ipsilesional	Right/ Contralesional	Left/ Ipsilesional	Right/ Contralesional	Left/ Ipsilesional	Right/ Contralesional
Healthy	0.68 (0.16)	0.01 (0.00)	0.64 (0.20)	0.46 (0.32)	0.52 (0.15)	0.49 (0.08)
Stroke	1.03 (0.51)	1.11 (0.67)	1.07 (0.45)	1.36 (0.63)	0.75 (0.20)	0.96 (0.49)
p-value	***0.048***	***0.000***	***0.01***	***0.001***	***0.007***	***0.01***
POST-training – Comparison stroke versus healthy						
	Left/ Ipsilesional	Right/ Contralesional	Left/ Ipsilesional	Right/ Contralesional	Left/ Ipsilesional	Right/ Contralesional
Healthy	0.81 (0.11)	0.01 (0.00)	0.69 (0.20)	0.46 (0.30)	0.64 (0.12)	0.52 (0.08)
Stroke	1.22 (0.64)	0.55 (0.52)	1.23 (0.83)	1.15 (0.91)	1.06 (0.82)	0.82 (0.80)
p-value	*0.06*	***0.006***	*0.06*	***0.03***	*0.12*	*0.26*
Comparison of PRE- versus POST-training (*p-value*)						

	Left/ Ipsilesional	Right/ Contralesional	Left/ Ipsilesional	Right/ Contralesional	Left/ Ipsilesional	Right/ Contralesional
Healthy	***0.001***	*N.D.*	***0.005***	*0.66*	***0.000***	*0.18*
Stroke	*0.36*	***0.02***	*0.46*	*0.49*	*0.17*	*0.44*

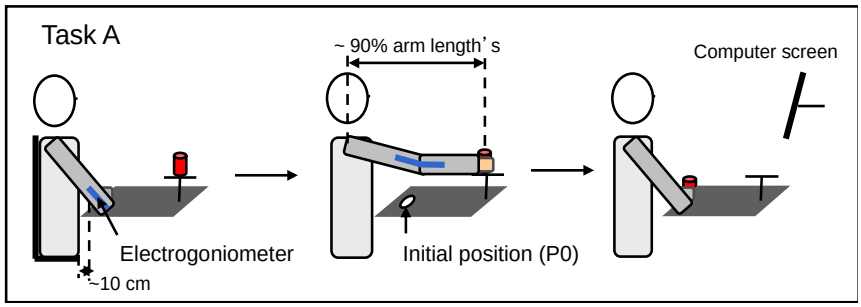

Fig. 1 Motor training paradigm – Task A

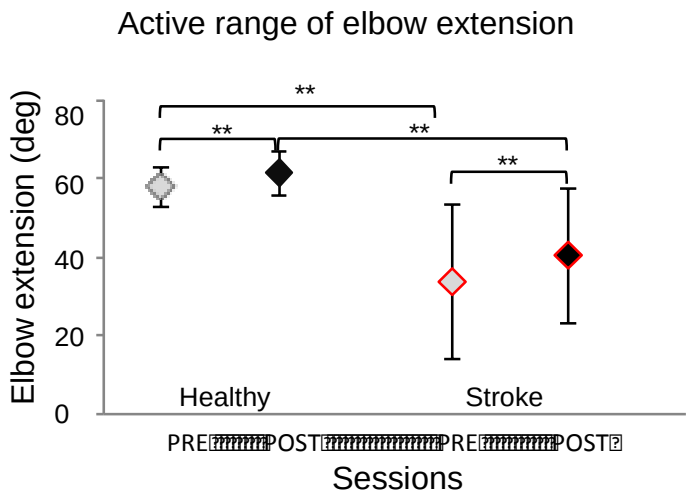

Fig. 2 Mean (SD) of elbow extension (deg) in PRE and POST sessions in healthy (grey/black diamonds) and stroke (grey/black diamonds with red contour) groups.

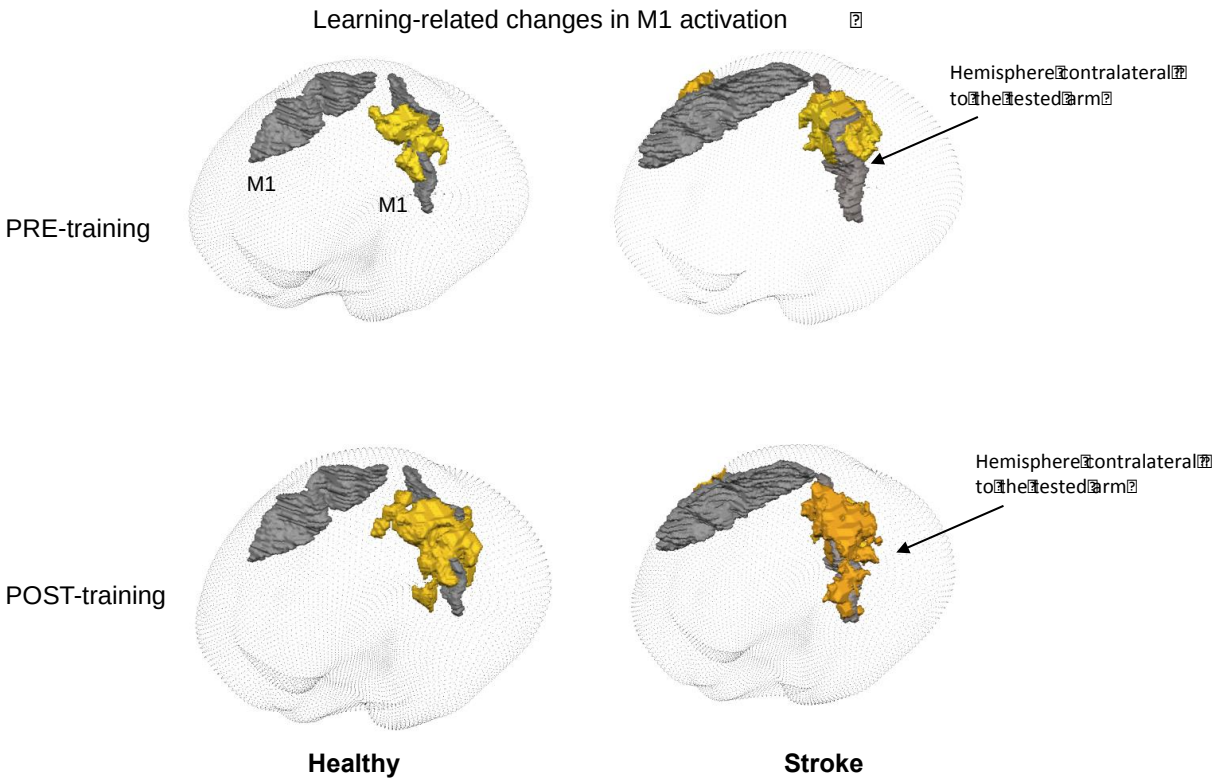

Fig. 3. Primary motor cortex (grey shadow) activation (yellow shadow) during a handgrip task in one healthy control (M, 61 years old; left panels) and one patient (M, 61 years old, infarct located in left basal ganglia and internal capsule, FMUE=31; right panels) in PRE-training (top) and POST-training (bottom) sessions.

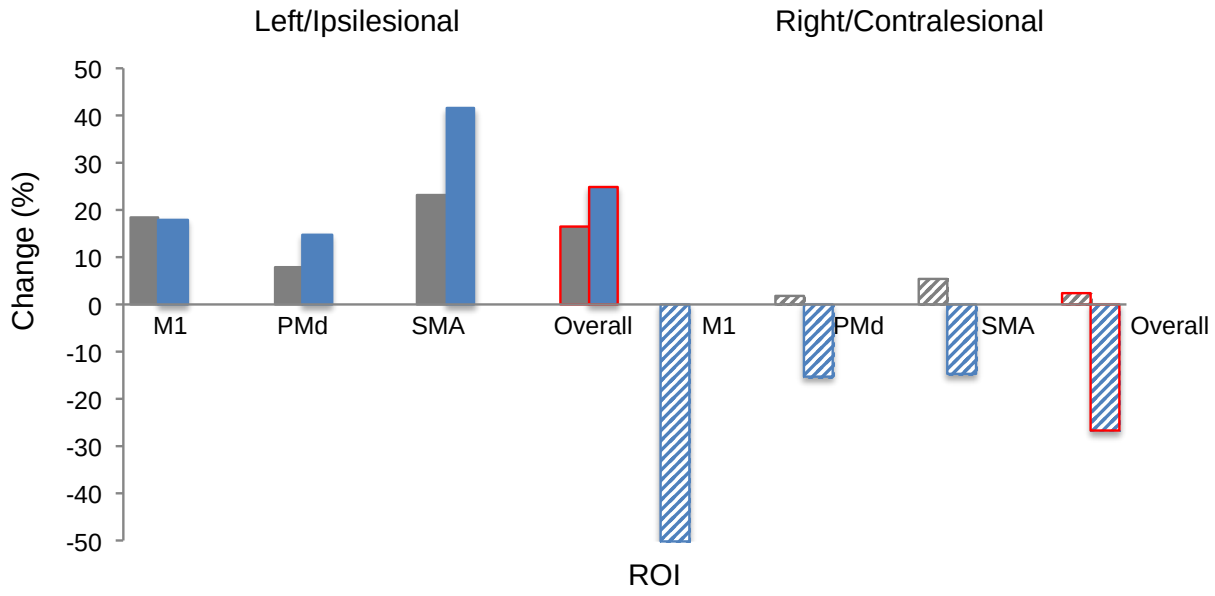

Fig. 4. Training-related changes (%) in motor-related activation in primary motor (M1), dorsal premotor (PMd), and supplementary motor (SMA) areas in left (ipsilesional) and right (contralesional) hemispheres in control (grey bars) and stroke (bleu bars) groups. Red-contoured bars represent the overall mean in all ROIs per hemisphere.

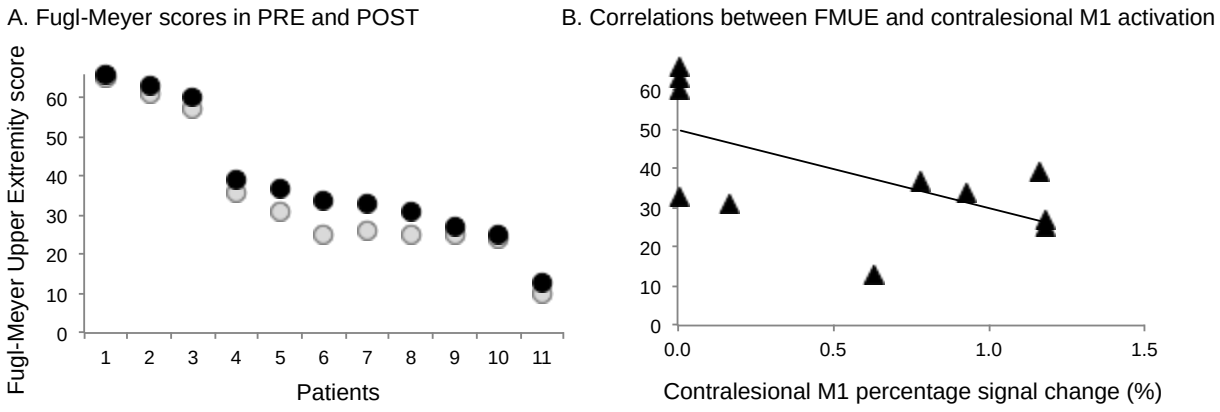

Fig. 5 A. Fugl-Meyer Upper Extremity (FMUE) scores in PRE (grey circles) and POST (black circles) in patients**. B.** Correlations between FMUE scores and motor related activation in contralesional M1 in POST-training session ($p<0.05$).

