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Valchev, Nikola Stanimirov

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Nikola Stanimirov Valchev

Paranimfen: Terence van Zoelen

Kanat Camlibel

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Promotor:

Prof. dr. ir. N.M. Maurits

Beoordelingscommissie:

Prof. dr. B.A. Conway

Prof. dr. H.C. Dijkerman

Prof. dr. K.L. Leenders

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Abbreviations

AC-PC – anterior commissure to posterior commissure (orientation for brain coordinates)

ActionExe – Action execution

ActionObs – Action observation

ADM – abductor digiti minimi (muscle)

Amyg – Amygdala

ANOVA – Analysis of Variance

BA – Brodmann area

BOLD – Blood Oxygen Level Dependent

BR – brachioradialis (muscle)

BV – BrainVoyager (software for fMRI data analysis)

CBF – Cerebral Blood Flow

CBV – Cerebral Blood Volume

cTBS – continuous Theta Burst Stimulation

CtrlExe – Control execution

CtrlObs – Control observation

dPM – dorsal Premotor (brain area)

EEG – Electroencephalography

EMG – Electromyography

EPI – Echo Planar Imaging

FDI – first dorsal interosseous (muscle)

FDR – False Discovery Rate

fMRI – Functional Magnetic Resonance Imaging

FWHM – Full width half maximum

GLM – General Linear Model

Hem – (Brain) Hemisphere

Hipp – Hippocampus

hOC3v, hOC4v – Brain areas in the ventral visual cortex.

hOC5 – Brain area, visual cortex V5.

ICC – Intraclass Correlation Coefficient

IFC – Inferior Frontal Cortex

IFG – Inferior Frontal Gyrus

IPC – Inferior Parietal Cortex

IPL – Inferior Parietal Lobule

iTBS – intermittent Theta Burst Stimulation

M1 – Primary motor cortex

MEP – Motor Evoked Potential

MN – Mirror Neuron

MNI – Montreal Neurological Institute (brain coordinate system)

MNS – Mirror Neuron System

MRI – Magnetic Resonance Imaging

MTG – Middle Temporal Gyrus

mV – millivolt

OP – Operculum parietale (brain area)

OSP – Optimal Scalp Position

PF (PFsm, PFm, PFop and PFt) – brain areas of the rostral inferior parietal lobule

P-P – Peak to Peak amplitude

PPI – Psychophysiological interaction (connectivity analysis)

rMT – Resting Motor Threshold

ROI – Region of interest

RS-fMRI – Resting state fMRI

RT – Reaction Time

rTMS – repetitive Transcranial Magnetic Stimulation

SD – standard deviation

SI – Primary somatosensory cortex

SII – Secondary somatosensory cortex

SMA – Supplementary motor area

SPL – Superior parietal lobule

SPM8 – Statistical Parametrical Mapping v. 8 (software for fMRI data analysis)

TE – Echo Time (fMRI)

TMS – Transcranial Magnetic Stimulation

TR – Repetition Time (fMRI)

vPM – ventral Premotor (brain area)

Chapter 1

1 General Introduction

In many ways the last frontier for mankind has moved from the faraway lands and stars to an object very close to all of us. Man has already travelled to almost all regions of the planet Earth and has gazed at almost all the objects in the sky. Yet, for a long time, we have ignored that getting to all the remote places, and observing the night sky finally boils down to the functioning of our brains. It can be seen as ironic that we need to use our brains to understand our brains. Yet, nowadays the brain has attracted the attention of many scientists. A vast amount of knowledge has been gathered on the functioning of the nervous system as a whole and on the functioning of the brain in particular. We know a lot about neurons, their organization and we have some knowledge about how they interact to produce our thoughts and behaviours. Yet, many neuroscientific questions remain to be answered.

As a precursor to modern neuroscience, phrenology in the XVIIIth and XIXth centuries aimed at finding a correspondence between the external features of the skull and internal brain structures on the one hand, and cognitive functions and personality traits on the other hand (Staum 1995). In contrast to this “localization” approach, many scientists adopted a more holistic perspective in which the human being and the brain as the organ that contains thought and soul are one undividable entity. In today’s

terms we can identify many of the principles of phrenology (Franz-Joseph Gall, 1758 – 1828) in the neuroscience studies which attempt to identify the functions of a certain brain area. On the other hand there are studies and scientists who adopt a more global view and study systems of interconnected and dynamic brain areas. Their views are closer to the holistic philosophical perspective of Marie-Jean-Pierre Flourens (1794 – 1867). From a modern point of view, however, none of the strict approaches of the XIX century is sufficient to explain the functioning of our brains. We nowadays assume that there are functionally segregated brain areas responsible for some specific aspects of cognitive processing, but that cognitive function in general can only be accomplished through the coordination between several segregated brain areas which are functionally integrated in one system. The challenge for modern neuroscience is to find ways to study this system of brain areas, that act both individually and in concert.

