ENGAGEMENT OF THE CORTICOSPINAL SYSTEM DURING ACTION OBSERVATION: COMPARING THE INTACT AND DAMAGED MOTOR SYSTEM

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I, Karine Gazarian confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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ABSTRACT

Stroke is the commonest cause of physical disability in the world and hemiparesis resulting from injury to the corticospinal system is a major contributor to that. Restitution of motor function is at least in part dependent on plasticity mechanisms, which might be aided by a number of interventions. For example, Action observation treatment (AOT) has been proposed to be a useful adjunct to motor treatment. Watching other people perform actions engages the motor system of the observer in the way that mirrors activity during action execution. Implemented together with motor training AOT might augment plasticity mechanisms in surviving brain regions leading to greater motor improvements. To date, however, outcomes of AOT have been variable and it is unclear which factors drive AOT related benefits in patients. One possible factor may be patients' ability to execute observed hand actions.

This thesis examined whether the ability to execute hand actions is necessary for engagement of the motor system during observation of those actions. Transcranial magnetic stimulation was used to assess excitability in the intact and damaged corticospinal system by measuring motor evoked responses in hand muscles. Functional magnetic resonance imaging was employed to examine magnitude and patterns of cortical activity during action observation in the intact and damaged motor system.

There was no relationship between the ability to execute hand actions (i.e. impairment) and engagement of the corticospinal system during observation of those actions. Instead, findings indicate that pre-stroke dominance of the paretic hand determines the response to action observation. Firstly, activity during action observation was greater in the dominant affected hand. Secondly, altered dexterity in the non-dominant hand (when dominant hand was impaired) led to changes in contralateral neural representations and in magnitude of brain activity during action observation. Finally, during action observation, corticospinal system excitability was markedly reduced with age in the non-dominant hand muscle required for dexterous execution in healthy individuals. These findings suggest that response to AOT is likely to be variable among patients: modulated by dominance of the impaired hand, use of the unimpaired hand and age of the patient.

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CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

The upper limb weakness or paresis, contralateral to the lesioned hemisphere, is one of the most prevailing disabilities following stroke, dramatically affecting quality of patient's everyday life (Kelly-Hayes et al., 1998). It is suggested that 60% of all stroke sufferers do not recover completely and remain with a permanent motor or sensory dysfunction, greatly impacting on the ability to use hand functions necessary to perform daily activities (Stein, 1998).

Hemiparesis results from lesions to the motor pathway - the projections originating in the cortex or the brainstem and synapsing on the motoneurons in the spinal cord (Lawrence and Kuypers, 1968). Among other descending motor pathways, the most prominent is the corticospinal tract (CST). Injury to the corticospinal tract leads to impaired movements of distal muscles, especially those that require fine motor control and finger individuation (Davidoff, 1990; Lawrence and Kuypers, 1968; Lemon, 2008; Lemon and Griffiths, 2005). The extent of functional impairment in the upper limb and the prospect of recovery depends on the integrity of the corticospinal tract after damage (Schulz et al., 2012; Stinear et al., 2014, 2006). If enough fibres survive, partial or even full recovery is plausible (Stinear et al., 2014).

Recovery of function after injury to the corticospinal tract is largely attributable to the reorganization of surviving brain areas. For instance, after damage to the hand areas of primary motor cortex, the thumb representation that would have been lost with the inju-

ry spreads into the adjacent region originally devoted to elbow and shoulder control (Glees and Cole, 1950; Nudo et al., 1996). Non-primary motor areas, as well as primary somatosensory region also support recovery of impaired function (Abela et al., 2012; Cramer et al., 2000; Frost et al., 2003). In addition, changes in the ventral and dorsal premotor regions of both ipsilesional and contralesional hemispheres have been associated with gains in motor function after stroke (Fridman et al., 2004; Johansen-Berg et al., 2002; Nishimura et al., 2007; Ward et al., 2003).

Furthermore, such restructuring of neuronal organization is dependent upon the task-specific use of the impaired limb after injury. The role of persistent practice of the affected hand contributes to neuronal plasticity in both healthy individuals and in patients. Professionals, such as pianists, whose job requires skilled control of individual fingers have more dense grey matter in the primary motor cortex than those who don't possess the skill (Gaser and Schlaug, 2003). In contrast, if after lesion to the hand area of M1 the animal is not using its affected limb, neurons responding to digit movement seize firing and adjacent regions do not take over (Milliken et al., 2013). However, with sufficient use of the limb and physical training, digit representation again spreads and output is regained (Milliken et al., 2013; Nudo et al., 1996).

Engaging the surviving motor system is key to motor recovery. On this basis, patients with loss of motor function undergo physical therapy (Krakauer, 2005). Movement, even if it is assisted, provides sensory feedback that is necessary for initiating response in the motor system and thus, for promoting recovery. Another way to prime the residual motor system during motor rehabilitation is by enabling patients to watch a healthy

movement while attempting to execute it themselves. This is called Action Observation Treatment (AOT) (Buccino, 2014; Small et al., 2013).

AOT is rooted in the discovery of mirror neurons - cells that fire when an animal pinches a food pellet to be eaten, but also when it simply observes somebody else pinching a food pellet (Gallese et al., 1996; Pellegrino et al., 1992; Rizzolatti et al., 1996). Importantly, mirror neurons are part of the corticospinal tract (Kraskov et al., 2009; Vigneswaran et al., 2013), and possibly other descending motor pathways. They were first identified in ventral premotor area (F5) of a macaque monkey, but subsequently were also found in the inferior parietal region (PFG) (Fogassi et al., 2005; Rozzi et al., 2008), dorsal premotor area (Tkach et al., 2007) and primary motor cortex (Tkach et al., 2007; Vigneswaran et al., 2013) - an extended network implicated in sensorimotor control. It is believed that after injury, engaging the remaining intact motor system through observation of actions together with motor therapy may facilitate neuroplasticity and lead to recovery of function. This notion is the basis of the AOT. The question is, can all patients benefit from the AOT equally?

The response of the motor system to observed actions – motor resonance - seems to be dependent on the initial motor repertoire of the observer. According to Uithol and colleagues, interpersonal motor resonance is possible when "both observer and executer have representation of […] action in motor areas", triggering observer's motor system when watching another's act (Uithol et al., 2011). Watching lip smacking will engage such as system, whereby watching a dog barking – will not (Buccino et al., 2004). Motor resonance is also greater if the observer is skilled at the particular action that he/she is presented with. For instance, in ballet, some dance moves are only appropriate to men

and some only to women dancers. The motor system of male dancers is more active when they watch male-only rather than female-only movements, and vice versa for female dancers (Calvo-Merino et al., 2006). What follows is that motor resonance seems to be sensitive to the ability to produce actions that are observed.

It appears possible that prolonged disuse of certain hand actions after stroke may lead to reduction of motor repertoire necessary for AOT to be beneficial. Following corticospinal injury, limited or eliminated use of individual fingers leads to changes in neuronal representation during movement. In monkey, constrained use of limb after lesion to M1 results in shrinking of M1 digit representation, as if eradicating it from the motor vocabulary (Milliken et al., 2013). As famously proposed, "use it or lose it". If the action is no longer in the motor repertoire of the patient, the AOT may not engage the system enough to promote recovery in that patient. The focus of this dissertation was, therefore, to explore *whether engagement of the corticospinal system through action observation depends on the ability to execute the observed action.*

In the course of this thesis I will be referring to the corticospinal tract as a system that can be manipulated through visual presentation of manual actions – action observation. This is not to say that hand motor impairment arises solely from injury to the CST, nor that observed actions only engage this and no other pathways. Pure CST lesions are extremely rare in humans, given that it constitutes only a small amount of white matter fibers that pass through the internal capsule. Nevertheless, the CST provides a perfect model for research. By now it is certain that mirror neurons exist in the corticospinal tract (Kraskov et al., 2009; Vigneswaran et al., 2013), while presence of these neurons in other motor pathways has not yet been confirmed. It is also relatively simple to pre-

dict lesioned CST based on the type of impairment. For instance, it is safe to suggest that the CST is damaged when a patient presents with impaired manual dexterity, such as inability to pinch or grasp an object (Lawrence and Kuypers, 1968). The extent to which the patient is still able to perform these actions is indicative of how much of the CST has survived (Schulz et al., 2012). Thus, by studying a group of patients with wide range of dexterity impairment, it is possible to clarify if the ability to execute observed action modulates the engagement of the CST during observation.

Broadly, the interest of this thesis arises from the field of restoration of motor function following damage to the corticospinal tract. In the coming pages I will first focus on the origins and pathway of the CST as well as on how engaging motor neurons of the tract can generate neuronal plasticity associated with recovery of function. Notably, by recovery I assume the following definition - "the individual's ability to perform movements using the same effectors and muscle activation patterns in the same manner as prior to" damage (Stinear et al., 2014), not to be confused with the ability to carry out required action by other means - adapted after injury - such as by using unaffected hand.

Subsequently, I will review literature specific to the topic of my investigation, in particular, how the intact and damaged motor system is activated during action observation. Here I will summarise current knowledge that action observation not only engages motor regions in healthy individuals, but is also successful in facilitating motor recovery in patients.

I will finish this chapter by outlining today's gap in knowledge. I will draw on evidence that in order to activate corticospinal system, observed action must be in the observer's motor repertoire. I will propose that inability to produce dexterous action after stroke

alters motor vocabulary of the observer and therefore may result in the insufficient engagement of the corticospinal system for benefits of the AOT to be observed.

1.2.1 Origins and path of the corticospinal tract

At the cortical level, CST originates primarily in six regions. In primates, the densest and most substantial concentration of corticospinal neurons (approximately 31%) are found in Brodmann area 4 - primary motor cortex (M1) and are essential for execution of movement. Uniquely to the CST, projections from the M1 include giant pyramidal Betz cells – the largest neurons of the central nervous system. Their size, diameter (up to 100 µm) and direct monosynaptic connections with neurons innervating muscles allows for speedy transmission of movement related information.

Another 29% arise from Brodmann area 6 – the supplementary motor area (SMA) and ventral and dorsal premotor regions (PMv and PMd), important for planning internally generated motor commands (Tanji and Shima, 1994), and in sensory guided, goal-directed movements (Hoshi and Tanji, 2000) respectively. Notably, the remaining 40% of CST neurons originate in the primary somatosensory cortex (S1) located in Brodmann areas 3, 1, and 2 of the postcentral gyrus, as well as in the superior parietal lobule (SPL) (Brodmann areas 5 and 7) and some parts of the cingulate gyrus (Haines, 2006). These numbers are generally taken from studies in monkeys and may differ in humans.

Recently, Diffusion Tensor Imaging (DTI) technique was used in several studies in order to quantify descending fibers in human population. For instance, in the study using 28 healthy participants (56 hemispheres) researchers looked at fiber tract volume descending from SMA, PMd, M1 and S1. It was concluded that 37.3% of CST crossing fibers originate from the M1, 32% from S1, 20.5% from SMA and 10.2% from PMd (Seo and Jang, 2013).

From the cortex, corticospinal fibers project down through the subcortical white matter, including internal capsule and the cerebral peduncle. In the medulla, the majority of axons of the CST cross over and terminate in the contralateral spinal cord, making either direct connection with motoneurons or synapsing on the interneurons (Rizzolatti and Strick, 2013). Therefore, injury to the CST above decussation level leads to paresis of the opposite side of the body, whereby damage below decussation results in impairment on the same side.

The CST primarily conveys signals essential for control of voluntary movement, especially that which requires dexterity and flexibility, such as typing or playing piano (Jang, 2014; Martin, 2005). Density of CS projections into the ventral horn is directly correlated with level of dexterity in primates (Heffner and Masterton, 1975, 1983). Thus, one of the most prominent consequences of damage to the CST is loss of manual dexterity and skilled actions that require independent control of digits, especially the thumb (Davidoff, 1990; Lawrence and Kuypers, 1968).

Nevertheless, motor control is not the only function of the CST, as classically thought. CST originates in several cortical regions, contains axons that differ in their diameter and conduction velocity and terminate in different parts of the spinal cord. It is thus believed that CST is responsible for multiplicity of functions, ranging from descending control of afferent/sensory inputs and excitation/inhibition of motoneurons to involvement in reflex, plasticity of spinal cord and autonomic control (Davidoff, 1990; Lemon, 2008; Lemon and Griffiths, 2005).

It must be noted that although the majority of CST axons project from the motor cortex, primary somatosensory region appears to be the second largest source of descend into

the spinal cord (Seo and Jang, 2013). Whereby axons from M1 in monkeys terminate "densely among the lateral motor nuclei supplying muscles of the arm, hand, and digits" (Lemon and Griffiths, 2005), projections from S1 terminate in the dorsal horn of the spinal cord. They are most likely to be "involved in the descending control of proprioceptive inputs generated by movement or sensory reafference" (Lemon, 2008). Consequently, lesions to CST may also result in failure to correctly interpret feedback from the upper limb manifested in impaired "tactile placing and inability to make rapid matching of tactile inputs to motor outputs" (Lemon and Griffiths, 2005).

1.2.2 Injury to the corticospinal system leads to impaired motor function

The amount of damage to the CST is directly related to the level of associated functional motor impairment (Schulz et al., 2012), and the integrity of the residual CST is predictive of the degree and timescale of recovery (Stinear et al., 2014, 2006).

To assess integrity of the CST, a combination of Transcranial Magnetic Stimulation (TMS) and neuroimaging has been used (Stinear et al., 2014, 2006). The former technique engages the corticospinal system by stimulating the primary motor cortex of the affected hemisphere and by measuring motor evoked potentials (MEPs) from contralateral muscles. Both, latency and amplitude of induced MEPs in the affected hand are informative of the CST integrity. Similarly, neuroimaging is used to assess the volume of the residual tract by means of previously mentioned DTI method, quantifying the structural damage of the white matter path. With these two techniques as well as with the behavioural measures within the first 72 hours of injury, it has been possible to predict potential recovery of function in patients with injury to the CST (Stinear et al., 2014).

Stinear and colleagues estimated that if MEPs can be induced in the impaired limb, full or notable recovery is predicted. In the absence of MEPs in the affected hand (indicating substantial damage to the tract), recovery depends on the residual volume of the ipsilesional CST. If enough fibers survive, recovery is limited, otherwise – it is unachievable (Stinear et al., 2014).

Evidently, there is a tight link between the integrity of the CST, the level of impairment and the degree of recovery. It appears that partial or even complete recovery is possible, providing substantial portion of the tract remains intact. Although, greatest gains in mo-

tor recovery are witnessed within the first 6 months after stroke (Grefkes and Ward, 2013), meaningful improvements can be observed as late as 3 years following injury (Stinear et al., 2006).

1.2.3 Post-injury functional recovery is based on neuroplasticity

1.2.3.1 *Neural strategies promoting recovery of motor function*

Motor recovery following injury, such as stroke is based on neuronal reorganization of perilesional brain, preserving essential functionality. Such restructuring is sensitive to time and persistence of use of the impaired limb.

Immediately after injury the motor system is underactive. Moving the affected hand just three days after stroke is associated with reduction of BOLD activity as compared to healthy volunteers (Rehme et al., 2011). Shortly after, however, movement of the paretic hand markedly increases activity in both hemispheres, surpassing one seen in controls (Marshall et al., 2000; Rehme et al., 2011; Ward et al., 2003). This escalation in engagement of the extended motor system corresponds to better recovery of motor function (Grefkes and Ward, 2013). As improvement progresses, motor related overactivity again normalises to the contralateral (ipsilesional) hemisphere resembling that of healthy controls. The degree to which recovery is achieved correlates with the level of normalisation (Rehme et al., 2011; Ward et al., 2003). It appears that within the first weeks after damage, significant neuronal adjustments are made, employing all relevant areas in both hemispheres. Once the restructuring is in place, brain function normalises, depending on the achieved motor gains.

Whilst spontaneous neuroplasticity is evident independent of physical involvement of the affected hand, use-dependent reorganization dramatically increases chances of functional recovery. Since motor areas in the brain are organised somatotopically, lesion in one part of the primary motor cortex may result in spreading of lost cortical representations to the adjacent territories and even non-primary motor regions. In this way, once a

thumb representation in the M1 is damaged, perilesional neurons start responding to thumb movement (Glees and Cole, 1950). Similarly, M1 region associated with elbow and shoulder control takes on a new function, such as movement of individual fingers (Nudo et al., 1996). Such redeployment, however, appears to be dependent upon task-specific use of the affected limb. Restricted movement of healthy animals or humans causes shrinking of cortical representations in the M1 (Liepert et al., 1995; Milliken et al., 2013). Conversely, task-specific physical practice of the impaired limb allows for recovery of function consistent with the enlargement of cortical representation (Nudo et al., 1996).

1.2.3.2 *The role of non-primary motor and primary somatosensory regions in recovery of motor function*

Crucially, non-primary motor regions also play a pivotal role in motor recovery. In 2005, Dum and Strick examined six monkeys to establish connectivity between regions containing digit representations in the primary and non-primary motor areas (Dum and Strick, 2005). They found that digit representations of the PMd and PMv were densely interconnected with the one in the M1, forming a network "concerned with generation and control of hand movement" (Dum and Strick, 2005). In the instance of lesioned primary motor cortex, rapid reorganisation within the premotor areas was associated with functional recovery. Similarly, Frost and colleagues studied spontaneous recovery after injury to M1 in five squirrel monkeys (Frost et al., 2003). After 12 weeks they reported marked expansion of hand representation in ventral premotor cortex. Change in the PMv correlated with the amount of hand representation damaged in M1.

Crucially, neuronal plasticity in the premotor regions after damage is associated with motor recovery. In Liu and Ruiller study, lesion to hand representation of the primary sensorimotor cortex of an adult monkey lead to complete loss of dexterity lasting 1-2 months. After 3-4 months, around 30% of prehension dexterity was recovered. Such improvement was abolished by administering musimol infusion to ipsilesional PMd and PMv areas, but not to the M1, implicating the role of these regions in recovery (Liu and Rouiller, 1999).

The influence of non-primary motor areas on motor improvement after injury is also evident in humans. In chronic well recovered stroke patients, TMS was used to temporarily disrupt activity within the PMd of the affected hemisphere. This caused slowing of contralateral finger movements in patients but not in a group of healthy volunteers (Fridman et al., 2004). These findings implicate recovery related reorganization within PMd of the ipsilesional hemisphere. Moreover, disruption of the PMd in the unaffected hemisphere caused similar slowing of digit movement in patients with more severe impairment, indicating additional rewiring within the contralesional PMd in order to facilitate impaired movement in those with greater constraints (Johansen-Berg et al., 2002).

It was also discovered that after lesioning of the M1, cortical connections of PMv changed as well (Dancause, 2005). Cortical rewiring was observed between PMv and 1st and 2nd Brodmann areas of primary somatosensory cortex. No significant alterations in connectivity were seen in other motor regions, including SMA, PMd and cingulate motor area. Dancause and colleagues proposed that changes in connectivity between PMv and S1 were “accompanied by functional recovery and expansion of the PMv hand rep-

resentation" and hypothesized that once cortical targets from PMv to M1 were destroyed, "intracortical axons seek new targets in surviving, intact tissue" (Dancause, 2005). Authors concluded that significantly increased number of PMv – S1 connections served as a compensatory mechanism important for achieving functional recovery after central damage (Dancause, 2005).

Clearly, the damaged brain undergoes significant changes after injury and the full scope of the underlying mechanisms is still to be unravelled. What is apparent, however, is the impact that perilesional brain has on recovery of motor function. Supporting the engagement of the surviving sensorimotor system could lead to increased neuroplasticity and potential restitution of function. Action Observation Treatment is proposed to be one of such solutions.

1.3 ACTION OBSERVATION TREATMENT - A MODEL FOR FUNCTIONAL MOTOR RECOVERY

Firstly, mirror neurons were described in the F5 area of the macaque monkey (Gallese et al., 1996; Pellegrino et al., 1992; Rizzolatti et al., 1996). An entirely accidental discovery that took the world by surprise. The existence of motor neurons firing congruently with executed pinch, but also with merely observed pinch of a food pallet was an unexpected finding. Many theories regarding plausible function of mirror neurons were instantly developed, but the debate continues into this date. While some firmly propose the role of mirror neurons in understanding of others' intents (Rizzolatti and Sinigaglia, 2010), others argue that activity in mirror neurons is driven by associative learning, developed with years of executing and observing one's own actions (Heyes, 2010a, 2010b).

Although there is little consensus among scientific community regarding the exact phylogenesis/ontogenesis of mirror neurons, the fact that neural cells residing within the motor pathway respond to action that is not executed, but merely observed, is promising considering the field of motor rehabilitation.

Mirror neurons are motor neurons that match observed motor act with executed similar or the same act (Rizzolatti and Fogassi, 2014). A number of corticospinal neurons descending from ventral premotor (Kraskov et al., 2009) as well as primary motor (Vigneswaran et al., 2013) regions in macaque monkey have been identified as mirror neurons, thereby implying their direct input into the spinal circuitry. It is therefore postulated that the engagement of 'mirror' part of the corticospinal system through action observation may facilitate post-injury plasticity and promote motor recovery (Buccino,

2014). Notably, a substantial number of the corticospinal mirror neurons has been identified as suppression mirror neurons in the premotor region F5 (Kraskov et al., 2009) and in the primary motor cortex M1 (Vigneswaran et al., 2013). These neurons show facilitation during action execution, however instead of firing during action observation their output is suppressed. It has been suggested that suppression mirror neurons play a role in the inhibitory mechanism preventing overt movement during observation (Kraskov et al., 2014).

Since mirror neurons respond not only during observation of real-life act, but also when pre-recorded action is presented on the computer screen (Rizzolatti and Fogassi, 2014), recruiting the spared CST through easily constructed videos of a motor act is a cost-effective adjunct to physical therapy that can also be implemented in home environment of a patient. Thus far, AOT has been used in motor rehabilitation of stroke and Parkinson's disease patients as well as of children with cerebral palsy (Buccino, 2014).

1.3.1 Mirror neurons and their dual property

In addition to monkey's ventral premotor (areas F5 and F4) (Caggiano et al., 2012, 2011; Gallese et al., 1996; Pellegrino et al., 1992), dorsal premotor (area F2) and primary motor regions (area F1) (Tkach et al., 2007), mirror neurons have been also identified in the inferior parietal region (PFG, as well as anterior and ventral intraparietal areas (AIP and VIP)) (Fogassi et al., 2005; Ishida et al., 2010; Rizzolatti and Craighero, 2004; Rozzi et al., 2008) (see figure 1). Altogether these regions form a parieto-frontal mirror network (Rizzolatti and Sinigaglia, 2010). In addition to this network, superior temporal sulcus (STS) was also proposed to be a part of the mirror neuron system (MNS). There are no mirror neurons in the STS, yet the area is activated during observation of biological movement and is suggested to provide sensory input into the MNS (Iacoboni and Dapretto, 2006).

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Figure 1. Subdivisions of the agranular frontal and posterior parietal cortices of monkey. AI – inferior arcuate sulcus; AIP – anterior intraparietal area; AS, superior arcuate sulcus; C – central sulcus; DLPF – dorsolateral prefrontal cortex; FEF – frontal eye field; IO – inferior occipital sulcus; L – lateral fissure; LIP – lateral intraparietal area; Lu – lunate sulcus; MIP – medial intraparietal area; P – principal sulcus; ST – superior temporal sulcus; VIP ventral intraparietal area; VLPF – ventrolateral prefrontal cortex. Figure from Rizzolatti and Fogassi, 2014.

1.3.2 Mirror neuron network in humans

1.3.2.1 *Evidence from neurophysiological studies*

The existence of similar mirror network in humans is difficult to confirm since single cell recoding in experimental settings is not used for obvious reasons. Nevertheless, it has been possible to demonstrate that human motor pathway also responds to observed action, engaging the musculature involved in execution of the observed motor act. The first convincing argument came from seminal TMS study performed by Fadiga and colleagues in 1995 (Fadiga et al., 1995).

During TMS, a small magnetic field is applied with a specialized coil over participants' primary motor area and a small electrical current is induced, painlessly penetrating the scull and triggering neuronal discharge that "drives synaptic inputs onto large populations of neurons throughout the cortex, including layer V corticospinal neurons" (Kleim and Schwerin, 2010). This process instigates activation of the tract and, eventually, a measurable twitch in the muscle - a motor evoked potential (MEP).

It has been suggested that although watching somebody perform a grasp does not elicit grasping in the onlooker, the activity in muscles that would normally be engaged during execution of the observed grasp is facilitated. The purpose of Fadiga's study was to establish if motor output was modulated by the observed stimuli. MEPs were measured from several hand muscles while participants observed (1) an object being grasped by the experimenter, (2) an object without any manipulation of it, (3) a moving hand without a presence of an object and (4) a dimming light. Authors found that most of the hand recorded muscles showed facilitation in response to the 1st and the 3rd conditions, but not to control stimuli, thus suggesting that corticospinal tract was differentially ac-

tive during observation of biological movement (figure 2). Moreover, it also appeared that the effect was muscle specific, since opponens pollicis (OP), a small muscle on the inside of the hand used to oppose the thumb, showed significant facilitation only during the grasp observation condition (condition 1), but not hand movement alone (condition 3).

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Figure 2. Mean motor evoked potentials during action observation in the experiment by Fadiga and colleagues **(figure from Fadiga et al., 1995).**

Subsequently, in the last twenty years, TMS has been employed to study various properties of action observation in relation to its impact on muscle excitability. The notion of automatic 'mirroring' in humans is now supported by evidence from numerous physiological studies showing covert engagement of agonist muscles during observation of related actions. Such studies have been able to demonstrate modulation within muscles by the type of observed action (Candidi et al., 2010; Cavallo et al., 2013; Fadiga et al.,

2005, 1995; Sartori et al., 2012; Senna et al., 2014; Urgesi, 2006), laterality of observed hand (Aziz-Zadeh et al., 2002), time-course of the observed event (Gangitano et al., 2004), as well as attributes of manipulated objects, such as shape or weight (Alaerts et al., 2012, 2010a). Often results indicated tight coupling between action production and action observation, since changes occurring within muscles during observation were analogous, albeit covert, to those during execution itself.

1.3.2.2 *Evidence from neuroimaging studies*

Asserting brain regions comprising the mirror neuron network in humans has been a challenging endeavour. Various neuroimaging techniques have been used, none coming close to single-cell recording performed in animal models. Nevertheless, here I will describe studies performed applying functional Magnetic Resonance Imaging (fMRI). In particular, I will outline findings obtained using three methods that I believe to be most useful in establishing mirror neuron mechanism in humans: (1) repetition suppression paradigm, (2) shared voxel analysis and (3) multivariate pattern recognition.

Using repetition suppression paradigm, several brain areas were attributed to the parieto-frontal mirror network, specifically inferior part of the precentral gyrus (PMv), inferior parietal lobule (IPL) and the posterior part of the inferior frontal gyrus (IFG) (Chong et al., 2008; de la Rosa et al., 2016; Kilner et al., 2009). Repetition suppression is an experimental model that allows identifying areas where activity is reduced if the same action is observed and executed consecutively irrespective of the order. Thus, only areas that contain mirror neurons with dual (motor/sensory) properties would exhibit the cross-modal adaptation effect (i.e. be suppressed with repeated stimulation). Recently, a debate arouse in light of new evidence that neurons in the F5 region in monkeys, where

mirror neurons were initially discovered, do not show repetition suppression (Caggiano et al., 2013). In response, Kilner and colleagues demonstrated that although mirror neurons in this region indeed did not show repetition suppression during initial observation trials, the response was evident after observing the same action at least seven times (Kilner et al., 2014).

Shared voxel analysis of the unsmoothed single-subject data is another reliable way to define individual voxels activated when participant performs as well as observes an action. In a study conducted by Gazzola and Keysers, brain activity was recorded in 16 healthy volunteers while asking them to observe complex hand actions and to execute similar actions on separate days (Gazzola and Keysers, 2009). Analysis was then performed on the unsmoothed data of each individual, allowing for an accurate estimation of regions involved in both observation and execution of similar action. The novel finding of this study was that not only classical mirror neuron areas (PMv, IFG and IPL) contained shared voxels, but also a number of other regions, including dorsal premotor, supplementary motor, superior parietal, primary and secondary somatorsensory, middle cingulate areas, as well as middle temporal cortex and cerebellum had shared mirror properties. Graphic representation of the relative contribution of these areas to the total number of shared voxels provides a clear picture that there are more than two major areas in the human brain recruited to similar extent during observation and execution of the same action (see figure 3). Unfortunately, the existence of voxels in which activity is shared between action observation and execution does not prove the existence of mirror neurons. After all, there are roughly 630 thousand neurons in a functional 3mm voxel (Aguirre, 2012). Nevertheless, this study is an important plausibility that mirror neuron

network may be much wider spread than initially thought. Mirror neuron presence in the fronto-parietal network, outlined in animals, does not prove absence of mirror neurons elsewhere in the brain. Such experiments have simply not been conducted yet.

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Figure 3. Diagram showing regions with identified shared voxels (sVx) that are active during observation as well as execution of similar action. Figure from Gazzola and Keysers, 2009.

Finally, multi-voxel pattern recognition is a method that aims to predict (or decode) whether action is observed or executed based on a voxel-wise pattern of brain activity. Such cross-modal decoding was found to be accurate in lateral occipitotemporal cortex, postcentral gyrus and parietal regions (Filimon et al., 2014; Oosterhof et al., 2010), as well as in premotor and inferior frontal areas (Filimon et al., 2014). Furthermore, spe-

cific information about a particular action, such as whether it is performed by grasping or pinching an object, can also be decoded independent of the modality (Oosterhof et al., 2010). Thus, a pattern recognition algorithm is able to predict if observed action is of pinching or grasping an object, using patterns of brain activity during execution of the same actions. The implication from these findings is that execution and observation of the same acts shares common neuronal representations.

1.3.3 Action Observation as a way to engage corticospinal system and promote functional regain after injury

1.3.3.1 *The engagement of the corticospinal tract during action observation compliments restitution of function following stroke*

Considering the above studies, there is strong implication that watching actions recruits motor system in humans. Shared voxel analysis showed that the same voxels were activated during execution and observation of similar action in origin regions of the corticospinal tract (Gazzola and Keysers, 2009), while Fadiga's TMS paradigm proved that corticospinal excitability is strongest in muscles normally involved in execution of the observed actions (Fadiga et al., 1995). Findings that engagement of the corticospinal tract was possible through action observation lead to clinical trials pairing AOT with physical training in order to stimulate recovery (Bang et al., 2013; Ertelt et al., 2007; Franceschini et al., 2012, 2010; Harmsen et al., 2014; Park and Hwangbo, 2015; Sale et al., 2014; Sugg et al., 2015).

For instance, Sale and colleagues performed a double-blind clinical trial involving 67 first-time stroke patients approximately 30 days after their insult (Sale et al., 2014). Patients were randomly assigned to the experimental or the control group and in addition to standard rehabilitation therapy received action observation intervention. Patients in both groups were exposed to 15 minutes of observation of video clips prior to their usual physical practice routine twice a day over the course of four weeks (five days a week). Patients in the experimental group observed sequences of everyday actions, such as drinking a cup of coffee, and were instructed to replicate the movement with their paretic hand. Participants were presented with one action a day, each time ascending in difficulty. That way, twenty actions were practiced over all of the sessions. In contrast,

patients in the control group watched sequences of still images and were asked to distinguish the odd one out. They were then instructed to execute the same action as patients in the experimental group. Motor function in all patients was then measured by Fugl-Mayer and Box and Blocks tests. The authors found that scores for both tests were significantly higher for patients in the experimental group not only after the four weeks of intervention, but also after 4-5 months following treatment. Notably, however, only left, but not right hemiparetic participants from the experimental group achieved significantly better results.

Importantly, not only patients with upper limb paresis benefit from action observation. Bang et al., were able to demonstrate that watching video clips of an actor walking on the treadmill prior to everyday physical practice improved walking in patients with lower limb weakness (Bang et al., 2013; Park and Hwangbo, 2015). Just as in the above study, 30 stroke patients were randomly assigned to two equal-sized groups. Patients in the experimental group observed 9 minute video clips with a model walking on the treadmill, whereas patients in the control group watched nature videos. Patients in both groups continued with their daily 30 minute walking practice after observation intervention. The progress was measured four weeks later and the dynamic balance, speed, endurance and knee flexion of all participants was assessed. The authors show a significant difference between the two groups on all of the measures, concluding that gait observation is a successful adjunct to motor training during recovery after stroke.

The two clinical trials clearly demonstrate the effect that action observation may have in recovery of the motor function in stroke survivors, leaving little doubt in its usefulness. A similar trial was conducted in the earlier experiment, merging the intervention with

fMRI technique to investigate the neural underpinnings of the effect (Ertelt et al., 2007). In this study 15 chronic stroke patients were randomly assigned to the treatment or the control group. Each received 4-weeks of physical training. In addition to conventional physical exercises, patients in treatment group were also subjected to Action Observation Treatment, whereby they were instructed to watch video sequences of daily life actions and to subsequently practice executing the same movements. Not only had the authors demonstrated significant effect of treatment in the group that received AOT in addition to physical training, but the difference between control and treatment group was also significant. Importantly, the improvement in the treatment group persisted 8 weeks post training. This motor improvement in the experimental group was also attributed to the increased activation in the bilateral ventral premotor (PMv) and inferior parietal areas (IPL) as well as bilateral superior temporal gyrus (STG) and supplementary motor area (SMA). These results were pivotal in attributing the benefits of AOT to the increased recruitment of the premotor and parietal areas.

In similar vein, Brunner et al. went on to test 18 sub-acute stroke patients observing and executing bimanual actions at: a) 1 to 2 weeks and b) 3 months post stroke (Brunner et al., 2014). 18 control subjects were recruited as well. The gist of the experiment was not to see if AOT can be beneficial, but how the response to action observation is affected immediately after stroke and within the first months of recovery. Again, the neuronal response was tested with the means of fMRI. Authors found that compared to the control group, activity in patients was reduced right after stroke, but increased after three months to the level of activity in healthy individuals (paralleling findings on motor recovery after stroke without AOT (Rehme et al., 2011). Most activated clusters were

found in the inferior temporal gyrus (ITG), thalamus, SMA, premotor and primary motor cortex (BA4 and BA6).

Crucially, success of the AOT was determined by the fact that although action observation modulates the motor system online, the effects were then sustained over several months (and perhaps even longer). Both, Ertelt's and Sale's research implies possible prolonged benefits of AOT, extending to 4-5 months after treatment (Sale et al., 2014). Presumably, the changes that are initially instigated by combined physical and observational intervention are then rooted within the motor system.

Using TMS, Stefan and colleagues were first to show that extended period of observation of a novel movement facilitated motor memory formation. They showed that watching a thumb moving in opposite to usual direction, increased the probability of then TMS evoked thumb movements falling in the newly learned direction (Stefan, 2005). Moreover, the same group later demonstrated that simultaneous physical training and observation of the same, congruent action significantly improved motor memory formation, as compared to physical training alone or when it was paired with observation of the incongruent actions (Stefan et al., 2008).

Similarly, both action observation and physical training increase finger abduction force (FAF) (Porro et al., 2007). Interestingly, prolonged observation of one hand increases FAF in both hands by approximately 30%, while physical training results in the similar 33% enhancement in the untrained hand and a higher 50% increase in the trained hand.

Notably, when observation and execution were combined, an increase in facilitation was apparent and gradual over the course of learning. This ongoing escalation was not pre-

sent during observation training alone (Sakamoto et al., 2009). What's more, when new skill was acquired with the help of observation, such as a sequence of novel handshapes, MEPs were enhanced during the period of learning, but decreased as learning progressed (Sakamoto et al., 2012; Zhang et al., 2011).

One study explored motor memory formation in stroke patients using the same paradigm as in the Stefan's experiment illustrated earlier (Stefan, 2005). Similar to the results in healthy population, Pablo Celnik and colleagues demonstrated that in chronic stroke patients combined action observation and physical training increased motor memory formation, as compared to physical training alone (Celnik et al., 2008). The group showed that the direction of TMS induced thumb movements changed from preferred to the trained one and concluded that action together with physical practice was a potentially useful rehabilitation technique.

1.3.4 The ability to produce actions alters engagement of the motor system during action observation

The use of AOT in therapy appears to be promising to those with loss of motor function after injury. Action observation can be a successful input into the motor system through engagement of motor pathway involved in production of observed action. If, nevertheless, the specific motor pathway for, say, *execution* of pinch is damaged, it is unclear whether *observation* of pinch can still engage the system. The uncertainty comes from evidence that observing actions that are not in the motor repertoire of the observer does not recruit mirror neuron network. For instance, in the seminal study by Buccino et al., it was demonstrated that independently of the model (man, monkey or dog), observation of actions that are in the motor schemata of an observer, such as biting or lip-smacking, resulted in the recruitment of premotor areas, whereas unused actions, such as barking, did not (Buccino et al., 2004).

In addition, there is ample evidence that the degree to which one is able to produce observed action alters the engagement of the mirror neuron network. For example, Beatriz Calvo-Merino and colleagues examined the effect of motor experience during observation by studying skilled male and female dancers recruited from the London Royal Ballet (Calvo-Merino et al., 2006). Certain moves in ballet are gender-specific and are only appropriate within the male or the female dance repertoire. Both types of gender-specific movements are equally visually familiar to both male and female dancers, yet motor expertise of the movement is only present in the dancer if it is appropriate for their gender. With this knowledge authors investigated the effects of observing movements that were or were not in one's motor repertoire, while maintaining the same

visual familiarity across all participants. They found that several areas, including dorsal premotor cortex and intraparietal sulcus (comprising part of the mirror neuron network) and cerebellum were activated more when observed action was in the motor schemata of the observing dancer.

Similar effects were further confirmed by numerous studies comparing neural activity during observation in novices and people with expertise in areas such as arching, tennis, piano playing and even smoking (Balser et al., 2014; Haslinger et al., 2005; Kim et al., 2011; Wagner et al., 2011). Conversely, Abreu et al. failed to find differential activity between expert basketball players and people naïve to playing or watching the sport. Nevertheless, they show evidence that experts were significantly more advanced in predicting erroneous throws just from watching the beginning of the movement whilst activating the extrastriate body area (EBA) to a greater extent than novices did (Abreu et al., 2012). This result suggests that basketball players may still simulate the observed throw, which provides them with predictive perceptual advantage.

Thus, the notion that perception and execution are tightly coupled through motor ability is also reflected in greater predicting abilities of individuals with motor familiarity of a given action (Abreu et al., 2012; Balser et al., 2014). To extend on these findings, Bischoff et al. conducted an experiment where participants were instructed to predict the direction of the flight of the tennis ball from mere pointlight displays. Subjects were unaware that some of their observed throws were in reality performed by themselves in earlier recording session. Authors found that participants performed better when they watched their own strokes than when they observed throws of the model, indicating that one's motor repertoire effects action perception (Bischoff et al., 2012).

Most of the above examples point to the greater neuronal activity whilst watching an action, providing that action is already within the motor repertoire of an observer. But what happens when the action was once within the motor schemata of an individual but is no longer practiced due to disability such as stroke? It may be that the degree to which the motor system is engaged during observation of an action depends on the ability to execute that action.

One attempt to address this question was made by Garrison and colleagues in a relatively small study (Garrison et al., 2013). 12 left hemisphere stroke patients with impaired dominant right hand and 12 right-handed healthy individuals participated in observation of grasping actions performed with either right or left hand. Actions were intentionally designed to be difficult or impossible to perform with the paretic hand. Analysis was then performed in the four regions of interest previously implicated in processing action observation: inferior frontal gyrus (IFG) pars opercularis (BA44), IFG pars triangularis (BA45), precentral gyrus (BA6) and supramarginal gyrus (area PF) bilaterally. When comparing between the two groups, Garrison et al. found that in both nondisabled and stroke groups observation related activity was significant in all ROIs (amongst other regions). Importantly, in the non-disabled group activity during observation of both hand actions was mostly lateralised to the right hemisphere, with exception of BA6, where observation of right hand actions was lateralised to the contralateral left side. In contrast, in the stroke group observation of the paretic right hand resulted in shifted lateralisation of activity to the left (ipsilesional) hemisphere, while remaining the same (contralateral) during observation of left hand - similar to the control group. In addition, comparison of neuronal activity during observation of paretic hand to the observation of

the intact hand resulted in greater response in the ipsilesional left hemisphere. Further correlational analysis showed that activity was greater in left BA44 and BA45, as well as right BA6 in patients with greater impairment, suggesting that activity was increased when observed actions were difficult to perform. Authors concluded: "observation after stroke promotes activation in ipsilesional cortical motor regions considered to be relevant to neuroplasticity" (Garrison et al., 2013). These findings point to significant changes in the engagement of the motor system during action observation after stroke.

Action Observation Treatment is proposed to be an effective addition to motor rehabilitation, enabling recovery of function. However, evidence shows that the degree to which AOT could be beneficial may depend on how well patients are able to execute observed actions. Watching actions that no longer are in the motor repertoire of the patient may not be an effective way of engaging the motor system and, hence, usefulness of AOT may be limited. A deeper understanding of how damaged motor system is activated during action observation is essential before the use of AOT in motor rehabilitation. The aim of this thesis is, therefore, to expand knowledge on how the ability to execute actions influences activity in the motor system during observation of those actions.

1.4.1 Research questions

I have addressed the following two questions in order to ascertain if lost ability to execute hand actions impacts on the engagement of the motor system during observation of those actions.

Question 1

Does watching hand that is congruent to the affected hand after stroke engage the motor system to a different degree than watching hand congruent to the unaffected one?

Question 2

Is there a relationship between the residual motor ability after stroke and the degree of motor system engagement during observation of hand actions?

To study these questions I have employed neurophysiological and neuroimaging techniques to probe the response to observed actions in a group of chronic stroke patients as well as in healthy individuals.

1.5 RESEARCH DESIGN AND HYPOTHESES

In the following chapter I will review research methods used in this thesis. In Chapter Three, Four and Five transcranial magnetic stimulation (TMS) technique was used to investigate the response to action observation in hand muscles of healthy volunteers and chronic stroke patients. Chapter Six and Seven were devoted to examining cortical engagement during action observation in healthy participants and in stroke patients, using functional magnetic resonance imaging (fMRI). General discussion completes this thesis in Chapter Eight.

Prior to addressing the questions of interest I paid considerable attention to establishing the correct paradigm. The reasons for that will become apparent in Chapter Three, where I describe three pilot studies conducted in search of suitable experimental design.

In Chapter Four I measured corticospinal excitability during action observation in hand muscles of 18 healthy participants. In order to obtain accurate depiction of muscle excitability in the intact corticospinal system, I tested volunteers twice, measuring response in their dominant right and non-dominant left hand. The aim was to replicate findings that watching hand actions engaged the corticospinal system in a muscle-specific way, facilitating response in agonist muscles in the execution of the observed action (Fadiga et al., 1995). In addition, I aimed to establish whether response in the left and right hand of the observer was dependent on which hand (left or right) was observed. Thus far, findings have been conflicting (Aziz-Zadeh et al., 2002 found hand specific facilitation, while Sartori et al., 2013 did not). Therefore, it was imperative to determine response to watching left and right hand in healthy individuals, prior to exploring if watching hand that was congruent to the affected or unaffected hand

modulated response in stroke patients. Finally, I explored if age of the observer influenced the engagement of the corticospinal system during action observation. The relationship between age and hand muscle response during action observation has not yet been studied and it was important investigation in this thesis as the majority of stroke patients are generally of older age.

In Chapter Five I, firstly, used data from Chapter Four in order to compare response to action observation in the intact left and right hand of healthy participants with that in the affected hand of 19 stroke patients. I then addressed the hypothesis of this thesis by: (1) exploring the engagement of the corticospinal system during observation of hand that was congruent or incongruent to the affected hand after stroke, and (2) establishing if there was a relationship between residual ability to perform actions and the response in hand muscles during observation of those actions.

In Chapter Six I examined cortical activity during action observation in 20 right-handed healthy volunteers and 22 right-handed stroke patients. Firstly, I have explored if watching left or right hand resulted in different activity in the motor system of the observers. Previously, hand-specific modulation of cortical activity was documented in several studies of healthy individuals (Shmuelof and Zohary, 2006; and Vingerhoets et al., 2012). The aim in this chapter was to replicate findings and to establish if activity, specific to which hand (left or right) was observed, was different in patients with one of the hands impaired after stroke. Moreover, I used multiple regression to test for the relationship between lost ability to execute actions and magnitude of activity in the motor system during observation of those actions.

In Chapter Seven I used data collected in Chapter Six to decode observed hand laterality and predict patients' motor function from patterns of brain activity during action observation. To this end, I used multi-voxel pattern analysis (MVPA), enabling to investigate differences between patterns of neuronal engagement rather than differences between activity in single voxels. Observed actions and effectors were previously decoded from patterns of activity while participants watched hand actions (Ogawa and Inui, 2011; Oosterhof et al., 2013), however, no studies have used MVPA to decode observed hand laterality from patterns of activity during action observation in stroke patients. In addition, I used multivariate pattern regression in order to predict residual motor function after stroke from patterns of neuronal activity during observation. Neuronal representations that are activated during action observation may be altered by loss of the ability to execute observed actions. My aim was to establish if this was the case by predicting motor function from patterns of activity during observation.

Finally, in Chapter Eight, I summarise all findings and offer a discussion of most fundamental results.

CHAPTER 2
INTRODUCTION TO THE RESEARCH METHODS

2.1 TRANSCRANIAL MAGNETIC STIMULATION

2.1.1 Background

> **SIR,-This note describes a novel method of directly stimulating the human motor cortex by a contactless and non-invasive technique using a pulsed magnetic field. (…). When the coil is placed on the scalp, over the appropriate region of the motor cortex, movements of the opposite hand or leg are easily obtained without causing distress or pain. The first cortical stimulations by this method were carried out with P. A. Merton and H. B. Morton at the National Hospital, Queen Square, London.(Barker et al., 1985, p.1106)**

These words, published in Lancet almost thirty years ago, marked the beginning of the Transcranial Magnetic Stimulation as we know it today. Although Antony Barker and his group in Sheffield University were the first to demonstrate the painless yet robust way of activating the muscles through magnetic stimulation of the brain, it was far from a new endeavour.

The effects of the magnetic stimulation of the cortex were reported by D'Arsonval in 1896 in the meeting of the Societe de Biologie in Paris, whereby he showed that he was able to induce the sensation of phosphenes and dizziness in subjects after placing their heads within an "intense alternating magnetic field of 110 V/30 Amp and 42 Hz" (D'Arsonval, 1896). The discovery that was left for the next fifteen years was subsequently reproduced by Sylvanius P. Thompson in 1910 and was still met with fair dose of scepticism. It was Knight Dunlap, residing at John Hopkins at the time, who had his doubts and who decided to follow up Thompson's findings by designing a coil of his

own (with some help). He believed the effect would likely to be due to the hum in the coil, but after completing the controlled trial with their very own equipment, Dunlap's group could only complement the findings of D'Arsonval and Thompson. More research has followed from other groups, including peripheral nerve stimulation by Bickford in the sixties, but the devices used did not permit an easy way to penetrate the brain magnetically and the artefacts from the coil were too big to allow measuring electrical activity in muscles. It really wasn't until early eighties, when Merton and Morton used an electrical stimulator over the vertebral column that produced motor evoked responses were measured. Still, the technique was too painful and was an unlikely candidate in clinical settings. So it's unsurprising that Antony Barker and his colleagues constituted a significant leap when they presented their magnetic stimulator at Queen Square. After the launch of Transcranial Magnetic Stimulation the use of this technique grew yearly at a tremendous speed, with a record number of over 900 papers published in 2013 (figure 4).

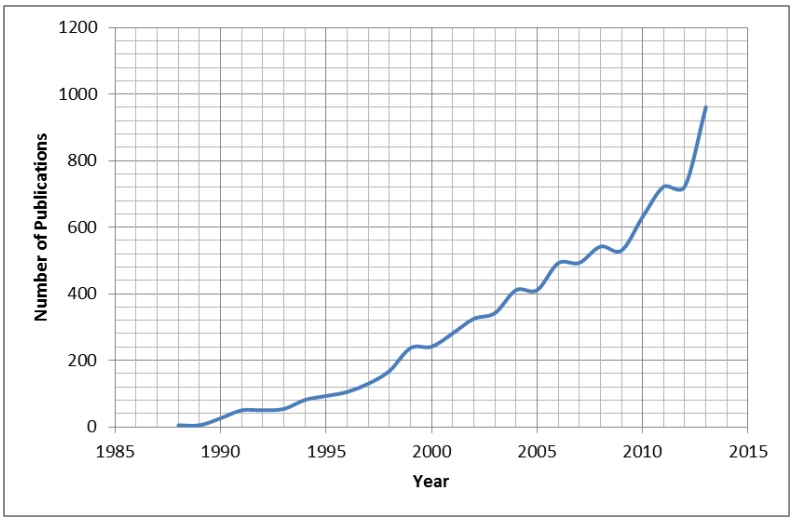

Figure 4. A mounting number of publications containing search words 'transcranial magnetic stimulation' in their title or abstract from 1987 to 2013 (search was completed using PubMed database).

2.1.2 Magnetic Stimulation of the motor cortex

Nearly two centuries ago, in 1831 Michael Faraday was credited for his discovery of electromagnetic induction (Day, 1999). A good lesson to publish your findings quickly, as there were at least two more candidates, Francesco Zantedeschi and Joseph Henry, who claimed similar results at the time, but weren't quick enough. Faraday knew that magnetic field can be created by electric current (based on Ørsted's discovery in 1820 and Sturgeon's first electromagnet in 1924), but he found that an electric field can also be produced by a magnetic field. He stated that if magnetic field would interact with an electric circuit an electromotive force (EMF) would be generated. Importantly, this induction of electric field and its force was dependent upon the motion of the magnet, or a magnetic flux.

Today Faraday's law of electromagnetic induction is the basis of TMS. When the electrical current is released into a coil, it produces a transient magnetic field, which then passes into the nearing medium, such as a human skull where electrical field is then generated. As soon as this field reaches the human brain, consisting of highly conductive neural tissue, the electrical current is propagated. The type of a coil used determines the spread of the stimulation effect. A 'figure-of-eight' or, more romantically, 'butterfly' coil, for instance, allows for the most focal stimulation by summating electrical fields induced by two currents flowing in two adjacent coils reaching their maximum level below the unction (Wassermann et al., 2008) (figure 5).

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Figure 5. The principle of the transcranial magnetic stimulations. Electrically charged coil induces a magnetic field, which penetrates the skull and produces electric current, exciting axons in the cerebral cortex, approximately 1-2 cm away from the coil. Figure from Ridding et al., Nature Review Neuroscience, 2007 (Ridding and Rothwell, 2007).

Albeit the way to stimulate the brain has been discovered, the effects of such stimulation on the brain are not easy to interpret. The brain is a complex structure comprised of billions of neurons carrying their own electric charges and is organized in columns and layers and folded into numerous gyri. It is also comprised of tissues, such as white and grey matter and of the cerebrospinal fluid (CSF) – all of which differ in their conductivity. Clearly, studying the responses of the brain by stimulating it from the surface is not as straightforward as one may hope. Notably, Mills concluded in his book on magnetic stimulation of the human nervous system, that the capacity of the current "to excite nerve cells depends upon its time course, magnitude and direction" (Mills, 1999). I will touch on these issues whilst focusing on the effects of the TMS on the corticospinal tract.

Stimulation of the motor cortex results in measurable response in muscles by exciting the ascending pathway of the corticospinal tract. This allows for quantification of the modulatory effect on the system elicited through presentation of the experimental stimuli and is an ultimate goal of part of this thesis. To begin with, understanding of the cytoarchitectonic organization of the motor cortex is important for one must take into consideration the convoluted structure of the brain to be able to address issues defined by Mills. The direction, the time course and the strength of the current – all play a detrimental role in the outcome and are guided by the anatomy.

2.1.3 The Cytoarchitectonic Organization of the motor cortex

The neocortex is structured in a way that increases efficient relay of information. It is comprised of layered columns of nerve cells and their projections that differ in their size and shape. Mostly, neocortex consists of six layers: layer I primarily hosts the dendrites of cells from the deeper layers, layers II and III contain small spherical and pyramidal cells and mediate intracortical connectivity, layer IV is the primary recipient of the sensory input, layer V is the main projector of the motor output and, finally, layer VI – a heterogeneous mix, hence called polymorphic or multiform layer (figure 6) (Kandel et al., 2012). In addition to the columnar organization, intrinsic inter-columnar connections run tangentially along cortical layers and due to their spread are also called horizontal fibre systems.

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Figure 6. S.R. y Cajal's Nissl-stained motor cortex of a human adult. Layer II is divided into 2 and 3 to distinguish between small and medium sized pyramidal cells. Thus 4 – corresponds to layer III, 5 – to layer IV, 6 to layer V, etc. Figure from the Comparative study of the sensory areas of the human cortex by Santiago Ramón y Cajal **(Santiago Ramón y Cajal, 1900)**.

In 1874, Vladimir Alexandrovich Betz discovered that layer V of the "paracentral lobule on the medial surface of the hemisphere" holds giant pyramidal cells (Finger, 2001). Later Sherrington demonstrated that destruction of these giant Betz cells has dramatic effects on movement in dogs (Finger, 2001). Betz cells reside within the primary motor cortex, Brodmann area 4, and together with smaller pyramidal cells in layer III project to individual muscles or to small groups of them (Mills, 1999). Stimulation of this area produces excitation of the corticospinal neurons and a motor evoked potential – a measurable response in the muscle (figure 7.1).

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Figure 7. Transcranial Magnetic Stimulation. During TMS a magnetic field (B) is generated in the coil inducing an electric field (E) (1). This electric field excites pyramidal cells in the layer V by affecting the transmembrane potentials and depolarizing cells resulting in the subsequent neural activity (2). Axon depolarization during magnetic stimulation. Figure adapted from Ruohonen et al. (Pascual-Leone et al., 2002).

2.1.4 Direction and Coil Orientation

Magnetic stimulation depolarizes axons that are in the plane of the electrical current, parallel to the coil, favouring axons with tangential orientation. Pyramidal neurons, however, are radially oriented and are hence activated indirectly (Wassermann and Zimmermann, 2012). In 1987 Meyer provided evidence that horizontal fibre systems in the primary motor cortex of humans run preferentially in orientation at the right angle to the precentral gyrus (Meyer, 1987). Therefore, the excitability of the motor cortex during TMS is also best when coil currents are perpendicular to the axis of the precentral gyrus. To add, the rotation around the midpoint of the figure-of-eight coil results in the "differential targeting of axon populations" (Wassermann and Zimmermann, 2012). As shown by Mills *et al.*, the optimal orientation of the figure-of-eight coil is at the 45◦ angle, perpendicular to the direction of the central sulcus (figure 8)(Mills et al., 1992).

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Figure 8. The largest response at a stimulus orientation of 45◦. Figure from Mills et al., Electroencephalography Clinical Neurophysiology, 1992 (Mills et al., 1992).