CHAPTER IV

MOTOR RELEARNING AFTER STROKE: CORTICAL FUNCTIONAL CONNECTIVITY REORGANIZATION AND TRUE RECOVERY

(in preparation for Neural Rehabilitation and Neural Repair)

Abstract

Background: Functional plasticity after stroke is attributed, at least in part, to neural reorganization in radiologically normal-appearing cortical motor regions. Non-invasive functional MRI provides a means to follow reorganization. Previous fMRI studies reported abnormal bilateral motor network activation and an altered functional connectivity within cortical motor system in stroke survivors. However, considerable variability in the evolution of motor-related activation has been described in patients with different rates of recovery and no prior study has longitudinally investigated the relationship between changes in functional connectivity within cortical motor system and recovery of premorbid movement pattern. Thus, by combining fMRI and kinematic modalities, we predicted that altered functional connectivity in both hemispheres would "normalization" with training and this would be accompanied by a "normalization" of their relations with elbow extension.

Methods: Eleven chronic survivors of a single subcortical stroke and twelve age-sex matched healthy controls underwent functional MRI, kinematic, and clinical (in patients) testing prior to (PRE) and immediately after (POST) a motor training. Participants were scanned performing a dynamic isometric handgrip task with the dominant (impaired) hand. Functional connectivity between primary (M1) and non-primary (dorsal premotor cortex, PMd, and supplementary motor area, SMA) motor areas within (intra-hemispheric) and between (inter-hemispheric) hemispheres was quantified by using Independent Component Analysis. Kinematic assessment (Vicon system) of elbow extension during a reach-to-grasp task and clinical measure of arm motor impairment (Fugl-Meyer Upper Extremity, FMUE, test) were administrated. Training consisted in a repetitive variable practice of a reach-to-grasp task with the impaired arm during a four-

week period (12 days, 90 repetitions/day). Functional connectivity and motor outcome differences between sessions and groups as well as their relationships were evaluated.

Results: PRE: Functional connectivity patterns were altered after stroke: intra- and inter-hemispheric non-significant positive relationships were found in patients while significant negative inter- (M1 ipsilateral to the trained arm to contralateral motor area) and intra- (ipsilateral motor areas) hemispheric correlations were found in controls. Between-group differences in functional connectivity reached statistical significance for ipsilateral M1-contralateral areas correlations. The relationships between connectivity and elbow extension were also altered in patients: all, but one, functional connectivity were significantly and negatively correlated to elbow extension, while non-significant correlations were found in controls. POST: Stronger and significant connectivity was found in the contralateral network in patients. There were no changes in the sign of ipsilateral or inter-hemispheric correlations with training. The correlation pattern between connectivity and elbow extension "normalize" with training in patients, i.e., significant correlations became non-significant.

Conclusions: Our preliminary data suggest that changes in connectivity strength within cortical motor system could be achieved with motor training even in the chronic stage of stroke. However, these changes were not accompanied by an inter-hemispheric "normalization" of inhibition/excitation. The relationships between functional connectivity and elbow extension were "normalized" with training. Although the training-related functional remodeling of motor networks within and between hemispheres in our patients is different from that reported in controls, this remodeling somehow is likely to underlie behavioral recovery.

Introduction

Human imaging studies have revealed that after stroke, paretic arm function is likely to be restored through the involvement of a distributed cortical motor network, including radiologically normal-appearing (or spared) motor areas in both injured (ipsilesional) and un-injured (contralesional) hemispheres (Crick and Jones, 1993; Doyon and Benali, 2005b; Floyer-Lea and Matthews, 2005; Hatakenaka et al., 2007; Pascual-Leone et al., 1995). However, successful recovery occurs in stroke survivors exhibiting relatively normal ipsilesional patterns of motor activation, whereas patients with larger stroke, who often show bilateral cortical activation, typically have less complete recovery (Calautti and Baron, 2003; Cramer, 2008b; Takahashi et al., 2008; Ward et al., 2003a). However, considerable variability in the evolution of motor-related activation has been described in patients with different rates of recovery. This variation could be explained by reorganization in the communication between motor areas, e.g., functional connectivity. Limited number of stroke studies have been published so far on this topic and the relationship between functional connectivity within motor system and behavioral recovery remains incompletely characterized (Gerloff et al., 2006). For example, low resting-state connectivity or "hypoconnectivity" (suggesting less efficient transfer of information) between ipsilesional primary motor cortex (M1) and supplementary motor area (SMA) is related to motor deficits (Calautti et al., 2001a; Calautti and Baron, 2003; Loubinoux et al., 2003; Marshall et al., 2000). This is further supported by "hypoconnectivity" among ipsilesional or contralesional M1 and ipsilesional SMA during unimanual (paretic arm) or bimanual movements (Grefkes and Fink, 2011b; Ward et al., 2003b). Moreover, pharmacological modulation of M1-SMA connectivity, supposedly increasing coupling strength between areas, has been associated with improved arm function (Sharma et al., 2009b). In contrast, high resting-state connectivity

between ipsilesional M1 and bilateral dorsolateral premotor cortex (PMd) was correlated with the level of motor impairment (Grefkes et al., 2008b). Anatomic connections between M1 and non-primary motor areas, such as SMA or PMd, are known to exist in non-human primates (Grefkes et al., 2008b; Mintzopoulos et al., 2009b; Rehme et al., 2011; Wang et al., 2011b). These secondary motor areas also contain neurons whose activity is related to execution of relatively simple movements similar to that of M1 neurons (Dum and Strick, 2002; Dum and Strick, 2005; Wang et al., 2010). In addition, corticospinal projections from each secondary area display a high degree of topographic organization (Boussaoud et al., 2005; Marconi et al., 2003; Mochizuki et al., 2004). Although we have very little direct information about anatomic connections in the human brain (Hoover and Strick, 1993), these findings indicate that function of any of these areas can only be understood in the context of a network, which acts in concert to generate motor command. Indeed, cross-sectional designs are useful to determine the relationships between functional connectivity patterns and impairment (Cadoret and Smith, 1997), but longitudinal assessment would truly define whether the changes in connectivity are associated with recovery. However, no prior study has investigated longitudinal changes in cortical task-evoked functional connectivity during a motor learning paradigm after stroke.

Another limitation in interpreting these results is the tool by which recovery is judged and/or defined. Most used outcome measures, e.g., Fugl-Meyer Upper Extremity scale, focus on task completion and tester's ratings of movement (i.e., low objectivity). Although these outcomes may provide valuable information regarding motor impairment after stroke, yet they do not provide precise quantitative data on movement strategies and thereby lack the sufficient sensitivity to characterize changes in movement strategies especially longitudinally over time (Boudreau et al., 2001; Dum and Strick, 1991). Indeed, recent studies have shown that using

clinical measure of impairment has poor ability to define recovery in the context of brain functional connectivity (He et al., 1993; He et al., 1995). These studies together with our studies (ref) suggested that movement kinematic assessment is an objective and robust measure of recovery following stroke. For example, changes in active range of elbow extension have been previously used as main outcome measure to quantify intervention-related recovery following therapeutic interventions (Crick and Jones, 1993). Thus, movement kinematics could be used for better interpretation of the alterations in functional connectivity patterns during the process of motor recovery longitudinally follow a motor learning paradigm.

We now investigate, through longitudinal studies in a homogenous stroke sample (subcortical lesion) and combining functional brain mapping and kinematic modalities, cortical functional connectivity dynamics and its relationship with recovery. By introducing this approach, the present study opens new ways to characterize stroke-related changes within cortical motor network. The first aim of this study was to characterize the changes in functional connectivity between M1 and PMd and SMA within and between hemispheres following an arm-focused motor leaning in patients as well as age-sex matched healthy controls. Since the connected areas in the existing network may play new roles after stroke, and therefore have different communications and based on previous studies mentioned above, we predicted that altered functional connectivity in both ipsi- and contralesional networks would “normalization” with training. The second aim was to investigate the relationships between functional connectivity and kinematically measured elbow extension during a reach-to-grasp task. Based on previuos studies, (see above) we expected altered correlations between the communication within motor system and the active range of joint motion. With training, we hypothesized that functional connectivity “normalization” would be accompanied by a “normalization” of their relations with elbow

extension. This would support the hypothesis that the expected changes in functional connectivity would represent an adaptive mechanism. Such understanding would be a significant addition to the current literature and fulfills several gaps that have not been addressed for years.

Materials and methods

Stroke patients and healthy controls

Ten stroke survivors and sixteen healthy controls (all right-handed) gave written informed consent. The study was carried out under approval of the Human Subjects Committee of the Kansas University Medical Center.

Patients were included if they had: 1) first ischemic subcortical stroke leading to arm paresis, at least 6 months prior to study enrollment. We have chosen subcortical lesion location because our regions of interest as well as their horizontal connections are left intact (Grefkes and Fink, 2011b). In addition, we could evaluate a homogeneous group with comparable lesion size and location. Lesion location and type were confirmed on T2-weighted images. We also studied the chronic state to avoid the confounding effect of rapid spontaneous recovery (Massie et al., 2011); 2) age 35-75 yrs, to minimize the confounding effects of age-related changes in the neural correlates of motor performance (Massie et al., 2011), 3) able to understand simple commands, 4) have no or mild attentional deficits (Cancellation test and the Line Bisection Test), or ideomotor (impaired ability of the sequencing and execution of motor tasks, i.e., the use of scissors to cut a paper), ideational (impaired ability to perform a series of acts although they may be able to perform the individual components of the series, i.e., making coffee) apraxia; 6) have a minimum level arm motor impairment (>10 on FMUE (Grefkes and Fink, 2011b)); and 7) have

no other neurological, neuromuscular or orthopedic problems that may interfere with data interpretation.

Patients were excluded if they have: 1) shoulder subluxation (by clinical palpation of the space between acromion and the head of the humerus, grading subluxation as none=0, minimal=1, or substantial=2 (Westlake and Nagarajan, 2011)); 2) verbal rating scale of the pain during shoulder passive lateral rotation, from none=0 to very severe=4 (Michaelsen et al., 2006). Participants with the scores > 1 for 1) and 2) were excluded; 3) unstable treatments for the study duration (i.e., surgical intervention, participation to other research studies); and 4) MRI exclusions, such as metallic objects in the head, cardiac pacemaker, epilepsy or convulsion history.

Age-, sex-, and academic-matched healthy controls, with normal T2-weighted images and without neurological and psychiatric disorders, were recruited.

Study Protocol

Participants underwent MRI and kinematic testing prior to (PRE), and immediately after (POST) a motor training period.

MRI Acquisition and Analysis

MRI data collection is carried out using a 3 Tesla Siemens Allegra scanner at the KUMC Hoglund Brain Imaging Center and consisted in structural and functional. Our experimental protocol has been detailed previously (Byrnes et al., 2001).