Asking the right questions is the first step towards finding their answer, but neuroscientists are also dependent on the tools that they have at their disposal. Phrenologists and physiognomists could only assess the external features of the skull and search for specific markings which would correlate with specific cognitive functions and personality traits. Very often studies were done post-mortem. Johann Kaspar Lavater (1741 – 1801), founder of physiognomy, stated that the face is the “magic mirror of the soul” and that by observing it we can see the soul. Instead of observing the face or skull, we now observe the brain. Highly sophisticated techniques to observe the brain in action became available to neuroscientists by the second half of the XXth century. Functional Magnetic Resonance Imaging

(fMRI) gave the possibility to precisely localize the brain areas that were activated during specific tasks. Transcranial Magnetic Stimulation (TMS) even allowed to interfere with the activity of selected brain regions and to test their importance for the execution of a given task. Some neuroscientific tools gave the opportunity to study other important aspects of human cognition: high resolution electroencephalography (EEG) and magnetoencephalography provided much better temporal resolution than fMRI. Each of these modern techniques has its strengths and weaknesses, but by combining them we can now answer new and ever more complicated neuroscientific questions.

In the last decades some neuroscientists have focused their attention on the brain's activity when we perceive and interpret other people's actions. We now know that this day-to-day behaviour that we all perform effortlessly and almost without noticing activates a whole network of brain areas and is part of a complex cognitive process, which ultimately permits us to "understand" the goals and intentions of other people. Rather than attempting to answer the question of how we "understand" the actions of the people around us, the research reported in this thesis tries to increase our comprehension of the functioning of the brain when we perceive other people executing simple hand actions, how the brain areas engaged in this process interact and what role each of these regions, and the primary somatosensory cortex in particular, plays in accomplishing this brain function.

1.1 Action perception and action understanding

We observe other people acting on a daily basis. We watch them move around, grasp objects and use them. As a simple example, watching somebody eat involves observing how the fork and knife are grasped, used to cut a piece of food, bring it to the mouth, chew and finally swallow it. All these actions can be "understood" at several different levels. In the framework of Hamilton and Grafton (2007) understanding can be focused on 1) the long term intentions of the actions (in the example: "to eat because the person is hungry"); 2) the short term goals necessary to achieve the long term intention ("cut a piece of food"); 3) the kinematics that describe the movement ("slide the knife across the food while using the fork as a support") or 4) the pattern of muscle activations required by the action ("which muscles to use and in what sequence in order to perform the action of cutting and holding the fork in place"). How exactly the brain is activated during each stage of this process is a question that still remains to be answered. A fascinating discovery in 1992 by Di Pelegrino and colleagues took a central role in many theories of action perception and understanding. They were the first to discover mirror neurons (MNs) in the macaque monkey brain. Later their existence was also confirmed in the human brain (Mukamel et al., 2010). These specialized neurons have the property of responding in two conditions: 1) when a person performs an action and 2) when a person observes a similar action being performed by another person. Although in the monkey brain only about 20% of the recorded neurons turned out to be MNs, researchers have since identified a network of areas in the human brain with mirror

neuron like properties. The areas of this network are homologous to the ones where MNs were found in the monkey brain and fMRI studies have shown that they are active both when participants execute and when they observe actions. Many theories have speculated on the role of this network in human cognition (Caspers et al., 2010; Catmur, Walsh, Heyes 2007; Friston, Mattout, Kilner 2011; Rizzolatti and Sinigaglia 2010). While the exact mechanism which enables us to extract information from the observed behaviours remains to be discovered, it is now generally thought that there is a system of areas in the brain which responds when we observe meaningful, goal directed actions of other agents.

1.1.1 Action perception network

In the human brain, rather than using single cell recordings researchers use fMRI to identify the brain areas which respond both when a person observes or executes actions of other people. This "mirror like behaviour" typically observed in the parietal and premotor cortices has given this network the name mirror neuron system (MNS) (Rizzolatti and Craighero 2004), or more recently parieto-frontal mirror circuit (Rizzolatti and Sinigaglia 2010). The latter authors include regions of the inferior parietal lobule (IPL) and the ventral and dorsal premotor cortices (vPM and dPM, respectively) in the network. However, when scanning subjects using fMRI while they observe and while they execute actions, typically more than just the premotor cortices and the IPL are found to be active in both tasks. This broader network has been referred to as "shared circuits" and includes the parieto-frontal mirror network and in addition the primary and

secondary somatosensory cortices (SI and SII) (Gazzola and Keysers 2009). Since we cannot assume that all areas in the shared circuits contain mirror neurons and that all identified activations are due to mirror neuron activity, it is important to study the system as a whole and also the role of each of its nodes during specific tasks which require action perception.