2.1.5 Time Course and Magnitude

In order for the nerve cell to propagate an action potential it needs to be depolarized above a certain threshold (figure 7.2). The strength and the duration of the electrical current impacting on the cell determine the likelihood of that cell's depolarization. Axons have lower thresholds than cell bodies and will depolarize at lower strength currents. Notably, there is a relationship between the strength and the duration in that if the current is to be shorter it needs to be stronger in order to excite the cell (Mills, 1999).

Both strength and duration can be modified depending on the magnetic stimulator and the technique used. Single pulse stimulation, used in the current experiment, is one of them. During single pulse experiments the coil of the stimulator is charged at certain intervals at the rate of usually no less that 5Hz. Other techniques, however, such as repetitive TMS (rTMS) will produce short trains of discharge at ether high (20Hz) or low (1Hz) frequency creating prolonged stimulation of an area. Latter technique is used to reduce (usually at a low frequency) or promote (at 10Hz or higher) natural excitation and is thus used therapeutically to treat depression, anxiety, schizophrenia or to boost plasticity, such as after stroke (Edwards et al., 2008; Feng et al., 2013; Haraldsson et al., 2004; Wassermann and Zimmermann, 2012).

The magnitude of the electrical current and the extend of the stimulated area is determined by the intensity of cortical stimulation and varies depending on the research question in mind (Wassermann et al., 2008). In studies of corticospinal excitability using single pulse TMS the intensity is measured by determining lowest stimulation strength – resting motor threshold (RMT) - over the primary motor cortex that produces response in the muscle of interest at least 50% of the time. This motor threshold reflects

the excitability of cortico-cortical axons regulated by the voltage-gated sodium channels. The intensity is further increased during the experiment in order to ensure consistent response in the muscle. The increase may vary, for instance, 10, 20 or 30 percent over the resting motor threshold (RMT) and may produce different outcomes in the same task (Loporto et al., 2013), further indicating importance of careful deliberation of an appropriate intensity for the needed effect.

2.1.6 D and I Waves

To add to the complexity of the TMS induced response, single stimulation of the primary motor cortex produces not one, but several volleys in the pyramidal tract (Amassian et al., 1987). The first is a D wave caused by a direct axonal excitation of the corticospinal neurons, the following I waves result from the indirect, synaptic excitation. Up to five I waves may occur successively and they can present without a preceding D wave. It is postulated that I waves are "generated by chains of intracortical neuron having constant loop times that resulted in the periodicity of I waves"((Mills, 1999) on (Amassian et al., 1987)). According to the D- and I- wave hypothesis, TMS excites the corticospinal neurons transsynaptically rather than directly, thus producing I-waves, rather than D-waves (Amassian et al., 1987). In addition, the amplitude of I-wave MEPs is sensitive to the changes in the corticospinal excitability elicited by a task performance or a conditioning stimulus. On the contrary, MEP size of D-waves (measure using Transcranial Electrical Stimulation) is not subject to change in excitability. Importantly, stimulation intensity as well as coil orientation also directly impact on the appearance of D and I waves. Latero-medial orientation and lower intensity elicit direct D-waves, whereby anterior-posterior (AP) and posterior-anterior (PA) coil orientation and higher intensity will preferentially recruit later I waves (Volz et al., 2014).

2.1.7 Using TMS after stroke

One major consideration when using TMS to study corticospinal excitability in stroke patients is the effect of lesion on conductance in the brain. In their review Wassermann et al. state that "regardless of where the current is "aimed", it will flow preferentially through areas containing cerebrospinal fluid, which has a much higher conductance than brain. Therefore, the current may concentrate at points that do not lie directly below the coil" (Wassermann and Zimmermann, 2012). The concern when testing the affected hemisphere of the stroke patient is that lesioned area is filled with the cerebrospinal fluid and thus may affect MEP response in an unpredictable fashion. It is important to keep this in mind when analysing the results.

To conclude, TMS has proven to be a useful tool to non-invasively study the excitability of the corticospinal tract. It provides the ground for investigation of cognitive processes and their effect on motor system. By employing this technique, I aim to explore how observation of simple hand actions impacts on the motor output of healthy individuals and patients with impaired motor function following stroke.

2.2 FUNCTIONAL MAGNETIC RESONANCE IMAGING

First studies using functional magnetic resonance imaging (fMRI) were published in 1992 (Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1992), merely 23 years ago. Today, using this technique results in approximately two thousand publications each year, markedly contributing to our understanding of brain processes in healthy and clinical populations. As implied from the name, functional neuroimaging is used to infer brain function by means of magnetic resonance imaging. More precisely, fMRI is devised to measure cerebral blood flow to regions that are in use by a cognitive or sensorimotor process.

2.2.1 Magnetic Resonance Imaging (MRI)

After the discovery of nuclear magnetic resonance (NMR) in 1945 (Bloch et al., 1946; Purcell et al., 1946), Erwin Hahn made an observation that decay of NMR was modulated by the chemical makeup of an object (Hahn, 1949). This finding became the basis of magnetic resonance imaging (MRI).

Perhaps it is simplest to describe MRI in the way that Small and Heeger did in their chapter on functional imaging of cognition, by outlining essential components of the MRI scanner: 1) a superconducting magnet, 2) a radio frequency (RF) coil, which is placed around participant's head, and 3) magnetic gradient coils (Small and Heeger, 2013).

1. Most tissue in the human body contains water and the amount of water is different depending on the tissue. Normally, water protons in the body continuously rotate in random directions. However, when placed in the powerful uniform magnetic field of the superconducting magnet, these water protons align vertically, 90 degrees to their original orientation. (Small and Heeger, 2013)
2. Then, rapidly alternating electrical current (radio frequency pulse) of the RF coil generates second magnetic field, also rapidly varying (following Ampere's law). This second magnetic field results in the spin of water protons (much like a wobbling spinning top), called precession. The precession of all water protons, in turn, generates a rotating magnetic field that changes with time, creating an alternating electric current – MRI signal - back in the RF coil (by same Faraday's principle mentioned in the section about TMS). The amplitude of this electric current decays at different rates, depending on the type of tissue water

protons are in. It is this decay that is measured with MRI, which results in different intensity image in distinct tissues (such as grey matter, white matter, cerebrospinal fluid, scull, etc.). (Small and Heeger, 2013)

3. Finally, magnetic gradient coils allow measuring MRI signal in thousand adjacent little volumes (voxels) resulting in a three dimensional volume of the brain. (Small and Heeger, 2013)

Initially, MR imaging was a very slow process, acquiring information at a rate of approximately 2 minutes per voxel. However, the development of echo-planar-imaging (EPI) in 1970s substantially sped up the process. (Huettel et al., 2004)

Notably, in the 1980s there were 12 MRI scanners worldwide, today – there are 25,000, with 2,000 scanners sold every year (Rinck, 2014).

2.2.2 Measuring Regional Cerebral Blood Flow (rCBF)

First attempts to measure the rate of cerebral blood flow in the living brain were recorded in late 1940s (Kety and Schmidt, 1948). Seymour Kety and Carl F. Schmidt had participants inhale nitrous oxide, while measuring the outflow of this gas from the jugular vein. In follow up studies, authors explored differences in the rate of the blood flow from the brain while participants were sleeping or awake. Although Kety and Schmidt only measured levels of activity from the entire brain, their studies became the foundation of fMRI (Small and Heeger, 2013).

A little earlier, in 1936, Linus Pauling, a chemist and a Nobel laureate, together with his student Charles Coryell discovered that haemoglobin molecules had magnetic properties (Pauling and Coryell, 1936). These properties, as it appeared, depended on whether the haemoglobin was bound to oxygen. While haemoglobin that contained oxygen had no magnetic moment, haemoglobin devoid of it (deoxyhaemoglobin) was paramagnetic. Forty years later, this seminal finding was exploited by Seiji Ogawa and colleagues, who showed that MRI could be used to detect changes in deoxygenated haemoglobin, which is linked to changes in neuronal metabolic rates (Small and Heeger, 2013). With greater activity in a brain region, such as during a cognitive task, metabolism there increases, resulting in the flow of oxygenated blood to the area. Blood flow is always greater than the consumption of oxygen within the region, which results in decrease of deoxyhaemoglobin and an increase in MRI image intensity (Ogawa et al., 1990). Such difference in signal as a function of the amount of deoxyhaemoglobin became the basis of BOLD (blood-oxygenation-level-dependent) imaging.

2.2.3 Hemodynamic response function (HRF)

The decrease of deoxyhaemoglobin within a voxel results in hemodynamic response function (HRF) - change in the MR signal generated by local neuronal activity (Huettel et al., 2004). Generally, neuronal activity is triggered within tens of milliseconds from presentation of the stimulus, yet changes in HRF are observable only 1 to 2 seconds later. Therefore, the temporal resolution of fMRI is several seconds. Three phases of HRF can be outlined: (1) initial dip, (2) overcompensation, and (3) undershoot (Buxton et al., 2004) (figure 9). Neuronal activity at first results in the increase of metabolic oxygen consumption, which in turn increases levels of deoxyhaemoglobin and a reduction of BOLD signal – initial dip – is apparent. Subsequently, demand of oxygen results in increased blood flow to the region. This blood flow is greater than the area can consume, which relatively decreases levels of deoxygenated haemoglobin, causing BOLD signal to rise – overcompensation. Signal increases above baseline approximately 2 seconds after the onset of neuronal activity and gradually reaches its peak at about 5 seconds. If neuronal activity persists over a period of time, this peak extends into a plateau. Eventually, when neuronal activity ceases, BOLD signal decreases to levels below baseline – undershoot – progressively increasing to its original level. Undershoot is thought to be related to differences in decline of blood flow relative to blood volume, resulting in the increase of deoxyhaemoglobin and reduced BOLD signal (Huettel et al., 2004). Notably, while haemodynamic response remains relatively constant in the same subject within the same region, it can differ between individuals and across different areas (Aguirre et al., 1998).

Figure removed due to copyright reasons

Figure 9. Three phases of hemodynamic response function (HRF). Figure from Ward (2010).

2.2.4 Using fMRI to measure cognitive and sensorimotor processes

During an fMRI experiment, participants are placed in the MRI scanner and instructed to perform a certain task. In the meantime, fMRI data are collected in the form of three-dimensional matrix of volume elements (voxels) sampled many times over the duration of experiment. The time course of each voxel is then extracted and differences in BOLD signal between participant's engagement in one as opposed to another task can be computed using tests of statistical significance. Such process entails that each voxel is always in the same unique position in the brain sampled at a consistent rate. This is not normally the case, thus in order to infer neuronal activity from fMRI, each voxel needs to be placed in the position whereby signal in it can be compared within the same subject over time and during different conditions, as well as between different individuals. The procedure by which this is achieved is called preprocessing, which deals with the experimentally unrelated variability in the data and prepares it for statistical analysis. Firstly, the data are realigned in order to correct for participant's head movement in the scanner; secondly, the data are transformed into a template anatomical space; finally, data are smoothed in order to increase statistical power (Friston, 2004)

2.2.4.1 *Spatial realignment*

When participants are placed in the scanner all care is taken to restrict movement of their head throughout the experiment. Nevertheless, it is impossible to stabilise this movement entirely, which could result in changes in signal detection in a voxel over time, confounding the data. Realignment is used to correct for head movement and is achieved by affine (rigid-body) transformations, minimising the difference between the reference scan (usually first scan) and all of the subsequent scans. During rigid-body

transformation images are superimposed on the reference scan by a combination of three rotations (by rotating image volume in x-y, x-z, and y-z planes) and three translations (by moving it along the x, y, and z axes) (Huettel et al., 2004). Sometimes, realignment is not enough and data needs further adjusting for remaining movement (Friston, 2004). In this case, data can be unwarped using field-maps, correcting for distortions in functional images due to inhomogeneity of the magnetic field. Distortion correction has been shown to increase coregistration accuracy between anatomical and functional images (Hutton, 2002).

2.2.4.2 *Co-registration of functional and structural (anatomical) data*

Functional or echo-planar imaging data can be coregistered onto individual's anatomical image by spatially aligning the images. This is performed in order to map subject's functional data into their own anatomical space.

2.2.4.3 *Stereotactic normalisation*

Following realignment, data are spatially normalised by warping it to fit into a standard anatomical space, such as Montreal Neurological Institute brain template ICBN 152 (Fonov et al., 2011, 2009), used in this thesis. In this way each voxel acquires a three-dimensional spatial coordinate (x, y and z), which in turn corresponds to the same voxel and coordinate in a template. Such normalisation enables comparison of neuronal activity in the voxel between several subjects as well as between different studies (Friston, 2004).

Normalisation becomes problematic in the pathological brain. For instance, stroke can result in deletion of normal cortical tissue, which can pose substantial problems during normalisation. In this thesis I have performed normalisation and lesion identification

using Automatic Lesion Identification (ALI) approach (Seghier et al., 2008). ALI normalisation is based on the unified normalisation-segmentation framework (Ashburner and Friston, 2005), which has been shown to be most effective when normalising lesioned brains (Crinion et al., 2007).

2.2.4.4 *Smoothing*

Smoothing is performed for several reasons: to correct for inter-subject anatomical variability, to improve signal to noise ratio, and to increase statistical power (Hopfinger et al., 2000).

Even after normalisation, functional anatomy across participants may still differ in that "areas of activity are rarely represented in exactly the same voxels" (Huettel et al., 2004). Low-pass spatial filters can be applied to "blur" or smooth the images. This is normally achieved by applying Gausian filter, which is shaped as a "bell-curve", because of its normal distribution. Under this filter, intensity in each voxel is spread to the neighbouring voxels. Depending on how narrow or wide the Gausian filter, the smoothing effect may range from over a few to many surrounding voxels. Usually, the width is expressed in millimetres at full-width-half-maximum (FWHM) value, typically from 6 to 10mm. In this thesis, all data were smoothed to 6mm FWHM.

By spatially smoothing functional data, voxels that had neighbouring active voxels yet were not active themselves are "switched on", while voxels that were active in isolation (likely due to noise) are "switched off". Smoothing also increases statistical power when analysing groups of participants. When data are spatially corrected, the spatial extent of active regions is increased, therefore, enhancing the chance of common regions of activity across individuals. (Ward, 2010)

2.2.4.5 *Statistical Parametric Mapping: mass-univariate approach*

Once the data are realigned, coregistered, normalised and smoothed, it can be analysed to make hypothesis related inferences. At the end, three-dimensional image - termed statistical parametric map (SPM) - is constructed, showing regions significantly affected by the experimental manipulation (figure 10). SPMs represent the overall outcome of the experiment and require several steps of processing before they can be achieved. During statistical analysis, each voxel's activity is analysed using univariate statistical tests, such as Student's T or F distribution, ANOVA or multiple regression. This is achieved by modelling imaging data using General Linear Model (GLM). Using GLM allows to "explain continuous (image) data in exactly the same way as in conventional analyses of discrete data" (Flandin and Friston, 2008).

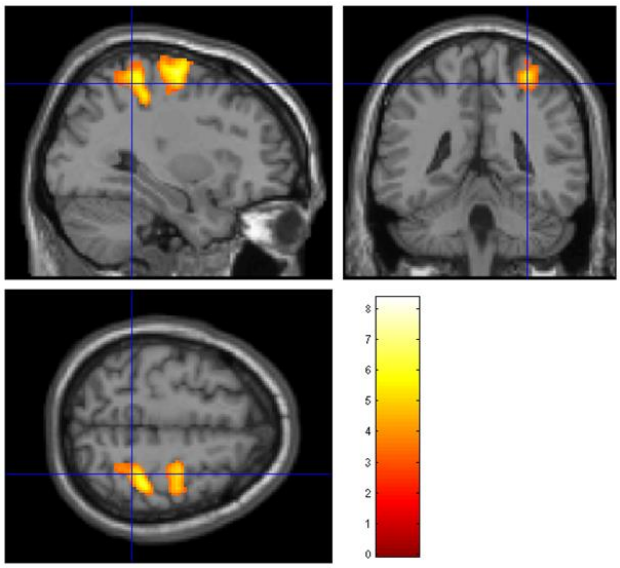


Figure 10. Example of a statistical parametric map (SPM).

The GLM is expressed as an equation $Y = X\beta + \varepsilon$, where Y represents collected data (observed response), X is an explanatory variable and ε is an error term (Friston et al., 1994). The explanatory variable X is a design matrix, comprising of effects of experi-

mental manipulation and of confounds. First columns in the matrix represent conditions in the experiment, which are followed by "series of terms that are designed to remove or model low-frequency variations in signal due to artefacts such as aliased biorhythms and other drift terms" (Flandin and Friston, 2008). Parameter weights β are estimates of relative contribution to the data from each regressor in the model. The final column in the design matrix represents activity in the whole brain.

Generally, two types of experimental design are used in fMRI, block or event-related. During the event-related design experimental stimuli are presented to the participant for a brief period of time. Block design, on the other hand, entails that stimuli are presented in blocks, usually of around 16 to 20 s, alternating with periods of rest. In this way, neuronal activity increases during presentation of the blocked stimuli and decreases with the onset of rest. Such design can be modelled as a boxcar function, whereby the presence of stimuli is expressed as "1" and the presence of rest as "0". Since haemodynamic response function (HRF) lags slightly behind the presentation of the stimulus, boxcar function for each condition is convolved with the canonical HRF (Boynton et al., 1996). Relative contribution of each column in the design matrix is then computed using standard maximum likelihood, whereby parameter weights are estimated. Finally, inferences about these contributions can be made using different statistical tests, such as t-test. This step requires an experimenter to define contrasts of interest, based on columns in the design matrix. For instance, to compare the difference in BOLD response between first and second condition, modelled as 1st and 2nd column in the design matrix, one would define a following contrast [1 -1 0 0 …] (Flandin and Friston, 2008). In such way, one

can also look at the interaction effects between several conditions [1 -1 -1 1 …], as well as common activations, conjunction, between two or more conditions [1 1 1 0 …].

2.2.4.6 *Random Field Theory*

One of the central issues with fMRI analysis is related to multiple comparisons. Statistical tests are performed on each voxel in the brain, however, the likelihood of false-positive results increases with the number of statistical tests (a typical dataset holds around 20,000 voxels). Thousands of voxels could appear as significantly important just by chance (Huettel et al., 2004). For this reason Random Field Theory (RFT) is used (Worsley et al., 1992). Essentially, RFT serves the same role as Bonferroni correction, but is less stringent. This method allows to adjust *p*-values in a small volume (region of interest), if one has prior expectation of effect particularly there - an anatomically constrained hypothesis. If no such prior knowledge exists – in case of anatomically open hypothesis - more stringent correction over the whole brain is used. RFT bases its calculations on resells (resolution elements, which depend on the amount of smoothing in the data), rather than voxels, therefore such correction is less conservative than that of Bonferroni.

2.2.4.7 *Second-level (random effects) group analysis*

Thus far described manipulations are performed on the data of each individual participant, also called *first-level* analysis. In the *second-level* analysis, data from multiple subjects in the experiment are combined. Second-level analysis is also called random-effects analysis, as it "treats the effect of the experimental manipulation as variable across subjects, so that it could have a different effect upon different subjects" (Huettel et al., 2004). In this analysis, only one image is used for each subject (i.e. a contrast de-

rived during first-level analysis), therefore, “error variance is computed using the subject to subject variability” (Friston, 2004). Using random effects analysis, one can infer that observed effect in the group of subjects is indicative of the effect in a larger population.

2.2.4.8 *Multivariate Pattern Recognition*

Above I have described methods employed to produce inference about experimental effects based on mass-univariate statistical analysis. It is “univariate”, since statistical tests are performed in each and every voxel of the imaged brain, it is “mass” because there are approximately 20,000 voxels involved in such computations (Huettel et al., 2004). This technique is a powerful way to establish regional contribution during specific cognitive or sensorimotor task, but it does not take into account the underlying communication between networks of regions or relationship between nearing voxels. Analysis that considers the interdependence of voxels and regions is multivariate by nature. One form of multivariate analysis in fMRI is that which investigates functional connectivity between brain areas during a given task or under specific circumstance (Friston, 2011). Functional connectivity explores how function is integrated in the brain by studying communication between regions. Another form of multivariate analysis is one that investigates the relationship between voxels under a particular condition, exploring patterns of neuronal activity.

Multivariate pattern analysis (MVPA) aims to decode information from patterns of activity in voxels, while employing powerful classification algorithms (Haynes and Rees, 2006; Norman et al., 2006). For instance, it has been possible to accurately predict the orientation of perceived visual stimuli from patterns of activity measured with fMRI

(Haynes and Rees, 2005; Kamitani and Tong, 2005). Similarly, Kendrick Kay and colleagues decoded which specific image was observed by their participants' from information in their primary visual cortex (Kay et al., 2008). Kay went on to predict that "it may soon be possible to reconstruct a picture of a person's visual experience from measurements of brain activity alone" (Kay et al., 2008).

Normally, MVPA is used to "predict a variable of interest (e.g. mental state 1 vs. mental state 2, or patients vs. controls) from pattern of brain activation/anatomy over a set of voxels" (Schrouff et al., 2013), which is achieved using machine learning models (Pereira et al., 2009). MVPA differs from univariate fMRI analysis in that: (1) "rather than predicting the time course of neural activity from a design matrix, we aim to predict parts of the design matrix from the time course of neural activity"(Brodersen, 2009), and (2) rather than analysing each voxel on its own, we consider patterns of co-activated voxels. Distinct neuronal populations may be engaged in coding information about two tasks within the same area. Such information may be missed during mass-univariate analysis, whereby presentation of two tasks could result in similar spatial average of BOLD response in a region (Mur et al., 2008). Therefore, MVPA affords sensitivity, which univariate approach may lack.

There are different ways to perform MVPA classification, although general principles are the same (for in depth reviews see Haynes and Rees, 2006; Norman et al., 2006). Here I will outline steps employed in this thesis by using Pattern Recognition for Neuroimaging Toolbox (PRoNTo) (Schrouff et al., 2013). PRoNTo was designed for use with Statistical Parametric Mapping (SPM) software (Wellcome Department of Imaging Neuroscience, UK (http://www.fil.ion.ucl.ac.uk/spm)).

Firstly, similarly to mass-univariate analysis, all data are realigned, corregistered, normalised and in some cases smoothed. Some researchers prefer data not to be smoothed, so as to retain fine grained pattern information. Then, data are modelled using GLM and contrasts of activity related to specific experimental condition are derived at a single subject level.

At this point, several features are selected for further decoding. Features represent three-dimensional areas where classification is performed, such as regions of interest where one expects accurate decoding between conditions. When no prior knowledge exists, whole brain may be used as a feature. Model is then specified, whereby conditions or groups to be decoded are outlined.

Subsequently, data are split into two sets: training and testing. The classifier algorithm learns to identify each condition or group from patterns of BOLD activity in defined features (i.e. ROIs) of the training set. The classifier is then tested on how well it can decode each condition or group from novel patterns of activity that are in the testing set. The accuracy with which the algorithm performs such decoding is expressed in percentage of correctly guessed test examples (when decoding between two conditions is at 50% accuracy, prediction accuracy is considered to be at chance).

In order to make use of the whole data, cross validation is used, whereby training and testing sets are repartitioned several times. Leave-one-subject-out (LOSO) cross-validation approach is most common in multi-subject designs (Ashburner et al., 2015). In this case data are repartitioned many times, in each instance leaving one subject out for testing and classifier is trained on the rest.

Finally, the significance of classification results (i.e. p-value) is achieved through permutation testing (Gaonkar and Davatzikos, 2013; Nichols and Holmes, 2002; Schreiber and Krekelberg, 2013). During this non-parametric procedure, the classifier algorithm is retrained and retested up to 1000 times using permuted labels for conditions (e.g. condition A is called condition B and vice versa). The outcome is then a meaningful p-value for classifier performance.

The discovery of functional magnetic resonance imaging has been a pivotal turn in the wide field of neuroscience, permitting the study of the human brain in the safe and non-invasive way. In this thesis I use fMRI to study how loss of motor function after stroke modulates neural activity during action observation.

2.3 PHYSIOLOGICAL MEASURES

Tests used to measure motor function of the affected and unaffected hand in each patient included Action Research Arm Test (ARAT) (Yozbatiran et al., 2008), 9 Hole Peg Test for finger dexterity (Mathiowetz et al., 1992), Box and Blocks test, assessing unilateral gross manual dexterity (Mathiowetz et al., 1985), Apraxia Screen of TULIA (Test for Upper-Limb Apraxia) (AST) (Vanbellingen et al., 2011), and finally pinch and grasp strength measurements were acquired with a dynamometer (Patterson Medical Ltd., Nottinghamshire, UK). Motor function testing instruction and scoring forms can be seen in the Appendix F. Motor scores obtained from patients included in both TMS and fMRI experiments can be found in Appendices A and B respectively.

2.4 COGNITIVE MEASURES

Patients were screened prior to their inclusion in the experiment. It was imperative to establish if patients were able to understand verbal and written instructions and if their vision was not impacted by stroke. To test for patients' spoken and written word comprehension, I used parts of Comprehensive Aphasia Test (Swinburn et al., 2004). Namely, sections on language comprehension, written and spoken word comprehension, written and spoken sentence comprehension and spoken paragraph comprehension were used. One patient did not complete cognitive scoring due to unfortunate time concerns. In this case, I made personal judgement that patient's comprehension was appropriate. In addition, patients were tested on their auditory verbal comprehension with a series of Yes/No questions. To examine if patients had unilateral spatial neglect, Mesulam's Symbol Cancellation (Mesulam, 1985). Examples of these tests can be seen in the Appendix E. All scores can be found in the Appendix C for patients included in the TMS experiment, and Appendix D for patients included in the fMRI experiment.

CHAPTER 3
CORTICOSPINAL EXCITABILITY DURING ACTION OBSERVATION - CHOOSING CORRECT STIMULI

3.1 INTRODUCTION

In this chapter I describe the process of appropriate stimuli selection and architecture of the experimental paradigm that subsequent experiments were built upon. The aim was to optimize the design of the paradigm that would be used consistently throughout this dissertation. It was imperative to perform careful selection of stimuli to ensure maximal engagement of the intact and damaged motor system during observation.

Corticospinal excitability during observation of actions depends on many factors. For instance, the size of motor evoked potentials in hand muscles is modulated by the type of observed grasp, by the weight and size of grasped object, as well as by precise timing of MEP recording within the cycle of observed action. Based on previously published literature at the beginning of my work I was aware that optimal response in muscles during observation is achieved:

- When recorded muscle is agonist in production of observed action (Candidi et al., 2010; Cavallo et al., 2013; Fadiga et al., 2005, 1995; Sartori et al., 2012; Senna et al., 2014; Urgesi, 2006).
- Just before observed hand interacts with the object, mirroring exact kinematics of observed action (Gangitano et al., 2004).
- When observed action is presented in the first person perspective (Alaerts et al., 2009; Maeda et al., 2002).

- When observed action would normally be effortful to execute, such as lifting heavier or bigger object (Alaerts et al., 2012, 2010a).

In this chapter three small pilot studies were conducted as ‘proof of concept’ and may appear insignificant. They, nevertheless, convey the fragility of motor resonance and its dependence on the type of stimuli and context. Each pilot experiment was built on somewhat disappointing results of the previous one. Finally, at the end of the third attempt, achieved results were satisfactory, allowing to build a novel paradigm that was used throughout this dissertation.

3.2 PILOT EXPERIMENT A

3.2.1 Aim

The aim in the first pilot experiment was to replicate muscle specific response to observed actions reported in literature and to determine an appropriate control condition.

3.2.2 Materials and Analysis

3.2.2.1 *Subjects*

10 healthy right-handed volunteers participated (4 females; mean age: 35.5 ± 11 years). All participants in the experiments described below provided their informed consent and were screened for adverse reaction to the TMS procedure based on the safety screening questionnaire by Keel et al., revised by Rossi et al. (Keel et al., 2001; Rossi et al., 2009). None of the participants reported any neurological condition. The experimental protocol was approved by the National Hospital for Neurology and Neurosurgery & Institute of Neurology Joint Research Ethics Committee.

3.2.2.2 *Experimental Design and Procedures*

Participants were seated comfortably in front of a computer screen at a 65cm distance, their right hand resting on the pillow positioned on their lap and underneath the desk out of view.

Five types of videos were presented on the screen (figure 11):

- Right hand pinching the lid of a jar to lift it and to put it back down.
- Left hand pinching the lid in the same action.
- Right hand grasping the jar by its side to lift it and to put it back down.
- Left hand grasping the jar in the same action.
- Large masking tape rolling to the jar, touching it and rolling back.

Each condition was presented as a pair of 4s videos, for instance, right hand pinch video would appear twice, separated by 1s interval with a fixation cross in the middle of the

screen. Two videos within a pair were either identical or differed slightly. The task for participants was to attend carefully and to respond verbally to the question that appeared at the end of each trial (a pair of videos) – “Same? Yes or No” (figure 12). After the question disappeared, short 1s rest period was marked by the same red fixation cross. An experimental run consisted of 30 such trials, presenting videos from all five conditions in a randomised order. There were 4 experimental runs in total.

All volunteers read an instruction sheet, explaining the task and completed a practice session before the beginning of the experiment.

Figure 11. Examples of stimuli used in experiment A. Stimuli were presented to the participants included power and precision grip with the left and right hand and the roll of the masking tape to the jar.

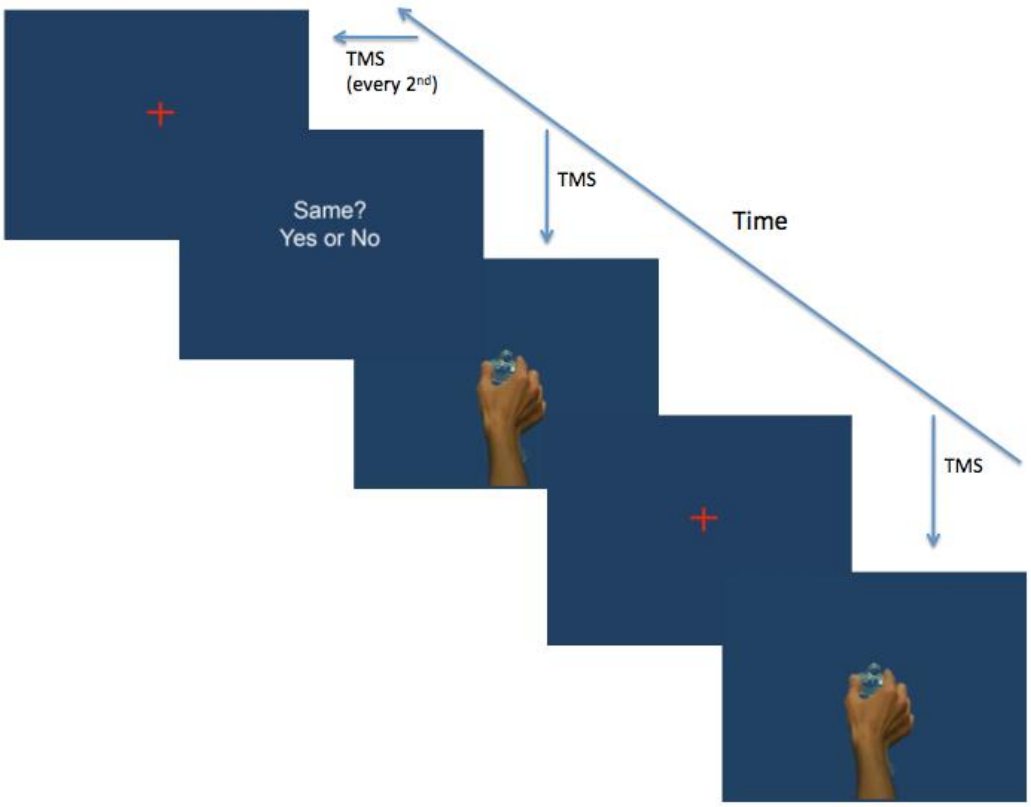


Figure 12. Experimental paradigm. Videos were presented in pairs with a following question of whether two actions were the same. Single pulse TMS was delivered at the 25th frame of each video and during every second rest period after the question.

Footage in all five conditions shared the same light blue background, same object – a jar, same velocity of movement and same time of point of interaction between the hand or a masking tape and an object. This was done to control for most of the confounding elements that could be modulating physiological response. To achieve this, I edited filmed material using Motion 2 software, part of the Final Cut Studio application package (Apple Inc.). Videos were made to be 100 frames each, 4 seconds long, with a consistent point of interaction between hand and object at the 25th frame, i.e. at the first second. The model in these videos was male and all actions were filmed from the egocentric perspective.

Participants performed the task in 4 experimental runs, each lasting approximately 10 minutes and comprising of 75 single-pulse TMS events. TMS was applied during observation of each clip as well as during every second rest period (15 per experimental run). Overall, 48 measurements were collected for each experimental condition and 60 measurements during rest. The pulses were not closer then 5s in time.

3.2.2.3 *Electromyographic (EMG) recordings and TMS*

MEPs were recorded from two hand muscles that act as agonists in either pinch or grasp. Bipolar surface electrodes were positioned in the belly-tendon montage over the first dorsal interosseus (FDI) and the abductor digiti minimi (ADM) muscles: the former located between thumb and index finger and predominantly involved in execution of pinch, whereas the latter largely employed during grasp by pulling little finger away from the other fingers. The raw EMG activity from these muscles was sampled at 5 kHz and amplified (CED 1401 data acquisition interface, Cambridge Electronic Design, Cambridge, UK). Band-pass filter was applied between 10 Hz and 2 kHz and notch filter was set at 50Hz. Data were thus digitized and stored for the subsequent offline analysis.

Single pulse TMS was applied to M1 by placing a figure-of-eight coil (9cm in external diameter) on the left hemisphere tangentially to the scull (approximately 5cm laterally and anteriorly to the vertex), with the handle pointing backwards and rotated 45 deg. away from the mid-sagittal line. The coil was connected to a Magstim BiStim2 stimulator (Magstim Company, Whitland, UK). The best point for stimulation (hot-spot) was set at the site on the scull where TMS pulse elicited largest MEP amplitudes in both muscles. Resting motor threshold (rMT) was defined at the stimulation intensity at

which MEPs of 50 μV were reliably induced at least 50% of the time (5 out of 10 times) in both FDI and ADM muscles. During the experiment the intensity used was 120% of the rMT to ensure consistent muscle activity.

TMS was triggered using a custom-made Matlab script (Matlab R2009b, MathWorks, Inc and Cogent toolbox, vislab), allowing for precise control of the TMS pulse timing with respect to the video display.

3.2.2.4 *Data Analysis*

Neurophysiological data were then analysed using Spike 2 software. Background EMG was analysed to exclude all trials with muscle activity above threshold, which was calculated by adding 2 standard deviations to the mean of the background activity in that muscle during the experimental run.

MEP values for each muscle were averaged across every run and condition. For a within subjects comparison, all values were normalized to the averaged values collected during rest period between the trials. Values were thereafter entered into the IBM SPSS statistical package for further analysis (Version 19.0. Armonk, NY: IBM Corp).

3.2.3 Results

As expected, observation of two types of grip had differential impact on the corticospinal excitability in two muscles. Size of normalised MEP amplitudes was significantly greater in the FDI muscle during observation of pinch (M = 0.96, SD = 0.12), compared to grasp (M = 0.89, SD = 0.1), t (9) = -2.71, p = 0.024. Similarly, greater MEP amplitudes were obtained in the ADM muscle during observation of grasp (M = 1.03, SD = 0.19), compared to pinch (M = 0.89, SD = 0. 22); t (9) = 3.70, p=0.005. What appeared puzzling from these calculations, however, was that overall size of normalised MEP amplitudes was below baseline (MEPs collected during inter-trial rest period).

Furthermore, when comparing measurements form the experimental conditions to those obtained during control condition (rolling of the masking tape), mean MEP amplitude in the ADM muscle was greater during observation of tape (M = 1.05, SD = 0.23) than that of pinch (M = 0.89, SD = 0.22).

In order to visualize mirror motor facilitation, mirror ratios were calculated (Catmur et al., 2011). MEP mirror ratio is determined by dividing MEP amplitudes collected during a particular condition from one muscle by amplitudes from another muscle. For instance, during observation of pinch I would expect FDI to ADM ratio to be greater than 1, as FDI is an agonist muscle during execution of pinch and is known to be facilitated during observation of pinch. However, I would also predict that the same ratio would be reversed during observation of grasp, as this time ADM, not FDI should be facilitated more. Action specific facilitation in muscles during pilot experiment A can be seen in figure 13.

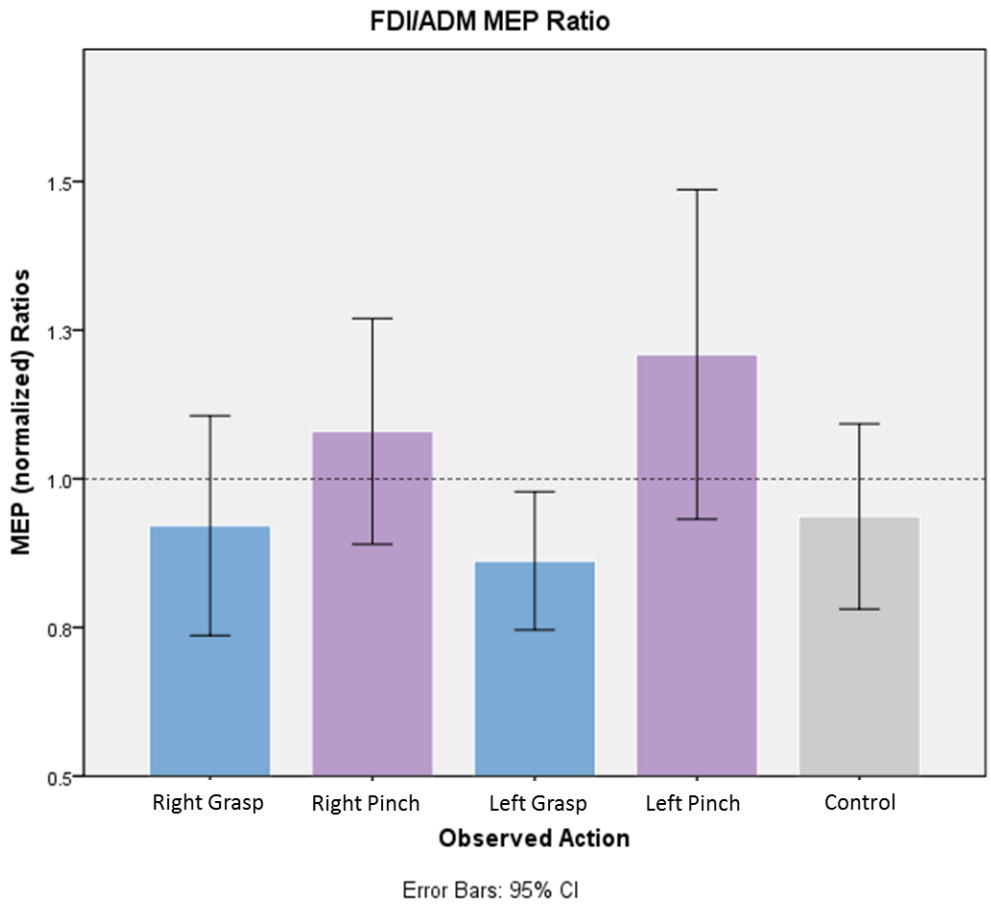


Figure 13. FDI/ADM MEP Ratio (pilot experiment A). This figure shows ratio of normalised MEP amplitudes between FDI and ADM muscle during observation of grasp actions (blue bars), pinch actions (purple bars) and control action (grey bar). Bars below 1 indicate greater ADM facilitation compared to FDI during a particular condition. Bars above 1 indicate the opposite (greater FDI involvement than ADM). This figure shows that observation of grasp indeed results in greater enhancement of MEP amplitudes in the ADM muscle, and observation of pinch results in larger response in FDI muscle. It is also evident that observing rolling masking tape produces higher MEP amplitude in the ADM muscle, which is an indication that this control condition is not suitable for the paradigm.

3.2.4 Discussion

3.2.4.1 *Summary of Results*

- Observation of different types of action, such as grasp and pinch elicits differential MEP facilitation in hand muscles.
- MEP amplitudes recorded during inter-trial periods do not reflect muscles at rest. Residual enhancement may still persist into the rest period contributing to its elevated MEPs.
- Non-biological movement, such as of a rolling masking tape, may elicit differential facilitation in muscles. Possibly due to affordability of an object. Masking tape appeared graspable due to its shape. Observing it may have produced facilitation in MEP amplitudes in the ADM muscle.

3.2.4.2 *Discussion*

Similarly to previously reported literature, my findings suggest differential patterns of muscle activity during observation of separate actions (Candidi et al., 2010; Cavallo et al., 2013; Fadiga et al., 2005, 1995; Sartori et al., 2012; Senna et al., 2014; Urgesi, 2006). ADM muscle, which is utilized during execution of grasp, showed higher covert MEP facilitation during observation of grasping a jar, as opposed to pinching a top of a lid. Whereas activity in FDI, an agonist muscle during pinch, was significantly more prominent during observation of pinch rather than grasp. Evidently, observation of motor actions alone has an effect on the corticospinal excitability.

An unexpected result was of greater facilitation in the ADM muscle during observation of control condition. It is plausible that presentation of the masking tape modulated ex-

citability in the muscle because of its affordability. Masking tape can be manually manipulated, in this instance grasped, suggesting that viewing a graspable item was sufficient to simulate necessary accompanying action.

This final point raised the question of whether the same object presented in both pinch and grasp conditions (a jar in this instance) is suitable to achieve optimal results, since by its mere presence it proposes affordability for both actions. I was interested in whether the mirror ratios could be enhanced by using objects suitable for either grasp or pinch, but not for both. The stimuli were re-filmed and re-edited to include two objects, each affording only one action. Further, the control condition was substituted with video clip depicting motion of the hand with no object present, to avoid introducing affordability. Finally, a baseline measure was added before the experiment for clear disambiguation of the activity at rest and during viewing of the stimuli.

3.3 PILOT EXPERIMENT B

3.3.1 Aim

The aim of this pilot study was to test the effects of pinch and grasp observation using objects that could be only grasped or only pinched, but not both. In addition, different type of control condition was included, accounting for biological motion in the absence of an object. This time baseline activity was collected before the experiment. Finally, activity was recorded from five hand and forearm muscles, two of which served as control muscles.

3.3.2 Materials and Analysis

3.3.2.1 *Subjects*

7 participants (3 females; mean age = 36.5 ± 10 years).

3.3.2.2 *Experimental Design and Procedures*

The experimental paradigm was identical to the one described in the previous experiment with several exceptions. The baseline recording of MEPs was added to the beginning of the experiment and the stimuli this time were different, comprising of six types of actions (figure 14):

- Right hand pinching a small marble to lift it and to place it back down,
- Left hand pinching the same marble,
- Right hand grasping a 4” ball to lift it and to put it back down,
- Left hand grasping the same ball,
- Right hand shaped into a fist (no hand opening) moving towards the centre of the screen and back,
- Left hand shaped into a fist performing the same action.

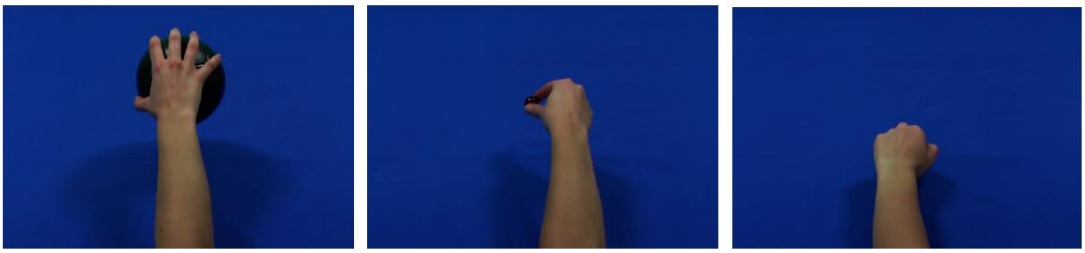


Figure 14. Examples of stimuli used in experiment B. Examples of stimuli presented to the participants included power grip of a ball, precision grip of a marble and movement of a hand forward and backward. All three actions were performed with left and right hand.

Before the beginning of the experiment 24 Baseline MEPs were collected at the interval of 5s. Participants were instructed to look at the red fixation cross centered on the screen. Experimental conditions were contained within 4 experimental runs, each lasting approximately 12 minutes and consisting of 90 single-pulse TMS events. 36 trials appeared per run in a randomised order. M1 was stimulated during observation of each clip, summing to 48 stimulations for each condition throughout the experiment. In addition, TMS was applied during every second rest period following disappearance of the question on the screen, amounting to 18 rest stimulations in one run.

3.3.2.3 *Electromyographic (EMG) recordings, TMS and Data Analysis*

During this experiment I collected muscle evoked data from five muscles. Apart from FDI and ADM muscles that were used previously, I included OP (Opponens Pollicis), the function of which is to permit thumbs touching other fingers , FCR (Flexor Carpi Radialis) and ECR (Extensor Carpi Radialis), both of which are essential in flexion and extension of the wrist, however not specific to either pinch or grasp (figure 15). All of these muscles are known to be involved in the actions that participants were watching. I hypothesized that MEPs elicited in OP would be largely similar to the ones collected in the FDI, and that FCR and ECR although facilitated, would not be differentially involved during observation.

In this experiment all values were normalized to baseline measurements collected at the beginning of the experiment. Remaining set up for recording, stimulation and data analysis were identical to the one described in the pilot experiment A.

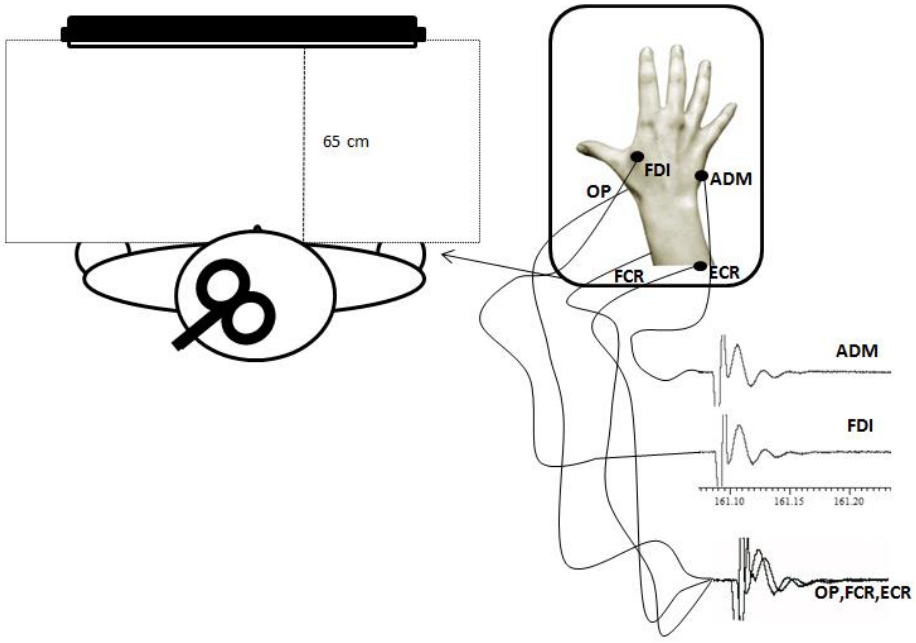


Figure 15. Experimental setup during pilot experiment B. Muscle activity throughout the experiment was recorded in five hand muscles: first dorsal inter-osseus (FDI), abductor digiti minimi (ADM), opponens pollicis (OP), flexor carpi radialis (FCR) and extensor carpi radialis (ECR).

3.3.3 Results

Facilitation of activity was observed in all muscles during observation of experimental conditions compared to baseline recorded at the beginning of the experiment.

Repeated measures ANOVA with factors (Observed Action (Pinch/Grasp) and Recorded Muscle (FDI/ADM) revealed significant interaction F (1, 6) = 15.643, p=0.007. Significant interaction was also found when the same ANOVA was performed using data from the OP muscle instead of FDI (as both of them are implemented in the execution of pinch) F (1, 6) = 29.539, p = 0.002.

A series of *post-hoc* t-test comparisons showed significant difference in normalised MEP amplitudes between observation of pinch and grasp in the:

- OP muscle – facilitation was greater during observation of pinch (M = 1.55, SD = 0.63) than grasp (M = 1.4, SD = 0.65), t (6) = -2.684, p = 0.036.
- ADM muscle – facilitation was greater during observation of grasp (M = 1.75, SD = 0.62) than pinch (M = 1.1, SD = 0. 27), t (9) = 3.86, p = 0.008.

There was no difference in MEP amplitudes between observation of pinch and grasp in the:

- FDI muscle – no significance.
- FCR and ECR – no difference (figure 16 shows normalised MEP ratios).

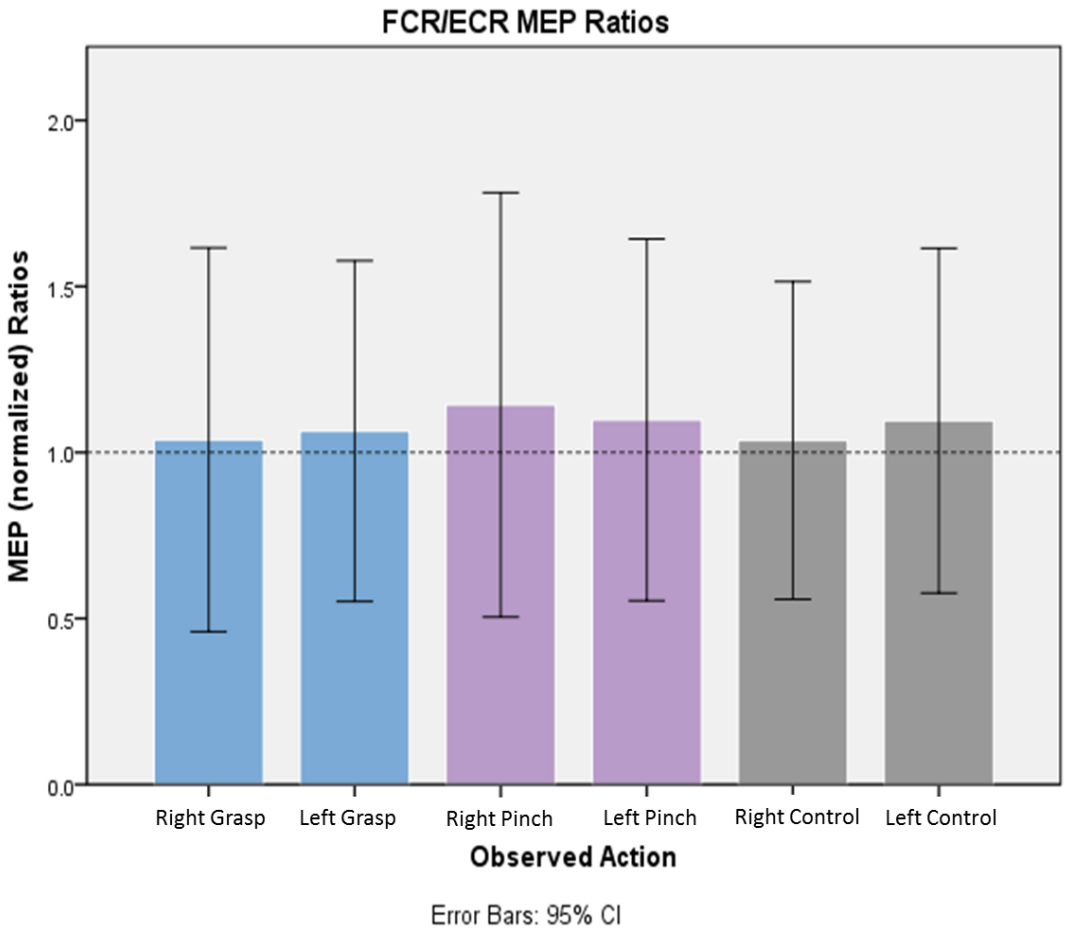


Figure 16. FCR/ECR MEP Ratio (pilot experiment B). Normalised MEP ratios (FCR/ECR) reflect absence of mirror motor facilitation in the control muscles.

3.3.4 Discussion

3.3.4.1 *Summary of Results*

- Baseline measurements yielded anticipated results showing facilitation in the muscles during experimental conditions.
- Observation of different types of action, such as grasp and pinch elicited differential MEP facilitation in hand muscles involved in execution of these actions. Facilitation was observed in the OP, FDI, ADM, but not in the FCR and ECR muscles.
- Observing pinching a small marble resulted in no facilitation of MEP amplitude in the FDI muscle. Although some facilitation was observed in the OP.
- Although significant interaction between action and muscle was present between ADM and FDI as well as ADM and OP, these interactions appeared to be driven by significantly higher response in the ADM muscle during observation of grasp.

3.3.4.2 *Discussion*

The difference between grasp and pinch observation was not present in the FDI muscle, but was prominent in both ADM and OP. This was likely due to the type of selected pinch stimuli, in this case lifting a small marble. Although it was clearly seen that the marble is pinched, it was also obvious from the video that the object was light in weight and pinching it would not require force application or great reliance on the FDI muscle. This accords with findings that weight of an observed object impacts on MEP sizes in muscles normally involved in manipulating that object (Alaerts et al., 2010a, 2010b).

Both pilot experiments A and B clearly demonstrate the importance of appropriate stimuli to study corticospinal excitability during observation. The effects were subtle and depended on the type of observed object, its affordability, the force and musculature required to manipulate it in real life. Experiment C was conducted to identify the pair of stimuli that would be best at disambiguating between grasp and pinch in FDI and ADM muscles.

3.4 PILOT EXPERIMENT C

3.4.1 Aim

The aim of the following pilot study was to identify the stimuli that resulted in greatest differentiation of activity in FDI and ADM. The results of the preceding two experiments outline the modulatory effect of the manipulated object in the observed action. Next, I presented participants with pinching and grasping of different objects in order to determine the stimuli that resulted in best interaction between type of observed action and recorded muscle. I wanted to pinpoint the pinch stimuli that would result in largest facilitation in the FDI muscle and the grasp stimuli that would have greatest effect on the ADM muscle. I aimed to make sure that if findings in my research showed lack of motor resonance in patients with impairment, it would not be due to inappropriate stimuli.

3.4.2 Materials and Analysis

3.4.2.1 *Subjects*

6 participants were recruited in this experiment (5 females; mean age = 28.5 ± 6 years).

3.4.2.2 *Experimental Design and Procedures*

The experiment consisted of 7 conditions, depicting 3 types of grasp and 4 types of pinch, all performed with the right hand. Grasp conditions consisted of grasping, lifting and putting down a 1) 4" black ball, 2) smaller jar and 3) large, heavier jar, both filled with objects to make them visibly heavier. Pinch conditions comprised of pinching, lifting and putting down a 4) small black marble, 5) clothes peg, 6) lid of a smaller jar and 7) lid of a larger jar, both of which were used during grasping conditions (see figure 17). Two baselines were collected, one before and one after the experiment. Just as in the earlier designs, same light blue background and same velocity of movement was used in all videos. Each clip comprised of 100 frames with the point of interaction between the hand and object consistently at the 25th frame.