A T1-weighted axial (3D-MPRAGE, matrix=256x256, slice thickness=1mm, no gap, TR=2300ms; TE=3ms) will be acquired to analyze functional MRI data. Proton density/T2-weighted images (slice thickness=5mm, no gap, TR=4800ms, TE=18/106ms) were collected to

confirm the presence of a single ischemic subcortical stroke and exclude any other neurological conditions.

Gradient echo blood oxygen level-dependent (BOLD) scans were acquired in 25 axial slices coincident with the PD/T2 series (TR=2000ms; TE=50ms; FOV=240mm; matrix=64x64; slice thickness=5mm; 0 skip; in-plane resolution=5x5mm^2; 100 time points). One BOLD scan was performed for the impaired hand and consisted of two alternating conditions: movement (20s) and rest (20s) conditions repeated five times (total time=3min 28s). A compatible custom-designed device that maintains constant pressure was used throughout movement and rest conditions (i.e. active grip followed by passive opening of the hand). In the movement condition, the participant performed a single brief handgrip task, repeated every 4s. To ensure similar performance across the participants, a target pressure (25% of handgrip maximal voluntary contraction, MVC) was displayed graphically, and participants performed the handgrip until the target pressure will be attaint, at which point the grip was released. We selected this task to broaden patient recruitment since handgrip returns earlier than fractioned finger movements (Duncan et al., 1992). In the rest condition, participants were instructed to lie motionless.

Before scanning: As mentioned before, the MVC was individually measured. Five practice trials were performed with the handgrip devise for familiarity purposes and responding to questions regarding the task.

During scanning: The participant was positioned supine in the magnet. Head stabilization was achieved by supporting the subject's head with pillows. In order to familiarize the subject with the task in that environment, another set of five practice trials were performed in the magnet. Participants received visual instructions through optical-fiber magnet-compatible goggles (Resonance Technology). If mirror or associated movements were detected (by visual

inspection), the acquisition was stopped and the subject was instructed accordingly that this movement is prohibited. If on a repeat scan there was continued mirror-movement, the data collection was aborted.

BOLD analysis: BOLD data was analysed using Brain Innovation (B.V., Maastricht, Netherlands). Motion correction estimating three translation and three rotation parameters was performed by a rigid body transformation to match each functional volume to the reference volume (the third volume, since the first two are discarded to avoid T1 saturation effects). These parameters were then inspected to estimate head movements (in our case, movements less than 5 mm were accepted). However, none of our participants moved the head more than 2 mm in any direction.

After movement correction, 3D spatial smoothing with a 4-mm Gaussian filter allows valid statistical inference according to Gaussian random field theory (Ward and Frackowiak, 2003). The time series in each voxel is filtered at 0.01Hz to remove low frequency confounds.

Movement and rest periods were modeled by a boxcar function with hemodynamic response modification (predictor movement). The general linear model was used to extract voxel-wise signal or percentage signal change (cluster threshold = 100 contiguous voxels; p(Bonferroni) = 0.01) and create a hand representation mask within M1, the anterior bank of the central sulcus with the caudal border lying in the depth of the central sulcus close to its fundus and anterior border abuts BA6; PMd, the anterior half of precentral gyrus and the anterior bank of the precentral sulcus; and SMA, the medial wall of the hemisphere from the top of the brain to the depth of the cingulate sulcus with the posterior boundary halfway between the extension of the central and precentral sulci onto the medial surface and the anterior boundary at the vertical line trough the anterior commissure.

To highlight the presence of functionally connected clusters within our ROIs (inter- and intra-hemispheric), we applied single-subject data driven Cortex-based Independent Component Analysis (ICA, Brain Voyager QX). ICA is a useful tool for following reasons: 1) spatial ICA finds systematically coherent brain regions without constraining the temporal domain; 2) ICA is available as a plug-in in BrainVoyager QX software (Fugl-Meyer et al., 1975); and 3) ICA detects task-related components within the selected ROIs. Further, lateralization in the spatial layout of these components was also detected (for more methodological details, please see (Bohannon and Andrews, 1990; Van Langenberghe and Hogan, 1988). Our regions of interest (M1, PMd, and SMA) were defined a priori based on anatomical landmarks (Cirstea et al., 2011b) (see also above). A polygon mesh representing the white/gray matter border has been obtained from a 3D anatomical data set to create the grey matter mask. Thus, the grey matter mask was projected into our pre-processed functional data set. Finally, the ICA was overlaid on the processed functional data, and the results within each hemisphere (intra-hemispheric) and between hemispheres (inter-hemispheric) were included in the model.

Kinematic acquisition and analysis

Kinematics of a reach-to-grasp task were recorded using four reflective balls positioned on arm (1-ulnar process; 2-lateral epicondyle; 3-acromion process of the ipsileateral shoulder; 4-acromion process of the contralateral shoulder; VICON System, Oxford Metrics; 3-6s at sampling frequency=100Hz). With no restriction of trunk movement, participants were seated with the impaired/dominant arm resting on a support placed ipsilateral to the tested arm and the contralateral arm rests alongside the body. From this position, participants were instructed to reach and grasp, at a self-selected speed, a cylinder placed in front of them on the midline of the

trunk (4-cm diameter). The height of the cylinder was adjusted to the shoulder level of each participant and at the maximal elbow extension (in stroke survivors, the target position was related with their passive maximal elbow extension). Participants responded to an auditory signal to perform the task and hold the hand in the final position (without lifting or displacing the object) until they received another auditory signal to come back to the starting position. No feedback regarding movement quality was given during the recording session. Blocks of 20 trials were recorded. Under normal conditions the selected reach-to-grasp task requires full elbow extension and no trunk movement. Thus, it allows us to identify the active range of elbow extension in stroke compared to healthy participants (Heller et al., 1987).

Elbow movement analysis: The vectors joining the appropriate IREDs were used to compute, using vector algebra, the elbow angles (flexion/extension, Fig. 1 (Friston et al., 1995)). Elbow extension values were determined from individual trials and mean (SD) are calculated for the 20 trials as the final output for each participant.

Clinical assessment of arm motor impairment

Since one of the major goals of this thesis was to investigate the relationships between functional brain changes and kinematics vs. brain changes and clinical assessments, we also evaluated in this paper these relationships. Thus, we performed clinical assessment of arm motor impairment was assessed using the FMUE scale, with a maximum (normal) score of 66.

Motor learning paradigm

Participants participated in 12 daily practice sessions for 4 weeks consecutively (3 times per week). Based on our previous results (Cirstea et al. 2003; Cirstea et al. 2006), the number of

repetitions (n=90) per session was considered “intensive” practice. The motor learning paradigm consisted of repetition of a reach-to-grasp task with the dominant (impaired in patients) arm.

Participants were seated in front of an adjustable height table so that the initial position is located approximately half of distance between the low-sternum and the umbilicus. The experimental seat height is normalized by placing it at a height equivalent to each patient’s lower leg length (Esposito et al., 2002; Formisano et al., 2004). The hip and knee joints are flexed at 90° and the feet make full contact with the floor. At the initial position, the dominant (impaired) arm rested on the table at approximately 10cm in front of the body (Fig. 2). The contralateral arm rested alongside the body. A cylindrical object was placed within a comfortable range for grasping on the platform located in front of the participant. The platform height corresponded to the shoulder height determined for each participant, and the platform distance was determined according to the length of the arm with the elbow in full extension (180°) and the hand comfortably grasps the object (about 90% arm’s length (Formisano et al., 2004)). The size (4, 6, 8 cm diameter, 10cm height) and weight (50, 100, 150 grams) of nine cylindrical objects were presented in a randomized order (90 trials). In response to an auditory signal, the participant reach and grasp the object with the whole hand, and move it back to the initial position at a comfortable self-selected speed. During training, participants received feedback about elbow extension angle (knowledge of performance, KP) by using an electrogoniometer (Exos, Inc., Woburn, MA, USA) located on their lateral epicondyle. Video images of elbow movement were presented on a computer screen after the end of the task (terminal KP). To minimize dependency on feedback, the KP was administrated with a decreasing frequency (faded KP) throughout the practice session (i.e., for the first 30 trials, KP will be given every trial, for the second 30 trials, every 2nd trial and for the last 30 trials, every 5 trials). This task was selected because it is not

entirely novel, and it should be re-acquired during recovery from stroke. In addition, this task involves the coordination of arm and hand movements. So our aim was not to train patients in a novel task but to identify whether intensive training of an altered movement component (i.e., elbow extension) improved movement quality and whether this improvement is underlined by changes in functional connectivity within the motor system. We note that this study was focused only on the reaching component of the trained task.

Statistical analysis

Demographic and experimental data from healthy and patients were compared using *t*-test for independent samples.

We used Pearson Rank Order correlation to quantify the putative interregional relationships between activated ROIs within hemisphere (intra-hemispheric) and between hemispheres (inter-hemispheric) for each session in every participant. Then, correlation coefficients differences between sessions within group, and between groups within session were assessed by using the Fisher-transformed correlation coefficient r (z-scores) with an online statistical package (http://vassarstats.net/rdiff.html).

We used factor analysis to group together the percentage signal change within each ROI and for each participant, into factor scores. Then, we used Pearson correlation, to quantify the contribution of the intra-hemispheric and inter-hemispheric components to elbow extension. All analyses were done using SPSS18 and a significant level of $P<0.05$ was used.

Results

Demographic and experimental data

Patients (n=11) and healthy controls (n=12) did not significantly differ with respect to age (mean ± SD, 57 ± 8yrs vs. 57 ± 5yrs, p=0.5) or sex (p=0.9). All patients had sustained a single cerebral infarction between 7 and 112 months previously (32±30 mo), leading to arm motor impairment (FMUE 35±18, range=66-10). The infarction site was determined on T2-weighted images. No patient had M1, PMd or SMA damaged by stroke. All but three had left-sided infarcts.

In our description below, laterality is referenced to the tested (right) arm in controls and the impaired arm in patients. Accordingly, hemisphere contralateral to the tested arm refers to left hemisphere in controls and to ipsilesional in patients.

PRE-training

Functional connectivity intra- and inter-hemispheric

Healthy-Controls: In controls, significant negative inter-hemispheric correlations were found between right M1 and left PMd and SMA and (right M1-left PMd, r=-0.74, p=0.006; right M1-left SMA, r=-0.86, p<0.001). Negative correlations were also found between left and right M1 (r=-0.563, p=0.057) and between primary and non-primary motor areas in the right hemisphere (M1-PMd, -0.41, p=0.2; M1-SMA, r=-0.45, p=0.1, Fig. 3A).

Patients: A different correlations pattern was found in patients. Specifically, non-significant positive correlations were found within and between hemispheres in our patients (Fig. 3B).

Patients vs. Healthy-Controls: Between-groups differences in functional connectivity reached statistical level only for the relationships between right (contralesional) M1 and all left (ipsilesional) ROIs (M1-M1, p=0.02; M1-PMd, p=0.005; M1-SMA, p<0.001) (Fig. 3B). In

accord with previous findings, in patients we found a decrease in correlation strength between M1 and SMA (r=0.29 vs. r=0.51) and an increase between M1 and PMd (r=0.54 vs. r=0.44) in the ipsilesional hemisphere, although non-significantly different from our controls (p=0.6 and p=0.7 respectively).