The areas composing the parieto-frontal mirror network have received quite some attention from researchers (Caspers et al., 2010; Molenberghs, Cunnington, Mattingley 2012). Studies have focused on the role of the premotor cortices and the IPL, and on their connectivity during action perception. The human inferior parietal lobule (IPL) is not only homologous to an area where mirror neurons were found in the monkey brain (Fogassi et al., 2005), but it has also been repeatedly shown using fMRI that this particular area is activated both when people observe and when they execute actions (Caspers et al., 2010). Taken alone these fMRI results cannot really prove whether in the IPL there is one population of mirror neurons that responds in both conditions or whether there are two distinct neuronal populations which overlap in space but are differentially activated by the tasks. However, Chong and colleagues (2008) used an adaptation paradigm to show that the IPL responds independently to specific actions regardless of whether they were observed or executed. The authors presented participants with sequences of actions to be observed or executed. They showed that participants' responses in the IPL were suppressed if the same action was observed first and then executed or vice versa. In the case when subjects first executed and then observed actions, observing the same action as the one just executed elicited less activity in the IPL as opposed to observing a novel action. This result shows that the

same neuronal population in the IPL reacts to the execution and observation of the same action and that activations measured from this region could be attributed to mirror neuron activity. Moreover, Buccino and colleagues (2001) showed that the activations in both the IPL and the premotor cortical areas are somatotopically organized, i.e. activations in those areas spatially correspond to the effector of the observed/executed action. These experiments show that 1) both the IPL and the premotor cortices are activated during both execution and observation (i.e. there is a functional connection between these areas) and 2) this "mirror like activity" suggests that the areas of the parieto-frontal mirror circuit most probably contain mirror neurons. Pobric and Hamilton (2006) went one step further in testing how crucial/essential the activation in the ventral premotor region is for action perception. The authors asked subjects to estimate the weight of a box they observed while being lifted. While performing the task, participants received inhibitory TMS pulses either to the inferior frontal gyrus (IFG) or the occipital cortex. Results showed that after inhibiting the IFG (but not the occipital cortex), participants are worse at estimating the weight of the box. Moreover, the same stimulation on the same region did not affect performance when participants were estimating the weight of a bouncing ball. This suggests that activation in the IFG, and possibly the whole parieto-frontal mirror network, is crucial for correct execution of the task.

As already mentioned, when the brain areas activated by both tasks of action perception and action execution are compared directly, we find more regions that behave in a mirror neuron like way. The somatosensory cortices have been repeatedly shown to be engaged in both

tasks (Gazzola and Keysers 2009). Moreover, it has been shown that SI displays “mirror like activity” in several tasks: it is active when people perceive pain and experience pain (Bufalari et al., 2007; Valeriani et al., 2008) and when they observe touch and experience touch (Keysers et al., 2004). In a recent experiment Bolognini and colleagues (2011) asked participants to perform a visual discrimination task with tactile stimuli (subjects watched videos of a finger approaching a hand and had to decide whether there was a touch or not). During the task inhibitory repetitive Transcranial Magnetic Stimulation (rTMS) was delivered either to SI, SII, or the occipital cortex (as a control site). Their results demonstrated that only rTMS over SI had a negative effect on the participant’s ability to detect an actual touch in the contralateral visual field. Stimulation over other areas, such as SII, only impaired visual processing. This shows that SI is not only active when touch is observed but also that its functioning is crucial to the detection of touch in the contralateral visual field.

Taken together, the results of Bolognini and colleagues (2011) and the fact that SI has anatomical connections to the premotor cortical areas and the IPL (Keysers, Kaas, Gazzola 2010), show that the somatosensory cortex must be considered as part of the network of areas active during action perception. Two questions that remain are whether this activity in SI is crucial for action perception and whether the functional connectivity of SI is stronger during the observation of other people acting.

To study the whole system of brain areas activated during action perception we propose to not only measure brain activity but also to interfere with it in a precise way. If we would only measure brain activation during action perception we would not be able to draw conclusions about

how essential the functioning of a certain brain area is for correct performance. By interfering with the activity in a target brain region we can determine the effects on the behavioural level in a specific situation, or on the activity in the rest of the system of interconnected brain areas. To achieve these goals we employed two neuroscience techniques, fMRI and TMS, both individually and in combination. fMRI permits to spatially identify brain activations, but does not allow to evaluate how crucial the activation in one particular area is for functioning of the entire system. On the other hand TMS allows interfering with a specific cortical region but provides no means of measuring the changes induced in the rest of the system. The combination of these two tools provides a powerful way of evaluating both how the stimulation of a target area affects overt behaviour and how an effect of stimulation in a target region affects activation in the rest of the studied system.