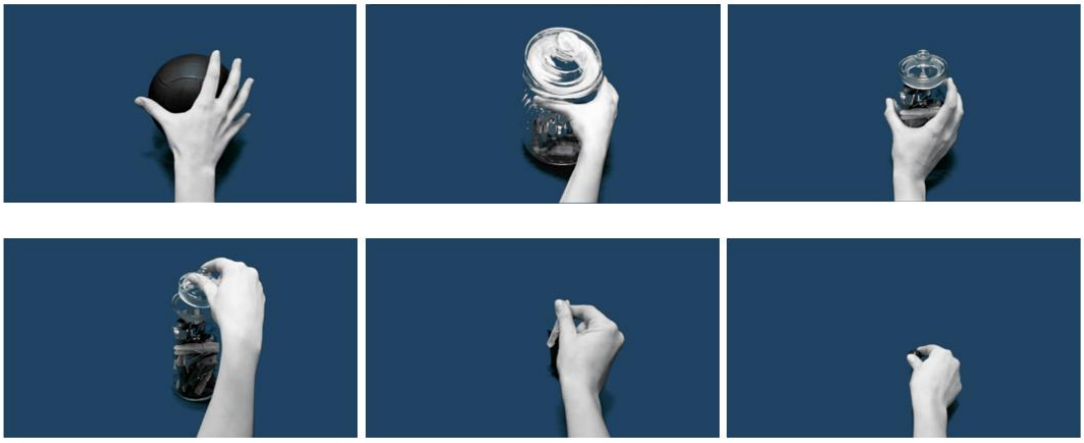


Figure 17. Examples of stimuli used in experiment C. Examples of the types of actions participants observed, including power grip of a ball, of a big jar or a small jar, as well as the precision grip of the marble and clothes peg, also the lids on big and small jars.

3.4.2.3 *Electromyographic (EMG) recordings, TMS and Data Analysis*

EMG recordings, TMS and Data Analysis were identical to those in previous pilot experiments.

3.4.3 Results

Consistent with previous findings, there was a differential activation in hand muscles based on the type of grip that was observed.

Following a series of paired t-tests, the most significant difference in both FDI ($t(5) = -5.155$, $p = 0.004$) and ADM ($t(5) = -3.476$, $p = 0.018$) muscles was found between grasping a ball (FDI*:* M=1.24, SD=0.436, and ADM: M=1.78, SD=0.78) and pinching a clothes peg (FDI*:* M=1.68, SD=0.49, and ADM: M=1.12, SD=0.54) (figure 18).

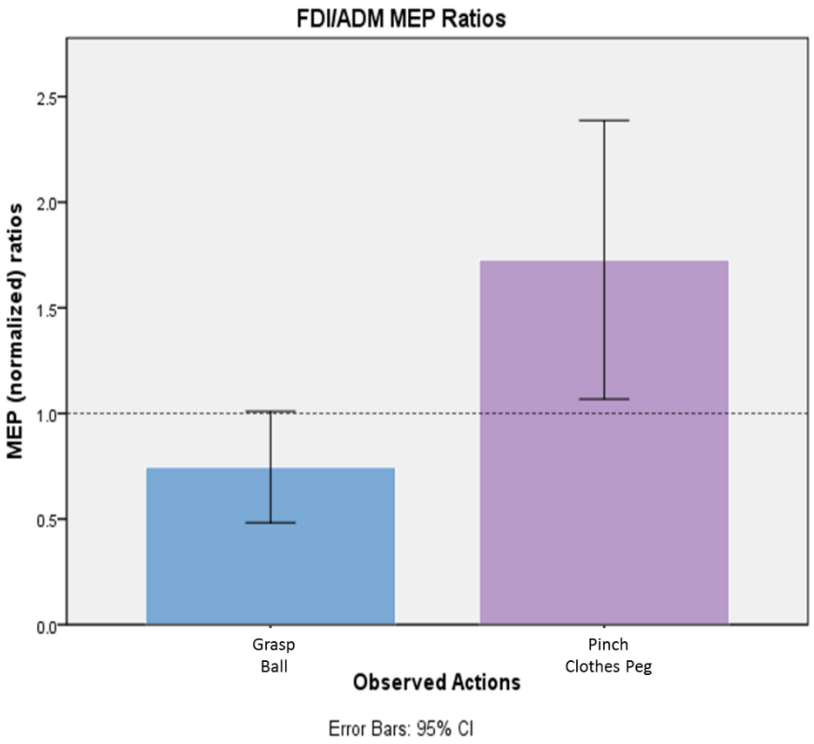


Figure 18. FDI/ADM MEP Ratio (pilot experiment C). Best mirror ratios between FDI and ADM muscles found during observation of grasping a ball (mirror ratio = 0.74) and pinching a clothes peg (mirror ratio = 1.73).

3.4.4 Discussion

3.4.4.1 *Summary of results*

- Best differentiation between FDI and ADM muscle for grasp and pinch actions was during observation of grasping a ball and pinching a clothes peg.

3.4.4.2 *Discussion*

It appeared that optimal facilitation in the FDI muscle can be achieved during observation of pinching a clothes peg and facilitation in the ADM muscle during observation of grasping a ball.

3.5 CONCLUSIONS

With my pilot experiments I have been successful in replicating the effects showed by other groups as well as optimizing them by selecting appropriate stimuli. In the process I became aware of the differences in the effect sizes caused by subtle variations in the observed stimuli. This was not readily seen from the published literature at the time. In addition to replicating previously reported results, it was imperative to also maximize the effect sizes. Selected final stimuli were used in experiments presented in the following chapters, aiming to establish if the ability to execute observed actions effects the engagement of the motor system during action observation.

CHAPTER 4
ENGAGEMENT OF THE CORTICOSPINAL TRACT DURING ACTION OBSERVATION IN HEALTHY INDIVIDUALS

4.1 INTRODUCTION

It is generally accepted that corticospinal tract (CST) excitability is modulated not only by action execution, but also by action observation. The use of transcranial magnetic stimulation (TMS) has made it possible to measure how observing actions influences activity in the corticospinal system. Specifically, a single TMS pulse to the primary motor cortex (motor 'hot spot') results in a motor evoked potential (MEP) in the target muscle. Changes in the size of the MEP are taken as evidence of altered corticospinal system excitability. This approach has demonstrated that action observation not only modulates CST excitability, but it does so in a muscle-specific and time-dependent fashion (Naish et al., 2014). The effects of action observation on muscle engagement are real but relatively subtle. As I showed in Chapter Three, these effects are sensitive to the choice of baseline or stimuli. The aim of this chapter was to replicate the muscle-specific effect reported in other studies and to address questions that remain unanswered or are inconclusive in published literature to date. In particular, I explored whether the observed hand laterality (i.e. watching left or right hand) resulted in differential response in left and right hand muscles of observers. Furthermore, I examined the relationship between age and CST excitability during action observation.

Several studies indicate that CST excitability is specific to the observed action, engaging agonist muscles to a greater extent than muscles not normally involved in the

execution of the observed action (Fadiga et al., 1995; Maeda et al., 2002; Romani et al., 2005; Sartori et al., 2012; Senna et al., 2014; Urgesi, 2006). This muscle-specific effect is thought to depend on several factors such as the precise timing of TMS pulse in relation to the observed action (Cavallo et al., 2014, 2013; Lepage et al., 2010) and whether the observed action is goal-directed or intransitive (Lago and Fernandez-del-Olmo, 2011; Naish et al., 2014). For instance, early stimulation of motor system results in non-specific CST excitability, while later stimulation (greater than 200 ms after the onset of the observed action) leads to clear muscle-specific facilitation (Naish et al., 2014).

In addition to increased MEPs in agonist muscles during action observation, some studies explored whether CST excitability was modulated by the laterality of observed hand (left or right). Results from these studies have been contradictory. Aziz-Zadeh and colleagues found a clear hand laterality-specific effect, showing that facilitation of MEPs in the right first dorsal interosseous (FDI) muscle was greater during observation of right rather than left index finger movement, and vice versa for the left side (Aziz-Zadeh et al., 2002). In contrast, Sartori and colleagues showed no laterality-specific effect in the abductor digiti minimi (ADM) muscle, demonstrating that facilitation was higher in the dominant hand of participants irrespective of which hand was observed (Sartori et al., 2013). It is unclear why findings are conflicting, except that both studies explored excitability in only one hand muscle. Furthermore, the observed action in the work of Aziz-Zadeh et al., was intransitive (movement of index finger) whilst Sartori et al., asked their participants to watch a thermos being grasped. So, differences in design may have contributed to different outcomes and it remains to be established if observed hand laterality influences CST excitability during action observation.

Moreover, to date there are no published studies exploring the relationship between age and CST excitability during action observation. It has been previously shown that dexterity is reduced with increased age (Martin et al., 2015). In addition, Sale and Semmler demonstrated that during grip execution MEPs were lower in the left hand of older as compared to younger participants (Sale and Semmler, 2005). Since FDI is also facilitated during observation of dexterous actions, it is likely that motor resonance in this muscle is modulated by age. To date, the majority of findings on CST excitability during observation are based on evidence collected from young healthy participants and the effect of age has not been explicitly addressed.

In this chapter I aim to replicate muscle-specific effect by establishing double dissociation using two agonist muscles normally involved in two distinct actions. I compare facilitation in the FDI (agonist muscle during pinch) with that in the ADM (agonist muscle during grasp) while healthy participants watch pinch and grasp actions. In addition, MEPs were recorded in both left and right hand of participants during observation of left and right hand actions. In this way, I aimed to establish if observed hand laterality is reflected in MEPs of left and right hand in both FDI and ADM muscles. Finally, participants in the following experiment ranged from 21 to 69 years old, allowing to explore if MEP facilitation in hand muscles is affected by observer's age.

Answering these questions in a group of healthy participants is important prior to addressing the primary hypothesis of this thesis and to establishing whether *impaired* ability to execute observed actions modulates motor resonance in patients.

4.2.1 Subjects

18 healthy right-handed volunteers participated (females=11; age range: 22 -69; mean age: ± 38 years) twice in this study. All participants in the experiments described below provided their informed consent and were screened for adverse reaction to the TMS procedure based on the safety screening questionnaire by Keel et al., revised by Rossi et al. (Keel et al., 2001; Rossi et al., 2009). None of the participants reported any neurological condition. The experimental protocol was approved by the National Hospital for Neurology and Neurosurgery & Institute of Neurology Joint Research Ethics Committee.

4.2.2 Experimental task

Volunteers were seated comfortably in front of a computer screen. The distance between the corner of the eye of the participant and the screen was 65cm. Both hands were resting on the pillow positioned on their lap and underneath the desk, out of view. Subjects were asked to watch pairs of videos (each lasting 3.36 s) which depicted experimenter's hand pinching or grasping an object. To ensure that participants were attending to the observed action, after each pair of video clips the question appeared on the screen instructing to judge whether seen clips were identical or not. Subjects responded verbally. Responses were recorded throughout the experiment for further analysis.

The experiment comprised three experimental and four baseline runs. The observation conditions within the experimental run were: 1) left pinch, 2) right pinch, 3) left grasp and 4) right grasp (figure 19). The object in the grasping condition was a black ball, 4 inches in diameter. The object in the pinching condition was a clothes peg fixated on the black upright cardboard for ease of manipulation. Each run consisted of 24 pairs of videos. During each trial a pair of videos from the same condition (example: left pinch) was shown followed by a question "Same? Yes/No" (figure 20). The clips were identical 50% of the time. In the remaining half of the trials the two videos differed very slightly, yet noticeably. Overall three similar video clips of the same action were used, thus each time the two movies differed in an unpredictable way.

A short 1s interval separated the two clips within a pair, during that time a red cross appeared on the screen and volunteers were instructed to keep their gaze fixated on it. The question following each trial remained on the screen for 2s and was followed by another

1s fixation cross period. Every condition was represented equally, i.e. appeared 12 times within each run and 36 times throughout the experiment. Muscle responses in the form of motor evoked potentials (MEPs) were recorded during observation of each video. To allow for some time-controlled rest, 1 minute break was introduced midway through each run.

During baseline runs, participants were asked to look at the fixation cross. 24 muscle responses were recorded within that time. The overall length of the experiment was close to 40 minutes with the variable set up time of approximately an hour.

All volunteers read an instruction sheet, explaining the task and completed a practice session before the beginning of the experiment. The practice session was not fixed and allowed enough time for participants to feel confident in their judgement.

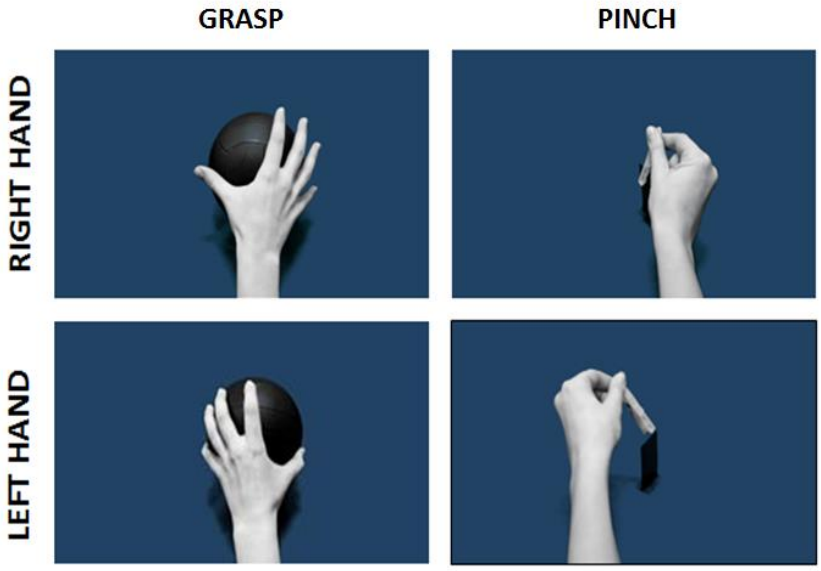


Figure 19. Experimental stimuli. Four action observation conditions were presented in pairs of short movie clips (from the top left): right grasp, right pinch, left grasp and left pinch

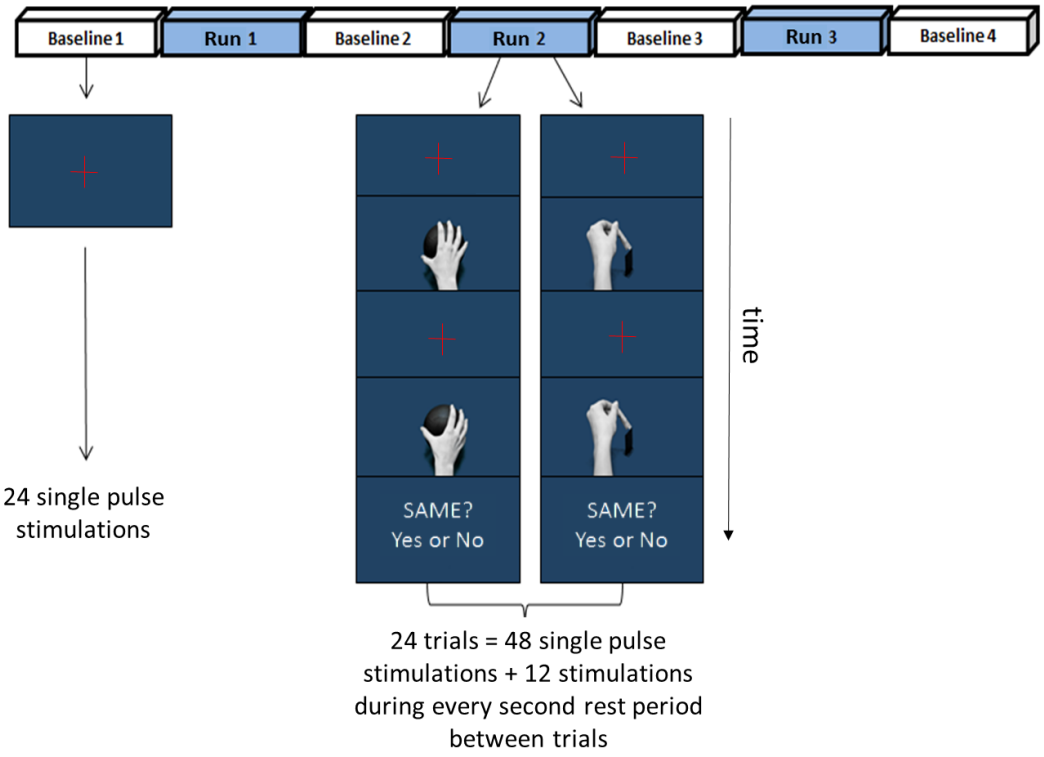


Figure 20. Experimental design. Three experimental and four alternating baseline runs comprised the experiment. During baseline, participants were instructed to fix their gaze on the red cross in the middle of the computer screen, while 24 stimulation pulses were delivered and MEPs were collected. During each experimental run 24 pairs of action movies were presented followed by a judgement question "Same? Yes/No" to which participants responded verbally.

Footage in all four experimental conditions shared the same light blue background, same velocity of movement and same time of point of interaction between the hand and the object. In doing so, I attempted to control for confounding elements unrelated to the type of action of interest and modulating physiological response. To achieve this, filmed material was subsequently edited using Motion 2 software, part of the Final Cut Studio application package (Apple Inc.). Videos were made to be 100 frames each, 3.6 seconds long, with a consistent point of interaction between hand and object at the 25th frame.

The model in these videos was female and all actions were filmed from the egocentric perspective.

4.2.3 Data Acquisition and Analysis

4.2.3.1 *Electromyographic (EMG) recording and TMS*

Data were collected from participants' right and left hands on separate occasions. The order was counterbalanced within the group. There was no set period between sessions, but it did not exceed 4 months.

MEPs were recorded from FDI and ADM muscles. The setup was exactly the same as in pilot experiments and has already been described in detail in Chapter Three section under the same heading.

In addition to the set up already tested in pilot studies, The Visor 2 neuronavigation system was also used in order to help monitor hot-spot location during stimulation (ANT Neuro HQ, Ent, Enschede, Netherlands). Deviation from the target by more than 5mm in distance and in angle was signaled online and prompted correct repositioning. The neuronavigation system was also used in subjects for whom structural MRI images were available (figure 21), permitting more accurate localization of individual hot-spot (hand area in M1). For participants whose anatomical scans were not obtained prior to testing, a template MRI image was used.

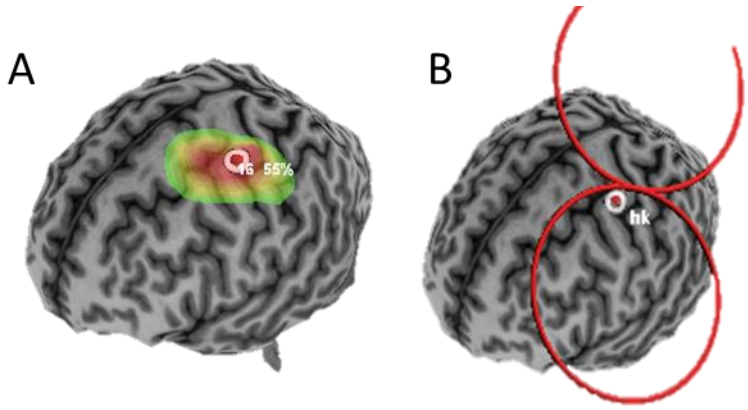


Figure 21. Example of participant's anatomical MRI scan reconstructed using Visor 2. The hot spot is clearly seen in the hand area of the primary motor cortex. The spread of stimulation is colour coded around the hot spot in image A. Image B shows the figure-of-eight coil position relative to the hot spot, which was maintained throughout the experiment.

4.2.3.2 *Data analysis*

Neurophysiological data were then analysed using Spike 2 software (Cambridge Electronic Design, Cambridge, UK). Background EMG was analysed to exclude all trials with muscle activity above threshold, which was calculated by adding 2 standard deviations to the mean of the background activity in that muscle during the experimental run.

MEP values for each muscle were averaged across every run and condition. For a within-subject comparison, all values were normalized to the averaged values collected during baseline periods preceding the runs of trials. Values were thereafter entered into the IBM SPSS (Version 20.0. Armonk, NY: IBM Corp) package for further statistical analysis. Some of the data were not normally distributed, which was solved by Log10 transformation of all of the data for further statistical analysis. The results in the figures presented further were back-transformed to reflect original means with adjusted variance.

4.2.3.3 *Statistical analysis*

For the statistical analysis, four factors were outlined: 1) Observed Hand (Left or Right); 2) Observed Action (Pinch or Grasp); 3) Recorded Hand (Left or Right) and 4) Recorded Muscle (FDI or ADM). A 4-way repeated measures ANOVA was performed to identify potential relationships between these factors. The interpretation of such results however, becomes too complex and inconclusive. Thus, further analysis was broken down into 4 2x2 repeated measures ANOVAs in order to reliably interpret the data. I present results in the order of relevance, addressing primary aims outlined in the beginning of this chapter.

4.2.4 Motor performance score

Each participant also undertook a set of standardized behavioural tests to measure upper limb motor performance. Tests included Action Research Arm Test (ARAT) (Yozbatiran et al., 2008), 9 Hole Peg Test for finger dexterity (Mathiowetz et al., 1992), Box and Blocks test (Mathiowetz et al., 1985), Apraxia Screen of TULIA (Test for Upper-Limb Apraxia) (AST) (Vanbellingen et al., 2011), and finally pinch and grasp force measurements acquired with dynamometer (Patterson Medical Ltd., Nottinghamshire, UK). The measurements were collected to exclude any motor dysfunction that could confound the results.

4.3.1 Behavioural Results

Response accuracy (RA) was significantly above chance in healthy participants (average RA = 85.5%, SD = 7.3). Subjects were tested on two occasions, average response accuracy was similar across both sessions (Right M1 stimulation: RA = 86.2%, SD = 5.8; Left M1 stimulation: RA = 84.9%, SD = 8.6).

4.3.2 Observing actions relative to baseline

Baseline values collected before each experimental run were used to normalise MEP amplitudes of the following run during which participants viewed hand actions. The change in amplitude during observation of actions as compared to rest was depicted. Greater MEP responses relative to baseline indicate facilitation in the muscle during the experimental condition.

Notably, facilitation in both muscles was evident during all conditions. The increase was highest in the right ADM muscle during observation of grasp marked by the rise of the average size in amplitude by 55% during observation of the right hand and 53% during observation of the left hand.

Overall, there was a significant increase in MEPs of the right hand muscles during observation of left pinch (FDI - p=0.002; ADM – p=0.001), right pinch (FDI - p=0.001; ADM – p=0.003), left grasp (FDI - p=0.032; ADM – $p < 0.001$), and right grasp (FDI - p=0.021; ADM – $p <$ 0.001). Similarly, significant facilitation was observed in the left hand muscles during observation of left pinch (FDI - p=0.009; ADM – p=0.012), right pinch (FDI - p=0.017; ADM – p=0.017), left grasp (FDI - p=0.027; ADM – p=0.011), and right grasp (FDI - p=0.044; ADM – p=0.005) (figure 22).

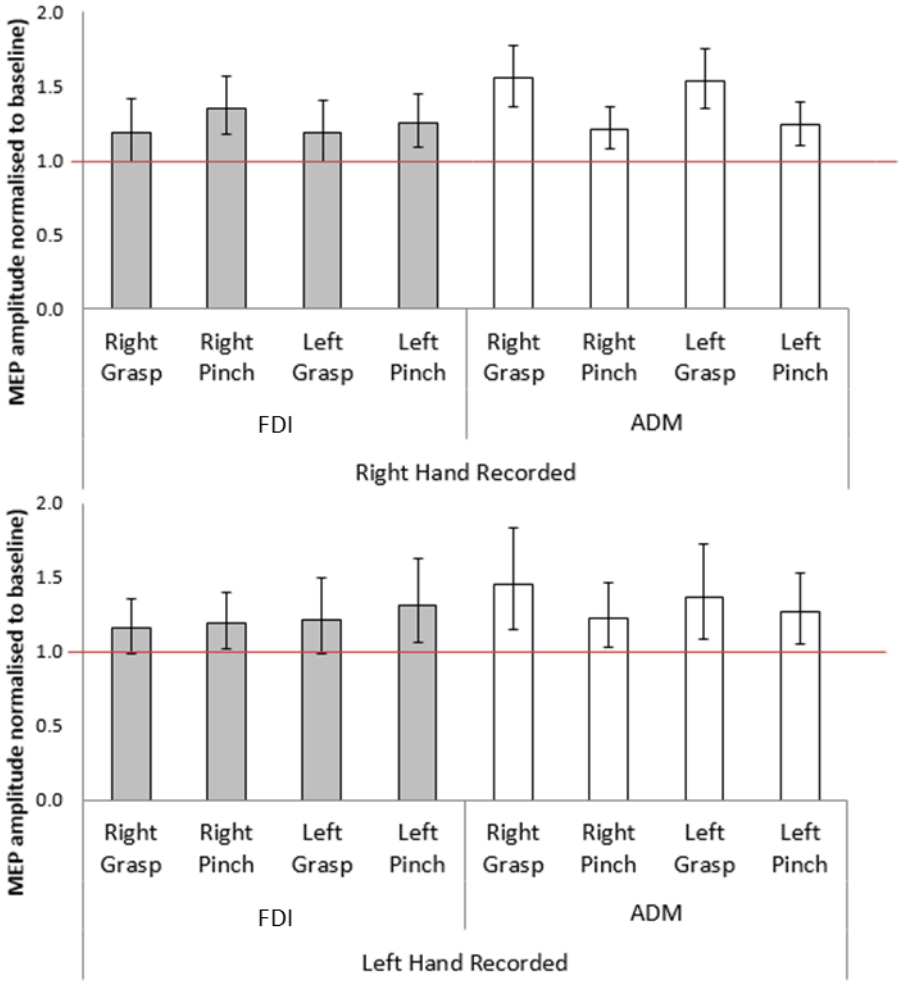


Figure 22. Facilitation in the hand (A – right and B – left) muscles FDI and ADM during observation of pinch and grasp actions. Y-axis marks an increase in the mean amplitude of the motor evoked potentials (MEPs) compared to baseline (indicated by the red line (Mean MEP of 1 denotes amplitude at baseline).

4.3.3 Main Effect of Muscle

Size of normalised MEP amplitudes in both FDI and ADM was significantly higher during observation conditions than during baseline runs. Moreover, it appears that overall activity in the FDI muscle was smaller than that in the ADM, as revealed by the significant main effect of muscle F (1, 17) = 5.508, MSE = 0.022, p = 0.031.

4.3.4 Action-specific facilitation

An interaction was observed between the Recorded Muscle (FDI/ADM) and the Observed Action (Pinch/Grasp), F (1, 17) = 40.916, MSE = 0.005, p<0.01 (see figure 23). In other words, the relative response in the two muscles was modulated by the type of observed action. In the FDI viewing pinch elicited higher facilitation than viewing grasp and, similarly, marked facilitation was present in the ADM during observation of grasp but not pinch. Overall, interaction seemed to be driven by response in the ADM muscle. To investigate this result further, interaction was broken down to account for the recorded and the observed hand laterality.

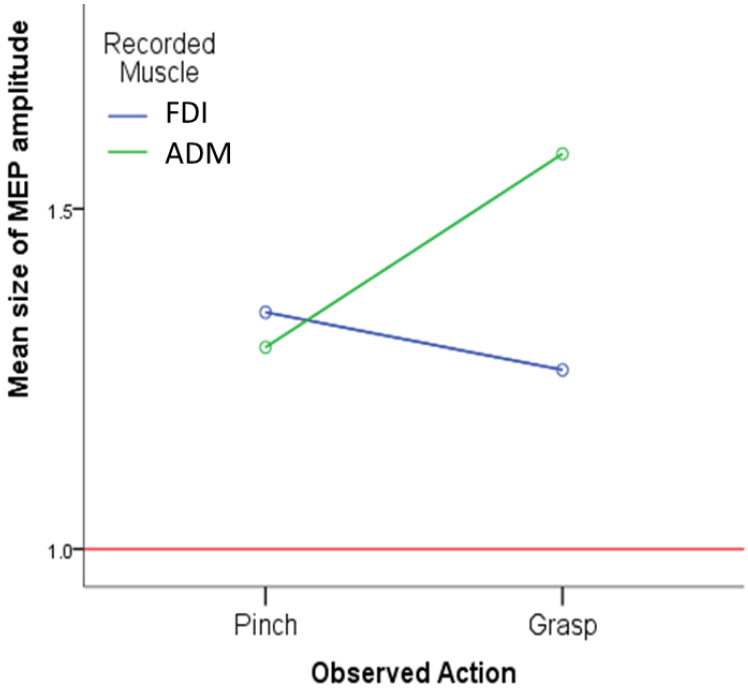


Figure 23. Mean MEP facilitation in FDI and ADM muscles. Facilitation in recorded muscles is modulated by the type of observed action as shown by the significant interaction between the Recorded Muscle and the Observed Action (p<0.01). Observation of pinch elicits stronger response in the FDI muscle and observation of grasp – in the ADM. Red line indicates baseline (MEP amplitude at rest).

The subsequent 4 2x2 ANOVAs were performed to test for motor resonance between observed action and recorded muscle in four different circumstances, two – when the observed hand and recorded hand matched (figure 24, A and D) and two when they did not (figure 24, B and C). The results show that although interaction between the Recorded Muscle and the Observed Action is present in all combinations, the strongest differentiation in motor evoked potentials was in the dominant right hand during observation of the same right hand, F (1, 17) = 26.982, MSE = 0.005, $p < 0.001$ (figure 24, A). Other significant interactions were found between right hand muscles and observed left hand actions, $F(1,17) = 15.947$, MSE = 0.004, p = 0.001(figure 24, B), between left hand muscles and observed right hand actions, $F(1,17) = 8.474$, MSE = 0.004, p = 0.010 (figure 24, C), and between left hand muscles and observed left hand actions, $F(1,17) = 6.771$, MSE = 0.003, p = 0.019 (figure 24, D).

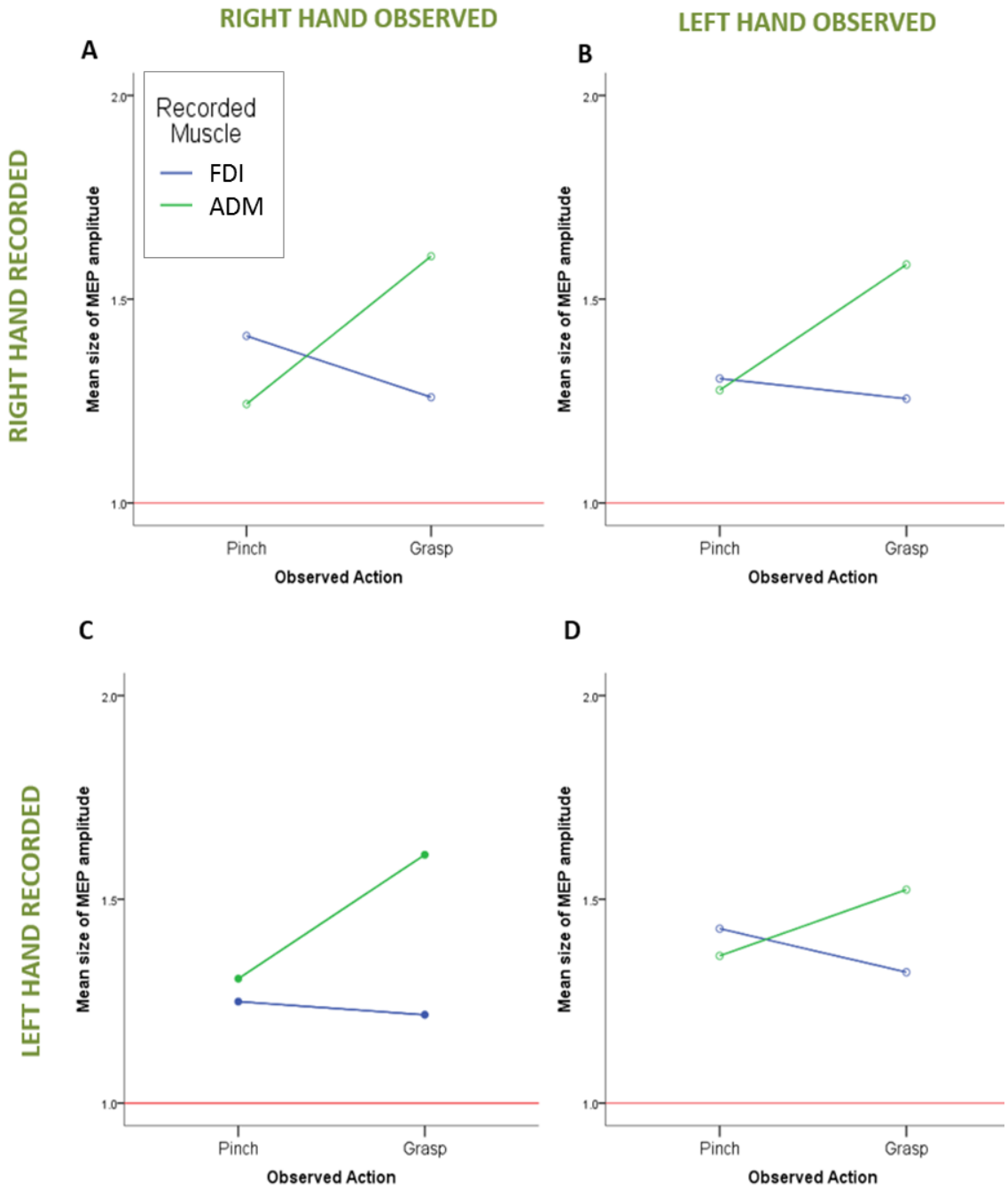


Figure 24. Recorded Muscle x Observed Action interaction based on the recorded and observed hand laterality. Recorded in the dominant right hand (A – observing right hand; B – observing left hand), and in the non-dominant left hand (C – observing right hand; D – observing left hand). Interaction was strongest when observed and recorded hand was right (A).

4.3.5 Laterality-specific facilitation

If there was laterality-specific effect, then observing actions performed with the right as compared to left hand would have produced greater motor evoked response in the matching right hand. An unexpected discovery was that this prediction was true for one muscle (FDI), but not for the other (ADM). Firstly, 4-way repeated measures ANOVA revealed significant triple interaction between factors of Observed Hand, Recorded Hand and Recorded Muscle $F(1,17) = 7.923$, $MSE = 0.002$, $p = 0.012$, suggesting that interactions between the Observed and Recorded hand are significantly different in the two Recorded Muscles. Subsequent analysis confirmed that the Observed x Recorded hand interaction is modulated by the muscle that was recorded from. Specifically, there was significant interaction between the Observed and Recorded hand when recorded in the FDI muscle, $F(1, 17) = 14.633$, $MSE = 0.002$, $p = 0.001$ (figure 25, A). This effect was not present in the ADM muscle $F(1, 17) = .716$, $MSE = 0.002$, $p = 0.409$ (figure 25, B).

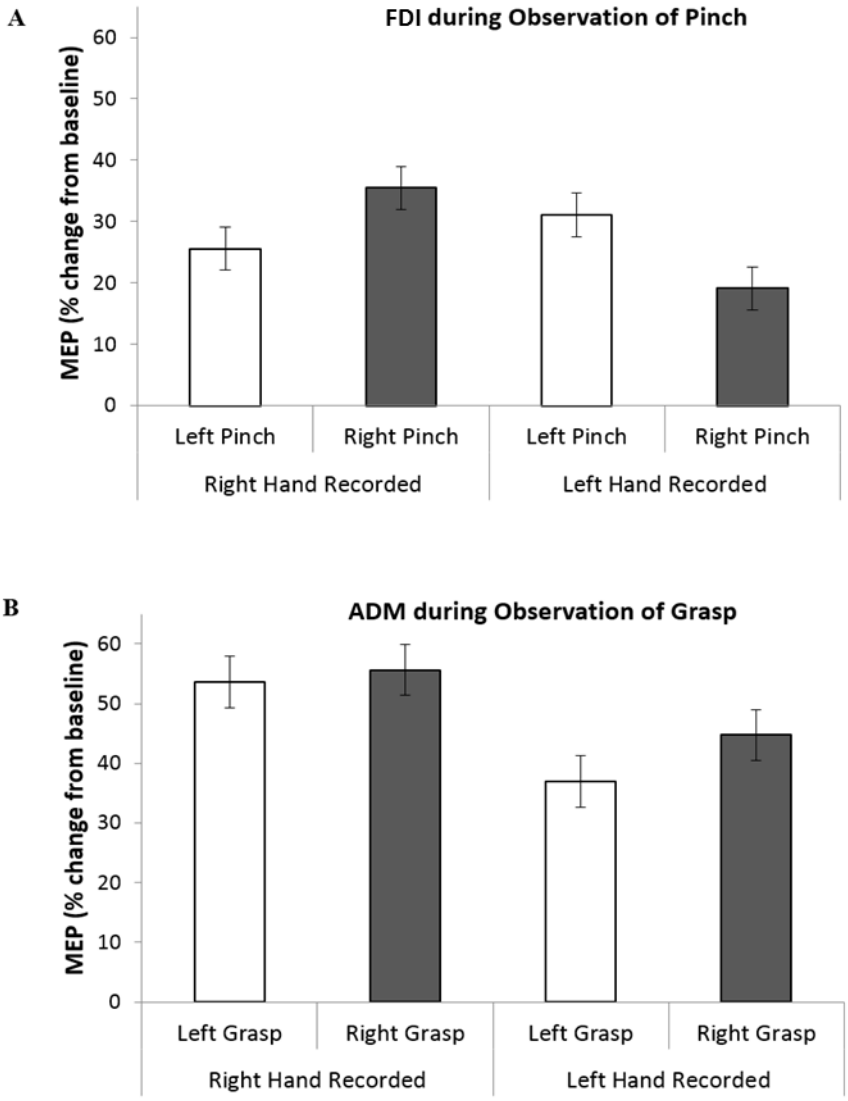


Figure 25. MEP facilitation in the FDI and ADM muscles of left and right hand. Facilitation in the recorded hand is modulated by the observed hand laterality only in the FDI muscle, where observation of left hand yielded greater increase in the amplitude of the motor evoked potential of the left FDI and observation of right hand had similar effect on the right FDI, resulting in the strong Recorded Hand X Observed Hand interaction (p=0.001) (A). This effect is not seen in the ADM muscle (B).

4.3.6 Age and action observation

When age was entered into the 4-way repeated measures ANOVA (described in the previous section) as a covariate, it became apparent that the main effect of recorded muscle was modulated by age (F (1, 16) = 10.125, MSE = 0.014, p = 0.006). To explore this effect further, I have split participants into two groups around their median age (32, n=9 in each group) and plotted results against each other (figure 26). A significant difference between groups was only observed in left (non-dominant) FDI during observation of right grasp (t (16) = 2.267, p = 0.038), right pinch (t (16) = 3.768, p = 0.002), left pinch (t (16) = 2.704, p = 0.016) and observation of left grasp approached significance (t (16) = 1.878, p = 0.079) (figure 26). In other words, the influence of age on motor resonance (as assessed by the facilitatory effect on MEPs) was driven by the decrease in facilitation in left FDI muscle of older participants.

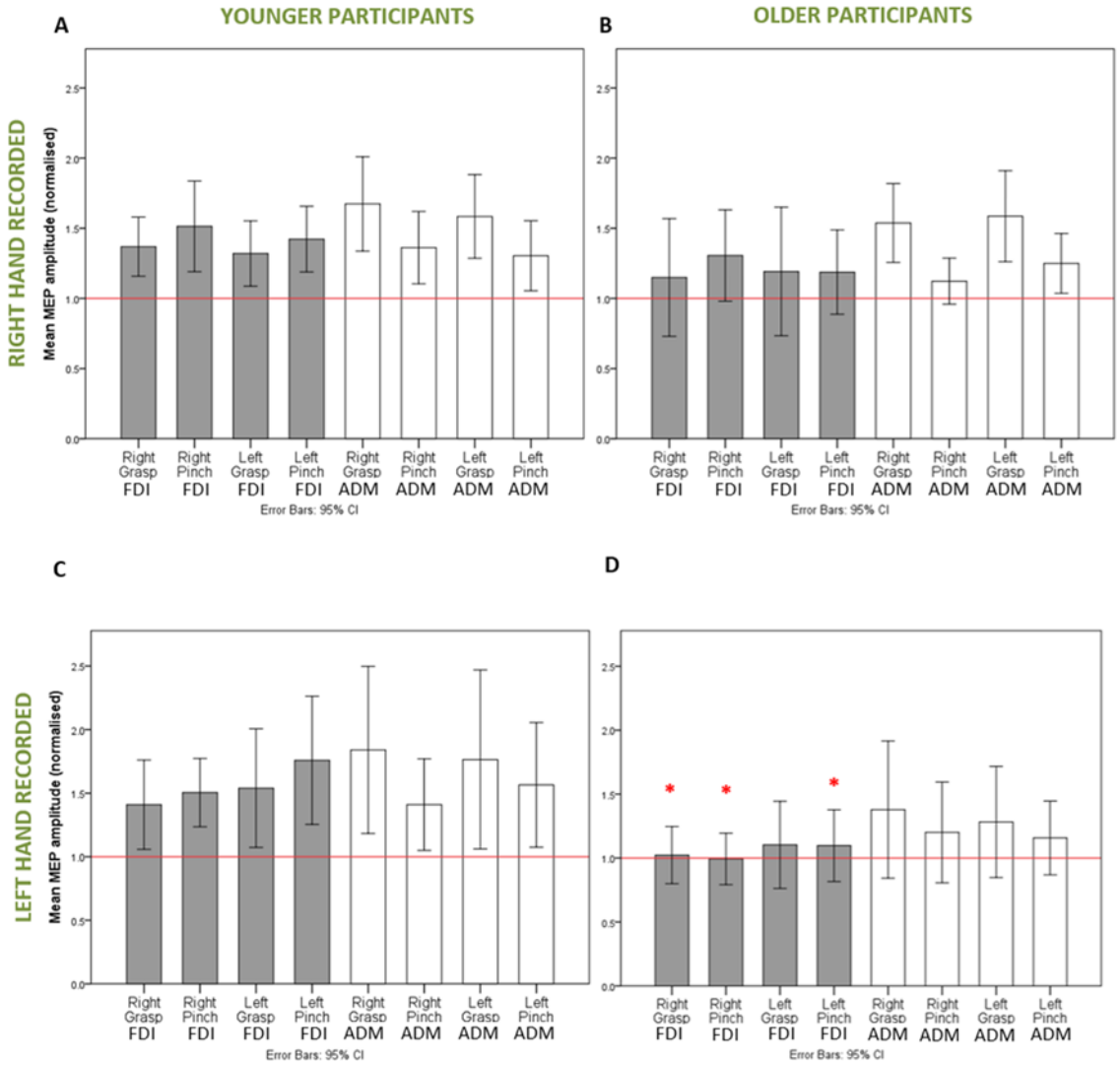


Figure 26. Difference in MEP facilitation between older (B and D) and younger participants (A and C), while recorded from the right (A and B) and the left hand (C and D). Grey bars show response in the FDI muscle; white bars – in the ADM. Red line indicates baseline; bars that are above the line show increased response during observation of actions. Although in the younger group facilitation is evident, it is not as marked in the older group, suggesting that age plays a role in the response of the motor system to observed actions. There is a significant difference between the older and the younger groups (marked with red asterisks) in the left FDI muscle during observation of left and right pinch and left pinch.

The lowest response, i.e. no facilitation on average was observed in the left FDI of older volunteers during observation of incongruent (right) hand actions. Drop in response of that muscle was also proved by significant negative correlation between age and size of MEP amplitudes in the left FDI muscle during observation of right pinch actions, $r = -0.496$, $n = 18$, $p = 0.036$ (figure 27). Age did not correlate with any other measures.

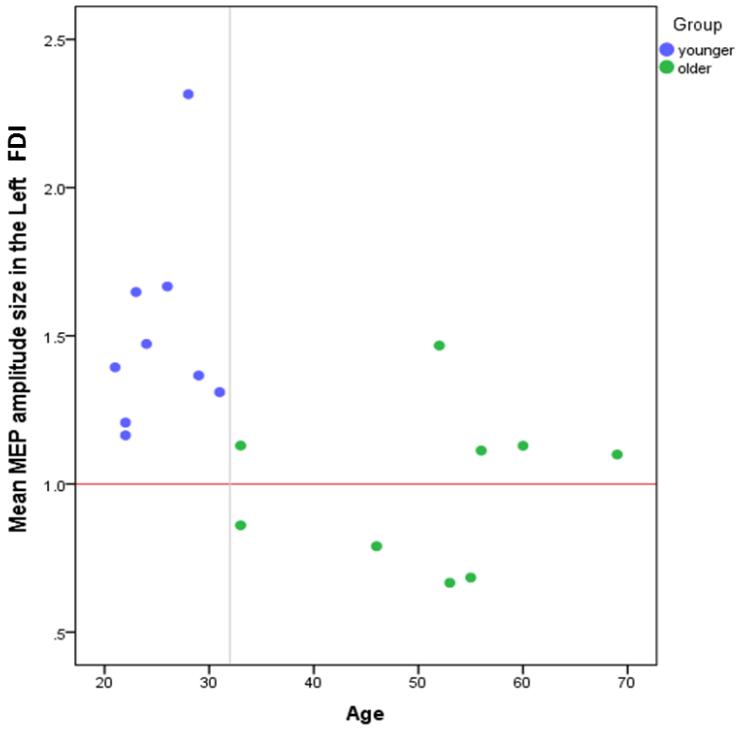


Figure 27. Correlation between age and the size of MEP amplitude in the left FDI during observation of right hand pinch. Red line indicates baseline level activity in the muscle. Grey line shows the median split (32 years). Strong increase during observation is clear in the younger group (blue dots), whereas in the older group (green dots) facilitation is lower and variable, in many cases below baseline level.

4.4 DISCUSSION

4.4.1 Summary of Results

1) I found that size of normalised MEP amplitudes in both FDI and ADM was significantly higher during observation conditions than during baseline runs.

2) Significant interaction between the type of observed action (pinch and grasp) and the recorded muscle (FDI and ADM) suggested that selected stimuli were successful at establishing motor resonance.

3) Significant interaction between the type of observed action and recorded muscles was independent of whether observed hand was congruent or incongruent to the recorded hand, although the largest effect was achieved in the right dominant hand muscles during observation of the same hand actions.

4) The size of response in each hand was modulated by the laterality of observed hand only in the FDI muscle during observation of pinch action. There was a significant interaction between observed and recorded hand, showing that MEP amplitude increased in the right hand when observed hand was right relative to left. Similarly, there was better facilitation in the left hand when observed hand was left as compared to right.

5) Finally, age appeared to be a factor in the corticospinal excitability during observation of pinch as measured by MEP amplitudes in the FDI muscle of non-dominant hand. I found markedly lower response in this muscle in the older group relative to the younger group during observation of all experimental conditions.

4.4.2 Discussion

Establishing how corticospinal system is engaged during action observation in people with intact motor capabilities is critical for addressing the key question in this thesis - whether impaired ability to execute observed action alters corticospinal engagement during observation. By measuring motor evoked responses in dominant and non-dominant hand muscles of healthy individuals I have established the following.

4.4.2.1 *Watching pinch and grasp results in a clear muscle specific MEP facilitation in both, dominant right and non-dominant left hand of participants.*

While participants watched grasp action, the response was higher in their grasp muscle (i.e. ADM), as compared to their pinch muscle (i.e. FDI) and while they watched pinch action the effect was reversed, now higher in their FDI as compared to ADM. Muscle specific facilitation to observed actions has been reported in several studies, beginning with the seminal experiment by Fadiga et al. in 1995, in which authors found that excitability was increased in the thumb opposing opponens pollicis muscle when subjects observed grasp, but not when they watched a moving or static hand (Fadiga et al., 1995; other studies showing muscle specific facilitation include Maeda et al., 2002; Sartori et al., 2012; Senna et al., 2014; Urgesi, 2006).

The muscle specific effect was significant independent of whether observed hand was congruent or incongruent to the recorded hand, although it must be noted that the strongest effect was found in the dominant hand while subjects were watching congruent dominant hand. Significant muscle-specific facilitation which was not dependent on

the observed effector was also found in the study by Senna and colleagues, whereby the effect in muscles used to perform observed action persisted even if action was performed with drastically different effector, such as grasping with a foot (Senna et al., 2014). Establishing a strong muscle-specific resonance in both hands of healthy volunteers was essential for probing the response in the affected hand of stroke patients.

4.4.2.2 *The average MEP amplitude is significantly greater when recorded and observed hand are congruent.*

Interestingly, such effect was only present in the FDI muscle during observation of pinch and not in the ADM. This could explain the differences in findings described in previous literature. In 2002, Aziz-Zadeh established a clear laterality specific effect when measuring MEPs during observation of moving index finger in the FDI muscle (Aziz-Zadeh et al., 2002), while Sartori and colleagues tested excitability in the ADM muscle during observation of whole hand grasp and reported effector-independent response (Sartori et al., 2013). Finding that observed hand laterality influenced facilitation in the FDI, but not the ADM muscle has not been reported previously and it was critical in consideration of next experiments that I carried out.

4.4.2.3 *Age of the observer influences CST excitability during observation.*

Significant correlation was outlined in the non-dominant FDI muscle, showing that activity in the muscle decreased in older participants. When average MEP amplitude was compared between a group of older and a group of younger volunteers, a significant difference was found during experimental conditions. This result indicates that independent of which hand or which action was observed, FDI excitability decreased with age. This effect was not found in the dominant hand or in the ADM muscle.

Although the association between age and hand muscle excitability during action observation has not been published, diminished activity in the same non-dominant FDI was reported during execution of various acts (Sale and Semmler, 2005). In the study, 20 participants performed finger abduction, precision, power and scissor grip while activity was recorded from their dominant and non-dominant FDI. Authors concluded that during performance of all actions MEPs were 30% lower in the non-dominant hand of older participants as compared to younger ones. Importantly, no age effect was found in the FDI muscle of the dominant hand. Sale and Semmler suggest that unaltered activity in the dominant FDI is due to its persistent use for skilled actions, such as writing, which is in contrast to less utilised non-dominant hand. Sale and Semmler's result matches that outlined in my experiments, suggesting that use-dependent changes in muscle excitability are also evident during action observation. Notably, not only the overall excitability was altered with age, but the facilitation during observation, i.e the difference between excitability while watching actions and during baseline. Therefore, the ability of the non-dominant muscle to 'resonate' with observed actions is reduced with age.

In conclusion, through this experiment I have established that a) robust muscle specific motor resonance is seen in both hands independently of which hand is observed, but that b) facilitation in the FDI muscle during observation of pinch is significantly greater when observed hand matches the recorded one, and that c) age related (possibly use-dependent) changes in the non-dominant hand alter excitability during observation, independent of which action is watched.

CHAPTER 5
ENGAGEMENT OF THE CORTICOSPINAL TRACT DURING ACTION OBSERVATION IN PATIENTS WITH MOTOR IMPAIRMENT

5.1 INTRODUCTION

Action Observation Treatment (AOT) is proposed to be a useful adjunct to physiotherapy in promoting recovery of motor function after stroke (Buccino, 2014; Small et al., 2013). Watching actions is known to engage the corticospinal system of the observer. The rationale is that observing actions 'primes' the motor system by increasing cortical excitability (and reducing inhibition) thereby increasing the potential for use-dependent plasticity. This in turn, it is believed, will increase learning and retention of practised tasks during physical training (Bisio et al., 2015; Celnik et al., 2008; Stefan et al., 2008; Zhang et al., 2011), thereby contributing to motor recovery. If proven beneficial, the use of complementary AOT during motor rehabilitation would be cost effective and easy to administer on wards or in patients' own homes.

Several clinical trials performed to date show a lasting positive effect of AOT on motor recovery after stroke (Bang et al., 2013; Ertelt et al., 2007; Franceschini et al., 2012, 2010; Harmsen et al., 2014; Park and Hwangbo, 2015; Sale et al., 2014; Sugg et al., 2015), although not all patients appear to benefit equally. In the recent trial involving 67 patients in the subacute stage after stroke, Sale and colleagues demonstrated that only those with right hemisphere stroke (left hand paresis) showed marked improvement following combined AOT and motor training (Sale et al., 2014). The reasons behind such a

difference in response to treatment, based on the injured hemisphere, remain unclear and suggest the need for further investigations before promoting the use of AOT in routine clinical practice.

CST excitability measured in muscles during action observation is known to be variable even in healthy participants. Such variability during observation appears to correspond closely with that during execution of observed actions (Borroni et al., 2005). In stroke patients, however, variability during action observation may be even greater, since the ability to execute observed hand actions is often compromised. It can be hypothesised, therefore, that MEP facilitation during action observation is dependent on the residual capability to execute the observed action.

Similarly, variability in the CST response to action observation may be due to a patient's experience of executing the observed actions. In their seminal study, Buccino and colleagues showed that watching actions that are not in the motor repertoire of human observers, such as a dog barking, did not engage their motor system (Buccino et al., 2004). Likewise, several studies outline the tight relationship between motor expertise and activity in the motor system during action observation. For instance, watching dance movements that are within dancer's motor repertoire engage the motor system to a greater extent than watching movements that are not routinely practiced by an observing dancer (Calvo-Merino et al., 2006). In fact, experts often show greater activity in their motor system during observation of actions that they are skilled at, including arching, tennis or piano playing, as well as smoking (Balser et al., 2014; Haslinger et al., 2005; Kim et al., 2011; Wagner et al., 2011). It is not known, however, if losing the ability to execute actions produces a reverse effect – if activity in the motor system de-

creases during observation of actions that are no longer in the motor repertoire of the observer. Addressing this question is essential before recommending AOT to be delivered conjointly with routine physical practice, as beneficial effects of such treatment may vary greatly among patients.

In this chapter I examined action observation induced corticospinal system facilitation in the affected hand of chronic stroke patients with a range of motor impairment in order to address the following questions:

1) *Is facilitation in FDI and ADM muscles during observation of hand actions dependent on integrity of the corticospinal tract?* I looked at the overall increase in excitability in recorded muscles during observation and compared it to that of healthy volunteers acquired in Chapter Four.

2) *Is muscle specific motor resonance affected by damage to the corticospinal tract?* Here I tested for the interaction between observed action and recorded muscle in order to explore whether greater MEP facilitation in agonist muscles during observation of pinch and grasp persisted in people with damaged corticospinal system.

3) Does overall size of response (above baseline facilitation) and muscle specific resonance (the interaction between observed action and recorded hand) correlate with the degree of motor impairment?

4) *Is the size of the MEP amplitude in the FDI muscle modulated by observed hand (impaired or unimpaired)?* In healthy subjects FDI response to observed pinch was higher when observed hand was congruent with the recorded hand (see Chapter Four). I therefore examined whether the damaged corticospinal system was engaged

differently while patients watched hand that was congruent to their affected hand (the same as their recorded hand) as opposed to when it was congruent to their unaffected hand.

5) *Did dominance of the impaired hand before stroke affect excitability during observation?* The dominance of the impaired hand after stroke has different functional consequences to patients. If prior to damage the impaired hand was dominant, the impact may be more functionally challenging than if it was non-dominant hand. Skilled hand actions that are usually performed unilaterally with the dominant hand, such as writing or eating are sometimes relearned with the non-dominant hand. In contrast, if the affected hand was non-dominant before stroke, the impact may be smaller as patients would continue to utilise their unimpaired dominant hand for skilled action performance. Therefore, losing the ability to perform dexterous action such as pinch may have more of an affect in the dominant hand than in the non-dominant hand, which may be influencing the corticospinal excitability during action observation.