Correlations between functional connectivity and elbow extension

Healthy-Controls: Negative correlations were found between right M1-left non-primary motor areas functional connectivity and elbow extension, albeit they did not reach statistical level (left PMd-right M1, r=-0.56, p=0.057; left SMA- right M1, r=-0.54, p=0.07). Overall, we found 6 negative and 3 positive functional connectivity-elbow extension correlations.

Patients: Only negative correlations were found in our patients and all, but one (ipsilesional M1-contralesional SMA), reached the significance level (see Table 1).

Patients vs. Healthy-Controls: Between-group differences in the functional connectivity-elbow extension relationships were found within (ipsilesional vs. left: M1-PMd, p=0.03, M1-SMA, p=0.04; contralesional vs. right, M1-Pmd, p=0.003) and between (ipsilesional-contralesional vs. left-right, M1-M1, p=0.01, M1-PMd, p=0.03) hemispheres.

Differences in relationships between functional connectivity-elbow extension and functional connectivity-FMUE

Patients: Similar negative relationships were found between functional connectivity and FMUE compared to those between functional connectivity and elbow extension. However, ipsilesional relationships did not reach statistical significance for FMUE (M1-PMd, p=0.06, M1-SMA, p=0.1). These correlations, although for a small sample, are the first demonstration of a stronger

relationship between functional connectivity within the motor system and a quantitative measure of movement quality than clinical measure of motor impairment after stroke.

POST-training

Functional connectivity intra- and inter-hemispheric

Healthy-Controls: Significant correlations between right M1 and left non-primary motor areas persisted (right M1-left PMd, r=-0.74, p=0.06; right M1- left SMA, r=-0.79, p=0.002) and a stronger negative correlation emerged in the right hemisphere, between M1 and SMA (r=-0.71, p=0.009) (Fig. 3). However, there were no significant differences between POST- and PRE-training values.

Patients: Stronger (significant) positive correlations were found within ipsilesional hemisphere (M1-PMd, r=0.74, p=0.01, M1-SMA, r=0.95, p<0.001) and between hemispheres (ipsilesional M1-contralesional M1, r=0.63, p=0.04; ipsilesional M1-contralesional PMd, r=0.68, p=0.02) (Fig. 3). A slight increase in functional connectivity between contralesional motor areas was alos observed (M1-PMd, r=0.54 in POST vs. r=0.46 in PRE). Ipsilesional M1-SMA correlation in POST-training was significantly different from PRE-training value (p=0.002).

Patients vs. Healthy-Controls: In addition to the PRE-training differences between-groups in functional connectivity, new significant differences were found in the POST-raining session. Specifically, contralesional correlations were significantly different form those in the right hemisphere in controls (M1-PMd, p=0.048; M1-SMA, p=0.02) and ipsilesional correlation between M1-SMA from that in left hemisphere (p=0.001).

Correlations between functional connectivity and elbow extension

Healthy-Controls: Interesting to note that the correlations between functional connectivity and elbow extension become positive, but one (left M1-rightPMd), following training. There were no significant changes in POST versus PREvalues.

Patients: Although training did not change the correlations' sign, i.e., negative, it altered their significance. M1-M1 connectivity - elbow extension was the only correlation reaching statistical significance in POST-training ($r=-0.63$, $p=0.04$). Thus, the other 7 significant correlations reported prior training became non-significant in POST-training. By comparing POST to PRE correlations between functional connectivity patterns and elbow extension, significant decrease in strength of correlations was found between elbow extension and right PMd-right M1 (PRE $r=-0.93$, POST $r=-0.33$; $p=0.01$) and right PMd-left M1 (PRE $r=0.86$, POST $r=-0.29$; $p=0.047$) connectivity.

Patients vs. Healthy-Controls: There were no significant between-group differences in the relationships between functional connectivity and elbow extension.

Discussion

Summary of results

The objective of this paper was to study the functional connectivity of motor-related brain areas during a motor learning paradigm, over a 4-week period in patients with stroke and healthy controls. The regions of interest were identified based on a motor skill-learning model (presented in the Chapter 1). We specifically investigated two issues: i) does altered functional connectivity within motor system recover (or "normalize) with motor training in chronic stages of stroke? and ii) do the relationships between functional connectivity and kinematic measure of motor impairment normalize with training or does "normalization" of functional cortical motor

connectivity relate to motor recovery? Our results show that motor learning is associated with changes in the level of integration between the motor and premotor areas even in the chronic stage of stroke. We found i) significant enhanced functional connectivity within the network contralateral to the trained hand (or ipsilesional) and between ipsilesional M1 and contralesional PMd following training in our patients. Despite of these changes, the balance between excitatory and inhibitory circuits has not been "normalized", i.e, lack of inter-hemispheric inhibition; and ii) a "normalization" of the relations between functional connectivity and elbow extension.

Functional connectivity within motor network in stroke patients before training

In accord with previous studies (Chari and Kirby, 1986; Cirstea et al., 2003b; J. Talairach, 1988; Subramanian et al., 2010), before training we found a decrease in connectivity strength or "hypoconnectivity" between M1 and SMA and an increase in connectivity strength between M1 and PMd within ipsilesional hemisphere, as well as a complete lack of intra-hemispheric inhibition between contralesional M1 and ipsilesional motor areas in our patients. If we consider the functional connectivity as a measure of the local efficiency of information transfer, it is likely that "hypoconnectivity" is related to low transfer of information while enhanced connectivity relates to high transfer. These changes are may be due to deletion of nodes or connections (Dean and Shepherd, 1997; Grefkes et al., 2008b; Mark et al., 1997; Sharma et al., 2009b). In an anatomically model, deletion of nodes produced widespread disruptions of functional connectivity (Mintzopoulos et al., 2009b; Rehme et al., 2011) that were consistent with effects reported in focal human brain lesions (Bullmore and Sporns, 2009). The mechanism underlying the lack of intra-hemispheric inhibition or shift of inter-hemispheric balance in cortical excitability toward unaffected hemisphere is not well understood. Since a subcortical stroke does

not affect the commissural fibers connecting the homologues motor areas in both hemispheres, a potential explanation is that the shift in inter-hemispheric balance results from indirect adaptive changes induced by thalamic or cerebellar pathways (Honey and Sporns, 2008). This disturbance has been linked with a down-regulation of GABA receptors function (Kaiser et al., 2007), or an up-regulation of N-methyl-D-aspartate receptors (Honey and Sporns, 2008) with an inadequacy of GABA-ergic transmission to balance overactive glutamate synapses (He et al., 2007a).

Correlations between functional connectivity and motor outcome in patients before training

A recent review suggested that kinematic assessment might add another dimension into understanding functional connectivity alterations following stroke (He et al., 2007a; He et al., 2007b). Our correlation analysis between functional connectivity and elbow extension revealed that the observed connectivity patterns have a negative influence on motor outcome and support the overall concept of the relationship between altered functional connectivity and motor output (Hagemann et al., 1998; Nowak et al., 2008; Que et al., 1999; Redecker et al., 2002). Specifically, significant negative correlations were found between most functional connections and active range of elbow extension (see Table 1). Moreover, our results are the first demonstration of a stronger relationship between functional connectivity within the motor system and a quantitative measure of movement quality than clinical measure of motor impairment after stroke.

Motor-learning changes in functional connectivity in healthy controls

After training, in healthy controls, only one significant correlation, between M1 and SMA in the hemisphere ipsilateral to the trained had (or right) was emerged after training. Thus, we did

not observe a major increase in functional integration in the motor network with practice. It is possible that the gradual built up of the trained movement in the motor network does not require sustained increase in functional connectivity. Alternatively, we cannot rule out the hypothesis that functional integration may increase/decrease in the associative network recruited during learning or within arm representation in the primary and/or non-primary motor areas. It is clear that interactions between these networks, motor and associative, are necessary during learned movements (Grefkes and Fink, 2011b; Westlake and Nagarajan, 2011), even though involvement of our regions belonging to the motor/premotor circuit was not significantly increased or decreased with practice.

Motor-learning changes in functional connectivity in stroke patients

As stated above, our analysis revealed enhanced functional connectivity between motor areas within hemisphere contralateral to the trained arm (or ipsilesional). Specifically, stroke survivors showed an enhanced positive connectivity (facilitation) between M1 and non-primary motor areas. These results are consistent with work derived from learning studies in healthy controls and animal models (Doyon et al., 2003; Doyon and Benali, 2005a; Friston, 2011; Grafton et al., 2002; Grefkes et al., 2008b; Grefkes and Fink, 2011b; Halsband and Lange, 2006a; Hazeltine et al., 1997a; Westlake and Nagarajan, 2011), showing a training-related increase in the cortical areas controlling the trained arm/hand. The increased importance of ipsilesional M1 within the motor network after recovery was also indicated by stronger functional connectivity of this area with contralesional motor areas (Seitz and Freund, 1997). Indeed, functional connectivity between M1s and between ipsilesional M1 and contralesional PMd reached statistical significance with practice, although positive relationships. These results are also consistent with

the assumption that recovery of motor function depends on reorganization processes within both ipsilesional and contralesional hemispheres.

Interesting to note is an increase in functional connectivity between contralesional PMd and both M1s, although did not reach statistical difference. This result is consistent with animal studies showing enhanced contralesional functional connectivity (Karni et al., 1995; Van Mier et al., 1999). Although we found an increase in connectivity of contralesional M1, the role of this area in motor recovery remains controversial and incompletely understood (Shadmehr and Holcomb, 1997). This debate is due, in part, to inconsistent results of TMS studies that found that inactivation of contralesional M1 improved (Doyon and Benali, 2005b; Floyer-Lea and Matthews, 2005), altered(Wang et al., 2010), or left unchanged (van Meer et al., 2010a) the motor performance of the impaired arm. In the healthy brain, a TMS-induced M1 "virtual lesion" results in increased excitability of the "contralesional M1" without changes in hand motor function(van Meer et al., 2010b). Therefore, it is conceivable that the increased contralesional M1 activation observed after stroke may reflect altered inter-hemispheric inhibition providing compensatory support for impaired hand movements in the presence of ipsilesional CST damage. The mechanisms underlying altered inter-hemispheric inhibition between M1 after stroke is not well understood, e.g., direct involvement of the commissural fibers connecting M1s after a cortical stroke (Grefkes and Fink, 2011b) or indirectly by thalamic or cerebellar pathways in subcortical stroke (Mansur et al., 2005). Some argue that the contralesional M1 recruitment reflects the recruitment of un-crossed CST fibers (Lotze et al., 2006; Takeuchi et al., 2005). Indeed, our recent findings demonstrate a significant relationship between contralesional M1 neuronal integrity and proximal impairment of the impaired arm (Werhahn et al., 2003a), as we would expect if the uncrossed pathways would be involved (Schambra et al., 2003). However,

contralesional M1 may reflect either a diffuse recruitment of the motor networks driven by higher orders areas during a task performance (Radlinska et al., 2012), or a dendritic overgrowth due to overuse of the healthy hand and unmasked by lack of transcallosal inhibition from the ipsilesional M1. The recruitment of this area also depends on the location and extent of injury(Calautti and Baron, 2003; Nelles et al., 1998; Nowak et al., 2008). Finally, increased activity in contralesional M1 is generally present in those with poor behavioral outcome (Craciunas et al., 2013; Foltys et al., 2003). Although the exact role of contralesional M1 in recovery remains elusive, its connection with ipsilesional M1 is critical for functional improvement (Calautti and Baron, 2003; Nair and Lincoln, 2007; Ward et al., 2003a).