1.2 Neuroscientific methodologies to investigate the brain in action

1.2.1 TMS

Transcranial Magnetic Stimulation (TMS) is a non-invasive technique through which cortical activity can be influenced both locally (directly targeted area) and remotely (by spreading the effect of the stimulation from the target area to other interconnected brain regions). Since its inception and first use by Barker and colleagues (1985) an ever growing body of research has focused on the effects of different TMS pulse sequences on cortical excitability (Rossi et al., 2009). There are several

ways in which TMS can be used to investigate human brain activity; the differences are mainly in the used stimulation intensities and the frequency at which pulses are delivered.

1.2.2 Single pulse TMS

Single pulse TMS is the simplest way of applying the technique and consists of delivering one pulse at a time. If applied over the cortical hand motor area of a participant, it can be used to assess the individual level of excitability of the primary motor cortex. After delivering the pulse a burst of activity is evoked in the motor cortex and the targeted muscle on the contralateral side of the body twitches slightly, generating a Motor Evoked Potential (MEP) which can reach an amplitude of several mV (Day et al., 1987). The correct positioning of the coil tangentially to the skull is essential. The magnetic field created by the TMS pulse decreases rapidly with the distance which means that subcortical structures cannot be stimulated directly, but the after effects (although poorly understood as a mechanism) are detectable in the corticospinal output, where series of volleys at a frequency of about 500Hz can be recorded after a single TMS pulse (Rothwell et al., 1991). The first volley is the result of the direct activation of the pyramidal tract neurons and is detectable only at higher intensity stimulation.

Of interest for the studies presented in this thesis are two applications of single pulse TMS. One is related to the measurement of the individual motor threshold at rest (rMT) and another one to the recording of MEPs during stimulus observation. To measure the rMT, single pulses of

TMS are delivered at different locations on the head of a participant and the intensity is gradually increased until a response area can be identified. The spot on the skull where the response as measured by the MEP amplitude is the most consistent and strong is defined as the optimal scalp position (OSP). After defining the OSP, the intensity of the TMS pulses is decreased until the level at which there is 50% probability to elicit a MEP of at least 50 mV in a completely relaxed muscle. This individual rMT is used as a reference to the strength all other types of stimulations (i.e. stimulation on different cortical regions or using different TMS sequences).

In an event related MEP recording, participants observe stimuli on a computer screen and at certain time points a single pulse of TMS with intensity of (typically) 120 to 130% of the rMT is delivered to the primary motor cortex. By comparing the magnitude of the MEPs recorded from the targeted muscles when participants are viewing stimuli or are at rest (baseline) we can estimate how much the stimuli themselves have provoked a change in the activation in the targeted brain area. Fadiga and colleagues (1995) provide a simple demonstration of this technique in the field of action perception, relevant to the research presented here. They have shown that the magnitude of the MEPs that are evoked by stimulation of the motor cortex is significantly increased when subjects observe grasping movements or arm movements compared to conditions during which they observe objects alone or detect a change in lighting conditions. This result shows, in a simple and direct way, that the human motor cortex changes its excitability when meaningful actions are being observed.

1.2.3 Repetitive TMS (rTMS)

When a train of TMS pulses is delivered to the brain, long lasting effects can be observed in cortical excitability. These effects depend on the intensity, frequency, train length, intertrain-interval, total number of pulses delivered, and also on the coil configuration, current direction, pulse waveform and position of the coil with respect to the cortex (for an extensive review of different TMS and rTMS sequences see Rossi and colleagues, 2009).

In the studies presented in this thesis we have used a relatively novel pattern of rTMS developed by Huang and colleagues (2005); theta burst stimulation (TBS). The main difference between TBS and conventional rTMS protocols is that with short stimulation durations long after-effects can be achieved. TBS consists of delivering bursts of 3 pulses at 50Hz repeated every 200ms at an intensity of 80% of the individual rMT, in this way mimicking the coupling of theta and gamma rhythms. Huang and colleagues (2005) explored two main modalities of TBS, intermittent TBS (iTBS) consisting of trains of 2s of TBS repeated every 10s for a total of 190s (600 pulses) and continuous TBS (cTBS) consisting of 40s of uninterrupted TBS trains (600 pulses). The effects observed by Huang and colleagues (2005) were opposite for iTBS and cTBS; iTBS caused an increase in cortical excitability where cTBS caused an inhibition, as reflected in an increase and decrease, respectively, in the amplitudes of the recorded MEPs. In the case of cTBS effects were detectable for 20 up to 60 minutes after stimulation.

In this thesis we use rTMS to induce a transient perturbation of the activity in a targeted brain region. In this way we can explore the causal contribution of the target area to optimal performance in a given task.