It is important to understand how damaged corticospinal system is engaged during observation of actions if considering the use of AOT in patients. Although watching actions may increase activity in the corticospinal system of the healthy observer, it may not be the case in every patient with lost motor function. As such, the benefits of AOT may be large for some, but not for others. Understanding whether motor impairment itself may affect motor resonance during observation will therefore fill an important gap in our knowledge.

5.2.1 Subjects

20 patients were recruited for this experiment (females=5; age range: 25-70; mean age: ± 53 years). 18 patients had suffered first ischemic stroke to either right (N =10) or left (N=8) hemisphere. 2 patients had haemorrhagic stroke (right N=1 and left N=1 hemisphere). Appendix A details each patient's demographics, lesions and functional motor scores. One patient was excluded from analysis due to flawed recording from the FDI muscle. Data from 19 patients was analysed and is presented below. All patients have provided informed consent and were screened for the adverse reaction to the TMS procedure (Keel et al., 2001; Rossi et al., 2009). The experimental protocol was approved by the National Hospital for Neurology and Neurosurgery & Institute of Neurology Joint Research Ethics Committee.

All patients completed Edinburgh Handedness questionnaire in which hand dominance prior to stroke was determined. Three patients were identified as left-handed. The handedness *per se* was not considered in the analysis, yet the dominance of the affected hand was.

5.2.2 Data Acquisition and Analysis

5.2.2.1 *Experimental task*

As detailed description of the experimental task and recording procedure were already outlined in Chapter Four, I will only mention them here in brief. Patients were positioned comfortably in front of the monitor with recording electrodes attached to their hand muscles. While participants watched pinch and grasp actions performed with either left or right hand, single pulse TMS was applied over their ipsilesional M1 and MEPs from their affected hand were recorded and stored for analysis. Patients were asked to maintain their focus by closely observing two consecutive actions from the same condition (for instance, left grasp) and say whether they thought the pair was identical or two videos were slightly different.

The Visor 2 neuronavigation system was used to locate and monitor hot-spot position during stimulation (ANT Neuro HQ, Ent, Enschede, Netherlands). Where patients' structural MRI scans were available, they were loaded and used to determine the best stimulation position guided by the place of damaged structure. A neurologist with experience in TMS studies in stroke population was usually present at this point.

5.2.2.2 *Data analysis*

As in the previous experiment, I used Spike 2 software (Cambridge Electronic Design, Cambridge, UK) to analyse background EMG and to exclude trials with muscle activity above threshold (mean background activity + 2SD). I then extracted MEP amplitudes for each trial from data of the two muscles. All MEPs were normalised to the mean amplitude of MEPs collected during baseline condition preceding each run. For instance,

MEP amplitudes of trials in run 1 were divided by the mean amplitude MEP collected before that run. Then, I averaged values for each muscle across every run and condition and data were then entered into Stata statistical package (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP) or IBM SPSS (Version 20.0. Armonk, NY: IBM Corp) for statistical analysis.

5.2.2.3 *Statistical analysis*

Firstly, I tested data for normality. Recorded ADM outcomes were significantly negatively skewed, thus I examined outliers and applied several transformations, albeit with no success. In addition, comparison between patients and controls showed that the two groups were not homogeneous. I therefore used multilevel mixed effects linear regression. This robust statistical model is complex, but allows violation to aforementioned basic assumptions. It also allows for scores of motor function, age, or affected hand dominance to be entered as covariates and examine their role in the effect. I will outline each method briefly in the results section, so it is easier to follow.

5.2.3 Motor performance score

Functional motor performance of an affected and unaffected hand was measured in each patient. Tests included:

- Action Research Arm Test (ARAT) (Yozbatiran et al., 2008),
- 9 Hole Peg Test for finger dexterity (Mathiowetz et al., 1992),
- Box and Blocks test (Mathiowetz et al., 1985),
- Apraxia Screen of TULIA (Test for Upper-Limb Apraxia) (AST) (Vanbellingen et al., 2011),
- Pinch and grasp strength measurements were acquired with dynamometer (Patterson Medical Ltd., Nottinghamshire, UK).

All measurements, except ARAT scores were then used in analysis to outline relationship between motor function and MEP facilitation during observation of actions. ARAT outcomes were not incorporated, as 18 out of 20 patients reached the maximum score. The use of the arm, which ARAT tests, was mostly preserved in the studied patients, as corticospinal tract must be relatively intact in order to permit sufficient MEP amplitudes through M1 stimulation, therefore patients with severely impaired arm use may have been excluded from the experiment.

The motor functional (MF) score for each test was calculated as percentage of function in the affected hand relative to the unaffected one. MF score of 0% suggests no residual function in the affected hand, while 100% means that function in the affected hand is well preserved (figure 28, B).

Principal Component Analysis (PCA) was performed to obtain a single upper limb outcome score for each patient (figure 28, A). PCA performs orthogonal transformation converting combinations of variables into linearly uncorrelated principal components (Jolliffe, 2002). A few principal components are created which are equal to or less than the number of the variables included. The first principal component accounts for the most variance between variables, and this is the score that will be used in further correlation analysis.

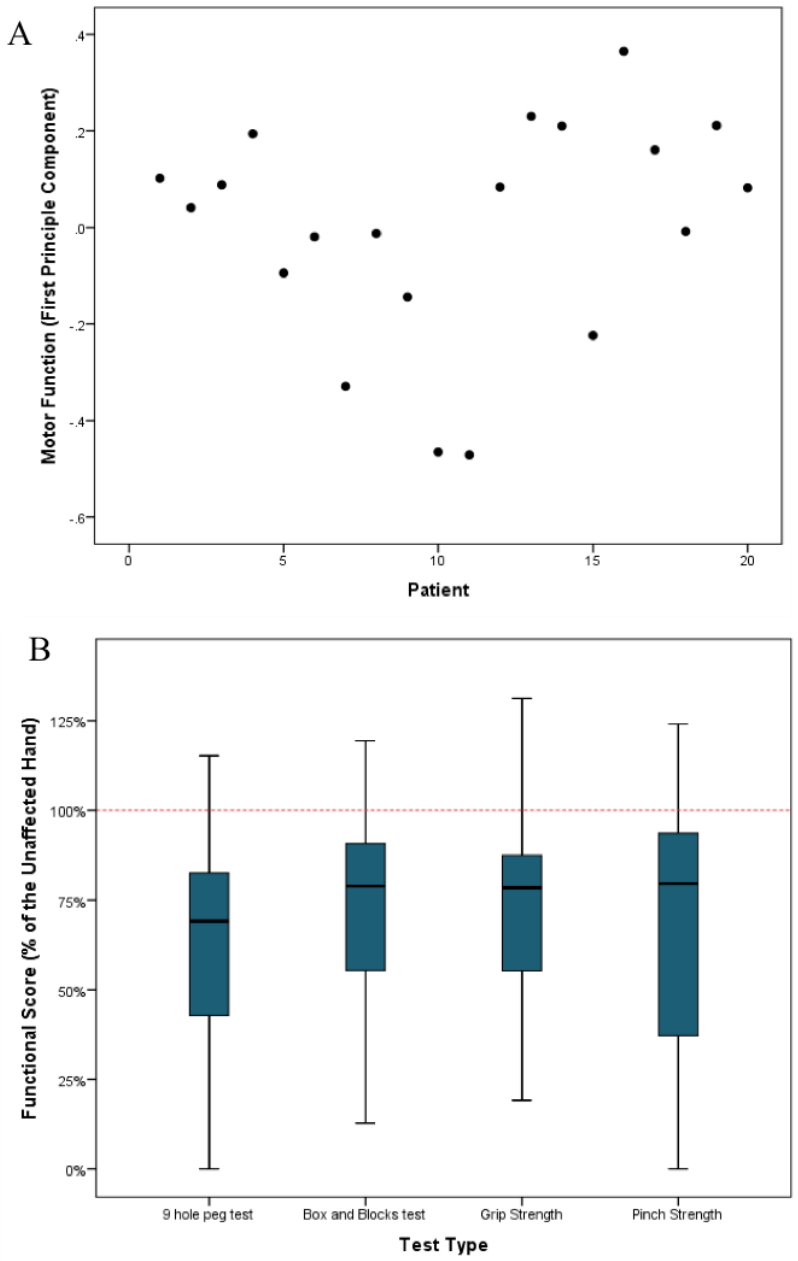


Figure 28. Motor function in patients (N=19). A) the first principle component of the four motor scores combined (9 hole peg test, Box and Blocks test, Grip and Pinch strength); B) the spread of all functional motor scores independently. Y-axis indicates how well the affected hand performs relative to the unaffected hand, whereby 0% is suggestive of no function in the affected hand and 100% - well preserved function.

5.3.1 Behavioural results

Response accuracy (RA) was significantly above chance in patients (average RA = 79.1%, SD = 11.9). RA was not significantly different than in healthy participants (average RA = 85.5, SD = 7.3). RA in patients did not correlate with their age or their motor function score.

5.3.2 Facilitation in FDI and ADM muscles during observation of hand actions depends on the integrity of the corticospinal tract

MEPs in patients were collected from the affected hand only. As such, it was impossible to compare patients and controls directly since in some patients left hand was affected and in others - right. Splitting patients into two groups would have compromised power of the effect. In order to maintain sufficient sample size I performed two group comparisons: 1) response in patients' affected hand vs response in the dominant right hand of controls, and 2) response in patients' affected hand vs response in the non-dominant left hand of controls.

5.3.2.1 *MEP size facilitation during action observation*

Overall facilitation during active conditions was not significantly higher than baseline.

5.3.2.2 *Comparing affected hand in stroke patients and right (dominant) hand in controls*

Facilitation in both muscles during all conditions was significantly higher in healthy participants as compared to stroke patients (Coef. =0.2761, z=2.63, P>|z| = 0.008) (figure 29). This difference persisted independently of: a) whether observed hand was congruent or incongruent to the recorded hand, b) whether observed action was pinch or grasp and finally, c) whether recorded muscle was FDI or ADM. It appears that in all instances MEP facilitation was significantly lower in patients.

5.3.2.3 *Comparing affected hand in stroke patients and left (non-dominant) hand in controls*

In parallel to the above finding, facilitation in both muscles was significantly higher in the non-dominant hand of controls than in the affected hand of patients (Coef. = 0.14275, z=2.01, P>|z|=0.044) (figure 29). This effect was also independent of the observed action, recorded hand or whether facilitation was recorded in the congruent or incongruent hand to the observed one.

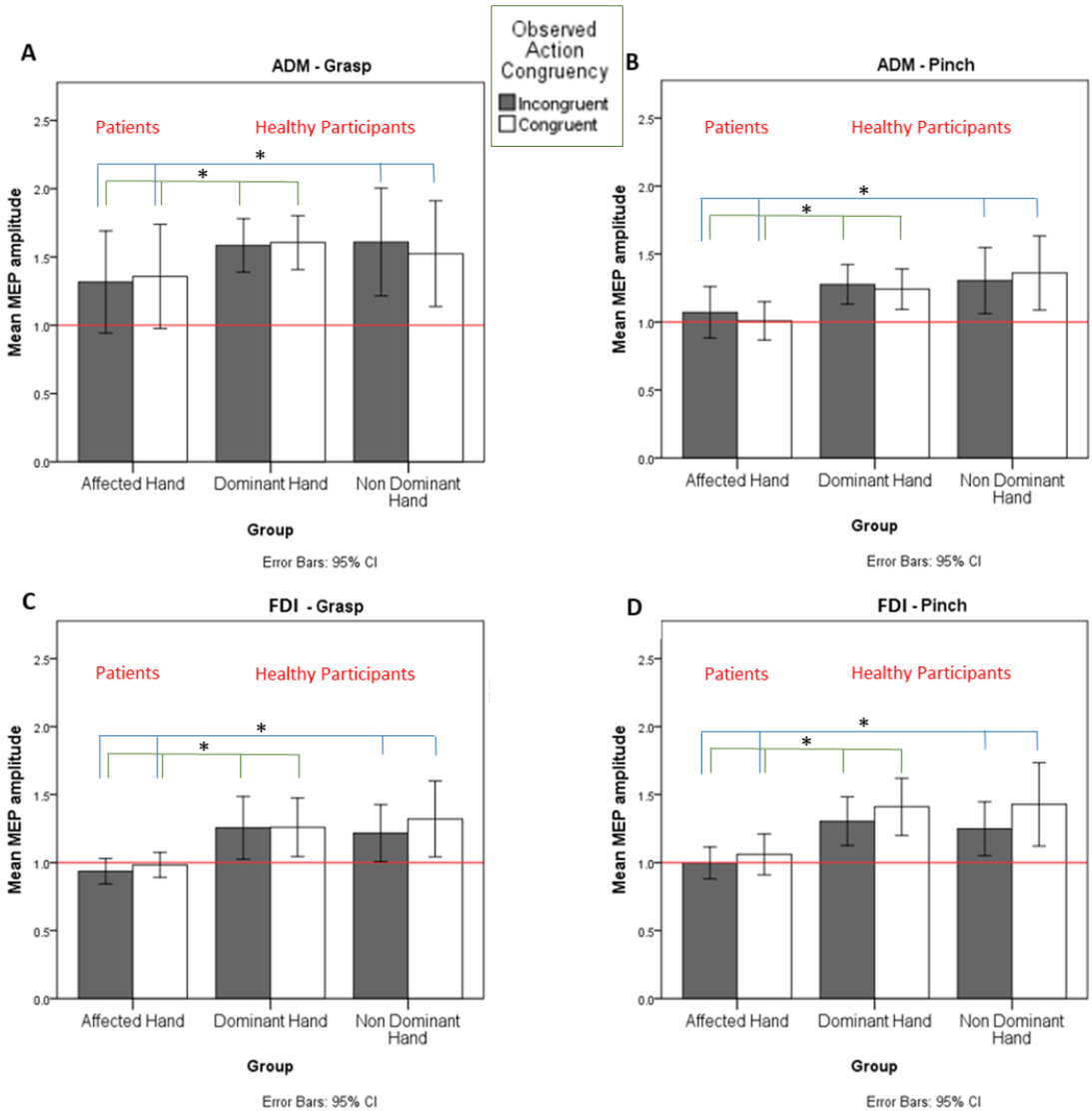


Figure 29. Mean MEPs during action observation in healthy individuals and in stroke patients. Normalised mean amplitude of muscle evoked potentials collected from FDI (C and D) and ADM (A and B) muscles in patients (Affected Hand) and controls (Dominant Hand and Non-Dominant Hand (note: the same controls were tested on two occasions)). The bars indicate observed action. The action is congruent when observed hand matched the recorded hand. In the case of patients, congruent actions were the ones that matched their affected hand. Red line shows baseline level. There is little if any MEP facilitation in the patient group.

Notably, the difference between patient and control groups was not due to differences in stimulation intensity (mean resting motor threshold, rMT, in patients (N=19) = 50% of the maximum stimulator output intensity (range from 30% - 82%), in controls (N=18) =

48% (left hand) and = 47% (right hand) (range 35% – 74%). Variability was larger in the patient group, but was unlikely to have been a cause of the main effect of groups. It appears that excitability during observation is overall reduced in patients, as compared to healthy controls.

5.3.3 Motor resonance is unaffected by damage to the corticospinal tract

In Chapter Four I found significant interaction between recorded muscle and observed hand in the group of healthy participants (figures 23 and 24). This interaction persisted, independent of the recorded or observed hand laterality. Significant effect was seen when recorded and observed hand were the same (congruent) as well as when they differed (incongruent). Here I tested for the same interaction in patients in order to establish their capacity for motor resonance. I therefore looked for a differential response to observed pinch and grasp in muscles (Recorded Muscle x Observed Action interaction) and if so was it modulated by observed hand (Congruent to the Affected/ Unaffected Hand). I hypothesized that motor resonance may be lost when hand congruent to the impaired hand was observed.

5.3.3.1 *Observing congruent to the affected hand*

There was a significant interaction between observed action and recorded muscle when hand congruent to the affected hand was observed (Coef. = 0.4251 z = 2.84, P |z| = 0.004) (figure 30, A). This interaction was not influenced by age or motor function after stroke. In addition, the interaction remained significant when separately accounting for grasp and pinch strength in patients.

5.3.3.2 *Observing congruent to the unaffected hand*

Similarly, significant interaction was present when observed hand was congruent to the unaffected hand (Coef. = 0.305955, z = 2.77, P |z| = 0.006) (figure 30, B). And just as during observation of hand congruent to the affected hand, this effect was not modulat-

ed by age or motor function post stroke. Similarly, grasp and pinch strength after stroke did not impact on the significance of this interaction.

It follows that similarly to healthy individuals, stroke patients' excitability in the two muscles was modulated by the perceived action. Importantly, this effect was independent of whether observed hand was congruent to the impaired hand in patients.

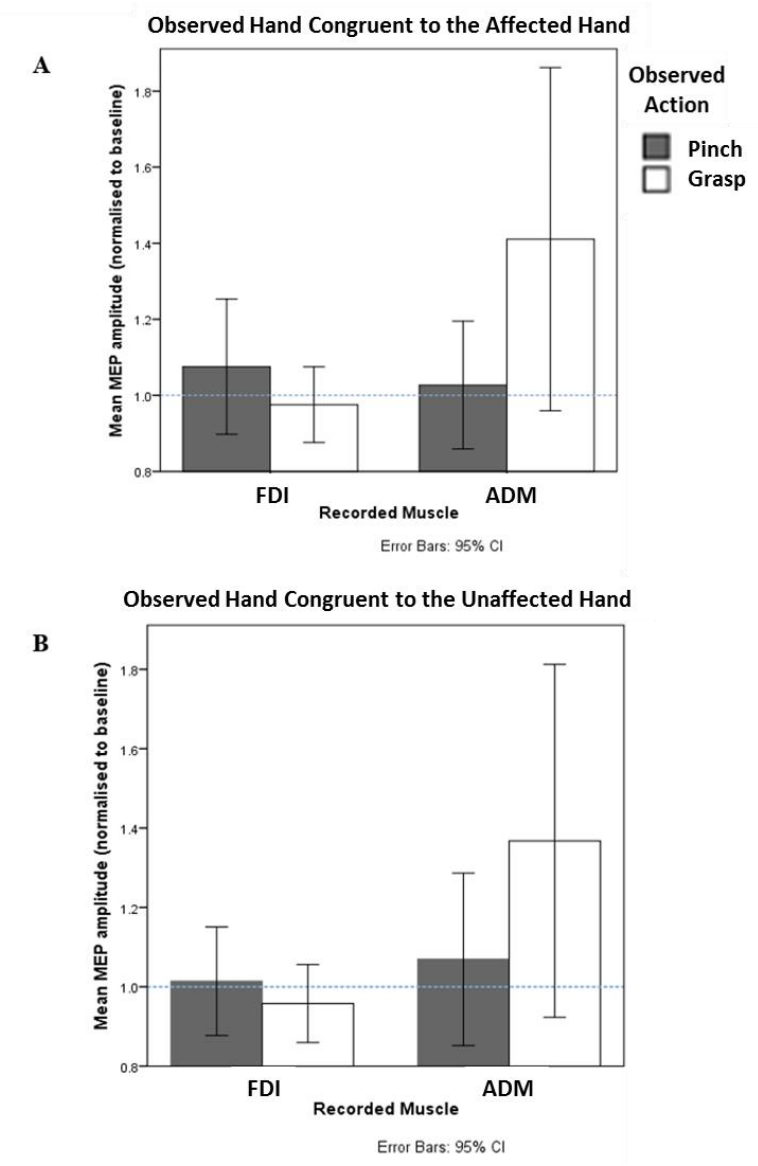


Figure 30. Mean MEP amplitude (normalised to baseline) during observation of actions performed with the: of A) hand congruent to the affected hand and B) hand congruent to the unaffected hand. Grey bars show activity during observa-

tion of pinch, whereas white bars – activity during observation of grasp. The action specific resonance persisted in both agonist muscles. Blue dotted line indicates baseline measure and clearly shows no marked overall facilitation.

5.3.3.3 *Individual variance during observation*

Crucially, there was no facilitation of MEPs during the observation conditions in patients. Since the variability in patients was high, it was useful to plot individual responses (figure 31). It became apparent that a few patients showed good resonance (comparable to that of controls) in the ADM as well as in the FDI muscles while watching their affected (recorded) hand actions. Yet for many patients motor resonance was not at all obvious. It raised the question whether significant interaction between recorded muscle and observed action was driven by a few patients in the group. I calculated a mirror ratio for each of the patients (FDI/ADM for each observed condition) to test for a correlation with the motor score in order to examine whether patients with greater ability to execute actions had better resonance, but found no such correlation.

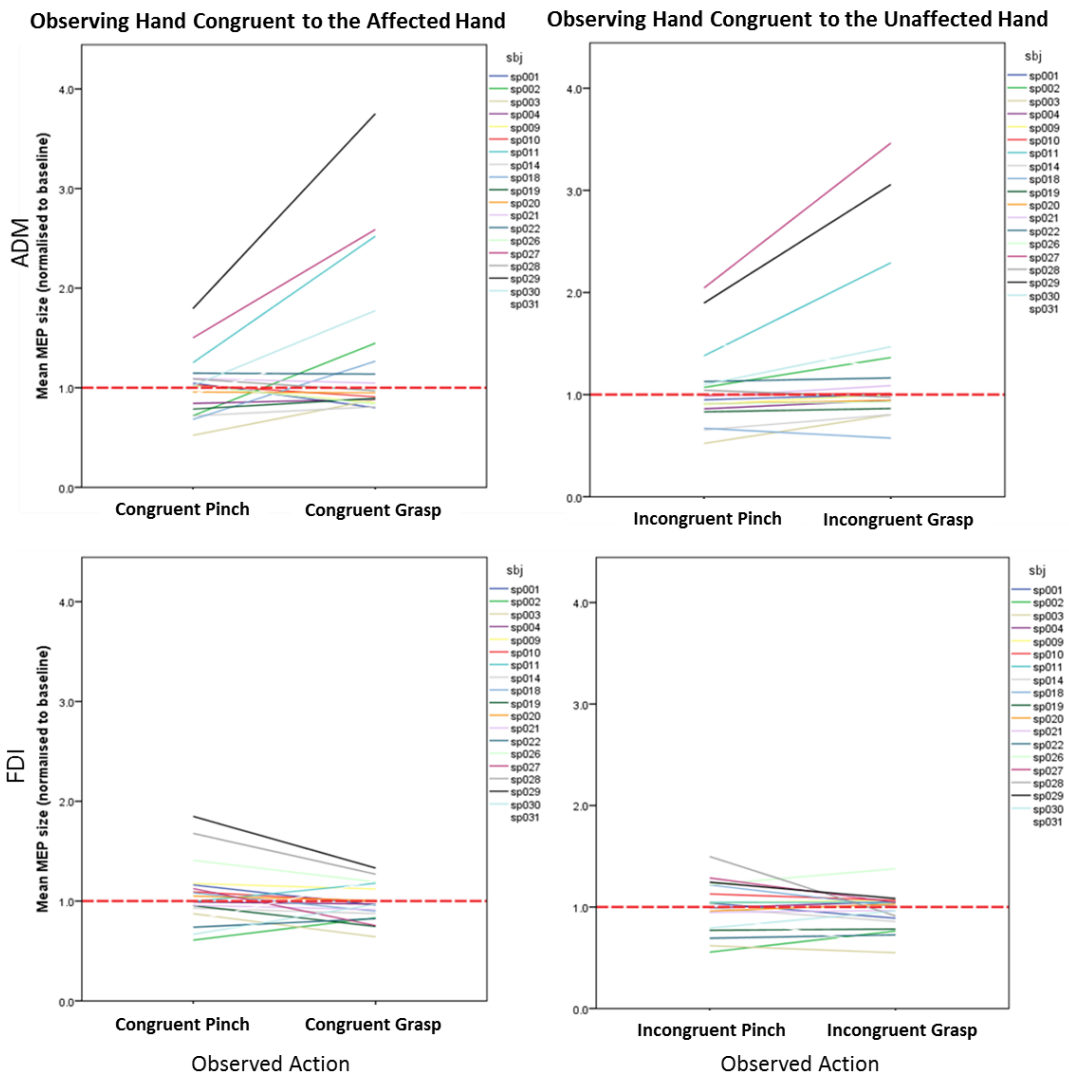


Figure 31. Individual Variability during Observation (N=19). Red dotted line indicates baseline. Responses above the line show observation associated facilitation. Motor resonance is apparent in the slope of each line, the steeper the slope the better the resonance. In the ADM muscle the line should be higher on the right hand side, signifying better response during observation of grasp. In the FDI muscle, the line should be higher on the left hand side – higher MEPs during observation of pinch.

5.3.4 Size of response is independent of motor impairment

Scores of motor function after stroke (derived from the principal component analysis) were entered as covariates into the model. In this group of patients function did not appear to have modulatory affect over the excitability during observation.

5.3.5 Facilitation in muscles during observation is not modulated by observed hand (congruent to the impaired or unimpaired hand)

Simple paired-samples t-test revealed no significant difference in FDI when observed pinch was performed with hand congruent to the affected as compared to when it was performed with hand congruent to the unaffected hand.

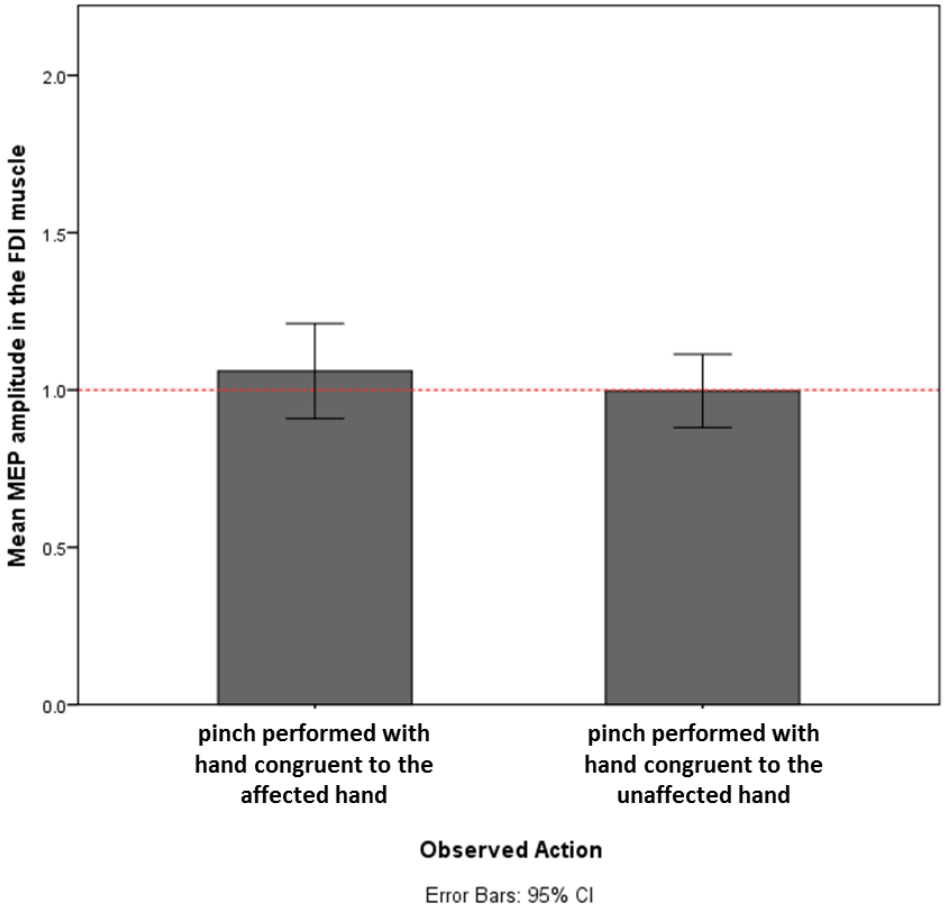


Figure 32. Mean MEP amplitude (normalised to baseline) during observation of actions performed with hand congruent to patients' affected or unaffected hand.

5.3.6 Dominance of the impaired hand before stroke affects excitability during observation

Finally, one of the factors in observation related facilitation in patients may be dominance of their affected hand prior to stroke. When dominant hand is impaired, performing everyday actions becomes very challenging. In contrast, patients whose non-dominant hand is impaired are faced with more sparing functional consequence.

Here, I used simple one-way ANOVAs to compare facilitation in the FDI muscle during observation of pinch between patients with dominant (N=9) and those with non-dominant (N=10) affected hand.

There was significant difference between MEPs recorded from dominant and non-dominant hand while watching pinch performed with congruent to their affected ($F(1, 17) = 4.989$, $MSE = 0.4$, $p=0.039$) and unaffected ($F(1, 17) = 5.120$, $MSE = 0.243$, $p=0.037$) hand. Higher facilitation was observed if affected hand was dominant prior to stroke (figure 33).

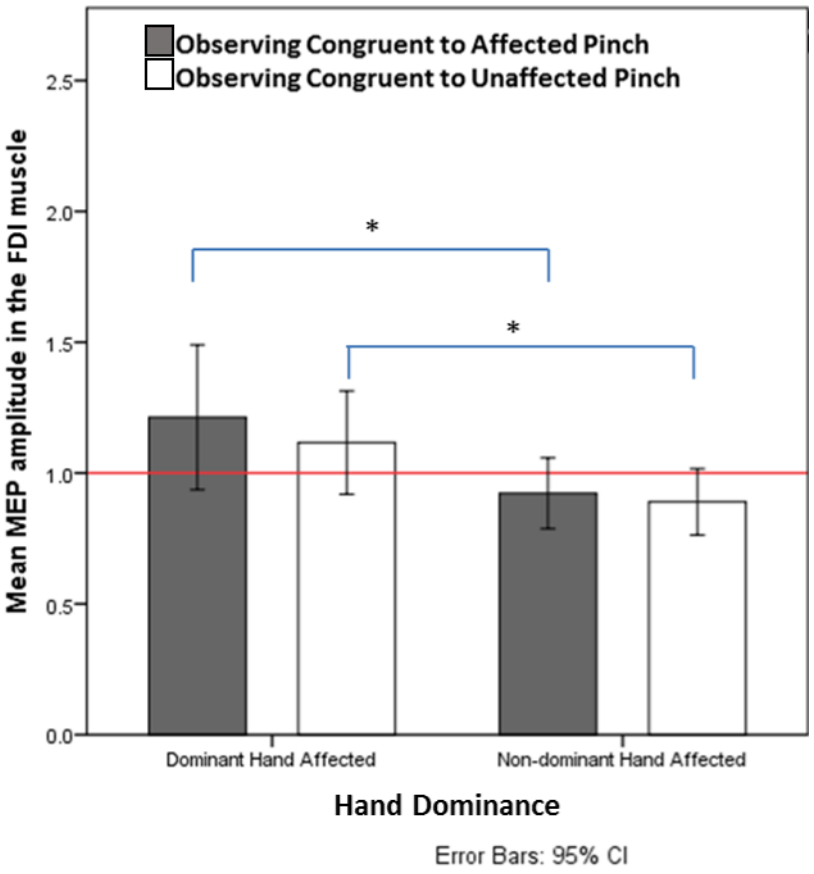


Figure 33. Mean MEP amplitude (normalised to baseline) in the FDI muscle during observation of affected hand pinch and unaffected hand pinch. Significantly higher activity is observed in the dominant affected hand. Red bar indicates baseline.

Subsequent plotting of individual scores reveals that best action specific resonance and overall facilitation is seen when the affected hand was dominant prior to stroke and the observed action was congruent to that hand, i.e. also performed with the dominant hand. In the non-dominant affected hand facilitation was mostly abolished (figure 34).

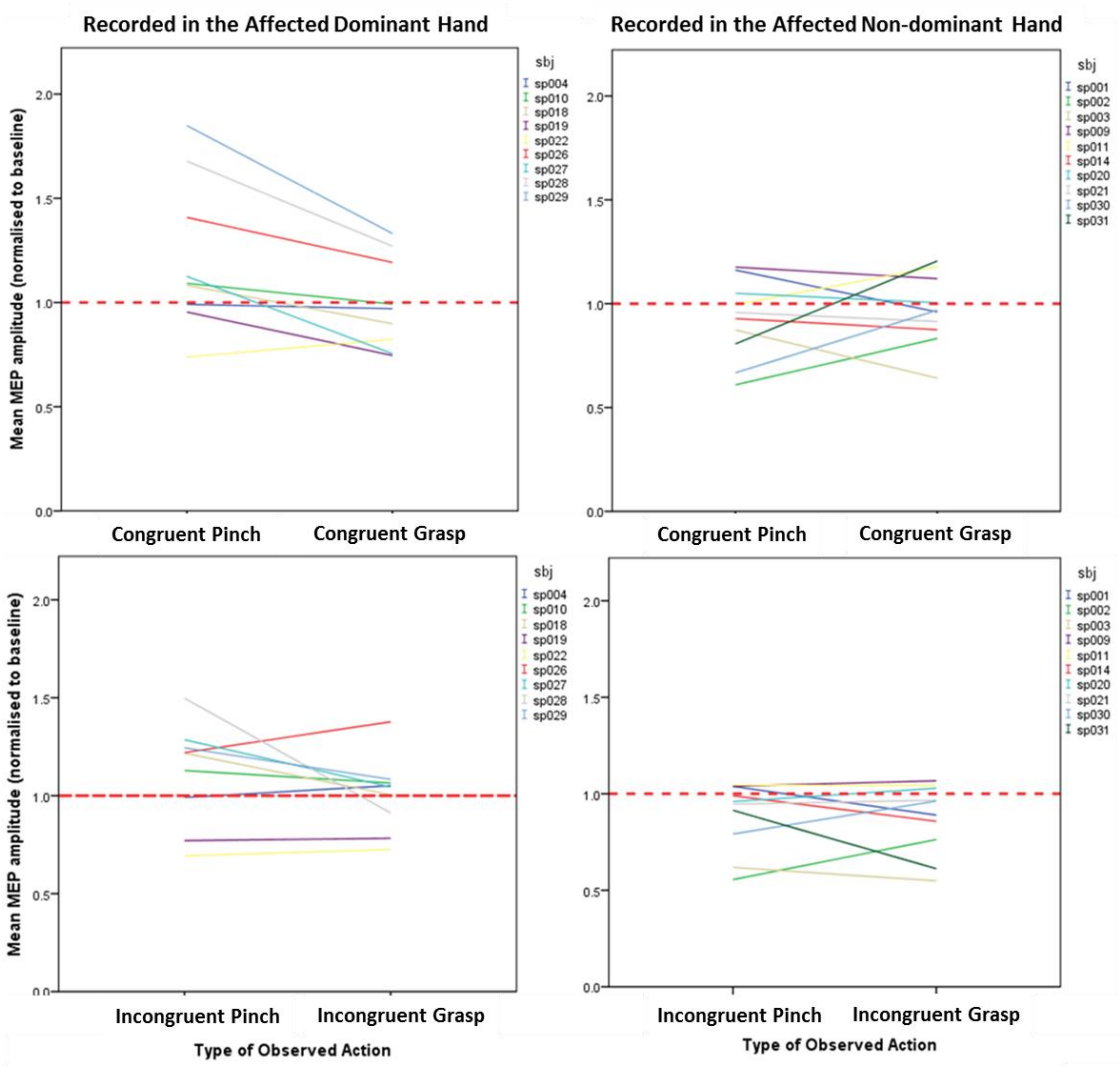


Figure 34. Individual response in the FDI muscle. Increase in the MEP amplitude size relative to baseline (red line) is highest when affected hand was dominant before stroke and when the observed action was performed with the dominant, albeit impaired, hand.

5.4 DISCUSSION

5.4.1 Summary of Results

1) There was no facilitation in FDI and ADM muscles during action observation in chronic stroke patients on average. The size of response was significantly lower than in healthy volunteers.

2) Interaction between Recorded Muscle (FDI/ADM) and Observed Action (pinch/grasp) in the affected hand was nevertheless significant and did not depend on laterality of observed hand (congruent to the impaired or unimpaired hand).

3) The size of response did not correlate with the functional motor score in this group of patients.

4) The size of MEP amplitude in FDI of the affected hand was independent from whether observed pinch was performed with hand congruent to the impaired or unimpaired hand.

5) Affected hand dominance before stroke significantly altered corticospinal excitability during observation.

5.4.2 Discussion

Present study directly explored how the ability to execute hand actions effects facilitation of motor evoked potentials during action observation in the affected hand of stroke patients. Two important novel findings were revealed in this chapter.

5.4.2.1 *The ability to execute observed action is not necessary for the engagement of the corticospinal system during observation*

In patients, just as in healthy participants, the interaction between observed action and recorded muscle was significant, independent of whether observed hand was congruent or incongruent to the recorded affected hand. This means that observing pinch, for instance, still engaged the pinch (FDI) muscle to a greater extent than it engaged grasp (ADM) muscle, and vice versa with observed grasp. The muscles of the affected hand, therefore, still 'resonate' with the specific action that is being watched.

Such conclusion may seem counterintuitive when overall no facilitation was apparent in the affected hand of patients during observation. Response to observed actions in FDI and ADM muscles was no greater than baseline and was significantly lower than in healthy participants. However, upon closer examination of individual responses it was apparent that facilitation was lacking in some, but not in all patients. This difference could not be explained by the ability to execute observed actions, i.e. motor impairment. There was no correlation between the motor function score and average facilitation of MEP size during action observation. Notably, lack of correlation between the MF score and facilitation in muscles during action observation may also be due to not sufficient range of MF scores, as patients with complete lack of function were not included in this

study. In patients with severely damaged integrity to the CST, motor cortex stimulation does not result in sufficient MEPs (Stinear et al., 2006), thus such patients were not able to participate in the current experiment. Nevertheless, in the particular group that was studied here, it appears that observing hand actions still engaged the corticospinal system, but overall variability in MEP facilitation during observation may have been dependent on another factor, as discussed further.

5.4.2.2 *Affected hand dominance before stroke plays a crucial role in the engagement of the corticospinal tract during action observation*

I tested the difference in average MEP amplitude in the FDI muscle between patients with dominant and non-dominant affected hand and I found that the difference between them was significant irrespective of whether they watched the hand that was congruent or incongruent to their recorded one. I was specifically interested in the FDI as this muscle is involved in production of skilled actions requiring dexterity and it is these action that are most compromised when the dominant hand is affected.

Crucially, I found that overall facilitation was above baseline in those with dominant hand impairment and it was below baseline (as if suppressed) in those with non-dominant affected hand. Although facilitation was not significantly above baseline in those with dominant affected hand, I believe a larger sample size (here there were 10 patients) may increase the effect.

It appears that ability to execute observed actions is not necessary for the system to be activated by observation. If however, Action Observation Treatment relies on facilita-

tion of corticospinal system during observation of the impaired movement to be trained, then it might be the case that only patients with dominant hand impairment will benefit.

There are two possible explanations of this result. Firstly, the dominance effect may be largely driven by age. In Chapter Four I showed that in healthy individuals the response in the non-dominant FDI was significantly lower in older as compared to younger participants, I also found a significant correlation between age and MEP size in the muscle during observation. People affected by stroke tend to be older and responsiveness in their non-dominant FDI may already be lower. Previously, Sale and Semmler found that during execution of actions, MEP size markedly diminished in the FDI of the non-dominant hand, but not in the dominant hand (Sale and Semmler, 2005), which is in agreement with results here. Authors suggested that such a decrease in FDI responsiveness reflects "neural adaptation that occur(s) throughout a lifetime of preferential hand use for skilled (dominant) and unskilled (non-dominant) motor tasks" (Sale and Semmler, 2005). It is possible that age-related reduced excitability in the non-dominant FDI muscle results in lack of facilitation during action observation, but more research needs to be carried out to test this hypothesis.

Although in my experiment both control and patient groups were matched in age range, the mean age in each group differed substantially (healthy - age range: 22 -69; mean age: ± 38 years VS stroke – age range: 25-70; mean age: ± 53 years). In my work healthy participants were tested before stroke patients were recruited, thus studies with closely matched wide range of age are necessary for ascertaining if reduced non-dominant hand response in patients is due to their older age.

Secondly, although age may play a role in the overall decrease in response of the non-dominant FDI, it is unclear why there should be a marked decrease from baseline during observation in patients with non-dominant hand impairment. MEP response during baseline was nearly significantly larger than during observation of the incongruent pinch ($p = 0.081$), resembling but not proving activity suppression during observation. Voluntary movement of one hand results in motor pathway excitability in another hand (Stinear et al., 2001; Woldag et al., 2004), yet it does not result in the simultaneous movement of that hand. It is assumed that suppression of movement occurs because of interhemispheric inhibition (IHI) (Ferbert et al., 1992; Kobayashi et al., 2003), whereby activity in the contralateral hemisphere to the moving hand sends information to the ipsilateral hemisphere through transcollosal connections and therefore suppresses the movement in the other hand. What's interesting about IHI, is that it appears to be asymmetric, being greater in the non-dominant hand during movement of the dominant hand (Lewis and Perreault, 2007). Increased motor system activity during action observation, especially of the incongruent dominant hand, may therefore increase IHI to the non-dominant hand, resulting in suppression. This idea would suggest that a thorough investigation of possible suppression in the non-dominant hand during observation is critical if AOT to be used in those with impaired non-dominant hand. Theoretically at least, prolonged action observation in such patients could do more harm than good.

To conclude, in this experiment my hypothesis that the ability to execute observed actions is necessary for the corticospinal system to be engaged during observation was not supported. I showed that observing actions still results in clear muscle specific responses, similar to those seen in healthy volunteers. However, although engagement of the

corticospinal system during action observation was not affected by the action that was observed or the level of motor impairment, it was strongly influenced by the affected hand dominance prior stroke. The results presented here lead to the hypothesis that those with impaired dominant hand have better chances of benefitting from AOT. Such hypothesis would contradict findings of Sale and colleagues who showed in their behavioural clinical trial that only patients with left (non-dominant) hemiparesis improved after AOT (Sale et al., 2014). Further in depth investigation of the effect of hand dominance on AOT is essential for predicting treatment outcomes in individual patients.

CHAPTER 6
BRAIN ACTIVITY DURING ACTION OBSERVATION AFTER STROKE: AN FMRI STUDY

6.1 INTRODUCTION

Action Observation Treatment is promising to be an effective tool in motor rehabilitation (Buccino, 2014; Garrison et al., 2010; Small et al., 2013), but it isn't certain if all patients can benefit from it equally. Previously, it has been shown that for the motor system of an individual to be engaged an observed action needs to be in their motor repertoire. In other words, the person watching an action needs to be aware of how it feels to execute it (Buccino et al., 2004). It is unclear if losing the ability to execute an action with one limb after stroke alters the general representation in the motor repertoire or the response of the motor system during observation. Is ability to execute an action necessary for the motor system to be activated during observation of that action? In this chapter I aim to further address this question with means of functional Magnetic Resonance Imaging.

I assessed brain activity during action observation by measuring blood-oxygenated-level-dependent (BOLD) response in the predefined regions of interest. This method allowed me to determine 1) whether watching hand congruent to the affected hand of patients elicited response significantly different than watching of the hand congruent to their unaffected hand, and 2) whether ability to execute the observed action altered the response in the motor system during observation of that action. In addition, based on results obtained using single pulse TMS described in the previous chapter, I explored

whether affected hand dominance before stroke influenced neural activity during observation. I also looked at how changes in the dominant and non-dominant hand of patients affect engagement of the motor system during action observation.

Using TMS I have established that in healthy individuals observing hand actions facilitates activity in the corticospinal tract as measured by elevated motor evoked potentials in hand muscles. It is likely that modulation in the corticospinal tract originates in areas that share direct or indirect connections with the primary motor cortex and that are activated by observation of hand actions. In my analysis, therefore, I focused on changes in neural activity in a set of regions that are linked to the motor cortex and are also known to be engaged by hand action observation. Specifically, I concentrated on the cortical origins of the descending corticospinal tract. In this way I tested modulations related to action observation only in regions that have influence over descending motor signals and are most likely to be involved in plastic changes associated with AOT.

In the following experiment I asked right-handed stroke patients and healthy participants to watch pinch and grasp actions performed with either left or right hand. As well as looking at the main effects of action observation in each group (healthy individuals and stroke patients), I also addressed the following specific questions.

1) *Is BOLD response modulated by observed hand laterality in healthy participants?*
   Prior to tackling the question of whether watching congruent to the affected as opposed to unaffected hand effects brain activity, it was important to establish the 'normal' response to observing left and right hand in a group of right-handed healthy individuals. Previously published findings are somewhat conflicting. While some indicate involvement of the contralateral hemisphere to the observed hand

(Rocca et al., 2008; Shmuelof and Zohary, 2006, 2005; Vingerhoets et al., 2012), Cabinio and colleagues showed mostly left lateralized activation when observing both left and right hand actions (Cabinio et al., 2010). Establishing the pattern of brain activity in response to my paradigm in healthy participants was important as it would be used in comparison with patients.

2) *Is BOLD response modulated by observed hand laterality in chronic stroke patients?* Here, I firstly looked at whether response to observed hand laterality in patients differed significantly from that in healthy volunteers. For instance, I tested if watching left compared to right hand elicited different effect in patients and controls. Secondly, I studied if watching congruent to the affected compared to unaffected hand differed in patients with left and right hemisphere stroke.

Previously, Garrison and colleagues studied 12 left hemisphere stroke patients to determine if activity during action observation was modulated by motor experience (Garrison et al., 2013). The authors found that watching hand congruent to the paretic hand as opposed to unimpaired hand led to greater activity in the ipsilesional inferior frontal, supramarginal and postcentral gyri. However, since only patients with a right paretic hand were studied, these results were not very different from those found in healthy individuals, whereby observing right as opposed to left hand lead to increased activity in the contralateral hemisphere (Shmuelof and Zohary, 2006, 2005; Vingerhoets et al., 2012). In the present study 22 right handed patients with stroke to either left or right hemisphere were recruited. In order to determine if *impaired* hand laterality modulated response to *observed* hand laterality, I tested for

interaction between the two, expecting to outline regions where watching paretic as opposed to unimpaired hand lead to significantly different activation.

3) *Is engagement of the motor system during observation modulated by the ability to execute observed actions?* Here, I studied if the ability to execute an action altered activity in the brain during observation of that action. In the following experiment, I used multiple regression analysis to explore the relationship between residual motor function in the affected hand and magnitude of BOLD signal during observation of actions performed with hand that was congruent to the affected hand.

While no association between hand dexterity and level of activity during action observation was found in healthy individuals (Bello et al., 2014), Garrison and colleagues reported a negative correlation between BOLD signal during action observation and motor function in stroke patients in left inferior frontal gyrus and right premotor cortex (Garrison et al., 2013). The authors concluded that activity in these regions was greater for actions that were more difficult to perform. While Garrison and colleagues studied one group of patients with right affected hand, here I further explored the relationship between motor function and brain engagement during observation in patients with either left or right paretic hand.

4) *Does affected hand dominance before stroke modulate the engagement of the motor system?* Following results in Chapter Five where facilitation in hand muscles during observation was markedly different in the dominant compared to non-dominant hand of patients, I aimed to explore the relationship between affected hand dominance and cortical response during action observation.

5) *Does motor function in the dominant and non-dominant hand of patients affect BOLD response during action observation?* Correspondingly to the previous question, I aimed to find out if dexterity and overall motor function in both dominant and non-dominant hands of patients correlated with brain activity during action observation.

6.2.1 Participants

22 chronic stroke patients (7 females; age range: 39 – 71, mean age: ± 55 years) and 20 healthy volunteers took part (10 females; age range: 22-74; mean age: ± 42 years). Detailed patient demographics and individual motor function scores are presented in Appendix B. All participants were right-handed as measured by the Edinburgh Handedness scale (Olfield, 1971). None had a diagnosed psychiatric or additional neurological condition. Each volunteer provided informed consent, in accordance with Helsinki Declaration. The experimental protocol was approved by the National Hospital for Neurology and Neurosurgery & Institute of Neurology Joint Research Ethics Committee.

6.2.2 Experimental Task

Participants completed two scanning runs during a single session. During each run, they were presented with 24 blocks of videos. Each block comprised four video clips depicting either a hand action (experimental condition) or moving scrambled blocks (control condition). Each block contained only one type of condition. Overall, four experimental conditions (observation of left pinch (LP), left grasp (LG), right pinch (RP) and right grasp (RG) and two control conditions (left (LC) and right (RC)) were presented (figure 36).

During two runs, each condition was seen 8 times by a participant. Videos within each 16.24 s block were separated by a 700 ms interval and were followed by a 3 s question displayed on the screen 'Same? Yes/No', as well as 2 s rest period with red fixation cross in the middle of the screen (rest duration varied slightly, as it was used to load images for the next block) (figure 35). The question prompted volunteers to decide whether they thought all four videos within the block were identical, or whether one of them was slightly different. During scrambled control task a white rectangle appeared on the screen for the duration of 1 frame, 33.6 ms. This rectangle either appeared in the same place in all four videos within the block or its location differed in one of the videos. Participants were then instructed to respond in the same way as during experimental conditions (i.e. to the question 'Same? Yes/No'). Subjects gave verbal responses that were recorded for further analysis. Half of the blocks within the run had identical videos and half contained an odd video within. The order in which they appeared was randomised. The order of an odd video within the block was chosen deliberately, whereby in

most of the cases the 4th video was different, rather than the 1st, 2nd or 3rd. This was purposely constructed in order to maintain participants' attention throughout the block.

All volunteers read the instruction sheet explaining the task and completed a practice session before the beginning of the experiment.

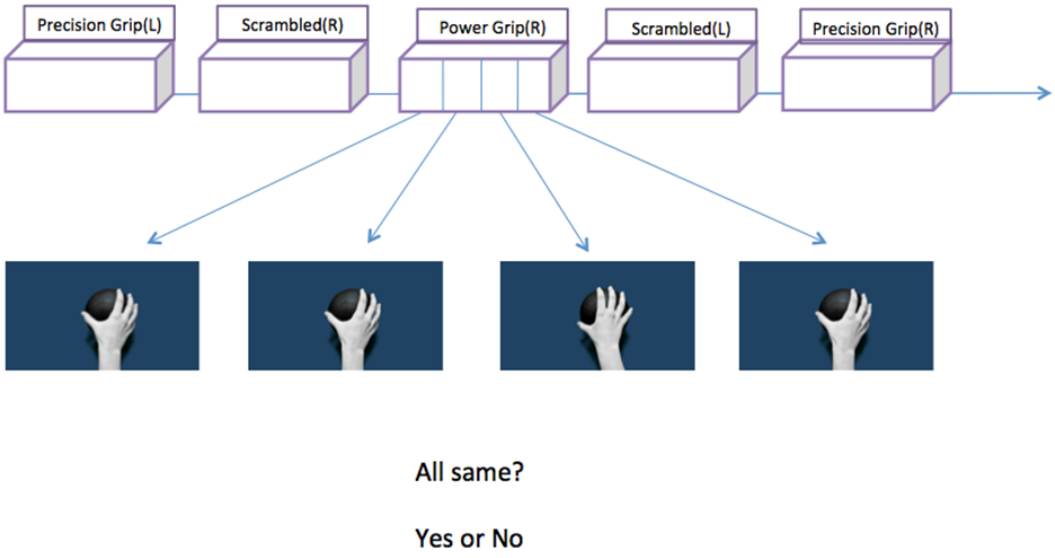


Figure 35. Experimental paradigm. Four action observation conditions (Left Pinch, Left Grasp, Right Pinch and Right Grasp) and two high-level control conditions (scrambled) were presented to viewers within block-design experiment. Each block comprised of four representations of the same condition, at the end of which the question prompted participants to judge whether observed videos were all same or different.

6.2.3 Stimuli

Footage in all four experimental conditions shared the same light blue background, same velocity of movement and same time of point of interaction between the hand and the object. The object in each video was also in a matching central position. Thus most of the confounding elements unrelated to the type of actions of interest were excluded. Scrambled control videos were derived from action stimuli by merging both grasp and pinch videos together and scrambling them beyond recognition. In this way the same background and shading as well as velocity of movement within the video remained the same as in the experimental condition. To achieve this, filmed material was subsequently edited using Motion 2 software, part of the Final Cut Studio application package (Apple Inc.). Videos were made to be 100 frames each, approximately 3.36 seconds long, with a consistent point of interaction between hand and object at the 25th frame. The model in videos was female and all actions were filmed from the egocentric perspective.

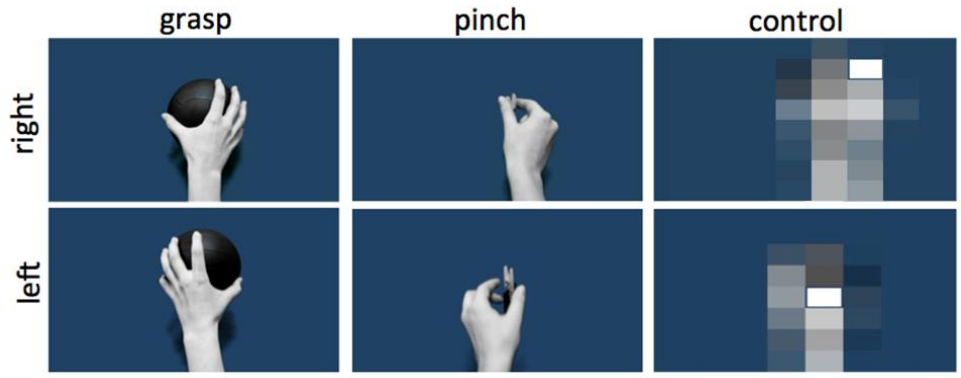


Figure 36. Example of stimuli used in the six conditions: right and left grasp, pinch and scrambled control.

6.2.4 Data Acquisition and Analysis

6.2.4.1 *Data Acquisition*

Both T1 anatomical and T2* weighted echo-planar images (EPI) were acquired on a 3T MAGNETOM Trio, A Tim System (Siemens, Erlangen, Germany) using a 12-channel coil. EPI (TR=68ms, TE=30ms, Echo spacing=500e-3ms, matrix size=64x64) volumes were collected by scanning 48 slices (2mm thick) in an ascending order covering the whole cerebrum. 190 volumes were acquired during each run, first 6 of which were discarded from further analysis in order to maintain the T1 equilibration effects (Frackowiak et al., 2004). Anatomical sagittal MRI images were collected using 3D MDEFT (Deichmann et al., 2004) sequence with an acquisition time of 12.51 m. Additionally, to correct for the geometric distortion in EPI, resulting from the inhomogeneous magnetic field, double-echo FLASH (GRE) sequence (TE1=10ms and TE2=12.46ms, and 3x3x2mm resolution and 1mm gap) was acquired in order to create B0 fieldmaps for subsequent use in SPM FieldMap Toolbox during the preprocessing stages of the analysis. During the entire scanning time, two grippers were positioned one in each hand of the participant and the force applied on grippers was monitored online as well as saved for further consideration. This way any force exerted by either hand was outlined and discarded from further analysis.