Learning-related changes in relationships between functional connectivity and motor recovery

An intrigued result was the "normalization" of the relationships between functional connectivity and elbow extension with practice. This suggests that although the training-related functional remodeling of motor networks within and between hemispheres in our patients is different from that reported in controls, this remodeling somehow underlies the behavioral recovery.

Limitations

One limitation of this study is that only cortical motor network has been covered. Thus, our results represent only a part of a puzzle of neural changes underlying motor learning.

Another limitation is related to methodology used to quantify functional connectivity. For example, contaminated BOLD signal may result in a noisy functional connectivity model, which makes functional connectivity quantification not robust.

Sample size is certainly a major concern. Further, the stroke category studied is quite selective (i.e., subcortical stroke) and limit our generalizability of our findings to other type of stroke. Although such selection criteria have some generalizability issues, it is our goal to establish the first stage that would offer a solid foundation of specific interpretable findings upon which future studies in this field

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views of the NIH or its institutes.

Table 1. Correlations between functional connectivity within cortical motor network and elbow extension in healthy and stroke participants before (PRE) and after (POST training). P-value represents the differences between groups. Differences between stroke vs healthy (z-transformation)

		PRE-training			POST-training		
		Healthy	Stroke	P-Value	Healthy	Stroke	P-Value
Ipsilesional/Left M1- Contralesional/RightM1	r	0.20	-0.78	0.01	0.01	-0.63	0.12
	P-value	0.53	0.00		0.97	0.04	
Contralesional/RightM1 - Ipsilesional/Left PMd	r	-0.56	-0.89	0.11	0.30	-0.52	0.07
	P-value	0.06	0.00		0.34	0.10	
Contralesional/RightM1 - Ipsilesional/Left SMA	r	-0.54	-0.81	0.28	0.31	-0.52	0.07
	P-value	0.07	0.00		0.33	0.10	
Contralesional/RightM1 - Contralesional/RightPMd	r	-0.17	-0.93	0.00	0.07	-0.33	0.40
	P-value	0.60	0.00		0.83	0.32	
Contralesional/RightM1 - Contralesional/RightSMA	r	-0.27	-0.71	0.20	0.26	-0.35	0.19
	P-value	0.39	0.01		0.42	0.29	
Ipsilesional/Left M1 - Ipsilesional/LeftPMd	r	0.21	-0.69	0.03	0.17	-0.43	0.19
	P-value	0.51	0.02		0.60	0.18	
Ipsilesional/Left M1- Ipsilesional/Left SMA	r	0.20	-0.66	0.04	0.18	-0.44	0.17
	P-value	0.53	0.03		0.57	0.17	
Ipsilesional/Left M1- Contralesional/RightPMd	r	-0.23	-0.86	0.03	-0.24	-0.29	0.90
	P-value	0.46	0.00		0.45	0.38	
Ipsilesional/Left M1 - Contralesional/RightSMA	r	-0.13	-0.53	0.35	0.11	-0.30	0.38
	P-value	0.68	0.09		0.74	0.36	

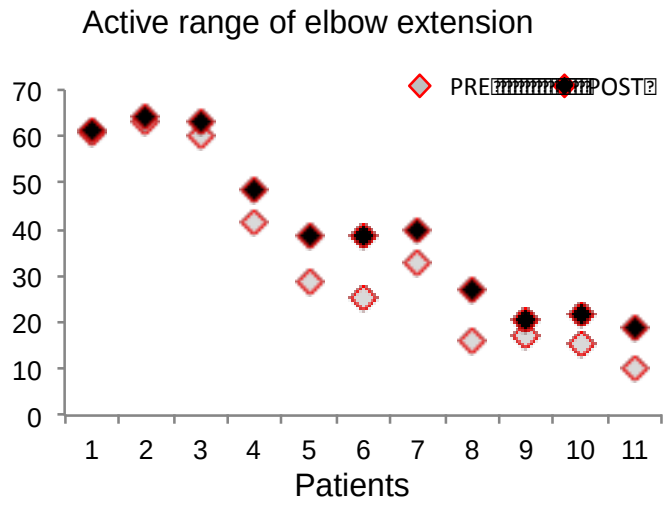

Fig. 1 Elbow extension (deg) in PRE (red-contoured grey diamonds) and POST (red-contoured black diamonds) sessions in stroke patients.

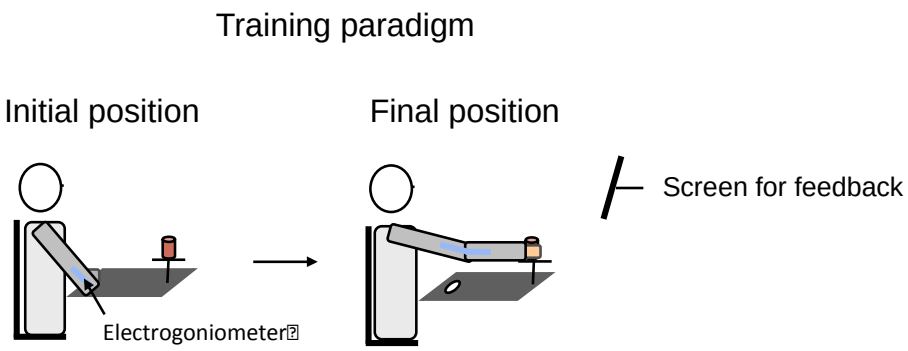

Fig. 2 Motor training experimental paradigm. The participant practiced a reach-to-grasp task of different objects placed in front of the participants at a confortable distance to reach. Faded terminal feedback about elbow extension was provided.

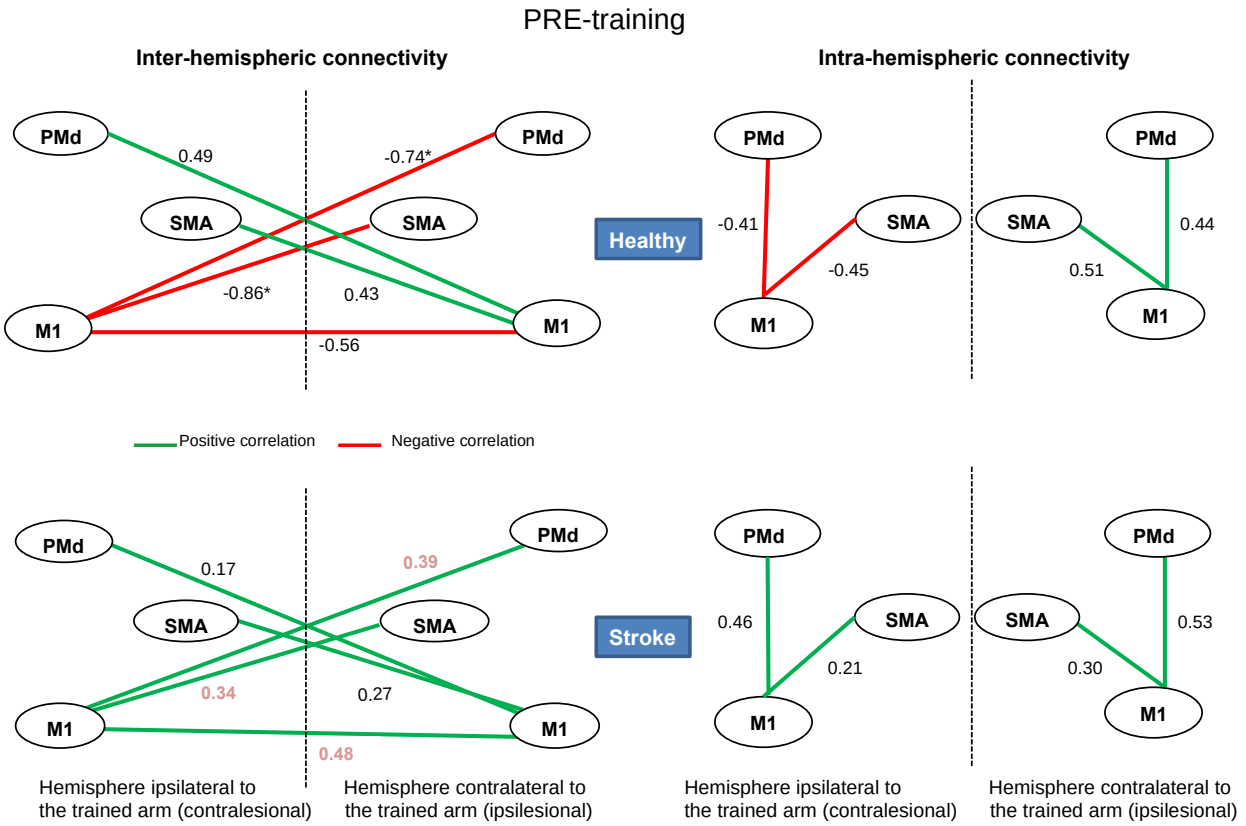

Fig. 3 Functional connectivity between primary motor cortex (M1), dorsal premotor cortex (PMd), supplementary motor area (SMA) between (inter-hemispheric, left panels) and within (intra-hemispheric, right panels) hemispheres in healthy (top panels) and stroke (bottom panels) groups in PRE-training session. Green lines signify positive correlations; red lines signify negative correlations; *signifies significant correlations ($p<0.05$); bold-pink numbers signify significant between-group differences ($p<0.05$).