Often this approach to the use of rTMS has been referred to as “virtual lesioning”. If perturbing the functioning of a target area affects the behaviour in a task, then this area is deemed essential for the cognitive processes in question. It should be noted that perturbing a region typically has a negative effect on subjects’ performance but this is not necessarily so. In some cases rTMS can result in an improvement of behaviour because of the interactions between different brain areas.

1.2.4 Functional Magnetic Resonance Imaging (fMRI)

Functional magnetic resonance imaging (fMRI) is a brain mapping technique that has been developed some 20 years ago. It is a modification of the structural or classical magnetic resonance imaging (MRI) technique. fMRI employs differences in magnetic properties of hemoglobin when its configuration changes from the oxygenated to the deoxygenated state (Kwong et al., 1992; Ogawa et al., 1992). Under normal, relative resting conditions it is assumed that the cerebral blood flow (CBF) and cerebral blood volume (CBV) are regulated by neuronal activity. However, a striking feature of the metabolic response to functional activation is that rising CBF/CBV uncouples from oxygen consumption (Fox and Raichle 1986). This uncoupling of CBF/CBV and oxygen consumption results in a decrease in deoxyhemoglobin concentration in the venous pool. It thereby provides a contrast that is used in fMRI studies. In this way, fMRI provides an indirect means of assessing neuronal activity. BOLD (blood oxygen level dependent) changes are typically only 1% above baseline. In order to detect these rather small signal changes, it is therefore important to repeat measurements a large number of times.

Generally, there are two possible study designs in fMRI: the block design and the event-related design. In a block design a single task condition consisting of many stimulus presentations of the same type is presented for about 15-30 sec, after which it is followed by a rest period or a different condition for the same amount of time. This sequence is typically repeated several times. In an event-related design, stimuli of different conditions are presented in (pseudo-)random sequence, which allows to study shorter lasting neuronal changes.

1.2.5 Combining TMS and fMRI

There are several ways to combine TMS and fMRI. The two techniques can be used simultaneously or one after the other, separated in time and possibly space. The simultaneous combination of fMRI and TMS allows researchers to interfere with a target brain area and measure not only the direct effect of this interference on the behaviour displayed by the subject but also on the cortical activity in the stimulated area as well as in other functionally connected areas. However, using TMS and fMRI at the same time implies several serious methodological and technical challenges. Special attention needs to be given to disentangling the direct effects of TMS stimulation on brain activity from the nonspecific consequences of the auditory and sensory stimulation associated with the delivery of the TMS pulse. In the work presented in thesis we use an off-line combination of fMRI and rTMS. First fMRI is used to guide the rTMS stimulation and then, in another session, we first stimulate the selected cortical area and immediately after we scan our participants using fMRI. We call this approach “perturb and measure”, since we first interfere with the

functioning of a cortical area and then we search for the effects of this perturbation in the whole system of interconnected regions active in a given task. We aim at identifying the effects of TMS stimulation on brain activity. To do so, we collect fMRI data both after participants have received active rTMS and sham rTMS during two different sessions in a randomized order. Afterwards we compare brain activations from the two sessions. It is important to note that the results of this particular combination of fMRI and rTMS can be viewed as a test of the causal relationship between brain areas. If we assume that two brain areas A and B are causally connected by A inhibiting the functioning of B during a certain task, then if rTMS is delivered over A the activity in B should be affected. Unfortunately, this simple example does not reflect the complex interactions between brain areas. Several alternative explanations can be given. First, an additional unknown area C can play a role in the change of activation observed in B after rTMS has been delivered over A. Second, the change in activity observed in B can be the result of a compensatory mechanism rather than an effect of the stimulation.

1.3 Overview of the thesis

In the studies presented in this thesis we use both fMRI and TMS, alone or in combination, to make use of their advantages and achieve a more global view of the system of interacting brain areas involved in action perception.

We address two main questions in this thesis. First we are interested in the functional connectivity of SI. To study this problem we combine cTBS stimulation and fMRI off-line. First we define the region in SI which is activated both by action perception and action execution. We do so by analysing the fMRI data of each individual subject. On two subsequent days we perturb the targeted area and immediately after measure the effects of our perturbation on the system as a whole during action observation and also during rest. Each subject receives both active cTBS and sham cTBS stimulations on two different days in a randomized order. By comparing the identified networks after active cTBS and sham cTBS stimulation we evaluate the connectivity of SI during action observation (Chapter 2) or during rest (Chapter 3).

Another key question regarding the studied brain region is its importance for the cognitive processes taking place during action observation. To address this issue we apply inhibitory cTBS to SI and then measure its effects on subject performance in a task (Chapter 4). In particular, we use the weight estimation through observation paradigm, since it has been shown to involve regions of the parieto-frontal mirror circuit (Pobric and Hamilton 2006). In the last study reported in this thesis we further explore the effect of weight estimation through observation on primary motor cortex excitability (Chapter 5). We measure MEPs from muscles which are directly observable in the actions (direct modulation of the primary motor cortex excitability) and muscles which are involved in the action but not visible to the subjects. The aim of that study is to explore the possibility of top-down influences on motor resonance as measured by the amplitude of the MEPs measured from visible and invisible muscles.