6.2.4.2 *Data Analysis*

All data were analysed using Statistical Parametric Mapping (SPM) software (SPM8, Wellcome Department of Imaging Neuroscience, UK (http://www.fil.ion.ucl.ac.uk/spm), carried out in Matlab R2010b (The Mathworks Inc., USA).

6.2.4.2.1 *Data Preprocessing*

Firstly, each subject's imaging data were realigned and unwarped using B0 field-maps, correcting for distortions in EPI due to inhomogeneity of the magnetic field. Distortion correction has been shown to increase coregistration accuracy between structural images and EPI (Hutton, 2002). Further, EPI data were coregistered to individual anatomical images. Resulting volumes were then normalized to standard space defined by the Montreal Neurological Institute brain template ICBN 152 (Fonov et al., 2011, 2009). Finally, all images were smoothed with 6mm full-width at half maximum Gaussian kernel in order to correct for inter-subject anatomical variability, improve signal to noise ratio and increase statistical power (Hopfinger et al., 2000).

6.2.4.2.2 *First Level fMRI Analysis*

Statistical analysis was performed in two stages. In the first stage, using a single subject fixed effects model, all conditions (LP, LG, RP, RG, LC, and RC) were modelled using a boxcar functions. The resulting covariates were convolved with a canonical synthetic haemodynamic response function, and were used in a general linear model (Friston et al., 1994) together with a single covariate representing the mean (constant) term over scans. The parameter estimates for each covariate resulting from the least mean squares fit of the model to the data were calculated, and statistical parametric maps of the t statistic (SPM{t}) resulting from linear contrasts of each covariate (Friston et al., 1994) were generated and stored as separate ‘contrast’ images for each subject.

Contrast images obtained from patients’ scans were subsequently masked to exclude lesioned areas from the analysis. Lesions were identified using Automated Lesion Identification approach described in detail further (Seghier et al., 2008).

6.2.4.2.3 *Second Level fMRI Analysis*

The data for this second stage of analysis comprised the pooled parameter estimates for each covariate. A number of analyses were performed to address my specific experimental questions.

1) An overall effect of action observation was calculated in comparison to both (i) rest and (ii) to the control condition.
2) Main effects of observed hand laterality and observed action in both patients and healthy volunteers were outlined. 2x2x2 mixed ANOVA was used with following factors: Group (patient/control), Observed Hand (left hand – LH / right hand - RH) and Observed Action (pinch - P / grasp - G). Main effects were computed and results were corrected for multiple comparisons (i) across the whole brain (family wise error), $p < 0.05$ and (ii) in regions of interest, using small volume correction (family wise error), $p < 0.05$ on the uncorrected ($p < 0.001$) data.
3) In order to determine whether responses during observation were modulated by whether observed hand was impaired in patients, 2x2 mixed ANOVA was also performed. This time two groups were outlined: Patients with left hand affected (LHA) and those with right hand affected (RHA). Factors in the analysis were: Group (LHA/RHA) and Observed Hand (LH/RH).
4) Multiple regression analysis was used to test for the relationship between motor function of the affected hand and BOLD response during observation of hand congruent to the affected hand in both LHA and RHA patient groups. This was achieved by performing regression using individual contrast images for each experimental condition and motor scores as covariates. As motor function score, I used

both dexterity score from 9 Hole Peg test and a combined motor function score using first principal component.

5) In order to test if BOLD signal during observation was modulated by dominance of the affected hand, a simple t-test was performed contrasting activity during left and right hand pinch observation between patients with dominant and non-dominant affected hand.
6) Finally, correlation between activity during observation of actions and motor function in the dominant right and non-dominant left hand was computed using multiple regression analysis. In regions where correlation appeared to be significant, 1st eigenvariate was further extracted and entered for bivariate correlation analysis using IBM SPSS statistics package (Version 20.0. Armonk, NY: IBM Corp). This was done in order to exclude patients with lesions in given ROIs from the analysis. 1st eigenvariate is the first principal component of the BOLD signal within a given region and is preferable to just using mean activity (Poldrack et al., 2011). For instance, if within the region some voxels are active while others are not, mean computation uses a combination of both, which could sum up to zero. 1st eigenvariate reflects the most common response.

6.2.4.2.4 *Region of Interest (ROI) selection*

ROIs were selected based on the *a priori* information from a large meta-analysis describing areas recruited during observation of hand actions (Caspers et al., 2010). 62 studies (data from 804 subjects) were used in order to determine a set of regions consistently activated during the viewing of hand actions. The resulting set of MNI

coordinates were then reported in the meta-analysis and used here to create appropriate ROIs of the network recruited during observation.

Some of the reported areas - such as superior temporal sulcus and visual area 5- were in regions known to process visual information serving as input into the motor system rather than being part of it. Since the aim of the study was to determine the effects of observed actions on damaged corticospinal system, these areas were excluded from the following analysis.

In addition, primary motor cortex was defined as an extra ROI. M1 was not outlined in the meta-analysis, however, several studies, including single cell recording in monkey and TMS in humans, suggest its possible involvement during action observation (Fadiga et al., 2005; Kraskov et al., 2014).

6.2.4.2.5 *ROI construction*

Majority of ROIs were constructed by firstly creating a 16mm sphere mask around a peak coordinate provided in meta-analysis (Casper's et al., 2010). This method produced sufficiently large areas to ensure inclusion of relevant information providing some inter-subject variability. Secondly, in order to avoid analysis of BOLD signal in the nearby but functionally distinct anatomical regions, ROIs were also combined with masks derived from the cytoarchitectonic probability maps in the SPM Anatomy toolbox v.1.8 (Eickhoff et al., 2005). The merging of the two masks into one ROI was performed using SPM MarsBar toolbox v. 0.43 (Brett et al., 2002).The overlap area was then used as a ROI in the following analysis (figure 37).

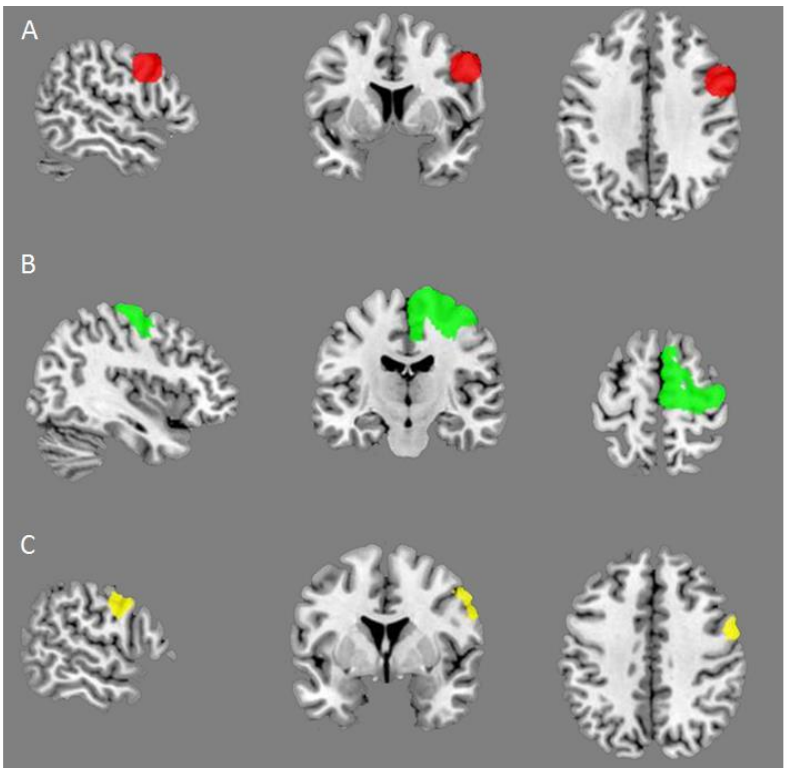


Figure 37. Construction of regions of interest. A) 16mm radius sphere mask centered on the coordinate from the meta-analysis (Casper et al., 2010); B) mask of the cytoarchitectonic probability map defined by SPM Anatomy toolbox v 1.8 (Eickhoff, 2005); C) Combination of A and B.

In total, 7 ROIs were used in each hemisphere: inferior frontal gyrus (IFG), ventral premotor cortex (PMv), dorsal premotor cortex (PMd), inferior parietal lobule (IPL), superior parietal lobule (SPL), primary somatosensory area (S1), and primary motor area (M1) (see table 1).

		Central Coordinate			Combination	
	Region of Interest	x	y	z	Cytoarchitectonic Map	Sphere
Left Hemisphere	Inferior Frontal Gyrus	-50	6	30	BA 44 + 45	16 mm
	Vantral Premotor Cortex	-50	6	30	BA 6	16 mm
	Dorsal Premotor Cortex	-26	-4	56	BA 6	16 mm
	Primary Motor Cortex	-38	-22	58	N/A	6 mm
	Primary Sensory Cortex	-53	-26	48	BA 2	16 mm
	Inferior Parietal Lobule	-58	-24	36	PFt	16 mm
	Superior Parietal Lobule	-26	-69	66	7A	16 mm
Right Hemisphere	Inferior Frontal Gyrus	52	8	36	BA 44 +45	16 mm
	Vantral Premotor Cortex	52	8	36	BA 6	16 mm
	Dorsal Premotor Cortex	36	0	54	BA 6	16 mm
	Primary Motor Cortex	41	-20	60	N/A	6 mm
	Primary Sensory Cortex	42	-34	46	BA 2	16 mm
	Inferior Parietal Lobule	60	-26	42	PFt	16 mm
	Superior Parietal Lobule	22	-62	64	7A	16 mm

Table 1. Description of regions of interest used in the analysis. Seven areas in each hemisphere were outlined. MNI coordinates state central voxels of each region, they were then used to create a 16mm sphere masks (except M1, where 6mm diameter was used). The sphere masks were then refined by overlay with the cytoarchitectonic probability map masks derived using SPM Anatomy toolbox v 1.8.

Notably, ROIs in the inferior frontal gyrus and ventral premotor cortex were derived using the same coordinate, however delineating them by applying a combined BA44 + BA45 cytoarchitectonic mask and BA6 mask respectively (figure 38).

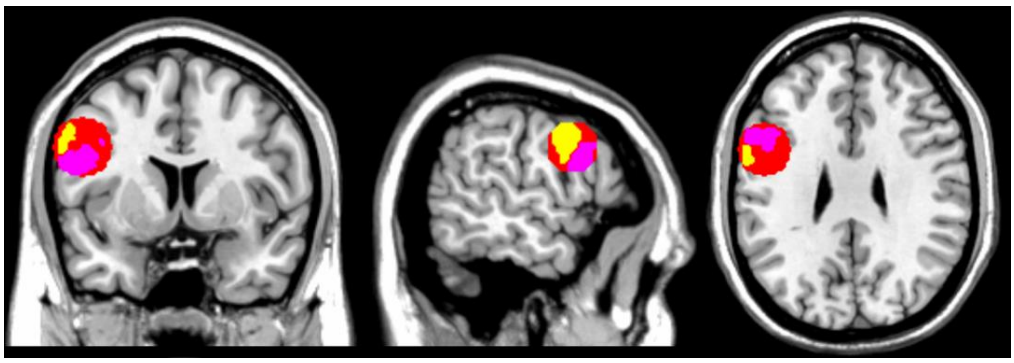


Figure 38. Constructing regions of interest for ventral premotor and inferior frontal areas. Ventral premotor (yellow) and inferior frontal (pink) regions of interest constructed using the same 16mm sphere around central coordinate (-50, 6, 30), delineating them by applying a combined BA44 + BA45 cytoarchitectonic mask (for inferior frontal gyrus) and BA6 (for ventral premotor cortex) mask respectively.

In addition, the peak coordinate for the left BA2 used in my analysis differs from that in the meta-analysis, as the coordinate provided by Caspers and colleagues did not reliably define S1 and, in fact, fell within the undefined cytoarchitectonic area (Caspers et al., 2010). In this case the peak coordinate was taken from the SPM Anatomy toolbox v 1.8 (Eickhoff et al., 2005) as the central coordinate for left BA2 region. Moreover, in the meta-analysis the coordinate was lacking for the left superior parietal lobule, and here was added for consistency. It was constructed in the same way as left S1. Finally, M1 region of interest was not mentioned in the meta-analysis and was defined using mean coordinates for the 'upper limb area' from previous work (Boudrias et al., 2012). In this case only 6 mm sphere was drawn around the coordinate, since the 'upper limb area' is small and does not comprise the whole of primary motor cortex.

6.2.4.2.6 *Lesions*

Binary lesion image for each patient was obtained by the means of Automated Lesion Identification (ALI) toolbox developed for SPM (Seghier et al., 2008). The toolbox produces enhanced segmentation of the T1 image. Grey and white matter segmented images of patients were then compared to those of healthy controls (voxel by voxel) by using the FCP algorithm. Voxels that significantly deviate from the normal range were defined as abnormal and assigned to the lesion. Binary mask was then constructed from the normal (value 1) and abnormal = lesioned voxels (value 0). These individual images (you can find all of them in Appendix G) were used to overlap lesions and ROIs. If more than 10% of the lesion resided within the region of interest, data from such region was not included in further analysis. 10% number was chosen arbitrarily, by assumption that region that is 90% intact could maintain valuable information. Bigger lesions may have compromised results. That being said, the choice may have been incorrect and analysis with and without inclusion of 10% lesioned regions could be performed to examine the difference. Summary of lesions in patients is presented in Table 2.

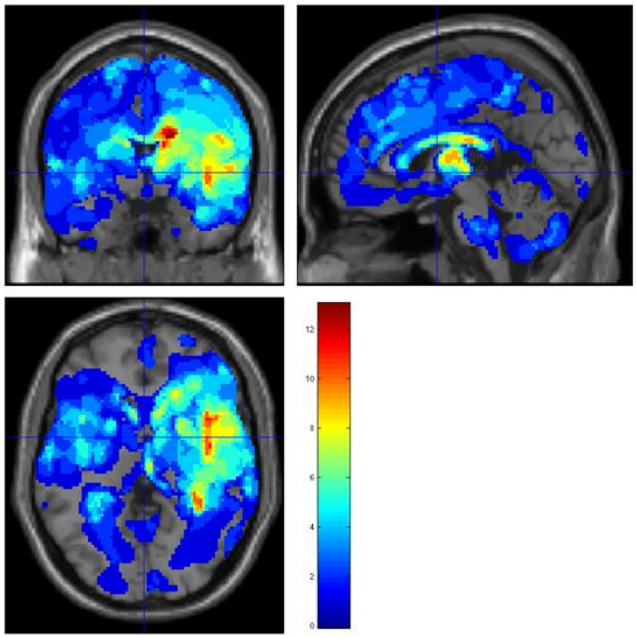


Figure 39. Lesion Overlap of all patients included in the analysis.

	Left Hemisphere							Right Hemisphere						
Region of Interest	S1	PMd	PMv	IFG	M1	IPL	SPL	S1	PMd	PMv	IFG	M1	IPL	SPL
Number of voxels	385	778	267	614	27	329	673	489	438	260	229	27	326	788
Patient 1											10			
Patient 2								489	438	260	221	27	326	62
Patient 3								181	135	11	174		198	
Patient 4								473	438	260	229	27	326	472
Patient 5														
Patient 6														
Patient 7								90	193	166	138	26	147	
Patient 8														
Patient 9														
Patient 10								372	147	80	91	27	326	
Patient 11					1							8		
Patient 12	190	772	251	568	27	294								
Patient 13	37	288			16		36							
Patient 14	171		5		27	41								
Patient 15													69	
Patient 16											28		249	
Patient 17								307	437	260	221	27	325	
Patient 18		42			8									
Patient 19														
Patient 20														
Patient 21	31	99	92	197			60							
Patient 22				33										
Number of patients included	20	19	19	20	18	20	22	16	16	17	15	16	14	21

Table 2. Overlap between region of interest and lesion (in voxels). Shaded cells denote areas where more than 10% of an ROI is lesioned. These ROIs were excluded from further analysis.

6.2.4.3 *Motor Performance Score*

Tests used to measure motor function of the affected and unaffected hand in each patient included Action Research Arm Test (ARAT) (Yozbatiran et al., 2008), 9 Hole Peg Test for finger dexterity (Mathiowetz et al., 1992), Box and Blocks test (Mathiowetz et al., 1985), Apraxia Screen of TULIA (Test for Upper-Limb Apraxia) (AST) (Vanbellingen et al., 2011), and finally pinch and grasp strength measurements were acquired with a dynamometer (Patterson Medical Ltd., Nottinghamshire, UK).

Scores from each test were subsequently entered into a Principal Component Analysis (PCA) to account for floor and ceiling effects amongst outcome scores (Jolliffe, 2002). PCA performs orthogonal transformation converting combinations of 5 motor scores into linearly uncorrelated principal components. First principle component score was calculated and used as a motor function score for each patient. This score as well as outcome of 9 Hole Peg Test (measuring only dexterity of a patient) and scores of pinch and grasp strength were used in regression analyses in this chapter. Notably, the dexterity score, as well as pinch and grasp strength outcomes were already included in the PCA analysis. Nevertheless, separating them in regression allow for different questions to be answered. In addition to establishing whether overall motor function correlates with brain response during action observation, I ask if there is a relationship between ability to perform fine/dexterous actions and activity during action observation.

6.3.1 Behavioural Results

Response accuracy was above chance in both patient (75.3%) and control (89.8%) groups. Performance in the patient group was variable (SD = 12.7) and significantly poorer than that in the control group (SD = 5.6), t (28.05) = -4.584, $p < 0.01$ (figure 40). In the patient group, response accuracy did not correlate with any of the motor function scores.

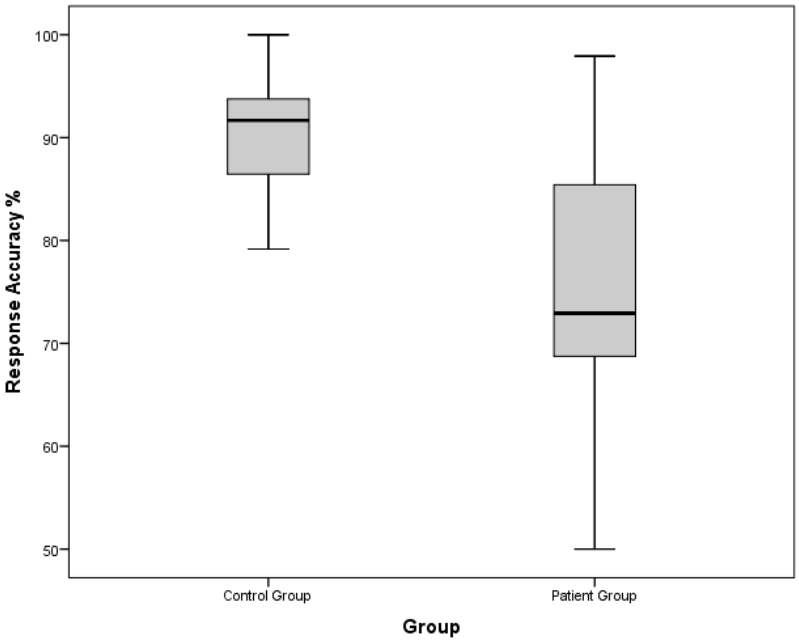


Figure 40. Group averages of behavioural scores. Response accuracy was significantly smaller in patients than in healthy volunteers, however, on average, both groups performed above chance on the task.

6.3.2 Observing hand actions engages regions of sensorimotor system in healthy participants and in patients

Overall, widespread activity was observed during presentation of hand action conditions, including well-established fronto-parietal action observation network (AON) (Caspers et al., 2010) (figure 41, A). Specifically, contrasting AO with rest resulted in greater activity in bilateral superior parietal lobule (SPL), bilateral primary somatosensory region (BA2), bilateral inferior parietal lobule (IPL), bilateral premotor cortex (BA6), right inferior frontal gyrus (IFG pars triangularis) and left IFG (pars opercularis). In addition to classical AON network, greater activity was also observed in bilateral supplementary motor area (SMA), bilateral Thalamus, bilateral Cerebellum (Lobule VIIb), and bilateral V5 (hOC5) (see Appendix H for detailed cluster descriptions).

Interestingly, contrast between observing hand actions (AO) and scrambled moving blocks (C) did not result in strong activation of the AON. In fact, after correcting for multiple comparisons across the whole brain, the difference between experimental and control conditions was only present in primary somatosensory region and in the visual areas V5, V3 bilaterally (figure 41, B and table 3). After correcting for multiple comparisons within ROIs, activity was higher during action observation as compared to scrambled moving blocks in primary somatosensory area (S1), in dorsal premotor region (PMd), inferior and superior parietal lobules – all bilaterally (figure 41, C). I found no significant difference in ventral premotor cortex (PMv) or inferior frontal gyrus (IFG).

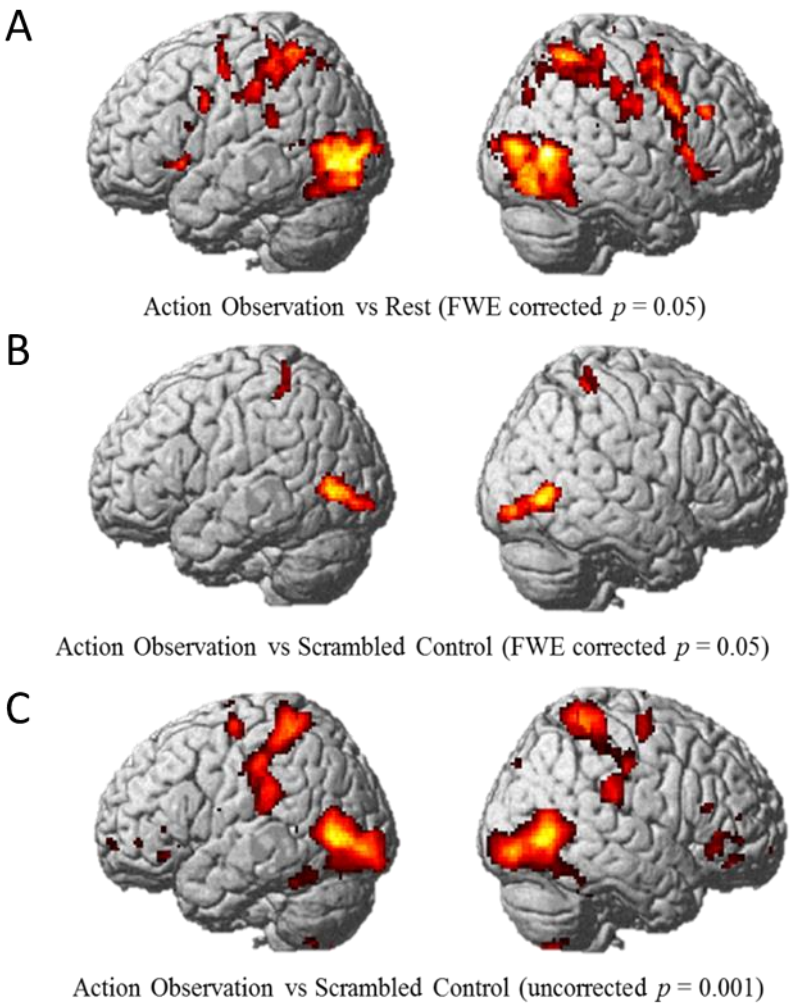


Figure 41. Engagement of fronto-parietal network during observation of hand actions in healthy participants. Contrasting activity between observation of actions and rest (A), as well as between observation of actions and of scrambled moving blocks (B (whole brain corrected for multiple comparisons (FEW, $p = 0.05$)) and C (uncorrected $p = 0.001$)).

Action Observation > Control (T> 7.13)		T	X	Y	Z	Anatomical Region	Cytoarchitectonic Map	
Cluster 1 (615 vox)	Maximum 01	13.89	48	-68	-1	Right Middle Temporal Gyrus	right	hOC5 (V5)
	Maximum 02	10.53	36	-88	-9	Right Inferior Occipital Gyrus	right	hOC3v (V3v)
Cluster 2 (521 vox)	Maximum 01	14.84	-46	-70	1	Left Middle Occipital Gyrus	left	hOC5 (V5)
	Maximum 02	12.12	-42	-80	-1	Left Middle Occipital Gyrus		
	Maximum 03	12.11	-38	-88	-5	Left Middle Occipital Gyrus		
	Maximum 04	8.24	-30	-94	-7	Left Inferior Occipital Gyrus	left	hOC3v (V3v)
	Maximum 05	7.91	-46	-78	11	Left Middle Occipital Gyrus		
	Maximum 06	7.65	-30	-92	1	Left Middle Occipital Gyrus		
Cluster 3 (146 vox)	Maximum 01	10.2	24	-44	59	Right Postcentral Gyrus	right	Area 2
	Maximum 02	8.9	32	-42	69	Right Postcentral Gyrus	right	Area 1
	Maximum 03	8.29	32	-36	49	Right Postcentral Gyrus	right	Area 2
	Maximum 04	7.86	34	-38	59	Right Postcentral Gyrus	right	Area 3b
Cluster 4 (89 vox)	Maximum 01	8.7	-30	-44	71	Left Superior Parietal Lobule	left	Area 1
	Maximum 02	8.59	-30	-46	59	Left Superior Parietal Lobule	left	SPL (7PC)
	Maximum 03	8.3	-34	-42	57	Left Superior Parietal Lobule	left	Area 2
	Maximum 04	8.07	-32	-46	65	Left Superior Parietal Lobule		

Table 3. Action observation > scrambled moving blocks blocks in healthy participants (FWE corrected p=0.05). Cluster size and peak coordinates in the MNI space are outlined. Descriptions of the anatomical areas and corresponding cytoarchitectonic maps are taken from SPM Anatomy Toolbox v.1.8 (Eickhoff, 2005).

Similar to healthy individuals, AO compared to rest resulted in a wide spread activation in patients. Small volume correction in regions of interest confirmed that just as in healthy controls, greater BOLD signal during experimental conditions was obtained bilaterally in superior and inferior parietal regions (SPL and IPL), primary somatosensory area (S1), dorsal and ventral premotor cortex (PMd and PMv), and in the inferior frontal gyrus (IFG) (figure 42, B).

Contrast between observing hand actions (AO) and scrambled moving blocks (C) resulted in similar activity in patients as in healthy controls. After small volume correction for multiple comparisons within regions of interest, activity was again greater during action observation as compared to scrambled moving blocks bilaterally in prima-

ry somatosensory area (S1) and in dorsal premotor cortex (PMd), as well as in left inferior and superior parietal lobule (SPL and IPL). Notably, just as in healthy participants no significant difference was found in the inferior frontal gyrus (IFG) or ventral premotor cortex (PMv) (figure 42, D). Difference in activity between AO and C that passed multiple comparisons across the whole brain are detailed in table 4.

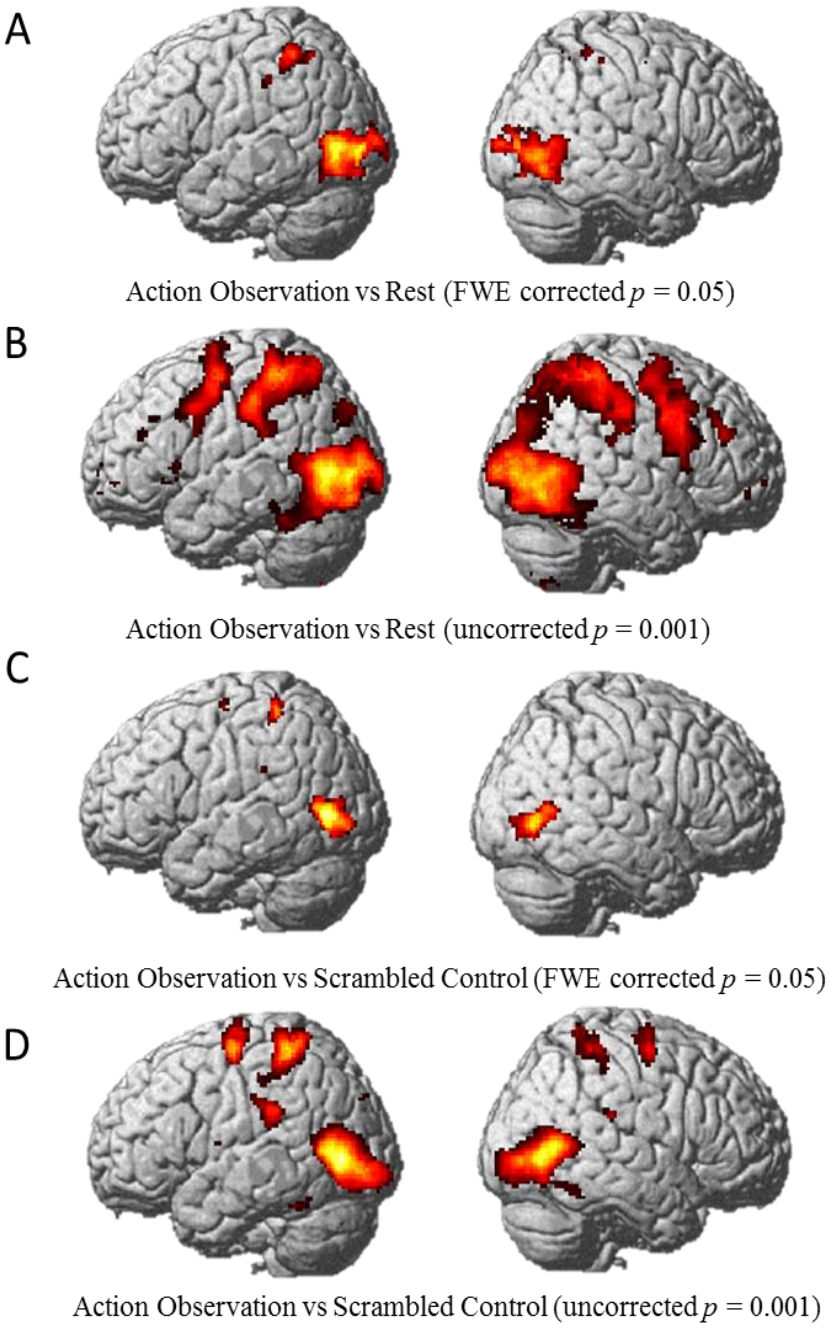


Figure 42. Engagement of fronto-parietal network during observation of hand actions in patients. Contrasting activity between observation of actions and rest (A (whole brain corrected for multiple comparisons (FEW, $p = 0.05$)) and B (uncorrected $p = 0.001$)), as well as between observation of actions and of scrambled moving blocks (B (whole brain corrected for multiple comparisons (FEW, $p = 0.05$)) and C (uncorrected $p = 0.001$)).

Action Observation > Rest (T > 6.69) (FWE corrected, p=0.05)		T	X	Y	Z	Anatomical Region	Cytoarchitectonic Map
Cluster 1 (596 vox)	Max 01	12.05	-46	-72	1	Left Middle Occipital Gyrus	left hOC5 (V5)
	Max 02	11.06	-42	-84	-3	Left Inferior Occipital Gyrus	
Cluster 2 (388 vox)	Max 01	14.1	50	-72	-1	Right Middle Temporal Gyrus	
	Max 02	10.15	50	-62	7	Right Middle Temporal Gyrus	right hOC5 (V5)
Cluster 3 (118 vox)	Max 01	9.66	-36	-42	65	Left Postcentral Gyrus	left Area 1
	Max 02	9.49	-32	-40	57	Left Postcentral Gyrus	left Area 2
	Max 03	8.5	-40	-44	59	Left Superior Parietal Lobule	left Area 2
Cluster 4 (14 vox)	Max 01	8.05	-26	-12	63	Left Precentral Gyrus	left Area 6
	Max 02	7.1	-32	-12	65	Left Precentral Gyrus	
Cluster 5 (5 vox)	Max 01	7.27	-64	-36	29	Left SupraMarginal Gyrus	left IPC (PF)
Cluster 6 (3 vox)	Max 01	7.12	30	-38	53	Right Postcentral Gyrus	right Area 2
Cluster 7 (2 vox)	Max 01	7.03	34	-32	45	Right Postcentral Gyrus	right Area 3a
Cluster 8 (1 vox)	Max 01	7.18	-14	-28	41	Left Middle Cingulate Cortex	left SPL (5Ci)

Table 4. Action observation > scrambled moving blocks in patients (FWE corrected (p=0.05)). Cluster size and peak coordinates in the MNI space are outlined. Descriptions of the anatomical areas and corresponding cytoarchitectonic maps are taken from SPM Anatomy Toolbox v.1.8 (Eickhoff, 2005).

6.3.3 BOLD response is modulated by observed hand laterality in both, healthy participants and patients.

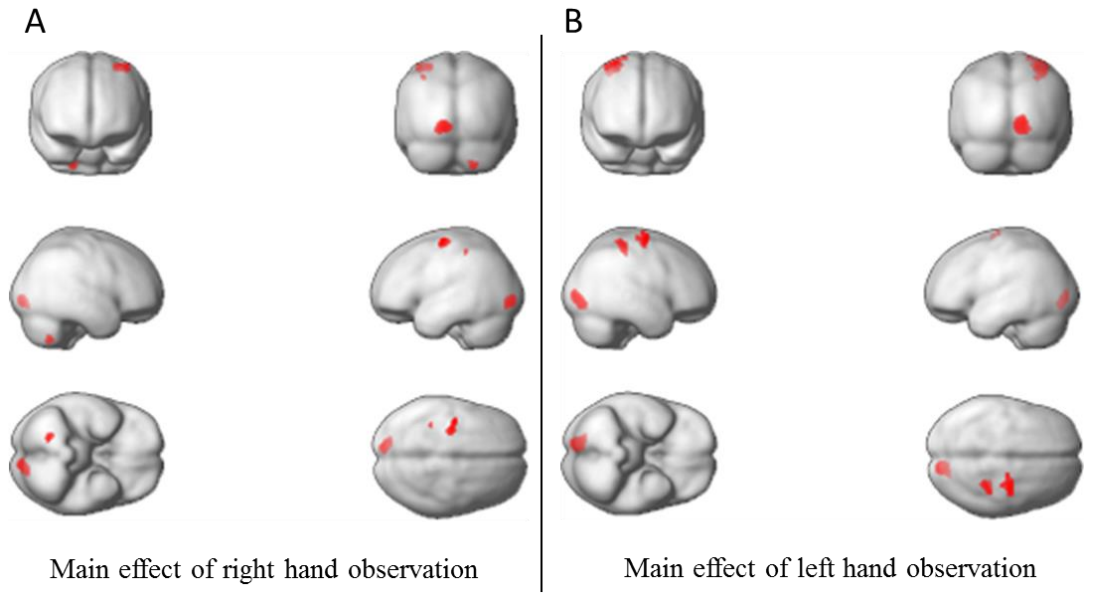
I used 2x2x2 (Group/Observed Hand/Observed Action) mixed ANOVA to test for the effect of observed hand laterality (left or right) in patients and healthy controls irrespective of observed action. There was no interaction between Group and Observed Hand, suggesting that there was no significant difference between patients and healthy individuals in the way observed hand laterality was processed.

In fact, strong differential processing of observed hand in several regions of AON was indicated irrespective of group or observed action. Analysis between observed effectors in both groups resulted in clear hemisphere dependent patterns of activation. During observation of left hand actions, BOLD activity was higher in *right* lingual gyrus, as well as *right* precentral gyrus (PMd) and *right* postcentral gyrus (S1). Whereas during observation of right hand actions, activity was larger in the left hemisphere: including *left* PMd, *left* IPL/S1 (in this case assigned to the primary somatosensory cortex area BA2) and *left* middle occipital gyrus (MOG), with the exception of the right cerebellum. Detailed description is provided below (see figure 42, A and B, and table 5).

Notably, when pinch and grasp strength were entered as covariates of no interest in the analysis, main effects of observed hand laterality remained unchanged.

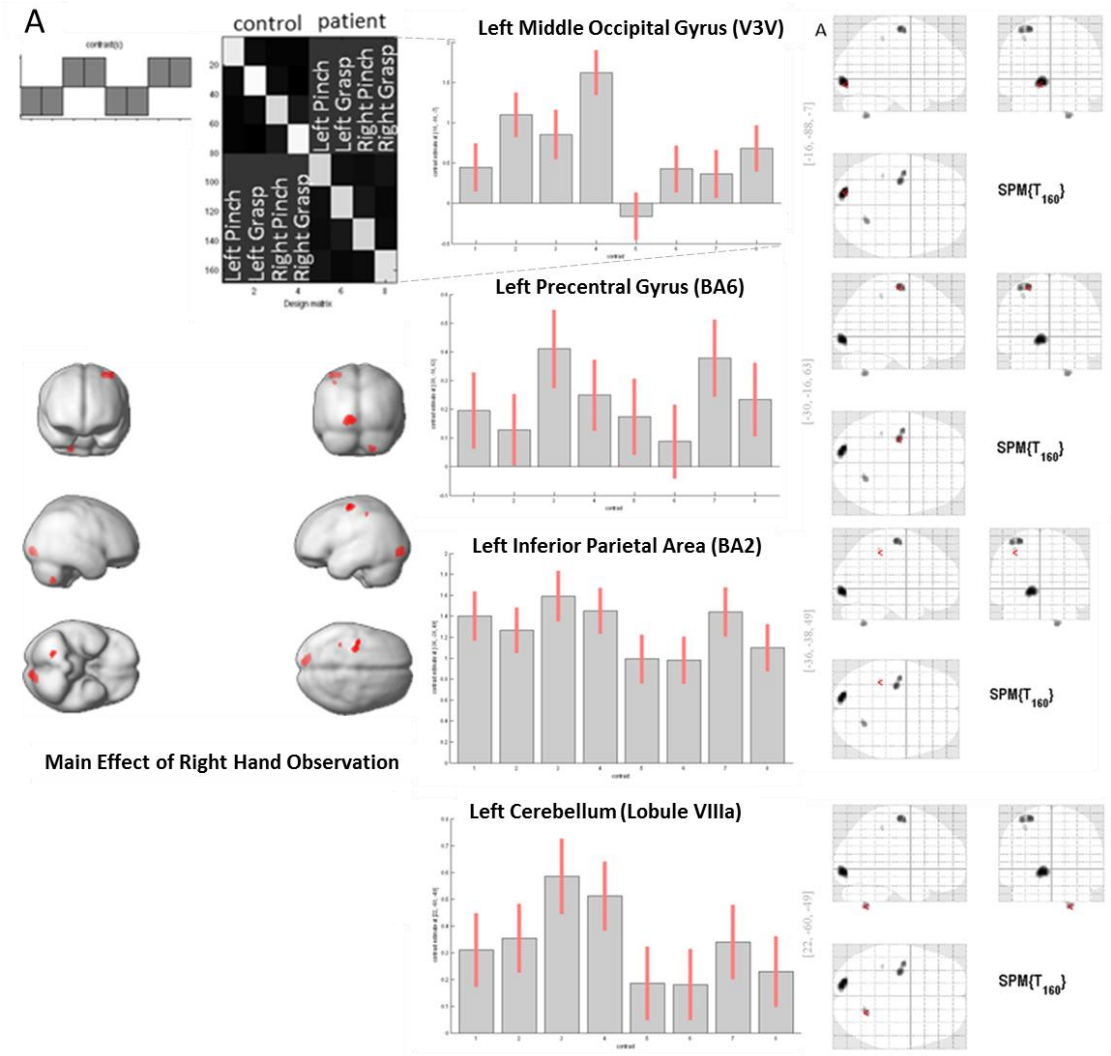
Positive effect of left hand observation (T>4.95)		T	X	Y	Z	Anatomical Region	Cytoarchitectonic Map	
Cluster 1 (354 vox)	Maximum 01	T = 8.34	16	-86	-5	Right Lingual Gyrus	right	Area 18
Cluster 2 (234 vox)	Maximum 01	T = 6.97	34	-12	65	RightPrecentral Gyrus	right	Area 6
	Maximum 02	T = 6.15	22	-14	73	Right Superior Frontal Gyrus	right	Area 6
	Maximum 03	T = 5.65	32	-18	67	RightPrecentral Gyrus	right	Area 6
Cluster 3 (138 vox)	Maximum 01	T = 6.26	40	-34	57	Right Postcentral Gyrus	right	Area 3b
	Maximum 02	T = 5.56	34	-34	51	Right Postcentral Gyrus	right	Area 3b
Positive effect of right hand observation (T>4.95)								
Cluster 1 (208 vox)	Maximum 01	T = 7.40	-16	-88	-7	Left Middle Occipital Gyrus	left	hOC3v (V3v)
Cluster 2 (118 vox)	Maximum 01	T = 6.64	-30	-16	63	Left Precentral Gyrus	left	Area 6
	Maximum 02	T = 6.35	-40	-10	61	Left Precentral Gyrus	left	Area 6
Cluster 3 (49 vox)	Maximum 01	T = 5.95	22	-60	-49	Right Cerebellum	right	Lobule VIIIa (Hem)
Cluster 4 (9 vox)	Maximum 01	T = 5.23	-36	-38	49	Left Inferior Parietal Lobule	left	Area 2

Table 5. Main effect of effector (FWE corrected (p=0.05) is separated into positive effects of left and right hand observation. Cluster size and peak coordinates in the MNI space are outlined. Descriptions of the anatomical areas and corresponding cytoarchitectonic maps are taken from SPM Anatomy Toolbox v.1.8 (Eickhoff, 2005).



Main effect of right hand observation

Main effect of left hand observation



Main Effect of Right Hand Observation

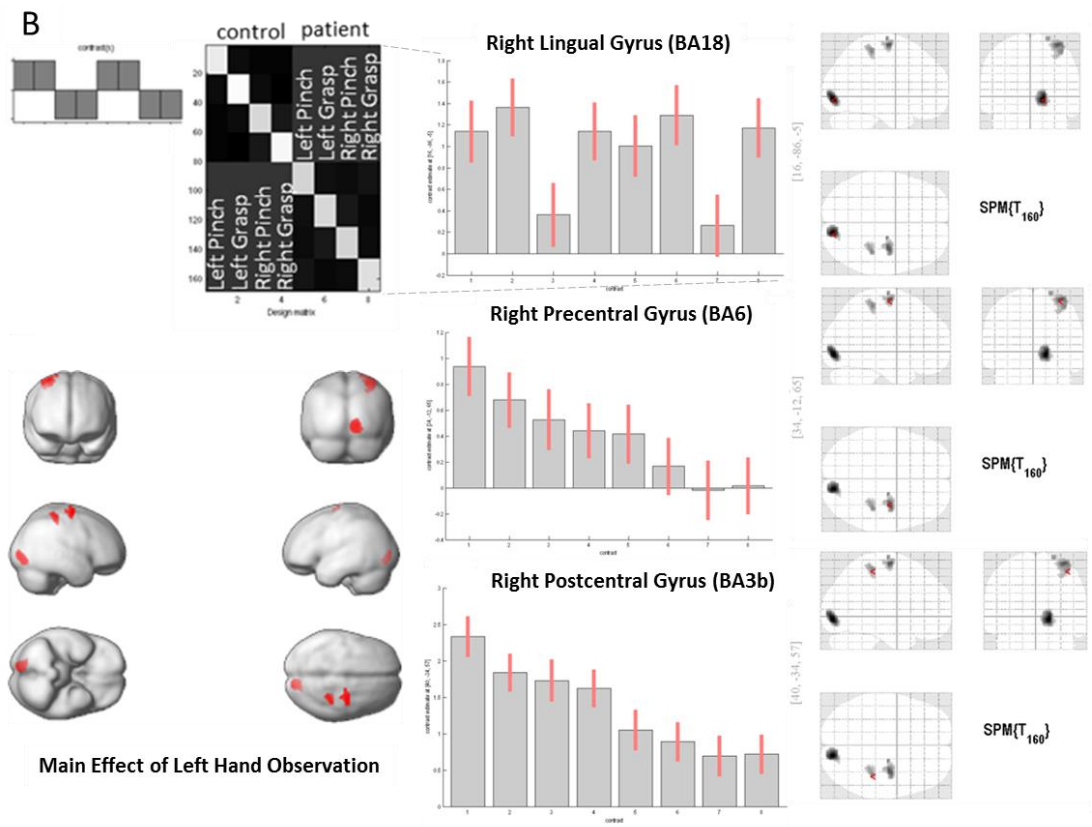


Figure 43. Main effect of observed hand laterality. Positive main effects of each condition: A) observed right > left hand actions, B) observed left > right hand actions. Parameter estimates from every peak voxel are shown to the left of the glass brain where the same coordinate is marked. The order of contrasts within the design matrix is as follows: Left Pinch (Control), Left Grasp (Control), Right Pinch (Control), Right Grasp (Control), Left Pinch (Patient), Left Grasp (Patient), Right Pinch (Patient), Right Grasp (Patient).

6.3.4 BOLD response is modulated by observed hand laterality in patients, but is independent of whether observed hand is congruent to the impaired hand

To test if laterality of impairment impacts on observation related change in BOLD signal, I have split patients into two groups: 1) 12 patients with left hand affected (LHA) and 2) 10 patients with right hand affected (RHA). In 2x2 mixed ANOVA I looked for interaction between group and laterality of observed hand. There was no significant effect even at the uncorrected level ($p<0.001$), suggesting that difference between observing left and right hand was not modulated by laterality of impairment.

6.3.5 The ability to execute actions does not alter brain activity during observation of those actions

Next, I used multiple regression analysis to test for the relationship between motor function of the affected hand and BOLD response during observation of the affected hand in both LHA (left hand affected) and RHA (right hand affected) patient groups. As motor function score, I used both dexterity score from 9 Hole Peg test and a combined motor function score using first principal component.

BOLD response did not correlate with motor function in regions of interest in neither of the patient groups. The relationship was insignificant even at the uncorrected level after correcting for multiple comparisons within ROIs. Notably, small sample size in each group might account for the insignificant result (12 patients had left hand affected, while 10 patients had right hand affected).

6.3.6 Engagement of fronto-parietal regions during action observation is modulated by dominance of the affected hand of the patient

Using single-pulse TMS, I found a marked difference in corticospinal excitability in patients depending on which hand was impaired after stroke. I found that CST excitability was significantly greater in patients with dominant affected hand as compared to those with non-dominant impairment. Based on these results, I have tested if BOLD response was also modulated by dominance of the affected hand.

Indeed, activity during observation was significantly lower in patients whose non-dominant hand was affected as compared to those with dominant hand impairment. Small volume correction for multiple comparisons within regions of interest at p = 0.05 confirmed significant difference in four regions of the right hemisphere – primary somatosensory region (S1), superior and inferior parietal lobule (SPL and IPL), and inferior frontal gyrus (IFG) (table 6 and figure 43).

Dominant > Non-dominant Affected			T	X	Y	Z	Anatomical Region	Cytoarchitectonic Map	
SVC in right S1	Cluster 1 (112 vox)	Peak 1	T = 5.06	48	-22	43	Right Postcentral Gyrus	right	Area 2
		Peak 2	T = 4.22	48	-30	45	Right Postcentral Gyrus	right	IPC (PFt)
		Peak 3	T = 3.62	48	-34	51	Right Inferior Parietal Lobule	right	IPC (PFt)
	Cluster 2 (10 vox)	Peak 1	T = 3.81	30	-34	47	Right Postcentral Gyrus	right	Area 3a
	Cluster 3 (2 vox)	Peak 1	T = 4.20	36	-34	53	Right Postcentral Gyrus	right	Area 3b
SVC in right IPL	Cluster 1 (44 vox)	Peak 1	T = 4.48	48	-26	41	Right Postcentral Gyrus	right	IPC (PFt)
		Peak 2	T = 3.36	56	-26	45	Right SupraMarginal Gyrus	right	IPC (PFt)
	Cluster 2 (9 vox)	Peak 1	T = 3.87	60	-14	25	Right SupraMarginal Gyrus	right	OP 4
SVC in right SPL	Cluster 1 (13 vox)	Peak 1	T = 4.47	34	-54	61	Right Superior Parietal Lobule	right	SPL (7PC)
		Peak 2	T = 3.96	34	-52	57	Right Superior Parietal Lobule	right	SPL (7PC)
	Cluster 2 (1 vox)	Peak 1	T = 3.57	32	-50	53	Right Inferior Parietal Lobule	right	SPL (7PC)
SVC in right IFG	Cluster 1 (58 vox)	Peak 1	T = 4.57	56	14	21	Right Inferior Frontal Gyrus (p. Opercularis)	right	Area 44
		Peak 2	T = 3.53	42	12	23	Right Inferior Frontal Gyrus (p. Triangularis)		

Table 6. Activity during action observation depending on whether dominant or non-dominant hand was impaired after stroke. Activity was lower in the patients with non-dominant affected hand (in the ipsilesional hemisphere). Cluster size and peak coordinates in the MNI space are outlined. Descriptions of the anatomical areas and corresponding cytoarchitectonic maps are taken from SPM Anatomy Toolbox v.1.8 (Eickhoff, 2005).

Conversely, there was no greater activity in patients with non-dominant affected hand as compared to patients with dominant hand impairment in the left hemisphere (figure 43).

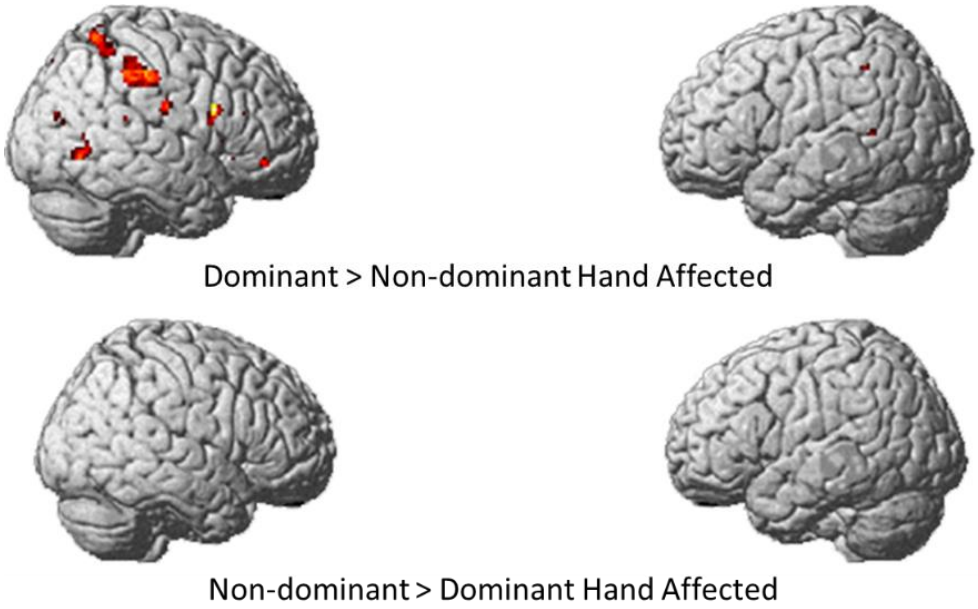


Figure 44. Watching both left and right hand pinch. BOLD activity in patients with affected dominant hand is higher than in patients with affected non-dominant hand irrespective of the observed hand (uncorrected, p=0.001). After small volume correction in regions of interest, significant difference was confirmed in the right S1 (peaking at 48, -22, 43), in the right IPL (peaking at 48, -26, 43), in the right SPL (34, -54, 61), and right IFG – area 44 (56, 14, 21).

Notably, there were more patients with damage to the right hemisphere (N=12) than with damage to the left hemisphere (N=10). Reduced activity may reflect greater overall injury.

6.3.7 Engagement of the fronto-parietal regions during action observation depends on motor function in dominant and non-dominant hand after stroke

I explored whether post-stroke motor function in the dominant and non-dominant hand modulates BOLD activity during observation. I was interested if - irrespective of which hand is affected by stroke - use dependent changes in dominant or non-dominant hand impact on brain activity during action observation.

Multiple regression analysis was performed to test for the relationship between motor function and brain activity during observation. Motor scores were entered as covariates in the analysis. Both, a combined motor function score (first principal component) and a dexterity score (derived from the 9 hole peg test) were used. The results were as follows:

1) There was significant negative correlation between combined motor function score of the dominant hand and BOLD response during observation in the right primary somatosensory (S1), right inferior parietal (IPL) and right inferior frontal (IFG) regions (figure 44).
2) There was significant negative correlation between dexterity score of the dominant hand and BOLD response during observation in the right S1, right IPL and right IFG.
3) There was no relationship between combined motor function score of the non-dominant hand and BOLD response during observation.

4) There was a significant positive correlation between dexterity score of the non-dominant hand and BOLD response during observation in the right S1, right IPL and right IFG.

It appears that activity in the S1, IPL and IFG in the non-dominant hemisphere were affected by motor function in both dominant and non-dominant hand, yet in the opposing directions. Whereby greater BOLD activity was associated with worse function of the dominant hand, but better function of the non-dominant hand.

The significance of these results was tested firstly using SPM multiple regression analysis, whereby small volume correction for multiple comparisons (p=0.05) within each ROI was performed on the uncorrected data (p=0.001). Secondly, 1st eigenvariates were extracted from each of the significant ROIs and tested in the IBM SPSS software for bivariate correlation, while excluding patients with identified lesions within the area.

6.3.7.1 *Worse dexterity in the non-dominant hand leads to a greater BOLD response in the non-dominant S1, IPL and IFG areas*

I found significant negative correlation between combined motor score and BOLD response during observation of right hand pinch in right S1, r (16) = -.766, *p* = .001; right IPL, r (14) = -.654, *p* = .011; and right IFG, r (15) = -.774, *p* = .001 (figure 45). Same significant negative correlation between dexterity score and BOLD response during observation of right hand pinch in right S1, r (16) = -.730, *p* = .001; right IPL, r (14) = -.576, *p* = .031; and right IFG, r (15) = -.722, *p* = .002. Notably, dexterity is a part of the combined motor score, thus the two are highly correlated between themselves. The results should not be read in comparison to each other, rather, dexterity correlation values can be further compared with those in the non-dominant hand.

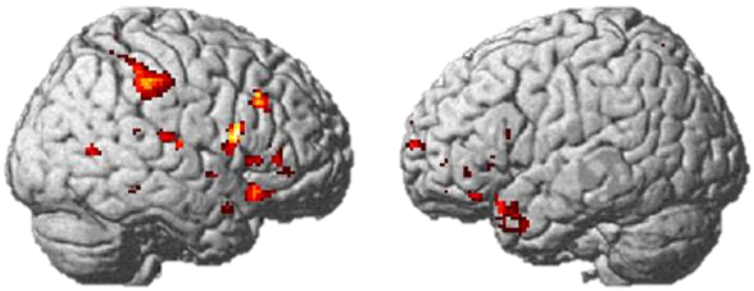


Figure 45. Significant negative correlation between dominant hand motor function and BOLD activity during observation of dominant hand pinch (uncorrected, p=0.001). After small volume correction for multiple comparisons within ROIs, correlation was significant in the right S1 (peaking at 42, -34, 41), right IPL (peaking at 46, -32, 41) and right IFG (peaking at 48, 12, 21).