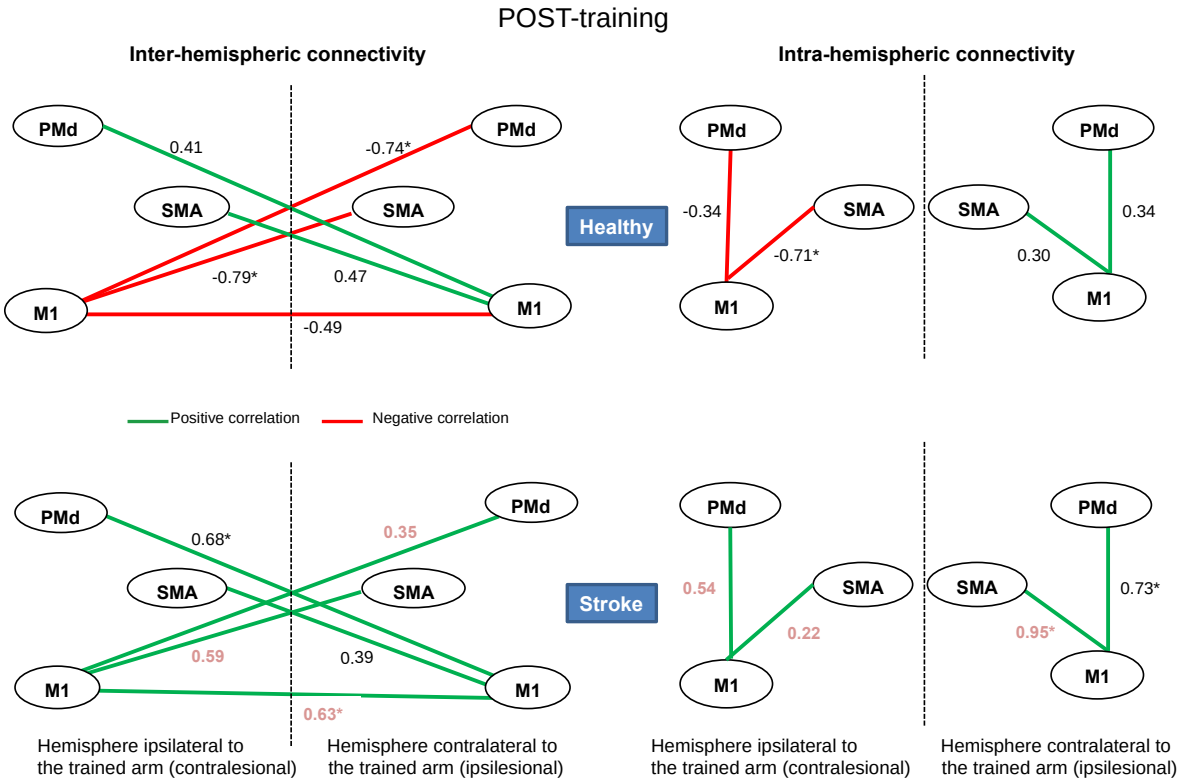

Fig. 4 Functional connectivity between primary motor cortex (M1), dorsal premotor cortex (PMd), supplementary motor area (SMA) between (inter-hemispheric, left panels) and within (intra-hemispheric, right panels) hemispheres in healthy (top panels) and stroke (bottom panels) groups in POST-training session. Green lines signify positive correlations; red lines signify negative correlations; *signifies significant correlations ($p<0.05$); bold-pink numbers signify significant between-group differences ($p<0.05$); green circle indicate between session difference (see M1-SMA in the ipsilesional hemisphere in patients).

CHAPTER V

CONCLUSIONS

Introduction

Our long-range goal was to investigate whether neural substrates underlying motor relearning in stroke are similar with those underlying motor learning in healthy controls. Specifically, this project was to determine whether the changes in cortical motor function at different levels, regional (micro-circuitry) and network (macro-circuitry) following an arm-focused motor training are similar in stroke and controls (see Chapters III and IV). Finally, we investigated whether these changes are related to recovery of the pre-morbid movement pattern or "true" recovery (see Chapters II-IV). To address these questions we used a multi-modal approach, including regional brain activation and functional connectivity within cortical motor networks (functional MRI) as well as kinematic and clinic metrics of arm motor impairment and recovery, in patients with chronic ischemic subcortical stroke.

In summary, we confirmed previous findings on cortical motor micro-circuitry in subcortical stoke (see Chapter I), i.e., increased motor-related activation bilaterally, particularly in patients with poor motor outcome, and we demonstrated for the first time the complementary role of kinematic metrics to the current clinical metrics of the arm motor impairment in understanding the neural micro-circuitry changes (Chapter II) and the evidence of similar neural micro-circuitry and behavioral changes following an arm-focused motor training in patients and age-sex matched healthy (Chapter III). These regional brain changes were also accompanied by macro-circuitry changes, i.e., changes in strength of functional connectivity within the cortical motor networks (Chapter IV). Finally, we found a "normalization" of the relationships between training-related micro- and macro-circuitries changes and "true" recovery with training (Chapters III and IV). The major findings are summarized below.

Cortical motor micro- and macro-circuitries in chronic subcortical stroke

In accord with previous subcortical stroke studies, we found an increased M1 (Cramer and Crafton, 2006; Gerloff et al., 2006; Ward et al., 2003a), PMd (Cramer, 2008a; Johansen-Berg et al., 2002a; Lindenberg et al., 2012; Murase et al., 2004; Perez and Cohen, 2009; Ward et al., 2003c), and SMA (Fridman et al., 2004; Johansen-Berg et al., 2002b; Mima et al., 2001; Nelles et al., 1999; Seitz et al., 1998; Weiller et al., 1992; Weiller et al., 1993a) activation. The activation pattern associated with impaired hand movement consistently included **contralateral (or ipsilesional) M1** (Chollet et al., 1991; Weiller et al., 1992). In our patients, the mean activation was significantly different from that seen in uninjured individuals, particularly in patients with poorer motor outcome, i.e., impaired selective movements of the fingers (Fig. 1B). Despite intense research on this topic, the specific mechanism by which ipsilesional M1 activation is increased in some patients remains the topic of some conjecture. Since the nervous system retains the ability to exploit the redundancy within the somatotopy of M1 to generate an output via the intact portion of corticospinal tract (Seitz et al., 1998; Weiller et al., 1993a), an excessive recruitment of this area could be found in an attempt to perform the task despite CST damage. Changes in somatotopic representation of the hand also occur in other areas, i.e., premotor areas (Cramer et al., 1997; Cramer et al., 2000), and might alter the anatomical connections between premotor areas and M1, potentially enlarging the motor output zone. This enlargement could also result from a simple disinhibition that has no relationship to actual motor performance (Johansen-Berg et al., 2002a; Nelles et al., 1999). In patients with poorer motor outcome, a wider sensori-motor recruitment, including deep central sulcus (or Brodmann area 4p) (Ward et al., 2003a) may be also explained by the change in the patient' perception of the task. Precisely, these patients perceive a simple task as a complex task. Although the effort levels

of the handgrip tasks were matched at 25% of their individual MVC, we did not control some aspects of cognitive performance, such as attention. Thus, the possibility that attention differences contributed to larger BOLD activations in ipsilesional M1 cannot be ruled out.

In line with previous studies (Newton et al., 2006; Sanes and Donoghue, 2000), we also found increased **ipsilateral (or contralesional) M1** recruitment during movements of the impaired hand. Indeed, in our patients, the mean contralesional M1 activation was about 13 times larger than the activation seen in controls. As in ipsilesional M1, this recruitment occurs mainly in patients with poorer motor outcome (Fig. 1A). This finding supports the hypothesis that a greater deficit is associated with a greater contribution of contralesional M1(Feydy et al., 2002a; Ghosh and Porter, 1988; Johansen-Berg et al., 2002a; Johansen-Berg and Matthews, 2002; Newton et al., 2006).

We also found increased **bilateral PMd activation**, as previously reported (Cramer and Crafton, 2006; Perez and Cohen, 2009; Ward et al., 2003a) (Cramer and Crafton, 2006; Perez and Cohen, 2009; Ward et al., 2003a). Moreover, contralesional PM activation is more prominent in patients with significant impairment (Fridman et al., 2004; Lotze et al., 2012). Similar results were found for SMA, i.e., increased activation in **ipsilesional and contralesional SMA**. These results are in accord with previuos findings (Carey et al., 2002; Gerloff et al., 2006; Johansen-Berg et al., 2002b; Seitz et al., 1998). These results should be viewed in the context of the anatomical structures and pathways of these areas. Although M1 motor pathways are critical, the premotor areas also contribute to motor control and might be recruited during motor recovery after stroke. The parallel nature of the direct (corticospinal) pathways from premotor areas and M1 emphasizes that PMd and SMA are, in some respects, at a similar level of hierarchical organization as M1(Lotze et al., 2006), although these projections to spinal cord motor neurons

are less numerous and less efficient that those from M1 (Johansen-Berg et al., 2002b; Riecker et al., 2010; Ward et al., 2006). Another possibility is the indirect (corticoreticulospinal) projections to cervical propriospinal premotoneurons, which have divergent projections to muscle groups operating at multiple joints(Calautti et al., 2007; Loubinoux et al., 2003). Finally, cortico-cortical connections between these areas might also play an important role in post-stroke recovery(Boudrias et al., 2010a; Boudrias et al., 2010b; Dum and Strick, 2002; Zemke et al., 2003).

After stroke, the motor system adapts not only in terms of what structures are engaged but also in **how these structures communicate between them.** In accord with previous studies (Dum and Strick, 2002; Maier et al., 2002; Mazevet et al., 2003; Stinear and Byblow, 2004), before training we found a decrease in connectivity strength or "hypoconnectivity" between M1 and SMA and an increase in connectivity strength between M1 and PMd within ipsilesional hemisphere, as well as a complete lack of intra-hemispheric inhibition between contralesional M1 and ipsilesional motor areas in our patients. If we consider the functional connectivity as a measure of the local efficiency of information transfer, it is likely that "hypoconnectivity" is related to low transfer of information while enhanced connectivity relates to high transfer. These changes are may be due to deletion of nodes or connections (Boussaoud et al., 2005; Marconi et al., 2003; Mochizuki et al., 2004; Sharma et al., 2009b). In an anatomically model, deletion of nodes produced widespread disruptions of functional connectivity (Grefkes et al., 2008b; Mintzopoulos et al., 2009b) that were consistent with effects reported in focal human brain lesions (Bullmore and Sporns, 2009; Rehme et al., 2011). The mechanism underlying the lack of intra-hemispheric inhibition or shift of inter-hemispheric balance in cortical excitability toward unaffected hemisphere is not well understood. Since a subcortical stroke does not affect the

commissural fibers connecting the homologues motor areas in both hemispheres, a potential explanation is that the shift in inter-hemispheric balance results from indirect adaptive changes induced by thalamic or cerebellar pathways (Bullmore and Sporns, 2009). This disturbance has been linked with a down-regulation of GABA receptors function (Honey and Sporns, 2008), or an up-regulation of N-methyl-D-aspartate receptors (Kaiser et al., 2007) with an inadequacy of GABA-ergic transmission to balance overactive glutamate synapses (Honey and Sporns, 2008).

Motor-learning changes in cortical motor micro and macro-circuitries in healthy controls

Since our major hypothesis was the comparison between training-related changes in cortical motor networks in patients versus those in healthy controls, in this section, we describe the training-related brain changes in a group of 12 controls.

We found increased movement-related activation in primary and non-primary motor areas in the hemisphere contralateral to the tested (trained) arm after training. These results are consistent and complement other work derived from learning studies in healthy controls and animal models (Hagemann et al., 1998; Halsband and Lange, 2006a; Hazeltine et al., 1997a; He et al., 2007a; He et al., 2007b; Nowak et al., 2008; Que et al., 1999; Redecker et al., 2002). As expected, the training-related increased activation was found only in the cortical areas controlling the trained arm/hand (Grafton et al., 2002). Indeed, ipsilsilateral primary and non-primary motor contributions showed slight increases of activation in activation. This result is also consistent with previous studies indicating that dominant cortices influences learning of both the dominant and non-dominant hand (Seitz and Freund, 1997).