Chapter 2

2 The functional connectivity of the left somatosensory cortex during action observation. A combined fMRI and cTBS study.

Adapted from the manuscript submitted as:

The contribution of somatosensory cortices to brain activity during action observation, a combined fMRI and cTBS study

Nikola Valchev, Valeria Gazzola, Alessio Avenanti and Christian Keysers

Abstract
The parieto-frontal mirror network, active both during action execution and observation is classically thought to include only premotor and posterior parietal areas. However, it has been shown that the primary somatosensory cortex (SI) is also activated during action execution and observation. Here we examine whether SI and the parieto-frontal mirror network are a single, interconnected sensorimotor network, or two independent networks processing observed actions separately. We use continuous theta-burst stimulation (cTBS) to perturb the activity in SI and fMRI to measure the effects on the system as a whole during action observation. We found interindividual differences in the effect of cTBS on the activity in SI. However, changes in activation in SI predicted changes of action specific brain activation in premotor nodes of the parieto-frontal mirror network, providing direct evidence that SI plays a role during action observation together with the premotor cortex. This suggests that during action observation the parieto-frontal mirror network together with the somatosensory cortex might provide an integrated, somato-motor representation of other's actions.

2.1 Introduction

The neural processes involved in witnessing other people's actions have received much interest in recent years. Functional magnetic resonance imaging (fMRI) has evidenced a complex network of regions activated while witnessing the actions of others or performing actions (for a review see Rizzolatti and Sinigaglia 2010). This network includes regions of the occipital and temporal lobe associated with vision and audition, and regions, agnostically dubbed 'shared circuits', involved both in action perception (observation or listening) and execution (Gazzola and Keysers 2009). Shared circuits include two groups of areas traditionally associated with different modalities. One group, associated with the motor system, includes in particular dorsal and ventral premotor cortices and the inferior parietal lobe, a network of areas also referred to as parieto-frontal mirror network (Rizzolatti and Sinigaglia 2010). The other group, mainly associated with the somatosensory system, includes posterior regions of the primary somatosensory cortex (Brodmann Area 1 and 2 in particular), and the secondary somatosensory cortex (SII) (Gazzola and Keysers 2009). Although SI is consistently activated during action observation across studies (Caspers et al., 2010) and contains the most consistent shared voxels across individuals (Gazzola and Keysers 2009), this region is not usually included in the parieto-frontal mirror network, and social neuroscience only recently started to realize that somatosensory cortices may play a key role in perceiving others in general (Adolphs et al., 2000; Bolognini et al., 2011; Bufalari et al., 2007; Keysers, Kaas, Gazzola 2010; Valeriani et al., 2008) and their actions in particular (Avenanti et al., 2007;

Caspers et al., 2010; Gazzola and Keysers 2009; Keysers, Kaas, Gazzola 2010). As neuroscience embraces that cognition results from the interplay of multiple regions, the challenge for a mechanistic understanding of action observation becomes to understand the interplay between the components of shared circuits. Relevant for the present study, there is evidence that SI has anatomical connections with posterior parietal and premotor regions commonly accepted as having mirror properties (Keysers, Kaas, Gazzola 2010). The critical question at hand, to help understand the neural basis of action observation, is therefore not whether such connections exist, but whether they are active during (hand) action observation as opposed to the observation of meaningless hand movements or objects alone.

Here, driven by our interest in the connectivity between SI and the parieto-frontal mirror network during action perception, we combine transcranial magnetic stimulation (TMS) to experimentally manipulate brain activity in SI to then test, using fMRI, if this perturbation has remote effects on the rest of the network (Driver et al., 2009; Reithler, Peters, Sack 2011; Siebner et al., 2009). To draw conclusions on the connectivity between SI and the rest of the network during action observation, we first define the exact cluster in SI activated during both action observation and execution for each individual subject. This specific cortical area is later targeted with TMS. We use a form of repetitive TMS called continuous theta-burst stimulation (cTBS) known to affect brain activity for a substantial amount of time (up to 1 hour) after only 40s of application (Huang et al., 2005; Huang et al., 2011). FMRI measurements can then be performed just after cTBS application to measure the effect of cTBS on brain activation without

the problems associated with applying TMS during scanning (e.g. interrupting scanning to deliver magnetic pulses, using larger head coils to accommodate the TMS coil etc).