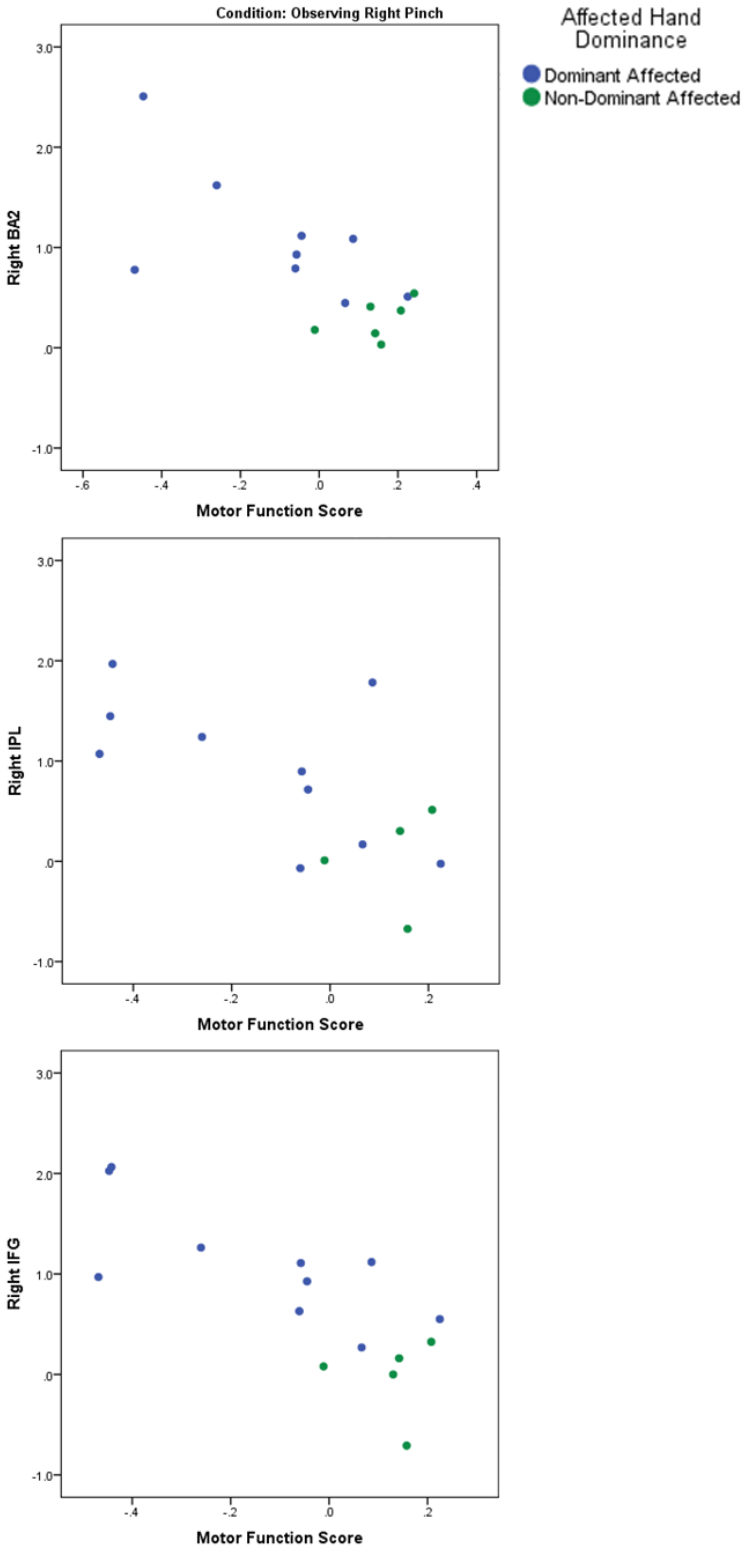


Figure 46. Negative correlation between motor function score in the dominant hand of patients and 1st eigenvariate of BOLD signal extracted from right primary somatosensory area (BA2), inferior parietal lobule (IPL) and inferior frontal gyrus (IFG).

Importantly, negative correlation was found in the same regions irrespective of observed action. Watching left hand pinch: right S1, r (16) = -.755, p = .001; right IPL, r (14) = -.741, p = .002. Watching right hand grasp: right S1, r (16) = -.874, p = .000; right IPL, r (14) = -.798, p = .001; and right IFG, r (15) = -.689, p = .018. Watching left hand grasp: right S1, r (16) = -.857, p = .000; right IPL, r (14) = -.778, p = .001; and right IFG, r (15) = -.677, p = .006.

Moreover, it appeared that correlation in right S1 and right IPL belongs to the same cluster, spreading across both regions, surrounding intraparietal sulcus (figure 46).

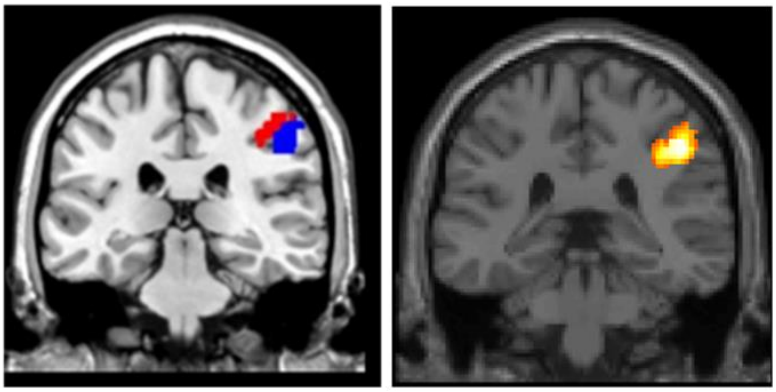


Figure 47. Regions of interest S1 and IPL border each other, but they do not overlap as each ROI is demarcated using the cytoarchitectonic map. Significant relationship between motor function and brain activity during observation in the right S1 and right IPL appears to stem from the same cluster spreading over both regions. Image on the left shows two ROIs overlaid on the brain template using MRIcron software (Rorden and Brett, 2000) (S1 – red, IPL - blue). Image on the right is of a cluster showing significant correlation between motor function score in the dominant hand and magnitude of BOLD signal during observation of right hand grasp.

6.3.7.2 *Better dexterity in the non-dominant hand leads to a greater BOLD response in the non-dominant S1, IPL and IFG areas*

In contrast, there was no correlation between combined motor function score of the non-dominant hand and magnitude of BOLD signal. Nevertheless, there was a significant positive correlation between non-dominant hand dexterity and BOLD response during action observation in the same network of regions - right S1, IPL and IFG after small volume correction for multiple comparisons in each ROI (figure 47). Upon extraction of 1st eigenvariate values for each patient in all three ROIs, I conducted correlation excluding patients with lesions in those areas. Observing left hand pinch still resulted in strong correlation in the right S1, r (16) = .650, p = .006; right IPL, r (14) = .645, p = .004; and right IFG, r (15) = .695, p = .004. Observing right hand pinch resulted in strong correlation in the right S1, r (16) = .675, p = .004; right IPL, r (14) = .752, p = .002; and right IFG, r (15) = .688, p = .005 (figure 48).

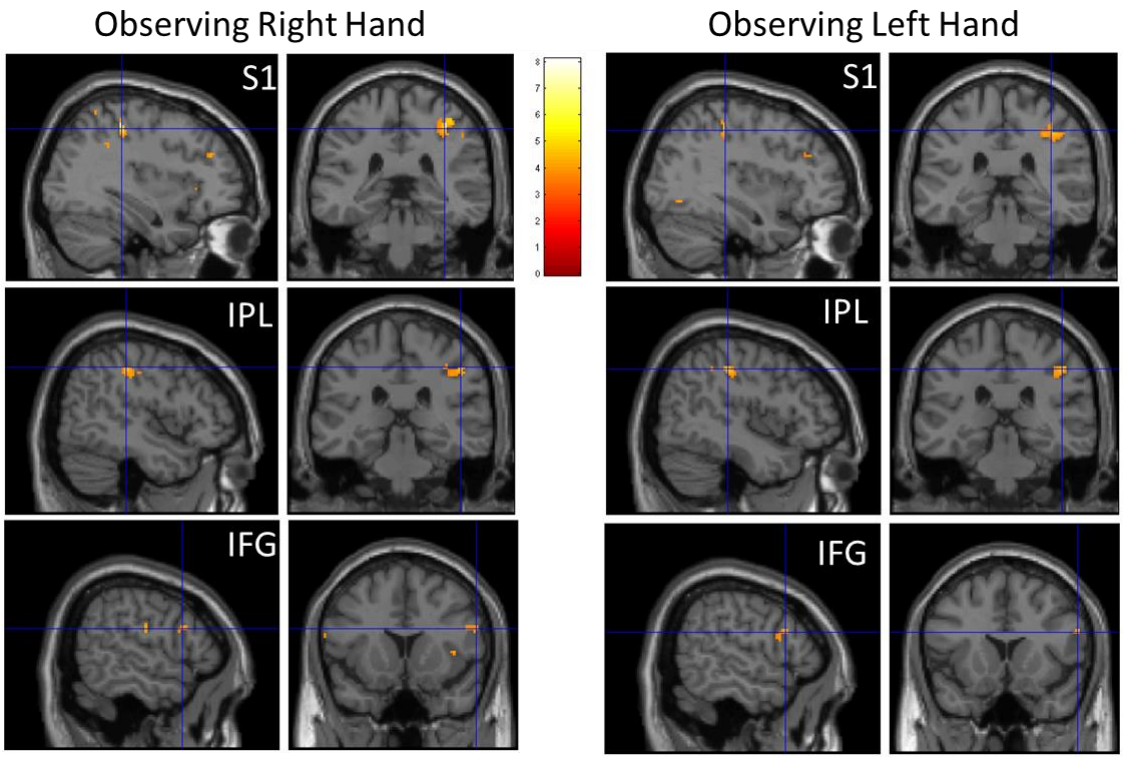


Figure 48. Significant positive correlation between dexterity in the left non-dominant hand and BOLD response during observation of left and right hand pinch found in the S1, IPL and IFG of the right hemisphere. Activation shown in these images is of uncorrected clusters (p=0.001). The crosshair on each ROI indicates a maximum coordinate clusters with significant result after small volume correction (p=0.05). Maximum coordinates: right S1 (observing right hand – 36, -34, 49, observing left hand - 38, -32, 47), right IPL (observing right hand – 48, -30, 45, observing left hand – 36, -30, 43), right IFG (observing right hand – 58, 14, 23, observing left hand – 58, 16, 23).

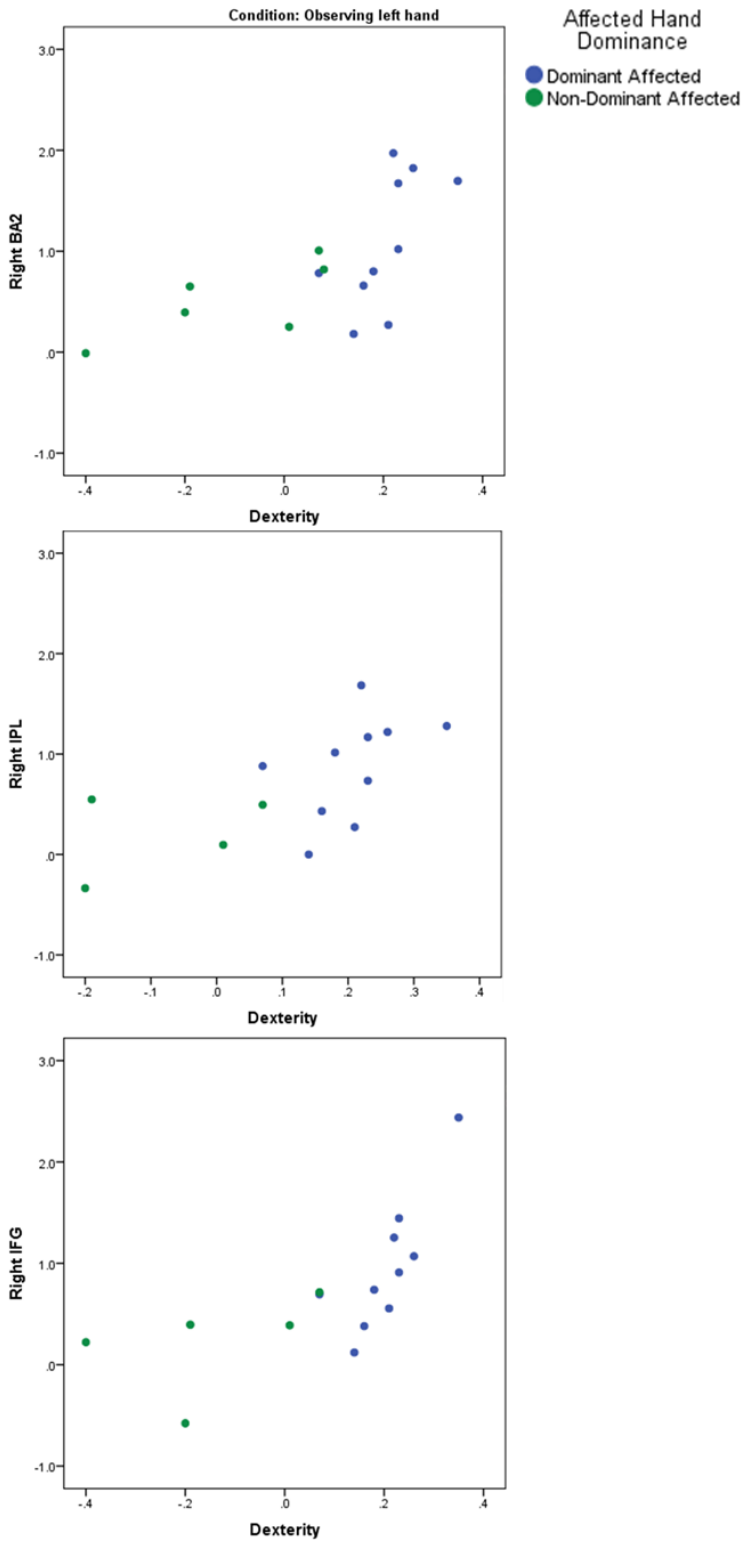


Figure 49. Positive correlation between dexterity in the non-dominant hand of patients and 1st eigenvariate of BOLD signal extracted from right primary somatosensory area (BA2), inferior parietal lobule (IPL) and inferior frontal gyrus (IFG).

Importantly, non-dominant hand dexterity and BOLD activity also correlated when patients watched left and right hand grasp. Observing left hand grasp resulted in strong correlation in the right S1, r (16) = .733, p = .001; right IPL, r (14) = .753, p = .002; and right IFG, r (15) = .662, p = .007. Observing right hand grasp resulted in significant correlation in the right S1, r (16) = .751, p = .001; right IPL, r (14) = .739, p = .003; and right IFG, r (15) = .540, p = .038.

Overall, during action observation, greater BOLD activity in the non-dominant hemisphere was associated with worse dexterity in the dominant hand and better dexterity in the non-dominant hand of patients (figure 49).

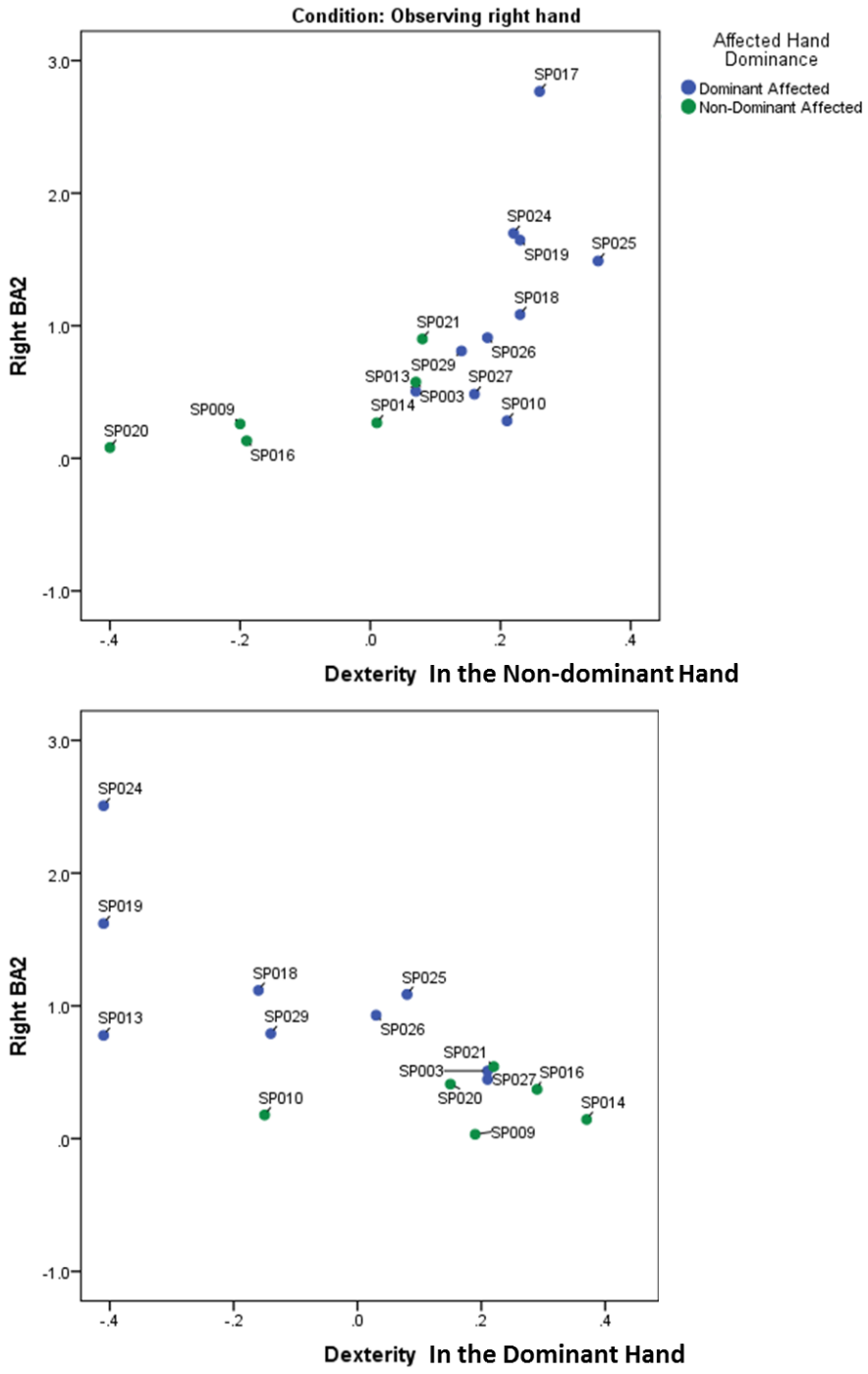


Figure 50. Negative and positive correlation in the same primary somatosensory region (BA2). Patients with worse dexterity in their dominant hand tend to have better dexterity in their non-dominant hand.

6.4 DISCUSSION

6.4.1 Summary of results

1) Although watching hand actions relative to rest elicited higher activity in the classical Action Observation Network, greater BOLD response was lost in the inferior frontal and premotor regions when observation of actions was compared to observation of moving scrambled blocks.
2) BOLD response is modulated by observed hand laterality in both, healthy participants and in patients.
3) BOLD response is modulated by observed hand laterality in patients, but is independent of whether observed hand is congruent to their impaired hand.
4) The ability to execute observed actions does not alter brain activity during observation.
5) Engagement of the fronto-parietal network during observation is modulated by the affected hand dominance before stroke. While activity in the non-dominant hemisphere is significantly lower in patients with non-dominant affected hand, activity in the dominant hemisphere is not altered in those with dominant hand impairment.
6) Greater activity during action observation in the non-dominant hemisphere is associated with worse dominant hand function and with better dexterity in the non-dominant hand.

6.4.2 Discussion

Firstly, comparing activity during observation of hand actions to that during observation of control scrambled blocks resulted in significant difference in the classical Action Observation Network, apart from ventral premotor region and inferior frontal gyrus. It is plausible that some features of movement were preserved in scrambled videos driving response in these areas. Indeed in a recent study, Gorbet and colleagues explored neural correlates of processing periodic, closed circuit visual motion, which is an essential component of rhythmic biological movement, such as walking or riding a bicycle (Gorbet et al., 2014). Such movement begins and ends in the same spatial location relative to the rest of the body. Researchers simulated this cyclical closed-circuit motion by designing radial frequency (RF) motion trajectories comprised of Gaussians moving in a closed trajectory (Gorbet et al., 2014). Three types of stimuli were produced: low-frequency visual motion trajectories that form consciously discriminable shapes, high-frequency trajectories that are behaviourally unidentifiable and static versions of trajectory shapes. Authors showed that recruitment of ventral and dorsal premotor cortex, as well as inferior parietal cortex was modulated by conscious recognition of the shape defined by the RF motion trajectory. Overall, BOLD signal was higher in premotor, posterior parietal, occipital and temporal cortex for low-frequency (consciously distinguishable) as compared to high-frequency motion trajectories. Authors suggested that recognisable RF motion trajectories evoke activity within the mirror neuron system, which may be similar to observing familiar motor actions and "reflect the level at which the motor system "understands" a particular RF trajectory and also the observer's conscious ability to identify the shape" (Gorbet et al., 2014).

Based on Gorbet's findings, the type of control stimuli used in present experiment may have driven activity within the mirror neuron regions during observation of moving scrambled blocks. In the current study, control condition comprised closed-circuit motion trajectories closely matching experimental videos of hand grasping and putting down an object. Although videos were scrambled so that biological effector could not be recognised, the closed-circuit motion was still apparent. It is worth noting that the majority of the fMRI experiments exploring mirror neuron system use still images or movie clips with no motion as their control stimuli, explaining the discrepancy between results of present work and that of others.

Further, results in this chapter can be summarized into three main findings: 1) the ability to execute observed actions is not essential for engagement of the system through observation; 2) affected hand dominance plays an important role in activation of the motor system during observation; and 3) changes in the non-dominant hand after stroke modulate BOLD response during observation in the fronto-parietal network of the contralateral hemisphere. Here I discuss each of these findings:

6.4.2.1 *The ability to execute observed actions is not essential for engagement of the system through observation*

My results suggest that brain responses to observed hand laterality (watching left or right hand) were similar in healthy individuals and in patients. Activity increased in S1 and PMd regions that were contralateral to the observed hand. While watching left hand pinch and grasp resulted in increased activity in the right hemisphere, watching right hand elicited greater response in the left hemisphere. Such higher contralateral activity in response to observed hand laterality has been previously reported in the superior pa-

rietal (SPL) and anterior intraparietal (aIPL) regions (Shmuelof and Zohary, 2008, 2006; Vingerhoets et al., 2012). While the role of PMd in processing observed hand laterality has not been documented yet, activity in S1 may be somewhat in keeping with findings of Shmuelof and Zohary (2008, 2006), as well as Vingerhoets and colleages (2012). Notably, findings in these studies do not directly suggest S1 involvement, but that of SPL and aIPL. One possible reason for dissimilarity in findings between current experiment and those of Shmuelof and Zohary (2008, 2006) and Vingerhoets et al. (2012) may not be the difference in activation, but difference in ascribing anatomical labels to the activation.

In order to make my findings testable by others, I have consistently employed labelling and ROI analysis using a standard Anatomy Toolbox probabilistic histological atlas (Eickhoff et al., 2005). This toolbox is freely available as part of the SPM software and has already been utilized widely by publishing neuroscientists. It is also common, however, to use other digital atlases or to label anatomical regions 'by eye'. Differences in labelling methods can lead to differences in interpretation of the same activations. In figure 50 I demonstrate how the coordinate of maximum activity taken from Shmuelof and Zohary (2006) study (defined by the authors as anterior parietal cortex) overlaps with my findings for the same contrast, labelled by the Anatomy Toolbox as primary somatosensory cortex (BA2).

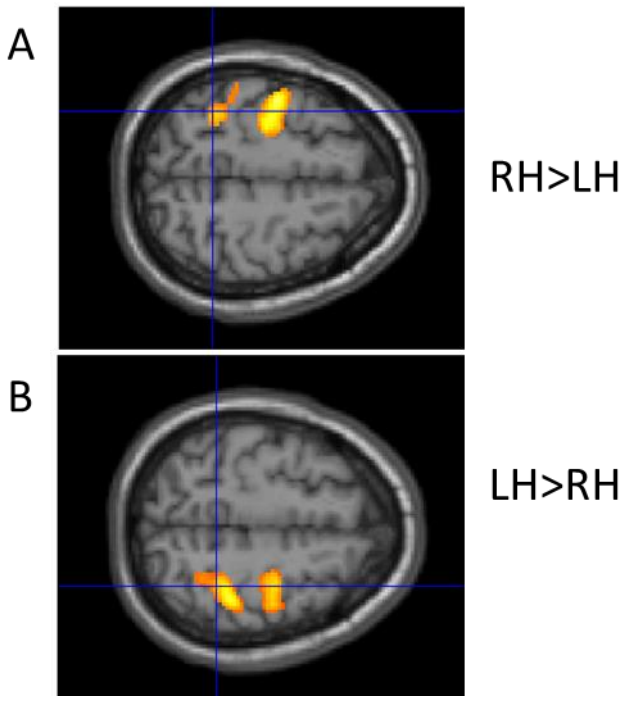


Figure 51. Overlap of findings between current study and that of Shmuelof and Zohary (2006). A) Main effect of right hand observation (p=0.001, uncorrected). B) Main effect of left hand observation (p=0.001, uncorrected). Cross-hair outlines centre of mass coordinate position reported in (Shmuelof and Zohary, 2006) during main effect of (A) right hand observation (x = -35, y = -45, z = 58) and of (B) left hand observation (33, -43, 57).

The Online Brain Atlas Reconciliation Tool (OBART) is very useful in comparing between brain regions that were defined using different digital atlases (Bohland et al., 2009). Using OBART's Multi-Atlas Labelling Tool I obtained a summary of brain regions from many digital atlases that overlap with my ROI representing left S1 (the tool only compares labelling in the left hemisphere). As a result, I found that what Anatomy Toolbox probabilistic histological atlas defines as primary somatosensory region (BA2), Tailarach Daemon gyrus-level atlas and Automatic Anatomical Labelling atlas define as inferior parietal lobule (figure 51). Hence, it is likely that previously published studies describing main effect of observed hand laterality find activation pertaining to the same region as found in this chapter.

Atlas	Region Name	% Contained In	% Contains	Composite
CYTO	Primary somatosensory cortex BA2 L	73.026%	12.131%	0.298
AAL	Parietal_Inf_L	68.421%	4.250%	0.171
TALg	Inferior Parietal Lobule	63.158%	1.474%	0.096
LPBA	L superior parietal gyrus	59.211%	1.543%	0.096
TG	superior parietal lobule, left	27.632%	2.815%	0.088

Figure 52. Summary of brain regions overlapping ROI of left primary somatosensory cortex created for analysis in this chapter. Summary was performed by Multi-Atlas Labeling Tool (part of Online Brain Atlas Reconciliation Tool), http://qnl.bu.edu/obart/label/ (Bohland et al., 2009).

Importantly, there was no difference in the effect of observed hand laterality between patients and healthy participants, suggesting that neural processes do no differ greatly when observed hand is congruent with the impaired hand. To confirm this result further I have tested if patients with an affected left hand processed an observed left as opposed to right hand differently in comparison to patients with an affected right hand. I found no such evidence. Finally, I have tested if impaired global arm/hand function or impaired dexterity influenced magnitude of BOLD response during observation of the affected actions. Again, there was no such relationship in patients with left or right affected hand. Thus, I concluded that the ability to execute observed hand actions is not essential for motor resonance in the motor system of patients. It may be that observed actions are still in the motor repertoire of patients, since although ability in one hand may be compromised, execution is still maintained in the other hand, possibly retaining neural representation of that action. Perhaps an alternative explanation is that patients still attempt to execute actions with their affected hand (most of the patients with greater impairment would also have undergone some physiotherapy) allowing for maintained neural representation of the affected act.

Notably, Garrison and colleagues studied 12 patients with affected right hand and found that activity during observation of pinch actions was modulated by their level of impairment (Garrison et al., 2013). The authors found a negative correlation between impairment and BOLD signal during observation in the left inferior frontal gyrus and right premotor cortex, leading them to conclude that watching actions that were difficult to execute increased activity during action observation. It is unclear why results in the current study were contradictory. A relatively small sample size was used in both experiments and should be increased in the future for better inference.

6.4.2.2 *Affected hand dominance plays an important role in activation of the motor system during observation*

The second key finding was that during action observation activity in the contralateral hemisphere to the affected hand - specifically the superior parietal lobule, inferior parietal lobule spreading into primary somatosensory region, and inferior frontal gyrus - was reduced in patients whose non-dominant hand was impaired after stroke. In contrast, in patients with dominant hand impairment activity in the dominant hemisphere was not altered as compared to those with intact dominant hand (affected non-dominant). The same effect persisted independently of which hand (left or right) was observed. Interestingly, these results match those found in Chapter Five, whereby facilitation of motor evoked potentials in the affected dominant hand was significantly greater than in the affected non-dominant hand while patients watched pinch actions. It appears that patients whose dominant hand is impaired may benefit from treatment using action observation more than those with affected non-dominant hand.

Structurally, IPL and IFG are connected via the third segment of superior longitudinal fasciculus (SLF III), which in humans is strongly lateralized to the right hemisphere (Hecht et al., 2015). It is possible that integrity of the right SLF III is essential for the increased activity during action observation. For instance, it has been shown that non-dominant Middle Cerebral Artery (MCA) stroke results in "severe visual and perceptual deficits" (Harvey et al., 2014), which could impact on the effect from action observation. Notably, in this experiment patients were screened for visual spatial neglect before the experiment. None had a profound deficit. In addition, task accuracy did not differ between patients with dominant or non-dominant hand impairment, suggesting that difference in BOLD response during action observation was not due to visual or perceptual deficits of those with right hemisphere lesions. Nevertheless, while damage to the dominant hemisphere does not result in reduced activity during action observation, damage to the non-dominant hemisphere does, which could lead to less benefit from AOT in those with non-dominant hand impairment.

Such a conclusion would contradict recent findings by Sale and colleagues (Sale et al., 2014). In their longitudinal behavioural study of Action Observation Treatment effects in a group of 67 stroke patients, the authors concluded that only patients with right hemisphere lesion (non-dominant hand impairment) benefited from action observation treatment. At first glance it is unclear why patients with lesser response to observed actions (as found in my study) would benefit more (as found in Sale's study), but is discussed in the next paragraph. My last set of results suggests that although action observation results in greater activity in patients with an affected dominant hand, their

functional improvement may be hindered by their increased everyday use of the spared non-dominant hand.

6.4.2.3 *Use-dependent changes in the non-dominant hand after stroke modulate BOLD response during observation in the fronto-parietal network of the contralateral hemisphere*

The third key result in this chapter was that during action observation activity in the non-dominant hemisphere was greater when patients' dominant hand function was worse and their non-dominant hand dexterity was better. Thus, motor resonance may be increased in the non-dominant hemisphere once patients are motivated to use their non-dominant hand for execution of dexterous skilled actions, usually following severe impairment in their dominant hand.

When the ability to perform essential everyday actions, such as brushing teeth, eating, or writing a note is compromised after stroke, necessary adaptation inevitably takes place. Normally, one of the consequences is that spared non-dominant hand is used more often to assist with such quotidian actions and with time skilfulness in this hand improves.

So called "learned non-use" of the affected hand followed by compensating use of the unaffected hand has been shown to hinder motor recovery and is the basis of constraint-induced movement therapy (CIMT) (Grotta et al., 2004). During CIMT, the intact hand of the patient is immobilized while the affected hand is subjected to physical training. If they have some residual movement to train, it is more likely that used dependent plastic changes in brain reorganisation will be focussed on ipsilesional motor cortex. From my

findings, it appears that action observation results in increased activity in the contralesional hemisphere of patients with dominant hand impairment, which instead of improving motor function of the affected hand may in fact hinder it. Above, I highlighted a study by Sale and colleagues, who discovered that motor function in patients with dominant hand impairment did not improve after Action Observation Treatment (Sale et al., 2014). I propose that increased use of the intact non-dominant hand may be one of the reasons for their negative result.

In conclusion, it must be noted that regions where greater activity is accompanied by better dexterity in the non-dominant hand match regions where activity is reduced in those with non-dominant hand impairment. The fronto-parietal network comprised of IFG and IPL has classically been described as mirror neuron system (MNS) (Rizzolatti and Craighero, 2004). It appears that damage to this system in the non-dominant hemisphere may result in reduced efficacy during action observation in those with non-dominant hand impairment. In addition, action observation may result in increased activation of the MNS in patients with compensating use of their spared non-dominant hand, which may hinder motor recovery. I propose that more careful examination of action observation effects should be carried out in larger groups of patients with dominant and non-dominant impaired hands prior to use of AOT.

CHAPTER 7
PATTERNS OF BOLD ACTIVATION DURING ACTION OBSERVATION ARE MODULATED AFTER STROKE

7.1 INTRODUCTION

A key question addressed in this thesis thus far was whether losing the ability to execute actions (i.e. as a consequence of stroke) reduces the engagement of the motor system during observation of those actions. One of the most common consequences after stroke is hemiparesis, leading to impaired execution of everyday activities with the affected limb. Upper limb impairment is often worse distally than proximally and manual dexterity, as well as the ability to use a pinch grip, are often difficult to restore after severe stroke. Action Observation Treatment (AOT) is proposed to be effective in augmenting rehabilitation of motor function in stroke patients, but it is unclear whether only some or all patients can benefit from the use of AOT equally.

Thus far, I have used TMS and fMRI techniques to explore if the ability to execute observed actions is necessary for the motor system to be activated by observation. In the previous chapter, I found that loss of motor function in patients did not hinder the engagement of the motor system during action observation as measured by the magnitude of fMRI BOLD signal in key brain regions. However, I also found that the dominance of the affected hand before stroke played a role in the magnitude of activity during observation, a finding that was independent of which hand was affected after stroke. I also showed that the dexterity in the non-dominant hand positively correlated with BOLD response during observation of pinch and grasp in the non-dominant hemisphere. I pre-

dicted that the use of non-dominant hand during dexterous movements in patients may alter neural representation of pinch activated by observation of a dexterous action. In the following chapter I expand on these findings by exploring patterns of BOLD activity rather than signal magnitude in response to action observation. I used data collected during the experiment described in Chapter Six to 1) decode observed hand laterality in healthy individuals and in patients and to 2) predict motor function and dexterity in dominant and non-dominant hand of patients from patterns of activity while they watched actions.

Exploring engagement of cortical regions in any given task by contrasting activity between conditions within each voxel is a commonly used method of analyzing functional imaging data sets. The question asked is of the magnitude of activity in the brain while participant is performing a task. We can attribute brain area to the task because of an overall increase in deoxygenation observed there. Although this approach is valid and in many ways preferable, it is also limited in the amount of information it provides. For instance, response to distinct conditions may be a cause of increased BOLD activity in the same region. However, it cannot be construed from such mutual excitation that activity is in fact identical. Separate neuronal populations may code information about two tasks within the same area, leading to a similar spatial average of BOLD response (Mur et al., 2008). A mistaken assumption of non-differential involvement of brain region during distinct mental tasks is thus easily made. Fortunately, there is a way to use neuroimaging data in combination with machine learning algorithms in order to decode 'representational content' of the area of interest.

Each type of observed action (pinch or grasp) or hand (left or right) may be represented differently in the same region. This representation may be decoded from neuroimaging data using a classifier method whereby an algorithm learns something about each representation during training sessions and is then tested on how well it can identify these representations from the new, never seen data. The accuracy score during the testing session is then computed, and if the classifier is capable of reliably decoding cognitive states in the area of interest, one may conclude that each condition in fact contributes to activating distinct neuronal representations in the same region.

The videos in my paradigm are of hand actions that are similar in nature (pinch and grasp performed with left and right hand), thus the likelihood of a comparable increase in response in some areas is high. Although I have shown that observed hand laterality is processed in S1 and PMd - 'working harder' in the contralateral hemisphere to the observed hand – standard general linear model (GLM) analysis may have missed areas that 'work equally hard', but hold distinct neuronal representations for observed left and right hand.

In the previous chapter I used mass-univariate analysis to determine if engagement of the motor system depends on motor ability after stroke. Here, I will describe the use of the multivariate multi-voxel pattern analysis (MVPA) method that I applied to data collected in the previous experiment. MVPA is an approach which uses machine learning methods and bases assumptions about task-specific effects on difference in patterns of activity rather than on magnitude of response within a region of interest (ROI) (Haynes and Rees, 2006; Norman et al., 2006). An additional benefit of MVPA is the use of multivariate pattern regression in order to predict a continuous value, such as residual motor

function or dexterity of patients, taking into consideration the relationship between voxels within the region of interest (pattern of BOLD activity), rather than magnitude of activity in individual voxels.

Thus far, there are no reports of MVPA use in research of action observation effects in patients. Using this sensitive approach in addition to mass-univariate analysis is a way to expand understanding of the relationship between motor ability after stroke and motor system engagement during action observation.

7.2 MATERIALS AND ANALYSIS

Data used in the following analysis was obtained during experiment described in the previous chapter, thus participants and experimental design description are identical to that provided earlier. The different approaches to analysis are outlined below.

7.2.1 Data Analysis

All analysis was implemented using Pattern Recognition for Neuroimaging toolbox (PRoNTo) designed for SPM (Schrouff et al., 2013). In order to predict which hand (left or right) was viewed by the participant, *pattern classification* was performed. For the purpose of predicting motor ability of patients from patterns of activity during action observation, *multivariate pattern regression* was used. Both, classification and regression were conducted using individual images derived using first level mass-univariate analysis (see previous chapter's Data Acquisition and Analysis section for details). Here I outline each MVPA method in detail.

7.2.1.1 *Classification*

In order to classify between conditions, I used the Support Vector Machine (SVM) algorithm in the PRoNTo toolbox. Images with estimated β values computed for each condition were used to train SVM to decode hand laterality in pinch and in grasp videos separately. Thus two types of classification were executed: 1) Left vs Right Pinch and 2) Left vs Right Grasp. Throughout the experiment each variation of action was seen by the volunteers eight times, thus there were 8 images with estimated β values per condition for each subject (160 images per condition for the group of healthy volunteers and 176 images for the patient group). Decoding was only performed in intact ROIs (i.e.

where patients did not have a lesion). In addition, classification between two control conditions (Left vs Right Control) was performed in the group of healthy participants in order to confirm that any significant classification is due to differences in processing observed effectors and not due to other attributes of the stimuli.

Classification in PRoNTo was based on the five step process (Ashburner et al., 2015). Firstly, data and design were specified by inputting β images for every subject. Secondly, features were outlined based on voxels included in classification. Here, each ROI (described in detail in the previous chapter) was considered as a discrete feature. Thirdly, models were specified based on each feature (all voxels from an ROI) and β values for conditions to be decoded. In this way, β images from condition A (e.g., Right Pinch) and from condition B (e.g., Left Pinch) were entered for classification in one of the selected ROIs (e.g., left PMv). Fourthly, data were partitioned into the 'training' and 'testing' sets, by using leave-one-subject out (LOSO) cross-validation approach, which is most common in multi-subject designs (Ashburner et al., 2015). Cross-validation is very important in the routine of decoding ensuring that classifier is tested on previously not encountered data. Data are repartitioned many times, in each instance leaving one subject out for testing and classifier is trained on the rest. Finally, the significance of classification results (i.e. p-value) was obtained through permutation testing (Gaonkar and Davatzikos, 2013; Nichols and Holmes, 2002; Schreiber and Krekelberg, 2013). During this non-parametric procedure the classifier was retrained and retested 1000 times using permuted labels for conditions. The outcome was thus a meaningful p-value for classifier performance.

7.2.1.2 *Multivariate Regression*

Multivariate regression was performed to predict dexterity scores in dominant right hand of patients from patterns of activity during observation. 22 patients were included in the analysis, some suffered stroke to their left hemisphere affecting their contralateral right hand, some – to their right hemisphere leaving their dominant hand intact. In addition, impairment in patients with left hemisphere stroke ranged from mild to severe paresis. In this way a good spread of motor ability was obtained and used for prediction. The dexterity score for each patient was obtained during 9 Hole Peg test, asking participants to position small pegs in the holes of the board in front of them (Mathiowetz et al., 1992). Each score indicated the number of pegs that participant was able to put into holes in one second. In addition, multivariate regression was also performed to predict dexterity in the non-dominant left hand of patients in order to outline possible practice effects after stroke.

The aim of multivariate regression was to predict level of impairment from data collected during observation of pinch and grasp, taking into account relationship between voxels within a region. This approach is different from the mass-univariate regression used in Chapter Six, where relationship between dexterity and level of activity was obtained in each voxel.

Defining a model for multivariate regression was a four step process. Firstly, contrasts from the condition (e.g., Left Pinch) were entered for each patient together with dexterity scores. Secondly, just as in classification, features were selected based on regions that I was interested in. Thirdly, prediction machine for the model was specified. PRoNTo provides three types: Kernel Ridge Regression, Relevance Vector Regression, and Gaussian Process Regression. In order to define the best prediction machine, I ran the

model using all three separately. Best fit was achieved using Relevance Vector Regression, which uses sparse Bayesian learning (Tipping, 2001). Finally, the model was run with 1000 permutations, allowing for conservative significance testing. Pearson's correlation coefficient and Mean Square Error (standard measure of goodness-of-fit for the regression model) were computed to estimate the relationship between actual values (targets) and those predicted by the model. 1000 permutation testing provided reliable confidence estimate. If correlation was significant, the conclusion was that one could reliably predict level of dexterity in a new patient from data of mere movement observation.

7.3.1 Can laterality of the observed hand be decoded from patterns of neuronal activity in healthy subjects?

SVM classifier was able to reliably predict observed hand laterality from patterns of BOLD activity of pinch action. Whether the pinch was performed with left or right hand was decoded accurately in right S1 (59.7%) and left SPL (58.1%) regions (table 7). 1000 permutations verified the result with p=0.005.

CLASSIFICATION ACCURACY (CONTROL GROUP)									
ROI		S1	M1	IFG	PMv	PMd	IPL	SPL	WHOLE BRAIN
Left vs Right Pinch	LEFT hemisphere	38.4	43.8	41.3	43.4	45.6	44.4	**58.1***	80.3
Left vs Right Grasp		36.9	46.6	33.8	41.6	44.7	37.8	36.9	55.6
Left vs Right Control		31.6	46.9	39.1	41.3	47.8	47.2	42.2	45.3
Left vs Right Pinch	RIGHT hemisphere	**59.7***	50.6	33.4	35.3	45.3	46.9	47.5	
Left vs Right Grasp		37.2	47.8	44.1	38.4	40	40	41.3	
Left vs Right Control		41.6	36.3	34.7	33.4	43.4	47.2	43.1	

Table 7. Classification Accuracy percentages in each ROI in healthy participants for decoding between: a) left and right pinch actions, b) left and right grasp actions and c) left and right control actions. Marked in red are classification accuracies significantly higher than chance (p=0.005), as verified by 1000 permutations**.**

7.3.2 Can laterality of the observed hand be decoded from patterns of neuronal activity in chronic stroke patients?

No. Classification between observed left and right hand was at chance in the patient group, irrespective of performed action (table 8).

CLASSIFICATION ACCURACY (PATIENT GROUP)									
ROI		S1	M1	IFG	PMv	PMd	IPL	SPL	WHOLE BRAIN
Left vs Right Pinch	LEFT hemisphere	43.4	44.1	34.1	48.8	44.1	41.3	41.2	65.9
Left vs Right Grasp	LEFT hemisphere	42.5	51.4	45.9	48.8	45.4	39.7	45.5	46.9
Left vs Right Pinch	RIGHT hemisphere	46.9	45.3	37.1	42.3	46.5	50	42.3	
Left vs Right Grasp	RIGHT hemisphere	47.3	53.1	31.3	34.9	46.9	40.2	41.1	

Table 8. Classification Accuracy percentages in each ROI in patients for de-coding between: a) left and right pinch actions, and b) left and right grasp actions. All classification accuracies were at chance level.

7.3.3 Can level of impairment be predicted from patterns of activity during observation of pinch and grasp actions?

The vector machine was accurate at predicting dexterity of patients from patterns of their BOLD activity in right PMv. Dexterity could only be predicted from observation of right hand pinch and grasp. During observation of right hand pinch, correlation (r) and Mean Square Error (MSE) between the predicted and actual dexterity score were 0.73 (*p*-value = 0.005) and 0.03 (*p*-value = 0.005) respectively (figure 53). During observation of right hand grasp correlation (r) and MSE between the predicted and actual dexterity score were 0.68 (*p*-value = 0.005) and 0.04 (*p*-value = 0.005) respectively (figure 54).

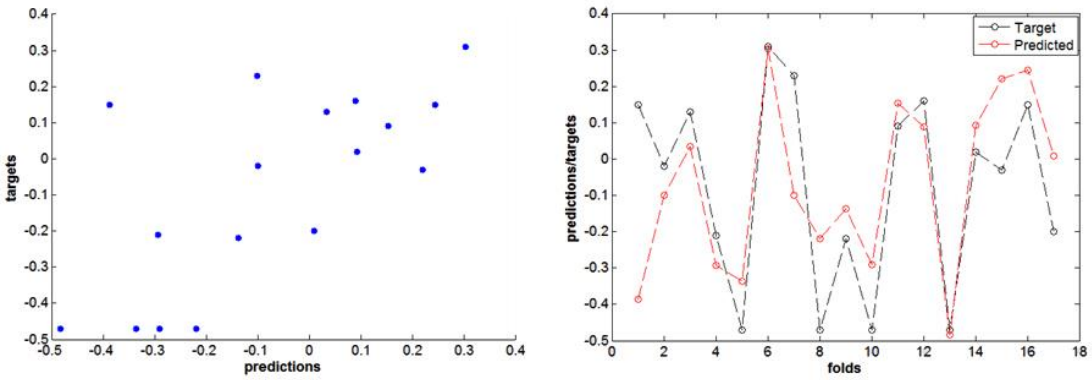


Figure 53. Pattern regression in the right ventral premotor cortex during observation of right hand pinch. Y-axis: targets indicate actual motor function scores; X-axis: predictions mean predicted motor function values given the data. 17 patients were included in this regression.

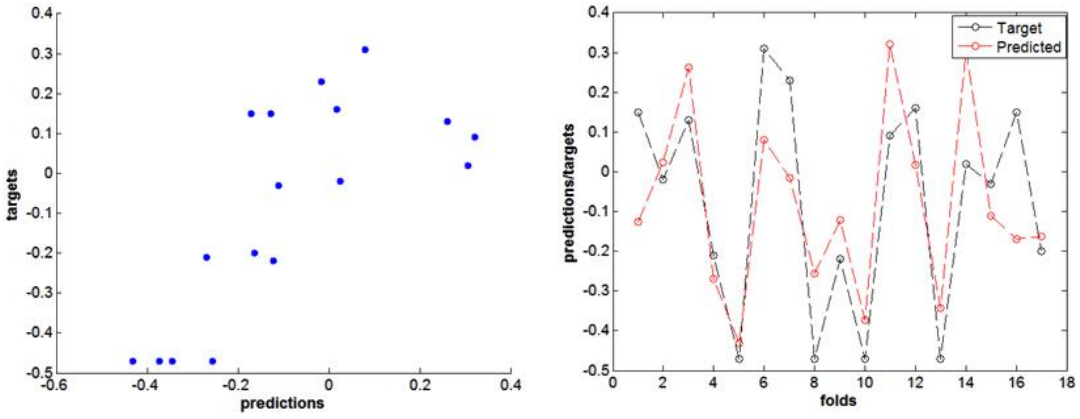


Figure 54. Pattern regression in the right ventral premotor cortex during observation of right hand grasp. Y-axis: targets indicate actual motor function scores; X-axis: predictions mean predicted motor function values given the data. 17 patients were included in this regression.

Finally, in the right S1, non-dominant (left) hand dexterity was accurately predicted from patterns of activity during observation of non-dominant hand grasp. Correlation (r) and MSE between the predicted and actual dexterity score were 0.68 (*p*-value = 0.005) and 0.02 (*p*-value = 0.005) respectively (figure 55).

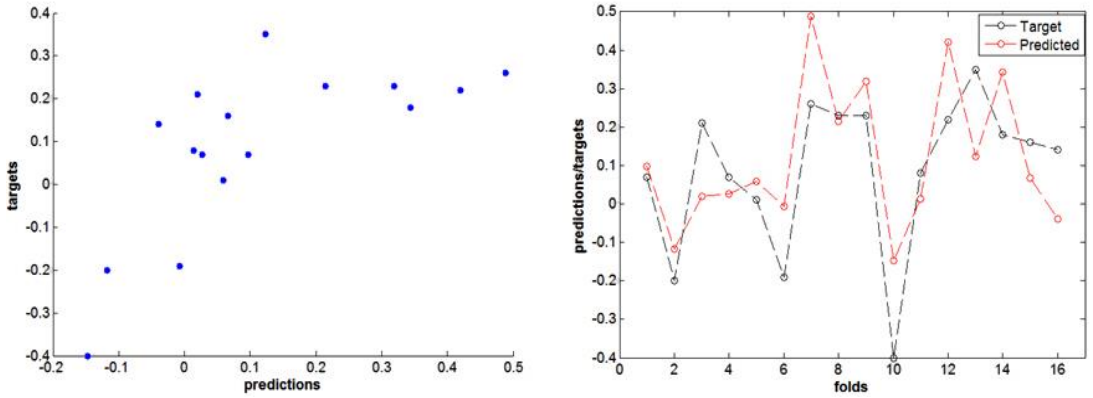


Figure 55. Pattern regression in the right primary somatosensory cortex during observation of left hand grasp. Y-axis: targets indicate actual motor function scores; X-axis: predictions mean predicted motor function values given the data. 16 patients were included in this regression.

7.4 DISCUSSION

7.4.1 Summary of Results

1. In healthy individuals, but not in stroke patients, laterality of the observed hand can be decoded from images obtained during observation of pinch, but not grasp actions.

2. Dexterity in the dominant right hand is reliably predicted from patterns of activity during pinch and grasp observation in the intact right PMv of patients.

3. Dexterity in non-dominant left hand is reliably predicted in the intact right (non-dominant) S1 of patients.

7.4.2 Discussion

In this chapter I have extended the findings reported in the previous chapter by exploring the patterns of BOLD activity during observation in healthy individuals and in stroke patients. I found that in healthy participants, the laterality of observed hand pinch, but not grasp, was encoded by separate neural populations in the left superior parietal lobule and right primary somatosensory region. In each of these regions, two distinct patterns of neural populations were activated during observation of left and right hand pinch. In stroke patients (in whom left SPL and right S1 were spared), it was not possible to distinguish between left and right hand pinch from patterns of activity in these regions. Furthermore, I showed that dexterity in the dominant hand of patients was reliably predicted from patterns of activity during observation in the right ventral premotor area, while dexterity in the non-dominant hand was predicted from patterns of activity in the non-dominant, right S1.

7.4.2.1 *Laterality of the observed pinch, but not grasp can be decoded in healthy individuals*

In Chapter Six I found that watching right hand increased BOLD signal in the left hemisphere dorsal premotor and primary somatosensory areas, and watching left hand increased BOLD response in the same areas in the right hemisphere (figure 42). Such findings indicate that observing each hand results in stronger response in the contralateral hemisphere, however it is unclear whether one neuronal representation is activated to a different degree, or if two distinct neuronal populations are engaged during observation of each hand. In this chapter I show that the answer differs depending on which action (pinch or grasp) is observed. If observed action is grasp, the same neuronal repre-

sentation is likely to be activated, yet to a stronger degree when observed hand is contralateral to the activated hemisphere. If, however, pinch is being observed, two distinct neuronal populations are engaged, also to a stronger degree in the hemisphere contralateral to the observed hand. There may be a fundamental difference in the way observed dominant and non-dominant hands are represented in the sensorimotor system of humans. Hand dominance defines the use of particularly dexterous hand actions, such as writing, sewing, eating, brushing teeth or putting out clothes pegs on the washing line. More crude grasping actions, on the other hand, are well practiced with either hand. For instance, it is easy to grasp a tin with the non-dominant hand, in order to open a lid with the dominant hand. In Chapter Four, I used TMS to measure motor resonance in the pinch and grasp muscles. I found that response in the pinch muscle (FDI), but not in the grasp muscle (ADM) depended on which (left or right) hand was observed. While it is difficult to compare TMS and MVPA results directly, they show that laterality specific engagement of the motor system during observation may be dependent on the observed action.

It is unclear why decoding was not accurate bilaterally in either S1 or SPL. This could be due to specific selection of ROIs chosen for this analysis, however both regions were reported previously to process laterality of observed actions (Shmuelof and Zohary, 2008, 2006, 2005), albeit not in MVPA paradigm.

Only one study so far attempted to decode laterality of observed hand. Ogawa and Inui performed a complicated experiment verifying the role of premotor and parietal regions in processing observed actions, observed hand laterality, perspective, objects and even size of the videos (Ogawa and Inui, 2011). Having so many questions lead to an overly

complex paradigm. Authors only performed classification in four regions: anterior intraparietal sulcus (aIPS) and PMv bilaterally. As control classification, decoding was also performed in white matter and visual cortex ROIs. Ogawa and Inui found no laterality specific classification in regions of interest. S1 and SPL areas were not tested in the described study, thus lack of similar results does not contradict findings in my experiment.

Moreover, observed hand laterality could not be decoded in patients. In order for the algorithm to distinguish between patterns of activity during observation of left and right hand, these patterns need to be consistently similar among subjects. It is likely that in patients neural representations for observed left and right hand pinch were altered by decreased use of their affected hand and adapted use of their unaffected hand. If so, such representations would be specific to each patient and would be impossible to decode in a group. Notably, classification was only performed in patients with spared regions of interest, so patterns of activity within ROIs were not altered by lesions.

7.4.2.2 *Action observation related representation in PMv is modulated by the impairment in the dominant right hand*

Using multivariate regression I aimed to predict each patient's dexterity from patterns of BOLD activity during observation of pinch and grasp actions. Instead of measuring the relationship between motor function and activity during observation in each individual voxel, multivariate regression predicts motor function taking into account the relationship between voxels in any given region of interest.

Very accurate prediction was achieved in the intact right PMv of patients when they were observing right hand performing pinch and grasp. This prediction did not depend

on which action was observed. This finding shows that PMv neurons that were responsive to observed actions were also sensitive to changes in the dominant hand dexterity. A study investigating structural brain changes during short-term motor learning task supports the role of right PMv in experience dependent engagement (Gryga et al., 2012). Five days of 20 minute practicing sequential pinch force task lead to changes in performance and in grey matter volume in healthy volunteers. Structural changes were observed in the left M1, right PMv and right dorsolateral prefrontal cortex (DLPFC). What's more, increase in grey matter volume was observed in those with greater behavioral gains. Importantly, participants with little or no behavioral advancement on the task after 5 days of learning showed either no change or a decrease in grey matter volume. The authors concluded that "practicing a motor skill is not exclusively accompanied by increased GM volume. Instead, bidirectional structural alterations explained the variability of the individual learning success" (Gryga et al., 2012). It is thus likely that diminished use of dominant hand may lead to structural adaptation in the PMv, modifying its engagement during observation of motor acts.

In the previous chapter I showed negative relationship between motor function in the dominant hand and magnitude of BOLD signal in the right S1. There was no indication of changes in activity in the right PMv. It appears that strength of engagement in this region during action observation is independent of structural changes within the region. So far it is unclear why. More research has to be carried out to clarify the relationship between right PMv involvement during observation and level of dominant hand dexterity.