Specifically, in M1, assemblies of neurons control specific movements of different joints and muscle groups (Karni et al., 1995; Van Mier et al., 1999). Thus, an assembly projects to several

pools of spinal motoneurons (Floyer-Lea and Matthews, 2005; Shadmehr and Holcomb, 1997). To control a multi-joint movement, reach-to-grasp movement in our case, different assemblies are interconnected via horizontal intra-cortical projections (Doyon and Benali, 2005b; Xerri, 2011). Movements and arm parts are represented multiple times and are intermixed with representations of related movements of parts forming a complex mosaic pattern. This pattern, called motor map, reflects the output of M1. During motor training, these maps are reorganized, i.e., enlarged (Cheney et al., 2004; Hund-Georgiadis and von Cramon, 1999; Nudo, 2008), increased signal strength (Keller, 1993), increased spiking reliability (Cheney et al., 2004). Such reorganization depends on restructuring of M1 microcircuitry. Structural modifications, as spine formation and synaptogenesis (Keller, 1993), and modulation of synaptic weights, i.e. long term potentiation (Cheney et al., 2004), form the basis of such changes in motor maps. For example, changes in local GABA concentrations induce unmasking of existing horizontal connections within the cortex, which allows rapid changes in motor representations (Kleim et al., 1998; Pascual-Leone et al., 1995). All these training-related changes support the notion of M1 as a locus of the long-tem acquired representation of specific motor skills, i.e., encoding novel (learned) mappings between limb motion and required muscle forces (Nudo et al., 1996a; Schieber, 2002).

However, changes in movement-related activation described here are fundamentally different than changes in evoked movements in response to cortical stimulation used to described learning-related map changes. In our case, increase in amplitude of movement-related activation indirectly reflects an increase in neuronal populations that control a movement. With training, the constituents of these populations could also be changed and morphological changes and/or alterations of connectivity are the neural substrates subserving the plastic changes in M1. Thus,

some newly generated synapses, that have functionally relevance for the learned movement, are formed, and synaptic transmission within horizontal connections are strengthened. Thus, the information from one region of M1 would be spread more effectively to other regions (Kargo and Nitz, 2004). These changes translate in a better connectivity among neurons across M1 resulting in activation in concert of the spinal motoneuron pools to enabling the performance of the trained movement. Some studies have suggested that, as learning proceeded, blood flow in M1 increased (Kleim et al., 2004b; Rioult-Pedotti et al., 2000). However, no significant changes were found in M1 activation when the rates of movements in the trained and untrained conditions were controlled (Floyer-Lea et al., 2006; Huntley, 1997).

It is likely that these changes are not specific only to M1. Synaptic plasticity in the horizontal oriented axon collaterals may operate thought many areas within the motor network to restructurate representation patterns. Thus, the neuronal plasticity in the primary neurons directly involved in projection pathways and horizontal connections is important contributor learning-related processes in the non-primary motor areas, such as PMd and SMA. A consistent increase in activation in SMA was found to occur with practice (Hatakenaka et al., 2007; Monfils et al., 2005; Rioult-Pedotti et al., 1998; Schlaug et al., 1994). Important to note is that such increase was reported particularly in the left hemisphere (Friston et al., 1992; Kawashima et al., 1994), which is in agreement with our results. Although some studies have suggested that, as learning preceded, activation increase in both left and right PM (Grafton et al., 2002; Jenkins et al., 1994), we found increased activation only in the left PMd. Since bilateral PM activation was described in early stages of motor learning, when spatial processing and high reliance on sensory feedback are critical for learning (Hazeltine et al., 1997b), we did not expected to find bilateral increased in PMd recruitment since data presented here have been acquired in late stages of motor learning.

However, a major input to the PMd is from the posterior parietal cortex (Grafton, 1992) and these cortico-cortical connections has been proposed to code reaching movements as the result of a combination of visual and somatic information. Learning-related changes in this network (Hund-Georgiadis and von Cramon, 1999) may also explain the changes in PMd described here.

After training, in healthy controls, only one significant correlation, between M1 and SMA in the hemisphere ipsilateral to the trained had (or right) was emerged after training. Thus, we did not observe a major increase in functional integration in the motor network with practice. It is possible that the gradual built up of the trained movement in the motor network does not require sustained increase in functional connectivity. Alternatively, we cannot rule out the hypothesis that functional integration may increase/decrease in the associative network recruited during learning or within arm representation in the primary and/or non-primary motor areas. It is clear that interactions between these networks, motor and associative, are necessary during learned movements (Grafton et al., 2002; Hazeltine et al., 1997b), even though involvement of our regions belonging to the motor/premotor circuit was not significantly increased or decreased with practice.

Thus, the final outcome of the trained task is a more extensive representation in primary and non-primary motor areas in the hemisphere contralateral to the trained arm.

Motor learning-related changes in cortical motor micro and macro-circuitries in stroke patients

The widespread recruitment of primary and non-primary motor areas during motor performance before training was followed by a contralesional reduction and an ipsilesional increase in this task-related recruitment over training. Only the reduction in the contralesional M1 recruitment (by 50.2%) was significantly for the group. Although half of our patients

recruited significantly less contralesional non-primary motor areas, there but there were no consistent effects across the group. In contrast, ipsilesional activation tends to increase as a function of training in half of our patients. Such `focusing' of brain activation has been reported before (Marshall et al., 2000; Calautti et al., 2001a; Feydy et al., 2002(Deiber et al., 1997; Halsband and Lange, 2006a; Inao et al., 1998; Tanne et al., 1995), but has never been described in relation to both the true recovery and the brain changes in a control group who underwent a similar motor training.

If contralesional motor recruitment is the consequence of impairment to ipsilesional corticomotoneuronal pathways, then the explanation for the reduction of this recruitment (by 17-50% from PRE- to POST-training) is that recovery of motor function is a direct result of restitution of ipsilesional anatomical link. Indeed, we generally found an increase in ipsilesional recruitment of both primary and non-primary motor areas, similar with learning-related cerebral reorganization observed in our healthy controls. A number of mechanisms may be involved in driving this reorganization.

Similar to controls, we found an increase (18%) in activation of M1 contralateral to the trained (impaired) arm in our patients. This is an intriguing finding since M1 output, in our patients is damaged and it is likely that this area already underwent a lesion-induced reorganization. Moreover, given that M1 is a key structure for the storage of motor engrams, relearning of a motor task may be hindered by “residual” elements of previously stored memory traces (Shadmehr and Holcomb, 1997) or dysfunctional spontaneous reorganization (Doyon and Benali, 2005a). We can interpret ipsilesional M1 increase as a reestablishment of previously damaged, but not destroyed output of this area. Alternatively, this enlargement is an attempt to access to undamaged fast cortico-motoneuronal pathways since the nervous system, although

injured, retains the ability to exploit the considerable redundancy within the somatotopy of M1 during learning, to generate an output via the intact portion of corticospinal tract (Doyon et al., 2003; Ward, 2006). Finally, neuronal plasticity is expressed not only in the primary neurons directly involved in projection pathways but also in the horizontal connections. (Liepert et al., 1998; Ward et al., 2003b). Overall, this result is in accord with a previous study showing that decreased hand, precisely the abductor digit minimi muscle, representation in M1 significantly increased after a period of 8-10 weeks of rehabilitation after stroke (Luft et al., 2004a). However, we did not find a decrease in hand representation before training in our group of patients. This could be explained by the differences in methodology, i.e., movement-related activation used in our study compared to evoked-movements in response to transcranial magnetic stimulation in the other study (see above), patient sample, chronic subcortical versus subacute cortical and subcortical stroke.

With regard to the recruitment of contralateral non-primary motor areas, similar changes, i.e., increase, albeit greater, was exhibited in our patients (PMd, 8.1% in controls vs. 15.0% in patients, SMA, 22.9% vs. 41.5%, see Fig. 4). Since experimental data suggest that these areas operate in a parallel rather than a hierarchical fashion with primary motor area, they are able to functionally substitute for each other (Krishnan, 2006; Schubring-Giese et al., 2007). Therefore, the over-increased recruitment of these areas could be explained by a recruitment of more ischaemia-resistant small diameter myelinated corticospinal fibers of these cortices to compensate loss of large diameter fibers from M1. Alternatively, changes in M1 topography might alter the anatomical connections between premotor areas and M1, potentially enlarging the motor output zone (Newton et al., 2006; Sanes and Donoghue, 2000). Indeed, anatomic connections between M1 and SMA or PMd have been well-characterized in non-human primates

(Fries et al., 1993; Grossman et al., 2002; Rioult-Pedotti et al., 1998; Strick, 1988; Traversa et al., 1997). These non-primary motor areas also contain neurons whose activity is related to execution of relatively simple movements similar to M1 neurons (Dum and Strick, 2002; Johansen-Berg et al., 2002a; Newton et al., 2006). In addition, corticospinal projections from each non-primary area display a high degree of topographic organization (Boussaoud et al., 2005; Dum and Strick, 2005; Marconi et al., 2003). Although we have very little direct information about anatomic connections in the human brain (Mochizuki et al., 2004), these findings indicate that function of any of these areas can only be understood in the context of a distributed network, which acts in concert to generate motor commands. In summary, these two premotor areas, PMd and SMA, are likely to remodel with training and thereby compensate for the injury of the M1 output.

Our analysis revealed enhanced functional connectivity between motor areas within hemisphere contralateral to the trained arm (or ipsilesional). Specifically, stroke survivors showed an enhanced positive connectivity (facilitation) between M1 and non-primary motor areas. These results are consistent with work derived from learning studies in healthy controls and animal models (Boudreau et al., 2001; Cadoret and Smith, 1997; Crick and Jones, 1993; Dum and Strick, 1991; Halsband and Lange, 2006a; Hazeltine et al., 1997a; He et al., 1993; He et al., 1995; Hoover and Strick, 1993), showing a training-related increase in the cortical areas controlling the trained arm/hand. The increased importance of ipsilesional M1 within the motor network after recovery was also indicated by stronger functional connectivity of this area with contralesional motor areas (Grafton et al., 2002). Indeed, functional connectivity between M1s and between ipsilesional M1 and contralesional PMd reached statistical significance with practice, although positive relationships.

Interesting to note is an increase in functional connectivity between contralesional PMd and both M1s, although did not reach statistical difference. This result is consistent with animal studies showing enhanced contralesional functional connectivity (Seitz and Freund, 1997; Van Mier et al., 1999). Although we found an increase in connectivity of contralesional M1, the role of this area in motor recovery remains controversial and incompletely understood (Karni et al., 1995). This debate is due, in part, to inconsistent results of TMS studies that found that inactivation of contralesional M1 improved (Floyer-Lea and Matthews, 2005; Shadmehr and Holcomb, 1997), altered(Doyon and Benali, 2005b), or left unchanged (Wang et al., 2010) the motor performance of the impaired arm. In the healthy brain, a TMS-induced M1 "virtual lesion" results in increased excitability of the "contralesional M1" without changes in hand motor function (van Meer et al., 2010a). Therefore, it is conceivable that the increased contralesional M1 activation observed after stroke may reflect altered inter-hemispheric inhibition providing compensatory support for impaired hand movements in the presence of ipsilesional CST damage. The mechanisms underlying altered inter-hemispheric inhibition between M1 after stroke is not well understood, e.g., direct involvement of the commissural fibers connecting M1s after a cortical stroke (van Meer et al., 2010b) or indirectly by thalamic or cerebellar pathways in subcortical stroke (Grefkes and Fink, 2011b). Some argue that the contralesional M1 recruitment reflects the recruitment of un-crossed CST fibers (Mansur et al., 2005; Takeuchi et al., 2005). Indeed, our recent findings demonstrate a significant relationship between contralesional M1 neuronal integrity and proximal impairment of the impaired arm (Lotze et al., 2006), as we would expect if the uncrossed pathways would be involved (Werhahn et al., 2003a). However, contralesional M1 may reflect either a diffuse recruitment of the motor networks driven by higher orders areas during a task performance (Schambra et al., 2003), or a dendritic overgrowth

due to overuse of the healthy hand and unmasked by lack of transcallosal inhibition from the ipsilesional M1. The recruitment of this area also depends on the location and extent of injury(Calautti and Baron, 2003; Nowak et al., 2008; Radlinska et al., 2012). Finally, increased activity in contralesional M1 is generally present in those with poor behavioral outcome (Craciunas et al., 2013; Nelles et al., 1998). Although the exact role of contralesional M1 in recovery remains elusive, its connection with ipsilesional M1 is critical for functional improvement (Calautti and Baron, 2003; Foltys et al., 2003; Nair and Lincoln, 2007).