Although in behavioural experiments, cTBS is generally assumed to have a net 'suppression' effect on the neural activity under the stimulation coil, the effect of cTBS is actually a complex combination of suppression and excitation (Gentner et al., 2008; Huang et al., 2011; Iezzi et al., 2008) and may be highly variable across individuals (Hamada et al., 2012; Ridding and Ziemann 2010). To take this variability into account, we identify remote brain regions within the shared circuits where activation during action observation changes in a synchronized way with the activation in the stimulated area of SI. By doing this on a single subject level we take into account the between subjects variability of the effects of cTBS on SI.

In our combined cTBS/fMRI experiment, we therefore measure brain activity using fMRI both while participants observe (i) short (3-4s) movie clips in which an actor interacts using the right hand with an object placed on a table (ActionObs) and, as a control condition, (ii) movies in which the same actor moves the hand close to, but without acting upon, the object (CtrlObs) (Figure 2.1A, Table S2.1). To ensure that we stimulate the part of SI which possibly contributes to the mental processes involved in action observation and execution, we first scan subjects while they perform the Action observation and Control observation tasks. On this same day participants also execute actions in the scanner (ActionExe) and perform a control task involving eye movements following the same visual stimuli (CtrlExe) as used during ActionExe (Figures 2.1C and 2.1D and Experimental procedures). By contrasting these conditions we define the cluster in SI

activated during the observation of meaningful actions (ActionObs) but not during the observation of a hand moving around an object (CtrlObs), and also activated during the execution of actions (ActionExe) but not during the following of visual stimuli on a screen (CtrlExe). On the next two days we again scan participants observing the ActionObs and CtrlObs videos but after cTBS (active cTBS, Day2 or 3) or, on a different day (Day3 or 2 respectively), with counterbalanced order, after the same stimulation protocol applied using a sham coil (sham cTBS) (Figure 2.1B). This sham coil also produces a sound and sensation on the skin, but with negligible neural effects. We then adopt a regression analysis approach. We evaluate the effect of cTBS on the stimulated part of SI individually and search for correlated areas in the shared circuits. Brain regions where activity has changed in a synchronized way with activity in SI after stimulation can be identified as functionally connected with SI. By calculating the regression on the contrast between the control videos and the action videos we can assess the specific functional connectivity of SI inside the shared circuits during the observation of meaningful hand actions.

2.2 Methods

2.2.1 Participants

Twenty-four participants took part in the study, but six failed to complete all three sessions (two because of excessive resting motor threshold (>64% of maximum stimulator output)[1], two because of voluntary drop-out, and two because of light headaches on Day2 — a sham cTBS session for both). From the remaining 18 participants, one was excluded because his stimulation point was too posterior due to a lack of activation in SI. The final group of 17 subjects (6 female, mean age 20.9 ± 1.95SD years) was right handed (Edinburgh handedness inventory mean score 82.2 ± 17.6, Oldfield 1971), had normal or corrected-to-normal visual acuity; had no neurological, psychiatric or other medical problem, nor contraindications to cTBS (Rossi et al., 2009) or fMRI, and were naïve to the purposes of the experiment. Full debriefing was provided only at the end of the third session. Participants gave their written informed consent and received monetary compensation (8€/h). Procedures were approved by the Medical Ethical Committee of the University Medical Center Groningen.

2.2.2 Resting motor threshold (rMT)

The individual rMT was determined by recording motor evoked potentials (MEPs) from the right first dorsal interosseous (FDI) by means of a TMSi-

[1] This limitation is due to the technical characteristics of the TMS machine used in this experiment. The frequency of the cTBS stimulation (50Hz) requires the capacitors of the machine to recharge at a rapid rate, which is not possible for stimulations of intensities of more than 51% (corresponding to 80% of the rMT of 64%).

Refa 16-channels amplifier (Twente Medical Systems international, Oldenzaal, The Netherlands). EMG signals were band-pass filtered (20 Hz-1.0 kHz) and sampled at 5 kHz. Pairs of silver/silver chloride surface electrodes were placed over the FDI muscle belly and associated joint, and a ground electrode was placed on the ventral surface of the right wrist. The TMS scalp position was chosen to produce maximum MEPs amplitude in the FDI muscle. The rMT was defined as the weakest stimulation inducing MEPs ≥ 50 μV with 50% probability (Rossini et al., 1994).

2.2.3 cTBS protocol

Bursts of 3 TMS pulses were delivered at 50 Hz, with each burst repeated every 200 ms (5 Hz) for a total of 600 pulses in 40s (Bertini et al., 2010; Franca et al., 2006; Huang et al., 2005). Active cTBS stimulation was administered with a 70 mm figure-eight stimulation coil connected to a Magstim Rapid2 (The Magstim Company, Carmarthenshire, Wales, UK). Sham stimulation was delivered with the same parameters but through a special placebo coil (The Magstim Company, Carmarthenshire, Wales, UK), which produces similar sounds and sensations on the skin as the coil that was used to deliver active cTBS but produces no effective stimulation. Pulse intensity was set at 80% of rMT, which corresponded on average to 47.35% (± 5.06SD) of maximum stimulator output.