7.4.2.3 *Patterns of activity in the right S1 obtained during action observation can be used to predict dexterity in the non-dominant hand in patients*

In the previous chapter I showed that in the non-dominant hemisphere activity in the primary somatosensory area (S1) spreading into the inferior parietal lobule (IPL), and in the inferior frontal gyrus (IFG) was greater in patients with better dexterity in their non-dominant hand. Here, using multivariate regression, I found that patterns of activity during action observation in the non-dominant S1 were also predictive of non-dominant hand dexterity in patients. This finding suggests that specific neural representations that are activated by action observation depend on dexterity. In contrast, dexterity could not be predicted from patterns of BOLD activity during observation in the right IFG or IPL. Therefore, changes in non-dominant hand dexterity in patients might have led to structural changes in the right S1 (but not IFG or IPL), activating altered neural representations during action observation.

Hand immobilization leads to structural changes and reduction in representation area in the S1 (Langer et al., 2012). When non-dominant hand is impaired, it often remains immobile, as patients shift to primarily using their dominant hand. It could be postulated that in those with worse dexterity in the non-dominant hand, the representation of hand in the right S1 is decreased. In contrast, when dominant hand is impaired, representation of the non-dominant hand in the right S1 expands, since essential everyday tasks are performed increasingly with the non-dominant hand. Learning to perform new skilled action with the non-dominant hand, such as playing a string instrument, is associated with enlargement in cortical digit representation in the right S1 (Elbert et al., 1995).

Why are no changes seen in the left S1? Losing non-dominant hand dexterity may not lead to better use of the dominant hand as everyday actions requiring manual dexterity could still be performed with the dominant hand. Hence, only post-stroke changes in the use of the non-dominant hand are reflected in the non-dominant hemisphere of patients. Further in depth investigations are necessary into the role of hand-dominance and associated functional changes after stroke in the context of action observation and Action Observation Treatment.

CHAPTER 8
GENERAL DISCUSSION

8.1 AIMS OF THE THESIS

The tenet of the Action Observation Treatment (AOT) proposed for post-stroke recovery of motor function rests on the assumption that the motor system during observation of actions is recruited in much the same way as during the execution of the same or similar action (Garrison et al., 2010; Rizzolatti et al., 2009; Small et al., 2013). It is believed that given the visual input the activity within the motor system could be driven, while continuous exposure to the stimuli together with physical training would promote experience induced plasticity and facilitate recovery of motor function. Although it is recognised that motor expertise leads to greater recruitment of the motor system during perception of actions (Calvo-Merino et al., 2005), it is not entirely understood how physical *impairment* effects engagement of the motor system targeted in motor rehabilitation. Several studies have eluded to the relationship between ability to execute actions and activity in the brain or muscles during perception of those action (Aziz-Zadeh et al., 2012; Garrison et al., 2013; Liepert et al., 2014). Nevertheless, results from these studies are inconclusive, with small samples and limited experimental designs. It is imperative to gain insight into how motor system is engaged during action observation, before embarking on AOT for all patients with hemiparesis. Such knowledge may serve to improve and tailor AOT to individuals and predict possible outcomes of training.

The aim of this doctoral work was to test if the ability to execute hand actions after stroke was necessary for the engagement of the motor system during observation of those actions. To this aim, I studied activity in hand muscles and in the cortex during

action observation in healthy participants and in chronic stroke patients. Overall, data from over 120 testing sessions were included in the results part of this thesis.

In the following sections I will briefly summarize results from each of the experiments, I will then lead into general discussion of the most important findings. Finally, I will conclude with remarks on limitations of my studies, general implications for the field of neurorehabilitation and suggestions for the future work.

8.2.1 Chapter 3: Corticospinal excitability during action observation - choosing correct stimuli

In Chapter Three I described the selection and testing of an appropriate experimental paradigm for probing activity within the corticospinal system during action observation. I used transcranial magnetic stimulation (TMS) to measure motor evoked potentials (MEPs) within hand muscles of participants during observation of pinch and grasp actions performed with left or right hand from the egocentric perspective. I aimed to determine whether strong motor resonance can be achieved in two agonist muscles, first dorsal interosseous (FDI) and abductor digiti minimi (ADM). I studied MEP facilitation in these muscles during observation of pinch and grasp relative to baseline and relative to each other. Specifically, I aimed to achieve motor resonance through interaction between Muscle (FDI/ADM) and Observed Action (Pinch/Grasp). While watching pinch induced higher response in the agonist muscle FDI, response was reversed with higher facilitation in the ADM muscle during observation of grasp.

In my first pilot study, pilot experiment A, I found that observation of grasp and pinch elicited differential MEP facilitation in hand muscles. Mean normalised MEP amplitude was significantly greater in the FDI muscle during observation of pinch ($M = 0.96$, $SD = 0.12$), compared to grasp ($M = 0.89$, $SD = 0.1$), $t(9) = -2.71$, $p = 0.024$. Likewise, greater MEPs were obtained in the ADM muscle during observation of grasp ($M = 1.03$, $SD = 0.19$), compared to pinch ($M = 0.89$, $SD = 0.22$); $t(9) = 3.70$, $p=0.005$. This meant that observation of pinch and grasp impacts activity within agonist hand muscles.

However, I also found that there was no facilitation above baseline, which was collected between videos, and thus was a poor measure of 'no activity' during observation.

Furthermore, the control condition of a masking tape moving towards and from the object resulted in facilitation in the ADM muscle. MEP amplitude in that muscle was more enhanced during observation of tape (M = 1.05, SD = 0.25) than that of pinch (M = 0.89, SD = 0.24); t (9) = 3.73, p = 0.006, perhaps suggesting affordability of the masking tape and unsuitability for the experiment.

In the follow up pilot experiment B I have adjusted the paradigm to: 1) include objects that could only afford one action (marble for pinching and ball for grasping), 2) add baseline before the experiment, 3) include biological effector without action as a control condition and 4) collect data from five muscles (two of which, flexor carpi radialis (FCR) and extensor carpi radialis (ECR), were control muscles).

I found significant Recorded Muscle x Observed Action interaction between FDI and ADM, while watching pinch and grasp F (1, 6) = 15.643, p=0.007. This interaction, however, was driven by differential facilitation in the ADM muscle (grasp (M = 1.75, SD = 0.62), pinch (M = 1.1, SD = 0. 27), t (9) = 3.86, p = 0.008), but not in the FDI. Based on literature showing that the weight of an object effects the degree of muscle facilitation during observation (Alaerts et al., 2012, 2010b), I concluded that a marble that I used in my stimuli may have been too light and requiring virtually no force during pinching, which could be reflected in no marked increase in MEP size in the FDI.

Although this was not the result I was expecting, two additional positive outcomes were recorded. Firstly, collecting baseline measurements before the experiment resulted in

marked facilitation of mean MEP size in all muscles during experimental conditions. Secondly, there was no differential facilitation between pinch and grasp observation in the control FCR and ECR muscles, further ascertaining that increase in MEPs during observation is specific to the action being watched at the moment of stimulation.

In the final pilot experiment C, I have filmed pinching and grasping of a variety of objects in order to select best stimuli for the experiment. The most significant difference in both FDI ($t(5) = -5.155$, $p = 0.004$) and ADM ($t(5) = -3.476$, $p = 0.018$) muscles was found between grasping a ball (FDI*:* M=1.24, SD=0.436, and ADM: M=1.78, SD=0.78) and pinching a clothes peg (FDI*:* M=1.68, SD=0.49, and ADM: M=1.12, SD=0.54). Facilitation was above baseline in both muscles during observation of both actions, which also signified that baseline was selected appropriately.

In conclusion, after lengthy exploration of the effects of experimental stimuli on covert activity in hand muscles during observation, I chose the following videos for my paradigm: pinching a clothes peg with 1) left and 2) right hand and grasping a ball with 3) left and 4) right hand. Baseline activity was collected before the experiment and between runs. Finally, I decided to exclude control condition from the paradigm as both muscles could control for each other.

8.2.2 Chapter 4: Engagement of the corticospinal tract during action observation in healthy individuals

In Chapter Four I tested the engagement of intact corticospinal system during action observation in a group of 18 healthy volunteers. Motor evoked potentials were measured on two separate days, once from the left and once from the right hand while participants watched pinching and grasping actions. I aimed to address these questions:

1) *Is there a substantial facilitation of MEP amplitudes during observation as compared to baseline?*

I found that size of normalised MEP amplitudes in both FDI and ADM was increased significantly during experimental conditions as compared to baseline. Overall, there was significant facilitation in MEPs of the right hand muscles during observation of left pinch (FDI - p=0.002; ADM – p=0.001), right pinch (FDI - p=0.001; ADM – p=0.003), left grasp (FDI - p=0.032; ADM – $p < 0.001$), and right grasp (FDI - p=0.021; ADM – $p < 0.001$). Correspondingly, significant facilitation was observed in the left hand muscles during observation of left pinch (FDI - p=0.009; ADM – p=0.012), right pinch (FDI - p=0.017; ADM – p=0.017), left grasp (FDI - p=0.027; ADM – p=0.011), and right grasp (FDI - p=0.044; ADM – p=0.005).

2) *Is there a clear interaction between the Observed Action (Pinch or Grasp) and the Recorded Muscle (FDI or ADM)?*

I established motor resonance during observation through significant interaction between the type of Observed Action (Pinch/Grasp) and the Recorded Muscle (FDI

/ADM), F (1, 17) = 40.916, MSE = 0.005, p<0.01. Response in the two muscles was modulated by observed action.

3) Does muscle specific motor resonance depend on which hand is observed?

The interaction between Observed Action and Recorded Muscle was independent of whether observed hand was congruent or incongruent to the recorded hand, although the largest effect was achieved in the right dominant hand muscles during observation of the same hand actions F (1, 17) = 26.982, MSE = 0.005, p < 0.001.

4) Is the size of response in each hand modulated by the laterality of observed hand? Is there an interaction between Recorded (Left/Right) and Observed (Left/Right) Hand?

Observed hand laterality modulated MEP response in each hand. Mean normalised MEP amplitude in the right hand was higher when right hand was observed. Likewise, facilitation was greater in the left hand when watching left hand. Interestingly, this observed hand-specific modulation was only present in the FDI muscle during observation of pinch action. Specifically, there was significant interaction between the Observed and Recorded hand when recorded in the FDI muscle, F (1, 17) = 14.633, MSE = 0.002, p = 0.001. This effect was not present in the ADM muscle F (1, 17) = .716, MSE = 0.002, p = 0.409.

5) Does age play a role in the size of response to the observed action?

Age appeared to be a factor in the corticospinal excitability during observation of pinch. Participants were split into two groups with the median age of 32 years. There was markedly decreased response in the non-dominant FDI in the older group relative to the

younger group during observation of all four experimental conditions. Significant difference between groups was only observed in left FDI during observation of right grasp ($t(16) = 2.267$, $p = 0.038$), right pinch ($t(16) = 3.768$, $p = 0.002$), left pinch ($t(16) = 2.704$, $p = 0.016$) and observation of left grasp approached significance ($t(16) = 1.878$, $p = 0.079$). In other words, the influence of age on motor resonance (as assessed by the facilitatory effect on MEPs) was driven by reduction in facilitation in non-dominant left FDI muscle of older participants. The lowest response was in the left FDI during observation of incongruent (right) hand actions. Decrease in response of that muscle was also proved by significant negative correlation between age and size of MEP amplitudes in the left FDI during observation of right pinch actions, $r = -0.496$, $n = 18$, $p = 0.036$. Age did not correlate with any other measures.

In conclusion, I have shown that the corticospinal system can be engaged using the designed paradigm. Motor resonance was established in the FDI and ADM muscles, showing greater involvement in agonist muscles of the observed action. Moreover, motor resonance was greater in the hand congruent to the observed hand, but only in the FDI muscle during observation of pinch. Finally, an important novel finding was of the age related decrease in motor resonance in the non-dominant FDI during observation of all experimental conditions.

8.2.3 Chapter 5: Engagement of the corticospinal system during action observation in patients with motor impairment

In Chapter Five I explored motor resonance in the affected hand of 19 chronic stroke patients. During the experiment patients' ipsilesional hemisphere was stimulated while motor evoked potentials were recorded from their affected hand. I addressed following questions:

1) *Is facilitation in FDI and ADM muscles during observation of hand actions dependent on integrity of the corticospinal* system*?*

My results show that on average there was no facilitation in neither of the muscles of patients' affected hand. This was significantly different from recordings obtained in the healthy group, whose facilitation was considerably higher in their dominant (Coef. =0.2761, z=2.63, P>|z| = 0.008) and non-dominant hand (Coef. = 0.14275, z=2.01, P>|z|=0.044) compared to that of the impaired hand of patients.

2) *Is action-specific response in the two hand muscles (motor resonance) affected by damage to the corticospinal* system*?*

Motor resonance was not affected by damage to the corticospinal system. A significant interaction between the type of Observed Action (pinch/grasp) and Recorded Muscle (FDI/ADM) was present when patients watched performing hand that was congruent (Coef. = 0.4251 z = 2.84, P |z| = 0.004) or incongruent (Coef. = 0.305955, z = 2.77, P |z| = 0.006) to their affected hand. Since overall facilitation was on average at baseline level in patients, such result was puzzling. Therefore, I plotted individual motor resonance (figure 31) and discovered that several patients showed increased response as compared

to baseline and good motor resonance. It is likely that MEPs in these patients were driving the interaction, while response from other patients was decreasing the average of overall facilitation. What made some patients 'resonate' more than others? For one, level of impairment did not seem to play a role as there was no correlation between motor function and mirror ratio (computed by dividing normalised MEP amplitudes from FDI by those from ADM for each observed condition).

3) *Does size of response and motor resonance correlate with degree of motor impairment?*

Overall size of response did not correlate with the degree of motor impairment in the recorded hand.

4) *Was size of the MEP amplitude modulated by observed hand (congruent to impaired or unimpaired hand)?*

In Chapter Four I showed that in healthy participants' FDI muscle the size of facilitation was dependent upon the laterality of observed hand pinch. For instance, when recorded from their left hand, MEPs were higher when observed stimuli was of a left hand, and vice versa when recording from their right hand. In patients I found no such difference. Their FDI response in the affected hand was on average not different from baseline and did not depend on whether they watched congruent hand to their affected or unaffected hand.

5) *Did dominance of the impaired hand before stroke affect excitability during observation?*

Importantly, the size of response was influenced by whether recorded affected hand was dominant or non-dominant before stroke. There was a significant difference between MEPs recorded from dominant and from non-dominant hands of patients while they observed congruent to their affected (F (1, 17) = 4.989, *MSE* = 0.4, p=0.039) and unaffected (F (1, 17) = 5.12, *MSE* = 0.243, p =0.037) pinch. No such affect was found in the ADM muscle during observation of grasp. Therefore, dominance of the affected hand may play a crucial role in recovery of motor function using AOT. Dexterity in the dominant hand is shaped through the most extensive practice in life, possibly promoting higher response during observation.

In summary, motor resonance in the affected hand of stroke patients is independent of the ability to execute observed action. Instead, the response in hand muscles is modulated by the dominance of the affected hand before stroke. These novel findings provide basis for more in depth investigation of the role of hand dominance in Action Observation Treatment after stroke.

8.2.4 Chapter 6: Brain activity during action observation is modulated after stroke: an fMRI study

In Chapter Six I conducted a neuroimaging study using the same stimuli as in Chapter Four and Five. With this experiment I aimed to outline cortical engagement during observation in origin regions of the descending corticospinal tract. 20 healthy participants and 22 stroke patients took part. Particularly, I looked to address next questions:

1) Is BOLD response modulated by observed hand laterality in healthy participants?

There was a main effect of observed effector (left or right hand) in the primary somatosensory region (S1) and in the dorsal premotor area (PMd) (family wise error (FWE) corrected at p=0.05). BOLD signal increased in these regions of interest when participants watched actions performed with the contralateral hand. Activity was greater in the left S1 and PMd during observation of right as compared to left hand actions. In contrast, activity was greater in the right S1 and PMd during observation of left as opposed to right hand actions. The same main effect was present in patients as well as in healthy individuals with no significant difference between groups.

2) *Is BOLD response modulated by observed hand laterality in patients?*

To explore if observing hand that was congruent to the affected or unaffected hand of patients lead to differential activity, patients were split into two groups based on injured hemisphere. I then looked for interaction between Group (Left Hand Affected/ Right Hand Affected) and Observed Hand (Left/Right) and found no significant effect. BOLD response during action observation was, therefore, independent of whether observed hand was congruent to the affected or unaffected hand after stroke.

3) *Is engagement of the motor system during observation modulated by the ability to execute observed actions?*

Furthermore, using multiple regression analysis I tested for the relationship between the ability to execute actions and BOLD response during observation of similar actions. In both groups of patients (Left Hand Affected and Right Hand Affected) there was no significant correlation between magnitude of BOLD signal and residual hand motor function or dexterity alone.

4) *Does affected hand dominance before stroke modulate the engagement of the motor system during action observation?*

BOLD response during action observation was modulated by the dominance of the affected hand. Activity was significantly lower in patients whose non-dominant hand was affected after stroke as compared to those with dominant hand impairment in the non-dominant hemisphere. Specifically, there was a significant difference between groups in the primary somatosensory region (S1), superior and inferior parietal lobule (SPL and IPL), and inferior frontal gyrus (IFG). In contrast, there was no reduced activity in patients with dominant affected hand as compared to those with non-dominant hand impairment.

5) *Does motor function in the dominant and non-dominant hand of patients affect BOLD response during action observation?*

Activity in primary somatosensory (S1), inferior parietal (IPL) and inferior frontal (IFG) regions of the non-dominant hemisphere was affected by motor function in both

dominant and non-dominant hands. Greater BOLD activity was associated with worse function of the dominant hand, but better dexterity of the non-dominant hand.

There was significant negative correlation between combined motor function score and dexterity of the dominant hand and BOLD response during observation in the right S1, right IPL and right IFG. Correlation was performed only in patients with intact mentioned regions. Negative correlation between magnitude of BOLD signal and dominant hand motor function as well as dexterity was significant during observation of:

a) Right hand pinch in the right S1- r (16) = -.766, p = .001; right IPL - r (14) = -.654, p = .011; and right IFG - r (15) = -.774, p = .001.

b) Left hand pinch in the right S1- r (16) = -.755, p = .001; right IPL - r (14) = -.741, p = .002.

c) Right hand grasp in the right S1- r (16) = -.874, p = .000; right IPL - r (14) = -.798, p = .001; and right IFG - r (15) = -.689, p = .018.

d) Left hand grasp in the right S1- r (16) = -.857, p = .000; right IPL - r (14) = -.778, p = .001; and right IFG - r (15) = -.677, p = .006.

Conversely, there was a significant positive correlation between dexterity score of the non-dominant hand and BOLD response during observation. Correlation between non-dominant hand dexterity and magnitude of BOLD signal was significant during observation of:

a) Right hand pinch in the right S1- r (16) = .675, p = .004; right IPL - r (14) = .752, p = .002; and right IFG - r (15) = .688, p = .005.

b) Left hand pinch in the right S1- r (16) = .650, p = .006; right IPL - r (14) = .645, p = .004; and right IFG - r (15) = .695, p = .004.

c) Right hand grasp in the right S1- r (16) = .751, p = .001; right IPL - r (14) = .739, p = .003; and right IFG - r (15) = .540, p = .038.

d) Left hand grasp in the right S1- r (16) = .733, p = .001; right IPL - r (14) = .753, p = .002; and right IFG - r (15) = .662, p = .007.

In summary, cortical engagement during action observation was independent of the ability to execute observed action. Watching left and right hand actions increased activity in the contralateral hemisphere to the observed hand in both, healthy individuals and in-stroke patients. Watching congruent hand to the affected one in patients did not result in significantly different activity than watching the same hand as their unaffected one. Moreover, there was no relationship between BOLD magnitude and residual motor function or dexterity in the affected hand. Nevertheless, there was a significant difference in the way observed actions were processed in patients with dominant and non-dominant hand impairment. Specifically, reduced activity in the non-dominant hemisphere was observed in patients with non-dominant as compared to dominant hand impairment, however, no reduced activity was evident in the dominant hemisphere of those with affected dominant hand. Finally, worse use of the dominant hand in patients (independent of which hand was impaired) and better dexterity in their non-dominant hand lead to increased BOLD signal in the non-dominant hemisphere of all 22 patients.

8.2.5 Chapter 7: Patterns of BOLD activation during action observation are modulated by impairment

In Chapter Seven I described using machine learning algorithms in order to analyse patterns of voxel activations during observation. I addressed these questions:

1) *Can observed hand laterality be decoded from patterns of BOLD activity during action observation in healthy individuals and in patients?*

I found that laterality of observed hand could be accurately decoded from patterns of activity in the right S1 and left SPL of healthy volunteers, but not in stroke patients (even when these regions were spared after injury). In healthy participants decoding accuracy was 59.7% in the right S1 and 58.1% in the left SPL. Although these numbers don't seem very high, their above chance significance was verified through 1000 permutations, resulting in *p* value of 0.005.

2) *Can motor function and dexterity in dominant and non-dominant hand of patients be reliably predicted from patterns of activity during action observation?*

Dexterity in the dominant right hand of patients was reliably predicted from patterns in the right ventral premotor region (PMv) during observation of pinch and grasp performed with dominant hand. During observation of right hand pinch, correlation (r) and Mean Square Error (MSE) between the predicted and actual dexterity score were 0.73 (*p*-value = 0.005) and 0.03 (*p*-value = 0.005) respectively. During observation of right hand grasp correlation (r) and MSE between the predicted and actual dexterity score were 0.68 (*p*-value = 0.005) and 0.04 (*p*-value = 0.005) respectively.

Dexterity in the non-dominant left hand of patients was accurately predicted from patterns of activity during observation in the right S1. During observation of non-dominant hand grasp correlation (r) and MSE between the predicted and actual dexterity score were 0.68 (p-value = 0.005) and 0.02 (p-value = 0.005) respectively.

8.3 GENERAL DISCUSSION

The aim of this doctorate was to determine if watching actions that stroke patients can no longer execute still engages the corticospinal system, allowing for Action Observation Treatment to be potentially beneficial in remedying motor impairment. AOT is based on the assumption that watching manual actions engages the motor pathway and thus leads to increased plasticity in the surviving regions of the motor system. There is evidence, however, that the extent to which the motor pathway can be activated depends on whether observed action is in the observer's motor repertoire (Buccino et al., 2004; Calvo-Merino et al., 2006a; Haslinger et al., 2005; Wagner et al., 2011). To date it is not clear if watching an action that has once been in the motor schemata of the patient, but was subsequently eradicated following brain injury, can still engage the corticospinal system. The answer to this question is a necessary prerequisite to the use of AOT for motor recovery.

Uithol and colleagues define interpersonal *motor resonance* as "resonance between observer and executor of action" (Uithol et al., 2011). Watching executer perform an act triggers response in the motor system of an observer. This response, or motor resonance, may be measured using various techniques. For instance, as shown in this thesis, the use of transcranial magnetic stimulation allows to assess covert muscle engagement during observation of actions, whilst functional magnetic resonance imaging measures changes in blood flow in cortical motor areas when participants watch hand actions. Here I have used both techniques to examine if the ability to perform observed actions modulates motor resonance during action observation. Using both TMS and fMRI I have firstly explored if MEPs in hand muscles or BOLD signal in the brain is greater

during action observation than during rest. Higher than rest activity indicates facilitation in the motor system induced by observing actions, i.e. motor resonance. I then tested the relationship between residual motor function in patients and this facilitation. In addition, during experiments using TMS technique I have tested for motor resonance in two ways. Firstly, as described above, I measured response in muscles during action observation relative to rest. Secondly, I have tested if this response was specific to the observed action depending on which muscle it was recorded from. For instance, watching pinch elicited greater response in the FDI muscle (normally used during execution of pinch) than in the ADM muscle (normally used during execution of grasp) and watching grasp resulted in higher activity in the ADM compared to the FDI muscle. This way of testing motor resonance is, in my belief, more accurate measurement of fine tuning between execution and observation within the motor system.

The primary conclusion from the work described in this thesis is that the engagement of the corticospinal system is independent of whether the patient is still able to execute observed action. Watching an action that is difficult to reproduce results in similar motor resonance in patients and in individuals with unimpaired motor ability. I found no evidence to suggest that watching hand congruent to the impaired one results in reduced or increased activity in patients as compared to observation of unimpaired hand or as compared to activity in healthy individuals.

However, several factors appeared to be critical to activating the motor system in patients through observation of manual actions. These factors should be an important consideration prior to recommending AOT as an adjunct to physiotherapy during rehabilitation.

1. The dominance of the impaired hand prior to injury determines whether the muscle will be facilitated or suppressed during observation.
2. In cases where non-dominant hand is affected, age of the patient may determine if corticospinal system can be engaged during observation of dexterous movements.
3. The extent to which the patient uses his/her non-dominant hand after injury (irrespective of which hand was affected) alters the neural representation in the primary somatosensory, inferior parietal and inferior frontal areas of the non-dominant hemisphere, resulting in greater activity in these regions in those with better dexterity.
4. Changes in patient's dominant hand dexterity after injury result in neural reorganization in the ipsilateral ventral premotor area. The same representation is activated during observation and is predictive of the residual motor function of the patient. Nevertheless, the degree of this region's engagement is independent of motor ability.

In the following sections I will discuss these findings in more detail.

8.3.1 Ability to produce observed actions does not alter the engagement of the corticospinal system during observation

The primary finding in this dissertation was one that refuted my initial hypothesis. In the beginning of this work I suggested that in order for the motor system to be engaged through mirror neuron activity, the observed action must be in the motor repertoire of the patient. The assumption was based on previous research showing that in people with no experience of producing an action, for instance barking, the motor system is not engaged during observation of that action (Buccino et al., 2004). Similarly in people with motor expertise of a certain action, such as specific dance moves, watching that action resulted in greater activity in the mirror neuron network and therefore their motor system (Calvo-Merino et al., 2006). On that basis, I predicted that watching actions that the patient could no longer perform would have little effect on their motor system. This did not turn out to be the case.

In order to address my hypothesis I focused on answering two questions:

1. Does watching hand congruent to the impaired hand result in decreased (or increased) corticospinal engagement during observation?
2. Does watching dexterous movement result in altered engagement of the motor system in patients with impaired dexterity?

Findings from both patient experiments using neurophysiological as well as neuroimaging measures showed that motor resonance in response to observed action was independent of the ability to produce that action and was similar to that in healthy individuals.

Firstly, using single pulse TMS I found that - just as in healthy individuals - there was a significant interaction between observed action and recorded agonist muscle in the affected hand of stroke patients. Corticospinal excitability was higher in agonist pinch muscle during observation of pinch and was higher in agonist grasp muscle during observation of grasp. This interaction was also significant in healthy individuals, thus findings suggest that motor resonance is retained in patients and action observation engages damaged motor system in a muscle specific way. Furthermore, this motor resonance was significant irrespective of whether patients' observed hand was congruent to their unimpaired or impaired hand. Therefore, although affected hand may be used less after stroke, watching it still activates motor system.

Notably, the overall excitability during observation was low in patients as compared to healthy adults. But low response did not dependent on neither patient's ability to produce observed action, nor on whether the observed hand was the same as their affected or unaffected hand after stroke. In the following section I will explain the reasons that I believe were driving low engagement in the clinical group.

Secondly, using fMRI, I have established that activity in the sensorimotor system depended on the laterality of observed hand in both healthy individuals and in stroke patients. Observing left as opposed to right hand resulted in increased activation in the contralateral right hemisphere and vice versa, when participants watched right hand - activity was greater in their left hemisphere. Moreover, I explored whether BOLD response was lower when hand congruent to patients' affected as compared to unaffected hand was observed. I found no interaction between the affected hand (left or right) and the observed hand (left or right) and therefore concluded that irrespective of the im-

pairment, patients retain motor resonance during observation. Not being able to use one of their hands did not affect BOLD signal during observation of that hand. Thus, the ability to execute observed action appears to be not necessary for the motor system to be engaged.

Next, I tested for the relationship between dexterity in either impaired left or impaired right hand and BOLD signal during observation of pinch. Production of pinch requires intact dexterity and observation of pinch facilitates excitability in the agonist pinch muscle. I aimed to examine if intact dexterity was necessary for the motor system to be engaged during observation of pinch. No significant relationship was present in patients with either left or right affected hand, once again indicating that the ability to execute observed actions is not related to the degree of motor system engagement during observation of those actions.

In conclusion, it appears that even though patients may lose ability to execute a certain action after stroke, watching that action sill engages their motor system. It is possible that although patient is no longer executing a particular action, they see it being performed with their spared hand and they attempt to execute it with their affected hand, thus the act is not entirely erased from the motor repertoire of the patient. It is likely that mere visual exposure to others executing an action can result in mirror neuron network activation. For instance, Aziz-Zadeh and colleagues found that congenital amputee, although unable to perform (from birth) actions she was instructed to observe, still exhibited activity in the mirror neuron system (Aziz-Zadeh et al., 2012). The authors postulated that life-long visual exposure to the observed movements had resulted in observational learning and thus increased activity.

Other research confirms that visual expertise modulates motor response during observation. One study showed that when healthy individuals watched dystonic handwriting (writer's cramp) their MEP amplitudes revealed significant muscle specific facilitation compared to observing healthy handwriting (Fiorio et al., 2010). This finding was attributed to similar results of heightened response during erroneous/ unexpected stimuli (Aglioti et al., 2008; Candidi et al., 2014). However, health professionals who were used to seeing dystonic movement in their everyday practice showed no difference in response between watching healthy and impaired handwriting (Fiorio et al., 2010). Although the dystonic action was not in the motor repertoire of practitioners, their motor system nevertheless was influenced by the prolonged visual exposure. Perhaps, even though particular action is no longer executed by a stroke patient, it is still in the motor repertoire, which is continuously activated through quotidian visual exposure to that action, either through performance of their own unaffected hand or that of others.

In contrast, Garrison and colleagues propose that ability to execute observed motor acts impacts on BOLD response in the mirror neuron network during observation (Garrison et al., 2013). They tested 12 stroke patients with dominant (right) hand impairment and showed that observation of hand that was congruent to their paretic hand resulted in greater response than observation of the intact hand in the ipsilesional left hemisphere. In addition, further correlational analysis showed that activity was greater in the dominant inferior frontal gyrus (BA44 and BA45), as well as in the non-dominant premotor cortex (BA6) in patients with greater impairment, suggesting that activity increased when observed actions were difficult to perform. Results from present experiments contradict those of Garrison and colleagues which may be due to methodological

differences. Firstly, patients recruited for experiments in this thesis had damage to either left or right hemispheres, and as I will describe next, there is a significant difference in the way mirror neuron network is engaged depending on dominance of the affected hand. Secondly, in Garrison's paradigm only actions that were "difficult or impossible to perform using the paretic limb" were presented to patients. Although authors conclude that negative correlation between motor scores of the paretic hand and activity in the mirror neuron regions signifies greater activity during observation of difficult actions, they did not test if the same correlation would persist during observation of easy to perform actions. In this thesis, I performed correlation between motor function and BOLD magnitude and found no significant relationship between the two during observation of paretic pinch. In contrast, I found that independent of which hand is affected after stroke, dexterity in the non-dominant hand correlates with BOLD signal during observation of both grasp and pinch performed with left or right hand. In my belief, such results are related to use-dependent structural changes in the motor system after stroke and not to observation of specific action.

In summary, findings in this thesis indicate that the motor system of patients is engaged during action observation independent of the ability to execute observed action.

8.3.2 Dominance of the impaired hand prior to injury determines motor resonance during observation

Although watching pinch could still engage the motor system of the patient who lost dexterity in his impaired hand, the degree to which pinch muscle can be facilitated during observation depends on the pre-stroke dominance of the affected hand. It appears that injury to the dominant hemisphere can in fact be to a patient's advantage. Importantly, observation treatment in patients with affected non-dominant hand may lead to suppression of corticospinal engagement. There is evidence that such asymmetry in activation induced during observation of actions is also present during execution. What's more, it is modulated by age.

While recording from the impaired hand of 19 patients I found that overall excitability in muscles was not much higher when they watched motor acts than when they watched a red cross. This was somewhat puzzling as action specific facilitation was still significant in the group. That meant that watching pinch elicited higher response in the pinch muscle than in the grasp muscle and vice versa when grasp was observed. Thus, motor resonance was still present in patients, but was not higher than baseline. These findings were not explained by motor ability, age or time since stroke. What appeared to make a difference was pre-stroke dominance of the impaired hand. After splitting patients in two groups, I found that facilitation during observation of pinch was significantly higher (and above baseline) in patients whose dominant hand was affected by stroke than in those with non-dominant impairment. Moreover, it seemed that in patients with damage to their non-dominant hemisphere corticospinal excitability may be suppressed (below

baseline) during observation, nearing significance when patients were watching dominant pinch.

Following these results I performed additional analysis of the fMRI data collected while patients watched pinch actions. I compared BOLD response in patients with affected dominant hand to that in patients with non-dominant impairment. I found that action observation resulted in reduced activation in the non-dominant hemisphere in those whose non-dominant hand was affected. In contrast, activity was not altered by hand preference in the dominant hemisphere. To further these findings, decreased activity in the non-dominant hemisphere was associated with the reduced use of the non-dominant hand after stroke.

Therefore, pre-stroke hand dominance affects the way the corticospinal system is engaged during observation and may be used to predict outcomes of AOT. In my study, those with impaired non-dominant hand showed reduced activity in their non-dominant hemisphere and decreased excitability in their affected muscle during observation. Those with impaired dominant hand showed no reduction in activity during observation and exhibited facilitation in their affected muscle during observation. Such asymmetry in activation during observation is likely reflective of the use dependent adaptation before and after stroke. Preference of one hand to perform skilled actions, such as writing, eating or brushing teeth is associated with structural changes in the motor system (Amunts et al., 1996; Volkmann et al., 1998). Such anatomical disposition thus may be advantageous after damage. Harris and Eng, for instance, studied ninety-tree left- and right-handed chronic stroke patients and found that level of impairment was modulated by hand dominance before stroke. Patients with affected non-dominant hand showed

greater impairment than those with affected dominant hand, irrespective of which hemisphere was affected (Harris and Eng, 2006). Authors suggested that "the propensity to use the dominant hand may lead to a better pre-stroke neuromuscular condition of the dominant hand (e.g., stronger muscles, more efficient motor unit recruitment) compared to the non-dominant hand" (Harris and Eng, 2006).

Perhaps one more possible explanation of reduced facilitation in non-dominant muscle during observation is interhemispheric inhibition (IHI). Voluntary movement of muscle in one hand results in motor pathway excitability to a homologous muscle on the opposite side of the body (Stinear et al., 2001; Woldag et al., 2004). It is believed that such facilitation is caused by neural pathways in the spinal cord (Muellbacher et al., 2000) and is also accompanied by an increase in interhemispheric inhibition in order to prevent simultaneous movement of homologous muscles (Ferbert et al., 1992; Kobayashi et al., 2003). The extent of IHI appears to be asymmetric, being greater in the non-dominant hand during dominant hand movement (Lewis and Perreault, 2007). It is plausible that by increasing corticospinal excitability, watching of hand movements contributes to this transcollosal inhibition, suppressing movement in the non-dominant hand to a greater extent.

8.3.2.1 *The relationship between age and non-dominant hand dexterity*

It is possible that not everyone with non-dominant hand impairment is disadvantaged. Participants recruited for above studies were mostly older as generally an older population (but not exclusively) suffers stroke. However, one of the most important findings in this thesis was that motor resonance in the non-dominant hand of healthy subjects also decreased with age.

I found that during action observation facilitation in the first dorsal interosseous (FDI) muscle was significantly lower in older healthy participants compared to that in younger ones. Such decrease in motor resonance was present during all observation conditions, but was largest when participants watched pinch performed with the dominant hand ($p = 0.002$). Moreover, the relationship between age and facilitation during dominant pinch observation was evidenced by the significant negative correlation in 18 healthy subjects ($p = 0.036$). While there are no reported studies exploring the effect of age on observation related excitability in the non-dominant FDI muscle, some supporting evidence can be found from literature concerned with dexterous execution.

Several experiments examined the relationship between manual dexterity and age. For instance, one recent study reported strong correlation between increased age and reduced dexterity in a sample of 107 adults (Martin et al., 2015). Nevertheless, much less is known about the impact of age on dominant and non-dominant hand dexterity. Sale and Semmler investigated age-related differences in the FDI muscle during left and right hand index finger abduction, a pinch, a grasp, and a scissor grip (Sale and Semmler, 2005). They found that activity during execution of left, but not right hand pinch decreased with age. The area of motor evoked potentials in the left (non-dominant) hand was significantly (30%) lower in old compared to young participants. Authors proposed that "differences in corticospinal control in the left and right hands of older adults may reflect neural adaptations that occurs throughout lifetime of preferential hand use for skilled (dominant) and unskilled (non-dominant) motor tasks" (Sale and Semmler, 2005).

The present findings closely match those of Sale and Semmler and point to the same corticospinal mechanisms engaged during pinch execution as well as observation. These results also show that if non-dominant hand is impaired following injury, the age may be a significant predictor of AOT success.

To conclude, in this work I was able to demonstrate for the first time that engagement of the corticospinal system during observation is greater in patients with damage to the dominant hemisphere and that this asymmetry may furthermore be modulated by age. Additional research with larger sample sizes will be necessary to determine the extent of the effect and its contribution to outcomes of Action Observation Treatment. Thus far, I hypothesise that usefulness of AOT will be dependent on the affected hand dominance.

8.3.3 Altered use of the non-dominant hand after stroke leads to changes in neural organization and activity in the primary somatosensory, inferior parietal and inferior frontal regions

When the dominant hand is severely affected after stroke patients may be driven to use their spared non-dominant hand for skilled manual acts like writing. Such enforced switch to utilize their non-dominant hand in a new way may contribute to altered neural representation in the sensorimotor system. In contrast, when the non-dominant hand is impaired after stroke, patients are less motivated to use their spared dominant hand more than before stroke. After all, writing or eating was already performed with their unaffected hand before damage and it is unlikely their habits for skilled unimanual actions would have changed dramatically. In this work I found that increased utilisation of the spared non-dominant hand resulted in plastic changes seen through increased activity in the sensorimotor system during action observation. I propose that use-dependent plasticity after stroke leads to alterations in the system seen during action observation and may be influential for the Action Observation Treatment success in a given patient.

I found that motor function in the non-dominant left hand of a patient can be reliably predicted from patterns of activity in the contralateral S1 during observation of pinch or grasp. Structural changes in the non-dominant S1 have been previously related with acquisition of new skilled ability, such as playing a string instrument (Elbert et al., 1995). Elbert and colleagues reported a significant enlargement in cortical representation of the left hand digits in the contralateral primary somatosensory cortex of string players. Violinists, for instance, use their non-dominant hand in a way that requires great manual dexterity. The amount of representation, reported by Elbert, correlated with years of

practice. Authors concluded that "representation of different parts of the body in the primary somatosensory cortex of humans depends on use and changes to conform to the current needs and experiences of the individual" (Elbert et al., 1995). Present study supports Elbert's findings and shows for the first time that use-dependent neural reorganization may also be accurately decoded from activity in S1 during action observation.

In addition, I showed that BOLD response in the non-dominant S1, inferior parietal lobule (IPL) and inferior frontal gyrus (IFG) during observation significantly correlated with measure of dexterity in the non-dominant hand of a patient. Correlation was performed in all patients, independent of which hand was impaired, which allowed for a wide range of motor ability. I found activity during observation increased with better non-dominant hand dexterity, suggesting that better use of the hand results in better motor resonance. Those with impaired non-dominant hand showed greater decrease in response, which is consistent with my TMS findings of below baseline corticospinal excitability in those with non-dominant impairment. Conversely, those with impaired dominant hand not only showed greater activity in the non-dominant hemisphere, but that this activity was associated with better (adapted) use of their non-dominant hand.

Just as primary motor cortex, primary somatosensory cortex is organized somatotopically with a proportion of the area devoted to afferent information received from hand receptors (Penfield and Boldrey, 1937). Inability to produce actions requiring dexterity, such as through 2-week immobilization of hand, results in shrinking of hand representation in both contralateral S1 and M1 areas (Langer et al., 2012). In contrast, increased use of hand increases contralateral somatosensory representation (Elbert et al., 1995, but

not Langer et al., 2012). Reduced BOLD activity in the S1 has also been documented as a result of dexterity deficit in patients with Parkinson's disease (Foki et al., 2015).

Part of S1, specifically Brodmann area 2, is also known to be activated when one watches a hand touching someone or something (Blakemore, 2005; Ebisch et al., 2008; Schaefer et al., 2009), possibly allowing the observer to infer haptic consequences of observed actions (Keysers et al., 2010). Correlation between dexterity in stroke patients and BOLD magnitude during action observation may thus indicate that watching another's actions activates altered neural hand representation resulting in greater response in patients with better use of the hand. It is unclear if better dexterity would also allow patients to better infer the feeling of the observed touch. Nevertheless, these findings once again point to a tightly calibrated relationship between observation and execution of manual actions.

While the relationship between right S1 and dexterity of the contralateral hand can be explained through structural changes in the region due to increased use of non-dominant hand, the role of right inferior frontal gyrus and inferior parietal lobule is less apparent. Both, IPL and IFG are known hubs of mirror neuron network and their involvement during action observation has been extensively documented (for reviews see Rizzolatti and Craighero, 2004; Rizzolatti and Fogassi, 2014). Yet the relationship between motor ability and the engagement of these regions during observation has been less often addressed.

A recent study by Bello and colleagues reported no relationship between right hand dexterity in healthy participants and activity in the left IPL during action observation (Bello et al., 2014). This is in contrast to my findings, although differences in design may ac-

count for conflicting outcomes. Firstly, while thirty-one healthy participants were used in Bello's study, the spread in their dexterity score may not have been sufficient to result in significant relationship in mirror neuron network. Here, I used a wide range of motor scores obtained from 22 stroke patients, which may have benefited the analysis. Secondly, the lack of relationship in S1 may have been due to the nature of stimuli used in Bello's design. S1 is thought to be activated only when observed hand is delivering a touch (Blakemore, 2005; Schaefer et al., 2009) yet videos in aforementioned study were comprised of intransitive actions and showed pinch devoid of an object.

Also, Garrison and colleagues found negative relationship between motor score in the impaired dominant right hand of 12 patients and activity in the left, but not right IFG during action observation (Garrison et al., 2013). They did not specifically test relationship between BOLD magnitude and non-dominant hand dexterity.

Structurally, IPL and IFG are tightly interconnected through the third segment of superior longitudinal fasciculus (SLF III). It has been suggested that SLF III "provides the ventral premotor region and adjacent Brodmann area 44 with higher order somatosensory input" (Makris et al., 2005). Injury to rostral IPL and underlying white matter results in patient's inability to imitate or mimic correct hand gesture – ideomotor apraxia (Makris et al., 2005). Recently, it has also been shown that in humans SLF III is strongly lateralised to the right hemisphere with frontal terminations mostly in the inferior frontal gyrus, rather than PMv, as previously thought (Hecht et al., 2015). Importantly, IPL receives projections from the S1 allowing for integration of visual, auditory and somatosensory information (Maunsell and van Essen, 1983; Pons and Kaas, 1986; Rozzi et al., 2008). It is thus plausible that better dexterity in the non-dominant hand results

in greater activity in structurally expanded S1, which in turn effects activity in IPL and IFG of the same hemisphere during observation. It has been postulated that during action observation S1 is activated precisely through these bidirectional connections with the IPL (Keysers et al., 2010). Therefore, reciprocal exchange of information between S1 and IPL, and between IPL and IFG may have resulted in altered activity in all three areas. Further research exploring connectivity between these regions is necessary to precisely establish the role of their relationship during action observation.

It is important to remember that S1 is densely interconnected with primary motor cortex (M1), so is IFG with ventral premotor region and hence also with M1 (figure 57). Findings from present experiment provide evidence that with greater use of the upper limb after stroke, the activity during observation may be facilitated in the sensorimotor system, in turn promoting plasticity and improving motor recovery.

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Figure 56. Connectivity between inferior parietal area (PF, PFG, VIP), inferior frontal gyrus (IFG), ventral premotor region (PMv), primary somatosensory region (BA1, 2, 3a and 3b) and primary motor cortex (M1). Adapted from Keysers et al., (2010). Modified to include inferior frontal gyrus (IFG).

Critically, although dense connectivity between S1, IPL and IFG and primary and secondary motor areas indicates plausible effect of AOT on motor outcome, it is unclear

whether increased activity in the contralesional hemisphere is beneficial to those with affected dominant hand. It has been proposed that the engagement of contralesional motor system may contribute to motor recovery in some, but not in all stroke patients and that exact influence of contralesional activity is still debated (Grefkes and Ward, 2013). Ideally, future longitudinal studies would explore the effect of action observation on cortical activity in both hemispheres in patients with dominant and non-dominant hand impairment.

8.3.4 Changes in dominant hand dexterity after injury result in neural reorganization in the ipsilateral ventral premotor area: evidence from activity during action observation

Using multivariate pattern regression, I found that dexterity in the dominant hand of a patient can be accurately predicted from patterns of BOLD activity in the ipsilateral (right) ventral premotor area. It appears that although watching dominant hand pinch or grasp results in the magnitude of activity that is independent of motor ability, the neural representations that are activated through observation are shaped by physical use.

Use-dependent structural changes have been reported in the right PMv, specifically, after a 20 minute practice of sequential pinch performed with the right hand over a five-day period (Gryga et al., 2012). In addition to changes in grey matter volume in contralateral M1, greater density was also found in the right PMv and right dorsolateral prefrontal cortex and was strongly associated with functional gains over the period of practice. Those with better behavioural scores at the end of the 5 days showed greater structural changes. It can be implied, therefore, that neural representations responsible for action execution in the right PMv are altered with increased or decreased use of the dominant hand. The same representations, or part of them, are then engaged during observation of matching hand.

It is still unclear why greater PMv volume in those with better dexterity did not also result in increased BOLD activity during observation, thus further investigation is still necessary to expand on these findings.

8.4 IMPLICATIONS FOR THE FIELD OF NEUROREHABILITATION

Injury to the descending corticospinal tract is a common consequence after stroke leading to hemiparesis - weakness of the hand contralateral to the damaged hemisphere (Kelly-Hayes et al., 1998). The degree of impairment and the rate of recovery depends on the residual integrity of the corticospinal system (Schulz et al., 2012; Stinear et al., 2014, 2006). Recovery can be boosted through the use of physical training, whereby engaging corticospinal system through exercise promotes neural plasticity allowing for the restoration of hand function (Milliken et al., 2013). Another way of enhancing plasticity through the corticospinal system engagement is through action observation, as watching others execute actions activates mirror neurons of the motor system (Rizzolatti et al., 2009). Combining action execution with action observation has been shown to be advantageous in motor rehabilitation (Buccino, 2014; Small et al., 2013). However, there is a lack of evidence that all patients with hemiparesis would benefit equally from addition of Action Observation Treatment (AOT) to their motor training. For example, one study showed that only patients with damage to their right hemisphere benefited from AOT, yet no reasons for such asymmetry were apparent (Sale et al., 2014).

Previously, motor resonance was shown to be dependent on the motor repertoire of the observer. Actions, such as barking, for instance, do not activate the motor system of a human (Buccino et al., 2004). It is plausible that after stroke, activation of the motor system through action observation depends on whether watched action is still in the motor repertoire of the patient. The original aim of this thesis was to establish if lost ability to execute hand actions affected the engagement of the motor system during action observation. If activity in the motor system during observation was indeed dependent on

whether observed action can be executed by a patient, it would be reasonable to assume that those with lesser impairment would benefit more from the AOT and those with greater impairment may not gain much. If, on another hand, the ability to execute observed action was not necessary for the engagement of the motor system, all patients may benefit equally, increasing validity of the treatment. In the work presented in this thesis I show that neither of these outcomes reflected the reality.

While lost ability to execute hand actions did not alter the activity in the motor system during observation of those actions, the engagement of the system depended on two things: 1) the dominance of the affected hand before stroke and 2) the degree of adapted use of the non-dominant hand when dominant hand was impaired.

Firstly, activity in the affected hand muscles was significantly lower if non-dominant hand was affected after stroke. Moreover, activity in the non-dominant hemisphere was reduced in these patients compared to those with dominant hand impairment. These findings suggest that patients with non-dominant hand impairment may benefit less from the AOT. In fact, activity in their affected hand may be suppressed during action observation, which calls for more experiments to properly understand the mechanism.

Secondly, it is unclear if those with dominant affected hand would benefit equally either. The advantage of using AOT may be dependent upon their adapted use of the non-dominant hand after stroke. Greater use of the non-dominant hand in everyday skilled actions in these patients leads to an increased activity in the non-dominant hemisphere, which may not be a desired consequence of treatment. In fact, it may be cancelling out the use of motor treatment as it would be enhancing contralesional rather than ipsilesional activity.

In the beginning I mentioned a study that concluded only patients with right hemisphere stroke (i.e. non-dominant hand impairment) benefited from the use of AOT during motor rehabilitation (Sale et al., 2014). Specifically, AOT resulted in dexterity improvement only in the non-dominant affected hand of patients. It appears possible that although patients with non-dominant hand impairment may be worse to begin with, their outcomes may be improved with combined motor rehabilitation and AOT. On the other hand, patients with dominant hand impairment may have learned to rely on their non-dominant hand for skilled action execution and it is this adaptation that may lead to activity in the non-dominant contralesional hemisphere during action observation resulting in hindered plasticity in the ipsilesional hemisphere and poorer functional outcomes.

Overall, it is still unclear whether using AOT is advantageous to patients with motor impairment, but it is now apparent that more in depth investigation needs to be carried out in order to ascertain that such novel treatment is not harmful. The effects of AOT may be dependent on the age of the patient, on the dominance of their affected hand, and on the adapted use of their unaffected hand. All of these factors should be taken into account when designing clinical trials or research studies that explore action observation treatment.

For instance, it would be important to establish if there is a relationship between the effectiveness of AOT and the adapted use of the unaffected hand after stroke. In this thesis use-dependent plasticity after stroke was not directly studied, however results point to possible relationship between the engagement of the motor system during action observation and motor function of the unaffected hand. The validity of these results may be revealed through the use of longitudinal AOT studies that measure motor func-

tion of both affected and unaffected hand from sub-acute to chronic stages after stroke. I hypothesize that increased use of the unaffected hand after stroke may lead to worse AOT outcomes.

Moreover, as shown in this work, motor resonance in the non-dominant FDI muscle is modulated by observer's age. Such effect was not present in the dominant hand or in the non-dominant ADM muscle. Stroke patients tend to be older, therefore, lack of facilitation during action observation in their non-dominant hand may not be related to damage in their motor system, but to their age and level of their dexterity before stroke. It is advisable to keep this result in mind when using TMS to assess benefits of AOT in rehabilitation.

Finally, in research presented in this thesis I show that watching hand actions results in greater excitability of the motor system in patients with dominant as opposed to non-dominant hand impairment. While majority of clinical trials investigating AOT only study treatment related changes in the affected hand (independent of its dominance) (Ertelt et al., 2007; Franceschini et al., 2012, 2010; Sugg et al., 2015), only one study distinguished between outcomes of patients with dominant from those with non-dominant hand impairment (Sale et al., 2014). I propose that in the future it is important to recruit patients with both dominant and non-dominant affected hand and make sure that the fact is reflected in the analysis. It is likely that the outcome of AOT is dependent on the affected hand dominance.

8.5 LIMITATIONS

Whilst conducting experiments described in this thesis, I have faced several important limitations.

1. Groups of healthy participants were not perfectly age matched with groups of patients in both TMS and fMRI experiments. Healthy participants were tested before patients were recruited to ensure that the paradigm was yielding necessary results. While all care was taken to recruit healthy individuals with age spread resembling that of potential patients, mean age within the healthy group was lower than in the patient group. In Chapter Four I showed that age played a role in the corticospinal system engagement during action observation, therefore better matched groups are necessary for future studies.
2. Pre-stroke dominance of the affected hand appeared to be an important factor in the engagement of the motor system during action observation. Although the patient group that I studied was substantial in sample size (22 patients), once broken down into groups according to hand dominance, the size in each group was small (10 vs 12 patients in each group). To further explore the effect of impaired hand dominance on the engagement of the motor system during action observation, the sample sizes in both groups must be increased significantly.
3. I used Anatomy Toolbox probabilistic histological atlas (Eickhoff et al., 2005) to define regions of interest for my fMRI analysis. However, it appears that what Anatomy Toolbox defines as one area, other digital atlases, such as Tailarach Daemon, may define as something else. Differences in labelling anatomy of activations leads to misconceptions of difference in results from other publications and further

misinterpretations. I used Online Brain Atlas Reconciliation Tool (OBART) to outline possible inconsistencies (Bohland et al., 2009). Nevertheless, results presented in this thesis were labelled using the same Anatomy Toolbox probabilistic histological atlas and anatomy was not verified manually.

4. Patient lesions were identified using Automated Lesion Identification (ALI) toolbox developed for SPM (Seghier et al., 2008), whereby grey and white matter segmented images of patients were compared to those of healthy controls. Since control group was on average younger, some structural differences outlined using this automatic approach were likely to be age related. Therefore, some structures that were not lesioned in patients were identified as lesioned and were not included in subsequent analysis. This could have resulted in smaller sample size of patients in any given region of interest, which could have affected the analysis.
5. The extent of damage appears to be different in patients with dominant and non-dominant hand impairment. On average there seems to be more damage to the right hemisphere than to the left hemisphere. Although sample size in each group is comparable (12 vs 10), difference in the extent of damage could have contributed to difference in overall engagement during action observation. Future studies with greater sample sizes in each group are essential.