In sum, in the chronic stage of stroke, the motor cortex has still the ability to undergo a remarkable reorganization even after 1180 movement repetitions. This reorganization is mainly reflected by increase in motor-related activation and strength of the functional connectivity within the network contralateral to the trained arm (or ipsilesional). We also observed a contralesional decrease in motor recruitment as well as strengthen of functional connectivity between the ipsilesional M1 and both contralesional M1 and PMd. Thus, we may conclude that learning-related brain changes, particularly for the hemisphere contralateral to the tested arm, in stroke were somehow similar with those in healthy controls.

Kinematic vs. clinical metrics of arm motor impairment and brain reorganization after stroke

We chose FMUE as one of our measures of arm motor impairment because this scale is one of the most widely used measures after stroke (Ward et al., 2003a). However, as stated above, this measure suffers from serious shortcoming: ceiling effort, no info about movement quality, observer ratings. The latter is a great threat of bias, particularly in trials in which a double-blind protocol is not possible (Gerloff et al., 2006).

We argued that using kinematic measures of motor deficit during a reach task would

minimize these shortcomings. Although reach requires coordination of multiple joints, this task could be considered as an easy task because it does not need to be learned. We focus on the elbow movement based on significant correlation between elbow kinematic measures and FMUE scores (Cramer and Crafton, 2006; Ward et al., 2003a). It is important to emphasize that we use this kinematic measure to identify "true" motor deficit. As expected, since our patients had moderate hemiparesis, we found significantly decreased active range of elbow extension compared to controls. This may reflect a deficit in motor control. Indeed, since reaching trajectory involving more than one joint consistently have invariant characteristics, such as straight paths and bell-shaped velocity profiles (Morasso, 1981), it is likely that reaching trajectory are planned in advance. Indeed, the left hemisphere is likely to play a special role in motor programming; one focuses upon its dominance for movements which are independent of sensory feedback and the other emphasizes its specialization for processing rapid temporal information (Cramer, 2008a; Lindenberg et al., 2012; Perez and Cohen, 2009). Most of our patients had the lesion located in the left hemisphere, and this could explain decrease the active joint range. Finally, limitations in elbow extension may have been caused by agonist muscle weakness (Gladstone et al., 2002) and/or antagonist muscle spasticity (Krakauer, 2005). However, all our patients have full passive range of elbow motion. It is thus unlikely that elbow limitation due to contracture could be responsible for the decreased elbow motion that we observed.

Before training, in agreement with our hypothesis, we found that activation of M1 contralateral to the tested arm (ipsilesional) correlated stronger with elbow extension than FMUE. It is interesting to note that we found this difference in correlations even though both measures quantify impairment and more importantly, are significantly correlated one with each

other. As previously shown (Cirstea and Levin, 2000a), we found a weak, non-significant, correlation between ipsilesional M1 and clinical measure of motor impairment in our study. In contrast, significant correlation has been observed between the activated clusters in ipsilesional M1 and "true" arm motor impairment (see Chapter II). In the subgroup of patients who underwent the training, we also found significant negative correlations between contralesional M1 and bilateral PMd activation and most functional connections and active range of elbow extension (see Chapters III and IV). Moreover, the relationships between functional connectivity and elbow extension were stronger (and significant) than those between functional connectivity and FMUE scores (see Results, Chapter IV). These results are the first demonstration of a stronger relationship between motor-related activation and functional connectivity within the motor system and a quantitative measure of movement quality than clinical measure of motor impairment after stroke.

After training, in healthy controls, we did not find significant correlations between learning-related brain changes and task performance. These findings are not consistent with prior transcranial magnetic stimulation studies in controls (Levin, 1996), which found correlations between increased size of representational maps in primary sensorimotor cortex and the performance of the trained task. However, as mentioned before, there is an enormous difference between movement-related activation described here and evoked movements in response to cortical stimulation. In accord with Shadmehr and Holcomb (1997), we interpret that brain reorganization of the representation of a motor skill increases stability of the representation of this motor skill rather a direct correlation with task performance.

In patients, we also found no significant correlations between both ipsilesional and contralesional activation of motor cortex and elbow extension performance. In other words, it is

likely a “normalization” or a return to “normal” pattern of these relationships. However, these patients also showed a significant decrease in motor impairment, clinically evaluated. Moreover, this improvement was significantly correlated with contralesional activation in M1. In other words, a decrease in contralesional M1 activation was related with clinical improvement. This correlation suggests a potential compensatory role of contralesional M1 activation in these patients. However, further studies are required to elucidate the role of this area in recovery in chronic stage of stroke.

An intrigued result was the “normalization” of the relationships between functional connectivity and elbow extension with practice. This suggests that although the training-related functional remodeling of motor networks within and between hemispheres in our patients is different from that reported in controls, this remodeling somehow underlies the behavioral recovery.

Although there is a lack of power to detect differences between the correlation coefficients, probably due to our small sample size, our data suggest that kinematic measures not only objectively identify the motor deficits but enhance the reliability of the fMRI data interpretation as well.

Experiment design - explanations

We selected the handgrip task based on the following reasons: i) since handgrip returns earlier than fractioned finger movements (Haaland and Harrington, 1989), we studied patients with the degree of impairment ranged from severe to none, and thus they are representative of a wide range of performance after stroke, ii) this task compares well with other clinical measures of arm function (Haaland and Harrington, 1994; Haaland et al., 2004), and iii) this task allows to

minimize movement artifact during scanner; none of our patients had moved their head more than 2 mm during the scanner. In addition, recent work on the somatotopic organization of M1 argues against divisions of modules controlling hand, elbow, or shoulder (Bourbonnais and Vanden Noven, 1989). Indeed, the cortical mechanisms controlling the hand are integrated with those of the elbow (and shoulder), as part of the system underlying reaching, prehension, and object manipulation (Bobath, 1990a). Moreover, the handgrip strength is closely associated with the strength of elbow flexors/extensors (Calautti et al., 2007) as well as with arm motor performance (Heller et al., 1987; Pascual-Leone et al., 1995) Thus, we were able to study the correlations between brain activation during a handgrip task, elbow kinematics during a reach-to-grasp task, and clinical test of arm motor impairment.

We have chosen subcortical lesion location because our regions of interest as well as their horizontal connections are left intact (Sunderland et al., 1989a). In addition, we could evaluate a homogeneous group with comparable lesion size and location.

We also studied the chronic state to avoid the confounding effect of rapid spontaneous recovery (Byrnes *et al.* , 2001). However, since restorative therapies are more effective when introduced earlier after stroke (Capaday, 2004; Devanne et al., 2002) such studies designed on motor learning principles are essential for identifying patients who will best benefit from such therapies.

We decide to select a reach-0to-grasp task for the motor learning paradigm, because this task is not entirely novel, and it should be re-acquired during recovery from stroke. In addition, this task involves the coordination of arm and hand movements. So our aim was not to train patients in a novel task but to identify whether intensive training of an altered movement component (i.e., elbow extension) improved movement quality and efficiency and whether this improvement is

underlined by functional changes within the motor system. Although we focused only on the reaching component of the task in the present work, grasp data has been also acquired.

We used Cortex-based Independent Component Analysis (ICA, Brain Voyager QX) to analyze functional connectivity within the motor system. This decision was based on: 1) spatial ICA finds systematically coherent brain regions without constraining the temporal domain; 2) ICA is available as a plug-in in BrainVoyager QX software (Bohannon et al., 1991); and 3) ICA detects task-related components within the selected ROIs. In addition, lateralization in the spatial layout of these components was also detected (for more methodological details, please see (Boissy et al., 1999; Sunderland et al., 1989b).

Limitations

There are some weaknesses to the present project.

Since lesion location affects neural reorganization patterns after stroke, all our patients had an ischemic lesion at the subcortical level. In addition, the focus on subcortical infarcts provides statistical power by minimizing patient variance. However, studying subcortical stroke limits our ability to explore the effects of infarct location on the relationships brain function-kinematics.

Our sample included left and right hemispheric strokes. Due to small sample size, the potential differences between brain activation-kinematics of left- versus right-sided stroke were not addressed in this study.

Another limitation of this study was certainly that not all areas involved in motor learning were fully covered. Thus, our results represent only a part of a puzzle of neuronal changes underlying motor learning. Especially, the hemodynamics of the cerebellum and the basal ganglia (Byrnes et al., 2001; Mercier and Bourbonnais, 2004) were not included in the present

study. Thus, enhanced activation in our regions of interest might reflect enhanced activation in these loops.

Further drawback of the study may be the lack of evaluation of the generalizability and persistence of the skill performance. Since this is an important topic in post-stroke rehabilitation, we will specifically address it in a further manuscript.

Performance during the session was controlled via video monitoring, but not continuously recorded an evaluated on-line by EMG, due to technical limitation in the scanner. Thus, it is possible that changes in muscle groups participating in performing the task rather than motor learning *per se* are responsible for changes in brain activation patterns. However, it is more likely that same muscles, but with increasing efficacy, are used to perform a task (Cramer, 2008a). In other words, the strategies used to perform a task are basically constant across the repetitions, particularly for the handgrip task (Cramer, 2008b).

Another limitation is related to methodology used to quantify functional connectivity. For example, contaminated BOLD signal may result in a noisy functional connectivity model, which makes functional connectivity quantification not robust.

Sample size is certainly a major concern. Further, the stroke category studied is quite selective (i.e., subcortical stroke) and limit our generalizability of our findings to other type of stroke. Although such selection criteria have some generalizability issues, it is our goal to establish the first stage that would offer a solid foundation of specific interpretable findings upon which future studies in this field.

Conclusion Statement

Despite of a huge number of studies on non-human and human primates on cortical reorganization in normal (learning) and pathological (stroke) conditions, we still have only limited knowledge why some patients recover more completely and others do not. It is also unclear whether improvement results from adaptive reorganization or behavioral compensation.

In conclusion, the present dissertation work support the hypothesis that motor system is plastic even in the chronic stage of stroke and these changes provide a substrate for recovery. In addition, kinematic measures may shed light on the relationship between plastic brain changes and motor strategies used during a goal-directed reach. These findings promote the use of neuroimaging and kinematic metrics to improve our understanding of the neural substrates underlying reorganization in remaining intact brain structures after stroke. Such an approach may further enable monitoring recovery or compensation based on this reorganization and evaluating new treatment regimes that assist motor recovery.

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