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APPENDICES

Appendix A

Patients included in TMS experiment described in Chapter 4: lesion site, demographics and motor function scores (expressed as percent of unaffected hand)

ID	Impaired Hand	Lesioned Hemisphere	Lesion Site	Age	Gender	Months Since Stroke	9HPT (%unaff)	Box & Block (% unaff)	Grip (%unaff)	Pinch (%unaff)	PC1 (%unaff)
Patient 1	Left	Right	MCA (whole)	70	M	25	87.7	88.5	72.2	80.4	0.10
Patient 2	Left	Right	MCA (whole)	53	M	99	65.4	77.9	60.3	101.9	0.04
Patient 3	Left	Right	MCA (whole)	51	M	5	73.9	94.0	73.2	79.7	0.09
Patient 4	Right	no scan		45	M	78	105.7	79.0	89.1	107.9	0.19
Patient 5	Left	Left	Cerebellar	57	M	40	32.5	54.6	71.5	81.6	-0.09
Patient 6	Right	rtical White Matter		54	M	114	44.4	66.1	85.9	77.7	-0.02
Patient 7	Left	Right	MCA (whole)	67	M	92	26.7	52.3	29.1	0.0	-0.33
Patient 8	Left		Subcortical White Matter	64	F	4	51.2	81.9	50.3	93.7	-0.01
Patient 9	Right	Left	MCA (whole)	53	M	47	41.3	70.8	83.3	0.0	-0.14
Patient 10	Right	Left	MCA (whole)	45	M	55	0.0	16.5	32.8	0.0	-0.47
Patient 11	Left	Right	Striatocapsular	40	M	27	0.0	12.8	34.4	0.0	-0.47
Patient 12	Left	Right	MCA (whole)	49	M	15	74.2	93.1	78.4	71.2	0.08
Patient 13	Left	Right	MCA (whole)	49	M	8	110.2	87.8	92.4	107.0	0.23
Patient 14	Right	rtical White Matter		39	F	207	68.0	81.3	122.4	120.4	0.21
Patient 15	Right	Left	Striatocapsular	55	M	14	79.0	47.8	19.1	18.2	-0.22
Patient 16	Right	rtical White Matter		70	F	89	115.3	95.1	131.2	124.1	0.37
Patient 17	Left	Right	MCA (anterior)	70	M	58	83.0	86.5	101.8	88.4	0.16
Patient 18	Right	Left	MCA (whole)	46	M	5	52.1	56.1	107.2	63.1	-0.01
Patient 19	Right	rtical White Matter		50	F	50	82.0	119.4	83.4	93.7	0.21
Patient 20	Left	Right	MCS (whole)	25	F	26	69.1	100.3	85.8	56.2	0.08

Appendix B

Patients included in fMRI experiment described in Chapter 6 and Chapter 7: lesion site, demographics and motor function scores (including first principle component score)

ID	Impaired Hand	Lesioned Hemisphere	Lesion Site	Age	Gender	Months Since Stroke	Left 9HPT	Left Box & Blocks	Left Grip	Left Key Pinch	Left ARAT	Left PC1	Right 9HPT	Right Box & Blocks	Right Grip	Right Key Pinch	Right ARAT	Right PC1
Patient 1	Left	Right	MCA (whole)	51	M	7	0.47	45	57.67	13.33	57	0.183	0.62	49	72.17	15.67	57	0.225
Patient 2	Left	Right	MCA (whole)	69	F	167	0	0	0	0	0	-0.361	0.54	35	28.87	6.00	57	0.018
Patient 3	Left	Right	MCA (whole)	62	F	121	0	0	0	0	0	-0.361	0.45	51	53.60	10.33	57	0.119
Patient 4	Left	Right	MCA (whole)	61	M	444	0	0	0	0	0	-0.361	0.45	20	53.16	12.33	57	0.043
Patient 5	Left	Left	Cerebellar	57	M	40	0.20	24	40.73	11.33	57	0.037	0.60	45	52.20	13.00	57	0.158
Patient 6	Right	Subcortical White Matter		54	M	115	0.61	48	56.83	12.33	57	0.207	0.26	32	42.36	8.67	57	-0.011
Patient 7	Left	Right	MCA (whole)	67	M	96	0.17	23	9.00	0	57	-0.134	0.62	45	28.33	5.66	57	0.062
Patient 8	Right	Subcortical White Matter		49	M	54	0.47	41	92.63	6.00	57	0.149	0	1	0	0	0	-0.469
Patient 9	Left	Subcortical White Matter		64	F	4	0.41	48	26.13	3	57	0.024	0.78	60	47.56	3	57	0.142
Patient 10	Left	Right	MCA (posterior)	53	M	11	0	11	4.07	0	44	-0.231	0.73	49	0	4.3	57	0.050
Patient 11	Left	Subcortical White Matter		56	F	18	0.21	23	37.10	5.33	57	-0.030	0.70	60	48.13	11.67	57	0.208
Patient 12	Right	Left	MCA (whole)	51	F	204	0.66	68	45.13	3.33	36	0.101	0	0	0	0	10	-0.442
Patient 13	Right	Left	MCA (whole)	53	M	48	0.63	49	104.20	7	57	0.227	0.25	35	75.30	0	57	-0.045
Patient 14	Right	Left	MCA (whole)	45	M	56	0.63	60	98.30	5.33	57	0.224	0	10	28.00	0	49	-0.260
Patient 15	Left	Right	Striatocapsular	40	M	27	0	4	45.30	0	16	-0.250	0.56	32	120.80	6.00	57	0.130
Patient 16	Left	Right	MCA (whole)	49	M	19	0.48	50	105.90	6.33	57	0.193	0.63	55	123.70	8.33	57	0.241
Patient 17	Left	Right	MCA (whole)	47	M	20	0	0	9.53	1.67	0	-0.330	0.80	62	93.20	6.66	57	0.246
Patient 18	Right	Subcortical White Matter		71	M	135	0.62	62	35.57	2.33	57	0.104	0	4	10.07	0	0	-0.447
Patient 19	Right	Subcortical White Matter		39	F	210	0.75	67	55.33	3.67	57	0.184	0.49	55	58.77	4.00	57	0.086
Patient 20	Right	Left	Striatocapsular	55	M	14	0.58	85	77.40	4.00	57	0.222	0.44	41	12.86	0.66	57	-0.058
Patient 21	Right	Subcortical White Matter		70	F	91	0.56	50	38.87	2.67	57	0.075	0.62	48	44.27	3.00	57	0.066
Patient 22	Right	Left	MCA (whole)	46	M	6	0.54	60	34.47	7.00	57	0.130	0.27	34	32.07	4.00	57	-0.061

Appendix C

Patients included in TMS experiment described in Chapter 4: written, spoken, and auditory comprehension scores, and test of visual spatial neglect

Patient	CAT - Language Comprehension (max 30)	CAT - Written Word Comprehension (max 30)	CAT- Spoken Sentence Comprehension (max 32)	CAT- Written Sentence Comprehension (max 32)	CAT- Paragraph Comprehension (max 4)	Auditory Verbal Comprehension (max 20)	Mesulem's symbol cancellation (max 60)
Patient 1	29	30	32	32		20	60
Patient 2	30	30	31	32		20	60
Patient 3	30	29		26	4	20	60
Patient 4	30	29		32	4	20	58
Patient 5	30		32	32	4	20	58
Patient 6	28	29	31	31	4	20	58
Patient 7	29	30	32	32		20	57
Patient 8	28	30	32	31	4	20	56
Patient 9	30	30	30	32	4	20	58
Patient 10	30	30	30	30	2	20	56
Patient 11	30	30	32	32	4	20	59
Patient 12	29	30	31	28	4	19	60
Patient 13	29	29	31	31	4	20	60
Patient 14	30	30	31	32	4	20	60
Patient 15	28	28	30	30	4	20	59
Patient 16	29	29	32	31	4	20	60
Patient 17	29	29	28	30	4	20	56
Patient 18	30	30	31	31	4	20	60
Patient 19	30	29	32	32	4	20	60
Patient 20	30	30	32	30	4	20	59

Appendix D

Patients included in fMRI experiment described in Chapter 6 and Chapter 7: written, spoken, and auditory comprehension scores, and test of visual spatial neglect

Patient	CAT - Language Comprehension (max 30)	CAT - Written Word Comprehension (max 30)	CAT- Spoken Sentence Comprehension (max 32)	CAT- Written Sentence Comprehension (max 32)	CAT- Paragraph Comprehension (max 4)	Auditory Verbal Comprehension (max 20)	Mesulem's symbol cancellation (max 60)
Patient 1	30	29		26	4	20	60
Patient 2							
Patient 3				32	4	20	54
Patient 4	29	31	29	31	4	20	52
Patient 5	30	30	32	32	4	20	58
Patient 6	28	29	31	31	4	20	58
Patient 7	29	30	32	32		20	57
Patient 8	30	30	30	32	4	20	58
Patient 9	28	30	32	31	4	20	56
Patient 10	30	29	32	30	4	20	56
Patient 11	29	29	30	31	4	20	60
Patient 12	28	26	26	26	3	20	39
Patient 13	30	30	30	32	4	20	58
Patient 14	30	30	30	30	2	20	56
Patient 15	30	30	32	32	4	20	59
Patient 16	29	30	31	28	4	19	60
Patient 17	27	30	30	30	4	20	59
Patient 18	30	30	32	29	4	20	57
Patient 19	30	30	31	32	4	20	60
Patient 20	28	28	30	30	4	20	59
Patient 21	29	29	32	31	4	20	60
Patient 22	30	30	31	31	4	20	60

Appendix E

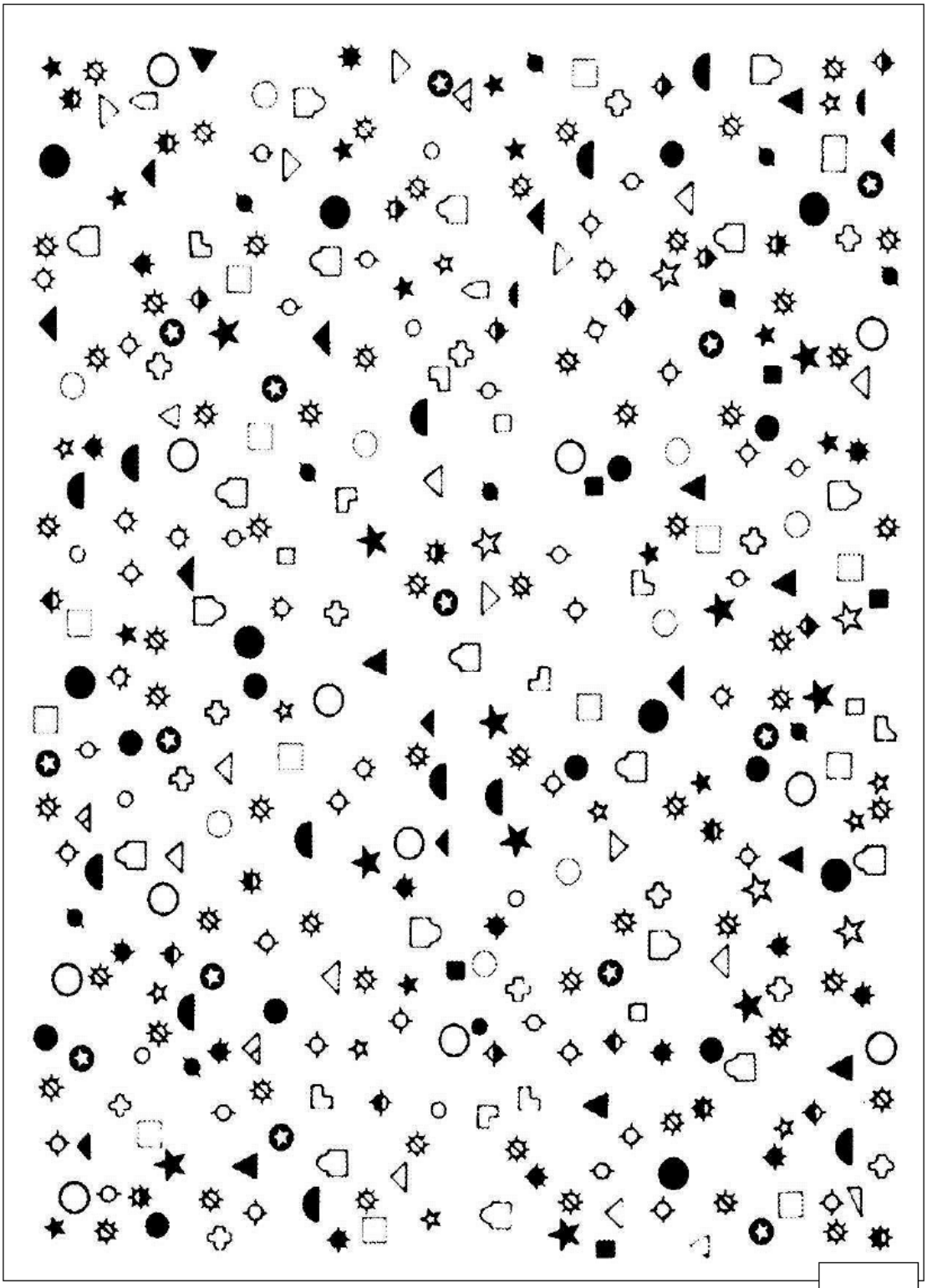
Examples of tasks used to measure patients' comprehension and to establish absence of visual spatial neglect.

AUDITORY VERBAL COMPREHENSION

Answers should be yes or no, establishing first whether the response will be verbal or gestural (including eyeblink). Avoid nodding or commenting on specific items. Self corrections by the patient are counted. If response is ambiguous then repeat the instructions and question. If response still ambiguous score 0. Score 3 for each correct answer.

1. Is your name Smith? (no should be correct) ☐
2. Is your name Brown? (no should be correct) ☐
3. Is your name _____? ☐
4. Do you live in Toronto? (no should be correct) ☐
5. Do you live in _____? ☐
6. Do you live in Windsor? (no should be correct) ☐
7. Are you a man/woman? (yes should be correct) ☐
8. Are you a doctor? (no should be correct) ☐
9. Am I a man? (yes should be correct) ☐
10. Are the lights on in this room? (lights are on) ☐
11. Is the door closed? (door is closed) ☐
12. Is this a hotel? ☐
13. Is this a hospital? (yes should be correct) ☐
14. Are you wearing red pyjamas? (no should be correct) ☐
15. Will paper burn in fire? ☐
16. Does March come before June? ☐
17. Do you eat a banana before you eat it? ☐
18. Does it snow in July? ☐
19. Is a horse larger than a dog? ☐
20. Do you cut the grass with an axe? ☐

Patient score ☐



Scoring system:

0 = incorrect
1 = correct after repetition of stimulus (R), self-correction (Sc) or significant delay (over 5 seconds) (D)
2 = correct with no assistance or delay

PART 1: LANGUAGE COMPREHENSION

7. COMPREHENSION OF SPOKEN WORDS (pp. 38–53 C&L)

I'm going to say a word.
I want you to point to the picture that goes best with that word.
Can you find me "mouse"? That's fine ... there is always a picture that is close but not quite right [point to "mouse" and "rabbit"] *... it's there to catch you out, so be careful.*

TIME for 5 seconds. DISCONTINUE if the person makes four consecutive failures.

	TARGET	MANNER OF RESPONSE	SCORE	PHONOLOGICAL DISTRACTOR	NO. OF FEATURES DIFFERENT & POSITION	SEMANTIC DISTRACTOR	UNRELATED DISTRACTOR
P	*mouse*			*house*	*3df I*	*rabbit*	*church*
1	ship	D Sc R	0 1 2	lip	2df I	boat	nose
2	goat	D Sc R	0 1 2	coat	1df I	sheep	dress
3	horn	D Sc R	0 1 2	horse	2df F	trumpet	cow
4	boat	D Sc R	0 1 2	bone	2df F	canoe	heart
5	dart	D Sc R	0 1 2	cart	2df I	arrow	horse
6	knee	D Sc R	0 1 2	bee	2df I	elbow	spider
7	pear	D Sc R	0 1 2	bear	1df I	apple	lion
8	tyre	D Sc R	0 1 2	fire	2df I	wheel	matches
9	leek	D Sc R	0 1 2	leaf	2df F	carrot	flowers
10	kettle	D Sc R	0 1 2	kennel	2df M	teapot	cage
11	hen	D Sc R	0 1 2	head	1df F	cockerel	foot
12	roof	D Sc R	0 1 2	hoof	2df I	chimney	tail
13	pine	D Sc R	0 1 2	pipe	3df F	palm tree	cigarette
14	bull	D Sc R	0 1 2	wool	1df I	cow	cotton
15	door	D Sc R	0 1 2	saw	2df I	window	hammer
			(/15) /30	/15		/15	/15

8. COMPREHENSION OF WRITTEN WORDS (pp. 55–70 C&L)

This is the same idea, only this time the word is written down for you there, I don't say anything. So which picture goes with that word [point to the word "rocket"]*?*

TIME for 5 seconds. DISCONTINUE if the person makes four consecutive failures.

	TARGET	MANNER OF RESPONSE	SCORE	PHONOLOGICAL DISTRACTOR	NO. OF FEATURES DIFFERENT & POSITION	SEMANTIC DISTRACTOR	UNRELATED DISTRACTOR
P	*rocket*			*packet*	*3df I*	*aeroplane*	*sleeve*
1	mug	D Sc	0 1 2	rug	2df I	cup	carpet
2	pin	D Sc	0 1 2	bin	1df I	needle	basket
3	cap	D Sc	0 1 2	cat	1df F	hat	dog
4	nail	D Sc	0 1 2	tail	2df I	screw	leg
5	wall	D Sc	0 1 2	ball	1df I	fence	toys
6	boots	D Sc	0 1 2	roots	2df I	shoes	branches
7	pen	D Sc	0 1 2	peg	2df F	pencil	safety pin
8	rose	D Sc	0 1 2	rope	3df F	tulip	chain
9	bag	D Sc	0 1 2	bat	2df F	case	owl
10	boy	D Sc	0 1 2	toy	2df I	girl	dice
11	pig	D Sc	0 1 2	wig	2df I	cow	hat
12	lock	D Sc	0 1 2	log	1df I	key	tree
13	sun	D Sc	0 1 2	nun	2df I	moon	mayor
14	book	D Sc	0 1 2	hook	3df I	newspaper	noose
15	grass	D Sc	0 1 2	glass	1df M	flowers	bottle
			(/15) /30	/15		/15	/15

9. COMPREHENSION OF SPOKEN SENTENCES (pp. 72–88 C&L)

This is similar—only this time I say a ***sentence*** *not a word. So you have to point to the picture that goes best with this sentence ... ready?*

TIME for 5 seconds from the moment of saying the target sentence.
DISCONTINUE if the person makes four consecutive failures.

	SENTENCE TYPE†	TARGET SENTENCE	SELECTION	RESPONSE	SCORE
P	NP VP (1)	**A The woman is sitting** B The woman is *standing* C The *man* is *standing* D The *man* is sitting	**A** B C D	D Sc R	0 1 2
1	NP VP (1)	**B The woman is drinking** A The woman is *eating* C The *man* is drinking D The *man* is *eating*	A **B** C D	D Sc R	0 1 2
2	NP VP (1)	**C The man is walking** A The *woman* is *standing* B The *woman* is walking D The man is *standing*	A B **C** D	D Sc R	0 1 2
3	NP VP (1)	**C She is laughing** A *He* is laughing B She is *crying* D *He* is *crying*	A B **C** D	D Sc R	0 1 2
4	NP VP NP (2) I/A	**D The man is eating the apple** A The *woman* is eating the apple B The *woman* is eating an *ice-cream* C The man is eating an *ice-cream*	A B C **D**	D Sc R	0 1 2
5	NP VP NP (2) I/A	**B The woman is painting a wall** A The woman is painting a *picture* C The *man* is painting a *picture* D The *man* is painting a wall	A **B** C D	D Sc R	0 1 2
6	NP VP PP (2) I	**A The dog is sitting on the table** B The dog is sitting *under* the table C The *boy* is sitting on the table D The *boy* is sitting *under* the table	**A** B C D	D Sc R	0 1 2
7	NP VP NP (2) R	**B The apple is under the shoe** A The *pen* is under the *paper* C The *shoe* is under the *apple* D The *paper* is under the *pen*	A **B** C D	D Sc R	0 1 2

	SENTENCE TYPE	TARGET SENTENCE	SELECTION	RESPONSE	SCORE
8	NP VP NP (2) R/A	**B The nurse shoots the butcher** A The *butcher* shoots the *nurse* C The *butcher chases* the *nurse* D The nurse *chases* the butcher	A **B** C D	D Sc R	0 1 2
9	NP VP NP (2) R/A	**C The singer hits the soldier** A The singer *photographs* the soldier B The *soldier photographs* the *singer* D The *soldier* hits the *singer*	A B **C** D	D Sc R	0 1 2
10	NP VP PP (2) R/P	**A The policeman is painted by the dancer** B The *dancer* is *chased* by the *policeman* C The *dancer* is painted by the *policeman* D The policeman is *chased* by the dancer	**A** B C D	D Sc R	0 1 2
11	NP VP PP (2) R/P	**D The butcher is chased by the nurse** A The *nurse* is *killed* by the *butcher* B The butcher is *killed* by the nurse C The *nurse* is chased by the *butcher*	A B C **D**	D Sc R	0 1 2
12	NP VP NP (2) R/A	**A The dancer paints the policeman** B The *policeman chases* the *dancer* C The *policeman* paints the *dancer* D The dancer *chases* the policeman	**A** B C D	D Sc R	0 1 2
13	NP (*PP) VP NP (2) R/E	**D The shoe under the pencil is blue** A (*shoe-under-pencil-blue*)** B The shoe *on* the pencil is blue C The *pencil* under the *shoe* is blue	A B C **D**	D Sc R	0 1 2
14	NP (*clause) VP NP (2) E	**A The carpet the cat is on is red** B The *red cat* is on the carpet C/D Irrelevant	**A** B C D	D Sc R	0 1 2
15	NP VP PP (2) R	**C The red pencil is under the shoe** A The red pencil is *on* the shoe B The *green* pencil is under the shoe D The red *shoe* is under the *pencil*	A B **C** D	D Sc R	0 1 2
16	NP (*PP) VP NP	**D The flower in the cup is blue** A The flower *under* the cup is blue B The flower *under* the cup is *green* C (*flower-in-cup-blue*)**	A B C **D**	D Sc R	0 1 2
	TOTAL			(/16)	/32

†Key: A = active sentence; P = passive sentence; R = reversible sentence; I = irreversible sentence; E = embedded sentence; ( ) = number of predicates; * = post-modifying; ** = using word order alone to comprehend this sentence

10. COMPREHENSION OF WRITTEN SENTENCES (pp. 90–106 C&L)

This time the sentences are written for you here—I don't say anything. When you're ready, point to the picture that goes with that sentence [point to the written sentence].

TIME for 5 seconds from when you've read the sentence to yourself.
DISCONTINUE if the person makes four consecutive failures.

	SENTENCE TYPE†	TARGET SENTENCE	SELECTION	RESPONSE	SCORE
P	NP VP (1)	**D The man is sitting** A The *woman* is sitting B The *woman* is *standing* C The man is *standing*	A B C **D**	D Sc	0 1 2
1	NP VP (1)	**C The man is drinking** A The *woman* is *eating* B The *woman* is drinking D The man is *eating*	A B **C** D	D Sc	0 1 2
2	NP VP (1)	**B The woman is walking** A The woman is *standing* C The *man* is walking D The *man* is *standing*	A **B** C D	D Sc	0 1 2
3	NP VP (1)	**D He is crying** A He is *laughing* B *She* is crying C *She* is *laughing*	A B C **D**	D Sc	0 1 2
4	NP VP NP (2) I/A	**B The woman is eating an ice-cream** A The woman is eating an *apple* C The *man* is eating an ice-cream D The *man* is eating an *apple*	A **B** C D	D Sc	0 1 2
5	NP VP NP (2) I/A	**C The man is painting a picture** A The *woman* is painting a picture B The *woman* is painting a *wall* D The man is painting a *wall*	A B **C** D	D Sc	0 1 2
6	NP VP PP (2) I	**D The boy is sitting under the table** A The *dog* is sitting *on* the table B The *dog* is sitting under the table C The boy is sitting *on* the table	A B C **D**	D Sc	0 1 2
7	NP VP NP (2) R	**A The pen is under the paper** B The *apple* is under the *shoe* C The *shoe* is under the *apple* D The *paper* is under the *pen*	**A** B C D	D Sc	0 1 2

	SENTENCE TYPE	TARGET SENTENCE	SELECTION	RESPONSE	SCORE
8	NP VP NP (2) R/A	**A The butcher shoots the nurse** B The *nurse* shoots the *butcher* C The butcher *chases* the nurse D The *nurse chases* the *butcher*	**A** B C D	D Sc	0 1 2
9	NP VP NP (2) R/A	**D The soldier hits the singer** A The *singer photographs* the *soldier* B The soldier *photographs* the singer C The *singer* hits the *soldier*	A B C **D**	D Sc	0 1 2
10	NP VP PP (2) R/P	**C The dancer is painted by the policeman** A The *policeman* is painted by the *dancer* B The dancer is *chased* by the policeman D The *policeman* is *chased* by the *dancer*	A B **C** D	D Sc	0 1 2
11	NP VP PP (2) R/P	**C The nurse is chased by the butcher** A The nurse is *killed* by the butcher B The *butcher* is *killed* by the *nurse* D The *butcher* is chased by the *nurse*	A B **C** D	D Sc	0 1 2
12	NP VP NP (2) R/A	**C The policeman paints the dancer** A The *dancer* paints the *policeman* B The policeman *chases* the dancer D The *dancer chases* the *policeman*	A B **C** D	D Sc	0 1 2
13	NP (*PP) VP NP (2) R/E	**D The shoe under the pencil is red** A (*shoe-under-pencil-red*)** B The *pencil* under the *shoe* is red C The shoe *on* the pencil is red	A B C **D**	D Sc	0 1 2
14	NP (*clause) VP NP (2) R/E	**B The carpet the cat is on is green** C The *green cat* is on the carpet A/D Irrelevant	A **B** C D	D Sc	0 1 2
15	NP VP PP (2) R	**A The blue shoe is under the pencil** B The blue *pencil* is under the *shoe* C The blue shoe is *on* the pencil D The *red* shoe is under the pencil	**A** B C D	D Sc	0 1 2
16	NP (*PP) VP NP R	**B The flower under the ~~cup is red~~** A (*flower-under-cup-red*)** C The flower *in* the cup is red D The *cup* under the *flower* is red	A **B** C D	D Sc	0 1 2
TOTAL					**/32**

† Key: A = active sentence; P = passive sentence; R = reversible sentence; I = irreversible sentence; E = embedded sentence; ( ) = number of predicates; * = post-modifying; ** = using word order alone to comprehend this sentence

11. COMPREHENSION OF SPOKEN PARAGRAPHS (p. 108 C&L)

I'm going to read you a short story. I want you to listen and then answer some questions. You should only say yes or no [point to yes/no on p. 108 C&L]. *Are you ready?*

Sally and Richard had been on the train for over three hours. They were tired and fed up. The train was already 45 minutes late, the buffet had closed so there was no food and the lady opposite was snoring.

No Practice.

a.	Were Sally and Richard travelling by car?	Yes/**No**	
b.	Were they on time?	Yes/**No**	
a.	Were they travelling by train?	**Yes**/No	
b.	Were they early?	Yes/**No**	**/2**

OK—here's another one. Are you ready?

The explosion in central London caused havoc. Initially terrorists were suspected but it turned out not to be a bomb. The cause was found to be a burst gas main that ignited when someone had thrown down a lighted cigarette. People three miles away heard the explosion and the damage is estimated at over a million pounds.

a.	Was the explosion in Leicester?	Yes/**No**	
b.	Was it caused by a bomb?	Yes/**No**	
a.	Was it in London?	**Yes**/No	
b.	Was the explosion caused by a gas main?	**Yes**/No	**/2**

TOTAL CORRECT **/4**

Appendix F

Motor Function Testing Instruction Sheet

Action Research Arm Test

(Yozbatiran et al, 2008)

There are 4 subscales. (grasp, grip, pinch, gross movement)

The tests in each are ordered so that if subject scores **3 on the first test**, no more tests need to be administered in that subscale, and the subject automatically scores top marks (all 3s) for all tests in that subscale.

If subject **fails the first test** (score 0) and **fails the second test** (score 0) of the subscale, the subject **automatically scores zero for all tests in that subscale**, and again no more tests needed to be performed in that subscale; and (3) otherwise the subject needs to complete all tasks within the subtest

Score:

3 = subject performed the test normally within 5 seconds;

2 = subject could complete the test but took abnormally long (5 to 60 seconds) or had great difficulty (wrong hand movement, wrong arm posture, wrong body posture);

1 = subject could only partially perform the test within 60 seconds (must initiate some form of arm movement that achieves holding or lifting the object);

0 = subject could not perform any part of the test within 60 seconds.

Instructions:

- Use the terms “grasp,”
- Set up according to diagram (paper by Yozbatiran et al, 2008)
- Perform each subscale for each hand (L then R) before moving to next subscale

- Test non-affected hand first
- See paper for guide on correct and incorrect performance
- Can do demonstration of action if needed
- Is allowed to practice to ensure they understand
- Time limit is 60 secs

9 Hole Peg Test

- Allow 15 sec practise for each hand
- Perform 3 trials per hand
- Dish side faces the hand that is being tested
- Start clock when subject picks up 1st peg
- Stop clock when subject releases last peg
- Subject only allowed to pick up 1 peg at a time
- If pegs fall out of the dish, pick up and replace if it doesn't distract the subject
- Restarts are allowed
- Allow 60 seconds to attempt completion

Box and Block Test

- Blocks start by the hand being tested
- Blocks must be moved one at a time
- Hand/fingers must cross the midline
- Count blocks as subject is performing, do not count incorrect trials i.e. if hand doesn't cross the midline or 2 at a time
- Allow 60 sec
- Test each hand once

Pinch and Grasp Dynamometer

- Make sure subject is only using thumb and tip of forefinger (pinch pulp)
- Pinch key – as if holding a key to unlock a door – only thumb and 1st finger, not 3rd.
- Pinch pulp – as if picking up a marble – only thumb and 1st finger, not 3rd
- Rest the dial on the table (pinch)

- Reset the needle
- Best/average of 3 trials

Apraxia Test

- Instructions on scoring sheet
- Demonstrate Imitation behaviours
- Do not demonstrate pantomime behaviours

Motor Function Testing Score Sheet

Action Research Arm Test – (Yozbatiran et al, 2008)		Score (0-3)	
Number	Item	Left	Right
	Grasp Subscale		
1	Block 10cm^3		
2	Block 2.5cm^3		
3	Block 5cm^3		
4	Block 7.5cm^3		
5	Cricket ball		
6	Sharpening stone		
	Subtotal (/18)		
	Grip Subscale		
7	Water Pour		
8	Move 2.25cm tube		
9	Move 1cm tube		
10	Washer over bolt		
	Subtotal (/12)		
	Pinch Subscale		
11	Ball bearing, ring finger and thumb		
12	Marble, index finger and thumb		
13	Ball bearing, middle finger and thumb		
14	Ball bearing, index finger and thumb		
15	Marble, ring finger and thumb		
16	Marble, middle finger and thumb		
	Subtotal (/18)		
	Gross Movement Subscale		
17	Hand to behind head		
18	Hand to top head		
19	Hand to mouth		
	Subtotal (/9)		
	Total (/57)		

9 Hole Peg Test

Trial	Left	Right
1		
2		
3		
Average		

Grip

Trial	Left	Right
1		
2		
3		
Best		

Box and Block Test

Left	Right

Pinch - Key

Trial	Left	Right
1		
2		
3		
Best		

Pinch - Pulp

Trial	Left	Right
1		
2		
3		

Subject:

Date:

Experimenter:

Klinik Bethesda
Neurorehabilitation
Parkinson-Zentrum
Epileptologie

INSELSPITAL
UNIVERSITÄTSSPITAL BERN
HOPITAL UNIVERSITAIRE DE BERNE

Apraxia Screen of TULIA (AST)

Name patient: **Test date:**
Name examiner:
Diagnosis (incl. lesion localization):

Imitation
General instruction: "Seven gestures are demonstrated in a mirror fashion, imitate them as precisely as possible"

	right	left
1. Bring thumb extended on forehead, other fingers point upwards		
2. Wipe dust from shoulder		

Additional instruction: "For the next five gestures, imagine holding a tool or an object in hand, don't use your fingers as a tool"

3. Drink from a glass		
4. Smoke a cigarette		
5. Use a hammer		
6. Use scissors		
7. Use a stamp to postmark		

Pantomime
General instruction: "Now gestures are asked. Listen very carefully and perform them as precisely as possible"

8. "Show as if someone is crazy" *		
9 . "Make a threatening sign" **		

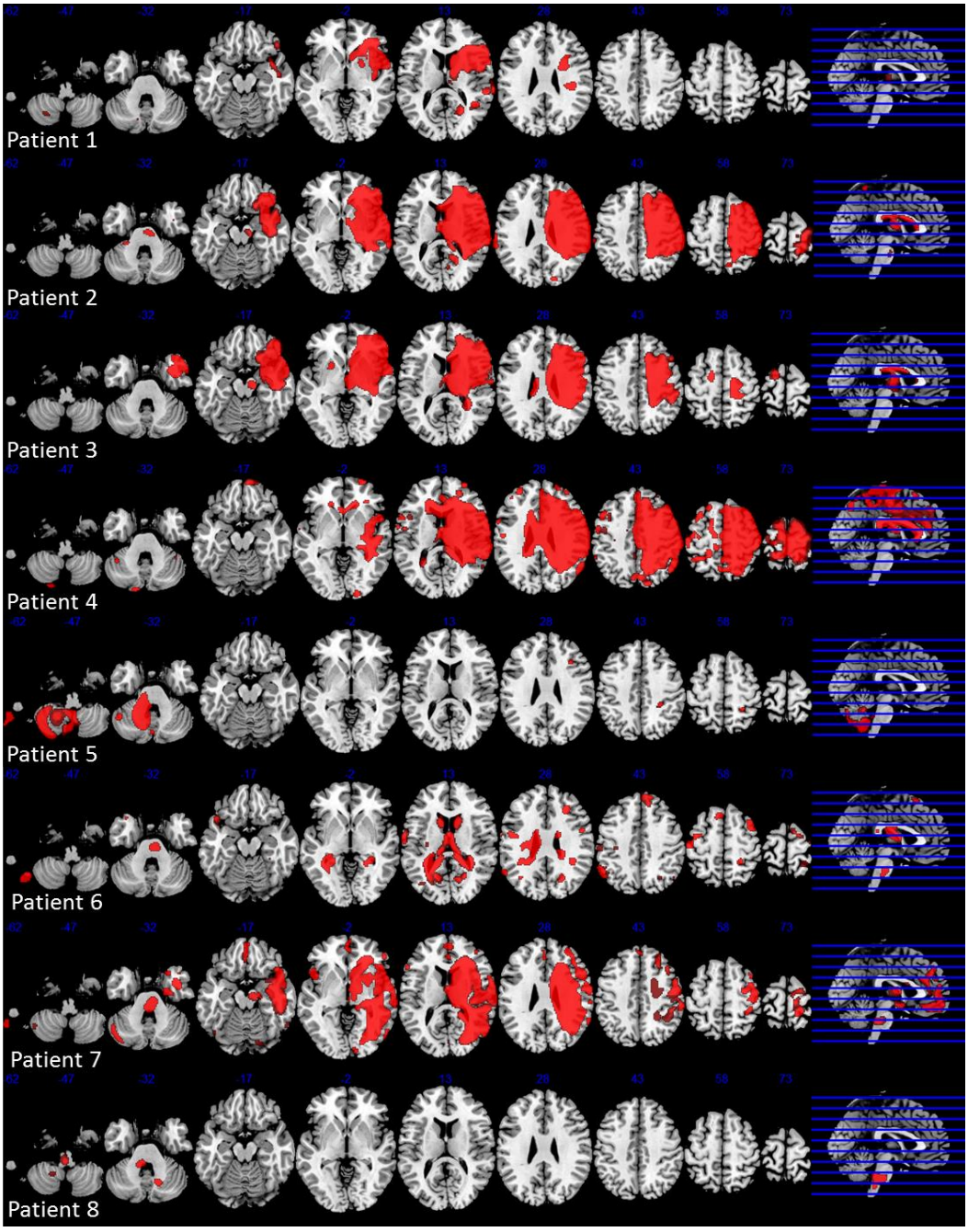
Additional instruction: "Again, imagine holding a tool or an object in hand, don't use the fingers"

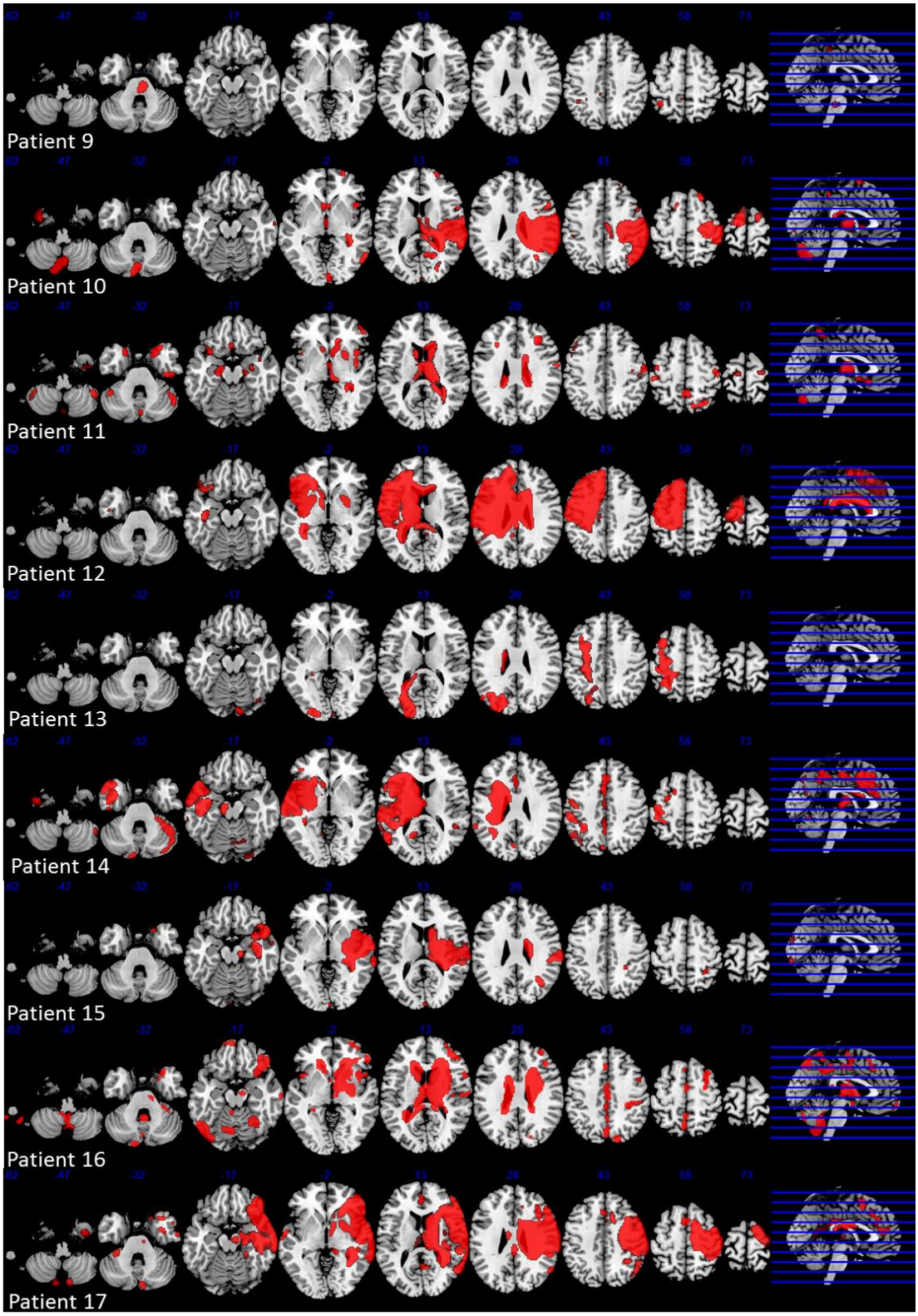
10. "Brush your teeth"		
11. "Comb your hair"		
12. "Use a screwdriver"		
Total Score		

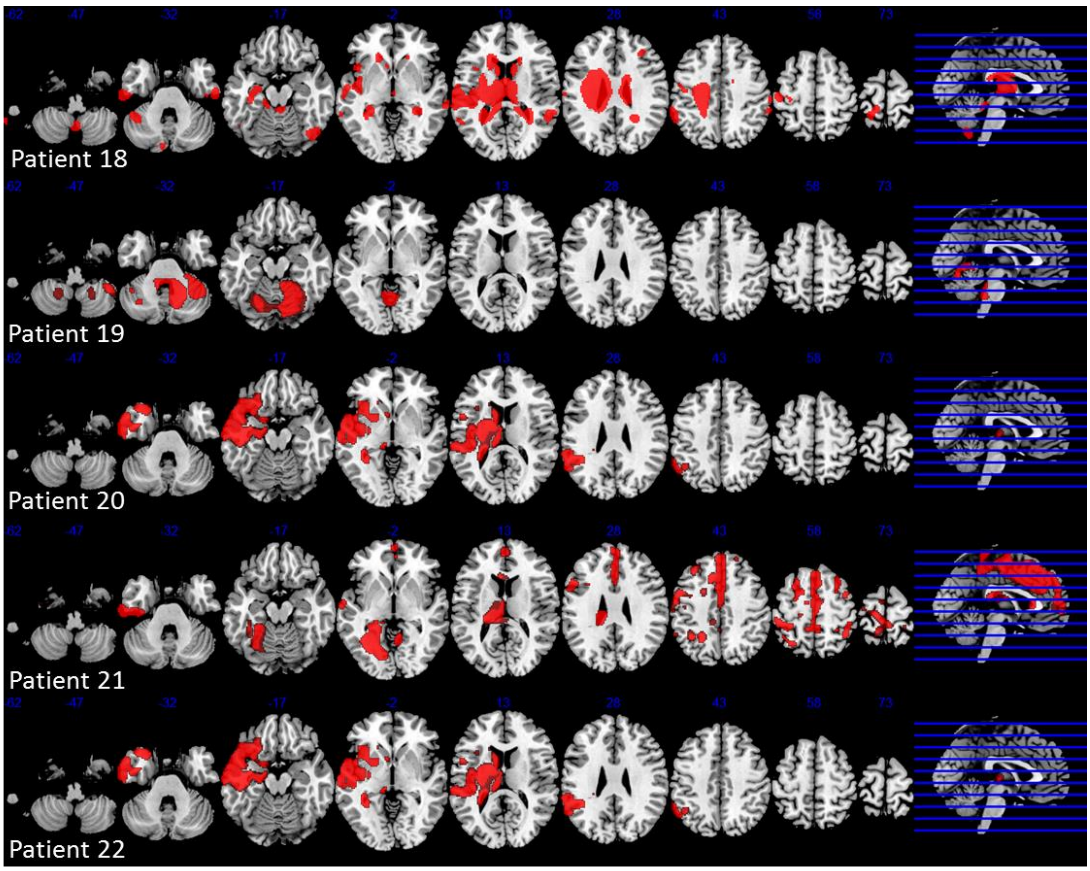
Item 1 = meaningless; Items 2,8,9 = intransitive; Items 3-7 and 10-12 = transitive
* repetitive tapping of the index finger at the temple (rotating movements of index finger are also correct).
** upraised clenched fist (upraised index finger or open hand are also correct).

Appendix G

Patients' lesions outlined using Automated Lesion Identification toolbox for SPM (Seghier et al., 2008). These lesions were masked out and data from these areas were not included in the analysis.




Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 14
Patient 15
Patient 16
Patient 17


Patient 18
Patient 19
Patient 20
Patient 21
Patient 22

Appendix H

Contrast Action Observation > Rest in healthy participants (FWE corrected within clusters (p=0.05). Cluster size and peak coordinates in the MNI space are outlined. Descriptions of the anatomical areas and corresponding cytoarchitectonic maps are taken from SPM Anatomy Toolbox v.1.8 (Eickhoff, 2005).

Action Observation > Rest (T > 7.06) (FWE corrected, p=0.05)		T	X	Y	Z	Anatomical Region	Cytoarchitectonic Map
Cluster 1 (2815 vox)	Max 01	16.52	54	-66	7	Right Middle Temporal Gyrus	right hOC5 (V5)
	Max 02	15.46	46	-66	1	Right Middle Temporal Gyrus	right hOC5 (V5)
	Max 03	15.46	44	-66	5	Right Middle Temporal Gyrus	right hOC5 (V5)
	Max 04	13.78	36	-78	-5	Right Inferior Occipital Gyrus	
	Max 05	12.71	34	-84	7	Right Middle Occipital Gyrus	
	Max 06	12.64	36	-86	9	Right Middle Occipital Gyrus	
	Max 07	12.09	54	-62	-3	Right Inferior Temporal Gyrus	
	Max 08	11.85	40	-76	-9	Right Inferior Occipital Gyrus	
	Max 09	11.75	34	-92	13	Right Middle Occipital Gyrus	
	Max 10	11.04	40	-82	-3	Right Inferior Occipital Gyrus	
	Max 11	10.66	24	-82	-9	Right Lingual Gyrus	right hOC3v (V3v)
Cluster 2 (2348 vox)	Max 01	17.51	-48	-72	-3	Left Inferior Occipital Gyrus	
	Max 02	15.03	-42	-80	11	Left Middle Occipital Gyrus	
	Max 03	14.71	-44	-68	5	Left Middle Occipital Gyrus	left hOC5 (V5)
	Max 04	13.81	-30	-90	5	Left Middle Occipital Gyrus	
	Max 05	13.42	-38	-82	5	Left Middle Occipital Gyrus	
	Max 06	13.35	-26	-84	-1	Left Inferior Occipital Gyrus	
	Max 07	13.22	-38	-80	-5	Left Inferior Occipital Gyrus	
	Max 08	12.94	-36	-86	1	Left Middle Occipital Gyrus	
	Max 09	11.86	-28	-94	11	Left Middle Occipital Gyrus	
	Max 10	10.39	-38	-74	-13	Left Fusiform Gyrus	
	Max 11	10.13	-36	-66	-15	Left Fusiform Gyrus	
Cluster 3 (1674 vox)	Max 01	17.21	30	-52	57	Right Superior Parietal Lobule	right SPL (7PC)
	Max 02	12.26	46	-24	39	Right Postcentral Gyrus	right Area 2
	Max 03	11.47	36	-44	55	Right Inferior Parietal Lobule	right Area 2
	Max 04	10.47	32	-46	45	N/A	right hIP3
	Max 05	10.46	32	-36	49	Right Postcentral Gyrus	right Area 2
	Max 06	10.29	34	-36	59	Right Postcentral Gyrus	right Area 3b
	Max 07	9.89	38	-28	39	Right Postcentral Gyrus	right IPC (PFt)
	Max 08	9.73	20	-60	61	Right Superior Parietal Lobule	right SPL (7A)
	Max 09	9.27	40	-44	47	Right Inferior Parietal Lobule	right hIP2
	Max 10	9.17	30	-30	45	N/A	right Area 3a
	Max 11	8.82	24	-68	51	Right Superior Parietal Lobule	right SPL (7A)
Cluster 4 (1599 vox)	Max 01	13.41	34	18	7	Right Insula Lobe	
	Max 02	13.23	38	-8	51	RightPrecentral Gyrus	right Area 6
	Max 03	12.22	42	-2	43	RightPrecentral Gyrus	
	Max 04	11.54	46	10	33	Right Inferior Frontal Gyrus (p. Opercularis)	
	Max 05	11.45	42	6	37	Right Middle Frontal Gyrus	
	Max 06	10.67	48	16	7	Right Inferior Frontal Gyrus (p. Opercularis)	
	Max 07	10.14	24	-8	57	Right Superior Frontal Gyrus	
	Max 08	10.01	42	24	-7	Right Inferior Frontal Gyrus (p. Orbitalis)	
	Max 09	9.93	48	0	45	RightPrecentral Gyrus	right Area 6
	Max 10	9.79	40	-2	65	Right Middle Frontal Gyrus	
	Max 11	9.69	24	-6	61	Right Superior Frontal Gyrus	
Cluster 5 (1385 vox)	Max 01	13.69	-34	-42	49	Left Inferior Parietal Lobule	left Area 2
	Max 02	12.78	-20	-68	47	Left Superior Parietal Lobule	left SPL (7P)
	Max 03	12	-26	-54	55	Left Inferior Parietal Lobule	left SPL (7A)
	Max 04	11.71	-40	-40	49	Left Inferior Parietal Lobule	left Area 2
	Max 05	11.54	-32	-46	57	Left Superior Parietal Lobule	left SPL (7PC)
	Max 06	11.17	-38	-50	57	Left Inferior Parietal Lobule	left SPL (7A)
	Max 07	10.39	-40	-28	43	Left Inferior Parietal Lobule	left Area 2
	Max 08	9.46	-30	-36	63	Left Postcentral Gyrus	left Area 3b
	Max 09	9.4	-50	-28	37	Left Inferior Parietal Lobule	left IPC (PFt)
	Max 10	8.38	-58	-16	35	Left Postcentral Gyrus	left IPC (PFt)
	Max 11	8.24	-50	-22	37	Left Inferior Parietal Lobule	left Area 2
Cluster 6 (670 vox)	Max 01	13.94	-4	2	57	Left SMA	left Area 6
	Max 02	13.05	-4	-2	65	Left SMA	left Area 6
	Max 03	12.55	-6	6	53	Left SMA	left Area 6
	Max 04	10.67	6	6	61	Right SMA	right Area 6
	Max 05	8.21	-26	-6	61	Left Superior Frontal Gyrus	
	Max 06	7.75	-18	-8	65	Left SMA	left Area 6
	Max 07	7.66	14	0	59	Right SMA	right Area 6

Action Observation > Rest (T > 7.06) (FWE corrected, p=0.05)		T	X	Y	Z	Anatomical Region	Cytoarchitectonic Map
Cluster 7 (296 vox)	Max 01	11.04	-28	22	3	N/A	
	Max 02	10.61	-42	16	-1	Left Insula Lobe	
	Cluster 8 (242 vox): mean						
	Max 01	10.46	-16	-72	-45	Left Cerebellum	left Lobule VIIb (Hem)
	Max 02	10.41	-10	-70	-43	Left Cerebellum	left Lobule VIIIa (Hem)
	Max 03	7.72	-20	-58	-53	Left Cerebellum	left Lobule VIIIb (Hem)
	Max 04	7.61	-22	-60	-51	Left Cerebellum	left Lobule VIIIa (Hem)
	Max 05	7.38	-16	-56	-51	Left Cerebellum	left Lobule VIIIb (Hem)
	Max 06	7.36	-8	-72	-21	Left Cerebellum	left Lobule VI (Hem)
	Max 07	7.33	-4	-70	-29	Left Cerebellum	left Lobule VIIb (Vermis)
Cluster 9 (195 vox)	Max 01	10.7	62	-12	31	Right Postcentral Gyrus	right Area 1
	Max 02	8.87	66	-10	35	Right Postcentral Gyrus	
	Max 03	8.46	66	-20	31	Right SupraMarginal Gyrus	right IPC (PFt)
	Max 04	7.74	56	-18	45	Right Postcentral Gyrus	right Area 1
Cluster 10 (194 vox)	Max 01	9.47	8	-72	-37	Right Cerebellum	right Lobule VIIb (Hem)
	Max 02	9.27	14	-74	-43	Right Cerebellum	right Lobule VIIb (Hem)
	Max 03	7.76	22	-64	-51	Right Cerebellum	right Lobule VIIIa (Hem)
Cluster 11 (193 vox)	Max 01	11.82	18	-28	5	Right Thalamus	right Th-Parietal
	Max 02	9.88	8	-22	-1	Right Thalamus	right Th-Prefrontal
	Max 03	8.53	10	-14	-1	Right Thalamus	right Th-Prefrontal
Cluster 12 (192 vox)	Max 01	9.03	8	20	47	Right SMA	
	Max 02	8.76	8	22	39	Right Middle Cingulate Cortex	
	Max 03	8.66	12	12	47	Right SMA	
	Max 04	8.28	8	30	43	Right Superior Medial Gyrus	
	Max 05	8.1	12	12	41	Right Middle Cingulate Cortex	
Cluster 13 (161 vox)	Max 01	10.86	-44	-10	49	Left Postcentral Gyrus	left Area 6
	Max 02	8.6	-40	-8	63	Left Precentral Gyrus	left Area 6
	Max 03	8.49	-40	-8	57	Left Precentral Gyrus	left Area 6
	Max 04	8.25	-30	-8	51	Left Precentral Gyrus	
	Max 05	8.23	-32	-12	47	Left Precentral Gyrus	
Cluster 14 (124 vox)	Max 01	9.57	-46	-36	23	Left Superior Temporal Gyrus	left IPC (PFcm)
	Max 02	9.2	-42	-32	23	Left Rolandic Operculum	left OP 1
Cluster 15 (123 vox)	Max 01	11.13	40	26	29	Right Inferior Frontal Gyrus (p. Triangularis)	
	Max 02	9.05	48	28	29	Right Inferior Frontal Gyrus (p. Triangularis)	
Cluster 16 (110 vox)	Max 01	9.82	28	-76	39	Right Superior Occipital Gyrus	
	Max 02	9.09	24	-72	33	Right Superior Occipital Gyrus	
Cluster 17 (98 vox)	Max 01	10.54	-56	2	31	Left Precentral Gyrus	left Area 6
	Max 02	7.84	-56	6	41	Left Precentral Gyrus	left Area 6
	Max 03	7.62	-46	4	37	Left Precentral Gyrus	
Cluster 18 (38 vox)	Max 01	10.06	-12	-20	37	N/A	
Cluster 19 (36 vox)	Max 01	9.14	22	-12	7	N/A	right Th-Premotor
Cluster 20 (15 vox)	Max 01	7.48	-12	-22	7	Left Thalamus	left Th-Prefrontal
Cluster 21 (13 vox)	Max 01	7.46	-22	-6	5	Left Pallidum	
Cluster 22 (11 vox)	Max 01	7.72	34	-12	-5	Right Putamen	
Cluster 23 (9 vox)	Max 01	7.49	-50	12	19	Left Inferior Frontal Gyrus (p. Opercularis)	left Area 44
Cluster 24 (8 vox)	Max 01	8.17	-12	-26	73	Left Paracentral Lobule	left Area 6
Cluster 25 (6 vox)	Max 01	7.41	-8	-16	-3	N/A	left Th-Prefrontal

Action Observation > Rest (T > 7.06) (FWE corrected, p=0.05)		T	X	Y	Z	Anatomical Region	Cytoarchitectonic Map
Cluster 26 (5 vox)	Max 01	7.29	-46	-54	9	Left Middle Temporal Gyrus	
Cluster 27 (5 vox)	Max 01	8.08	-18	0	-3	Left Pallidum	
Cluster 28 (4 vox)	Max 01	7.78	-12	-64	61	Left Precuneus	left SPL (7A)
Cluster 29 (4 vox)	Max 01	7.31	-48	-48	9	Left Middle Temporal Gyrus	
Cluster 30 (3 vox)	Max 01	7.9	12	-26	73	Right Paracentral Lobule	right Area 6
Cluster 31 (3 vox)	Max 01	7.48	-18	-12	49	N/A	
Cluster 32 (2 vox)	Max 01	7.12	-16	-28	3	Left Thalamus	left Th-Temporal
Cluster 33 (1 vox)	Max 01	7.2	14	-8	59	Right SMA	
Cluster 34 (1 vox)	Max 01	7.09	16	-74	55	Right Superior Parietal Lobule	right SPL (7P)
Cluster 35 (1 vox)	Max 01	7.09	14	14	35	Right Middle Cingulate Cortex	
Cluster 36 (1 vox)	Max 01	7.36	-20	-78	33	Left Superior Occipital Gyrus	
Cluster 37 (1 vox)	Max 01	7.23	60	-36	21	Right Superior Temporal Gyrus	
Cluster 38 (1 vox)	Max 01	7.09	-32	-36	17	N/A	
Cluster 39 (1 vox)	Max 01	7.18	16	10	-1	Right Pallidum	