2.2.4 MRI data acquisition

All images were acquired with a Philips Intera 3T Quaser with an 8Ch synergy SENSE head coil. Functional images: 28 AC-PC aligned axial

gradient-echo slices, 4.5 mm thickness, no gap, 3.5 x 3.5 mm in plane, interleaved slice acquisition, single shot EPI; TE = 28 ms, TA= 1.28 s, TR= 1.33 s. T1-weighted structural scans: TR = 7.657 ms, TE = 3.54 ms, flip angle= 8 deg, FoV: x1; x2; x3; 1x1x1 mm voxel size.

2.2.5 Observation task

A set of 36 distinct ActionObs and 36 matching CtrlObs clips (see Figure 2.1A and Table S2.1) were recorded using a digital video camera (Sony DSRPDX10P), elaborated using Adobe Premiere (www.adobe.com) and presented using Presentation (Neurobehavioral systems, Davis, CA). All stimuli started with the actor's right hand entering from the right side of the screen. To vary the kinematics, two female and two male actors (balanced across conditions) acted in the movies. Three movies of the same category formed a 10s block (two movies of 3 s and one of 4 s in each block) and 12 blocks of each condition were presented in a semi-randomized fashion (i.e. no more than 2 repetitions of the same condition in a row). Blocks were separated by an 8-12 (random) s fixation cross. At the end of the ~8 minutes session subjects had to answer four questions, that tested whether subjects watched the movies carefully or not.

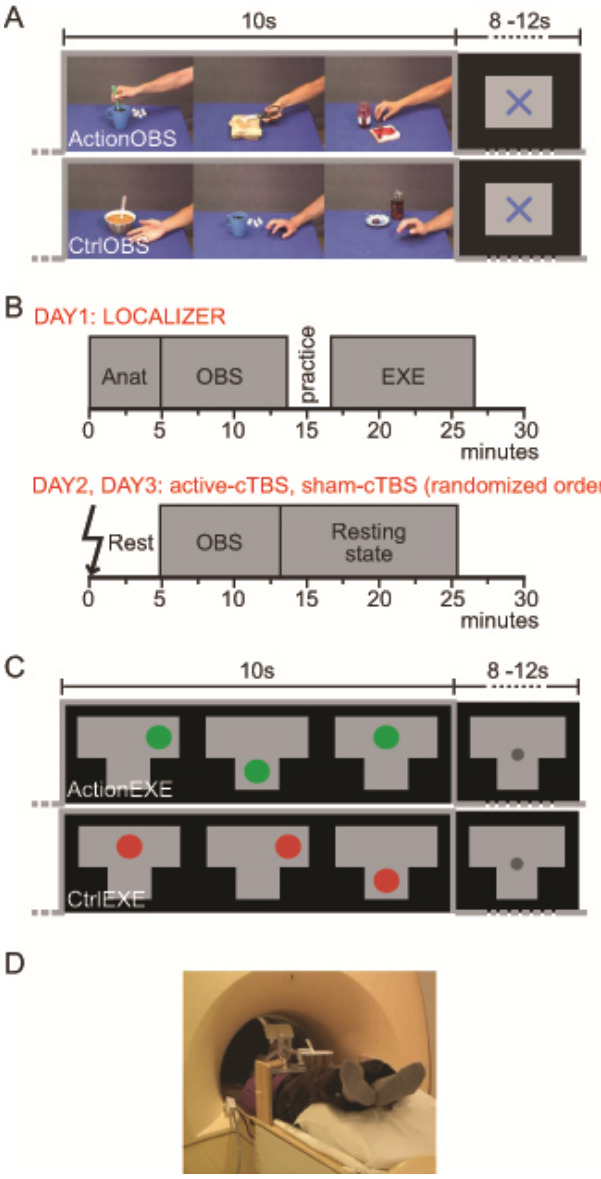

Figure 2.1. Experimental stimuli and design. (A) Timing with example frames of an ActionObs and CtrlObs block. (B) Timeline of the three experimental days. (C) Instruction given to subjects during the ActionExe and CtrlExe tasks. (D) A photograph of the experimental set-up during ActionExe. (D) A photograph of the experimental set-up during ActionExe.

2.2.6 Execution task

A spoon in a bowl, a wine glass and a coffee cup were positioned on three different locations of a T-shaped table placed over the participants' torso (see Figure 2.1C and 2.1D). During each 10s block subjects were required to use the spoon to scoop soup from the bowl, to swirl the wine glass and to grasp the coffee cup, all with their right hand and in a randomized order. Instructions to subjects were projected on a screen which was visible to the subject through mirrors: a green dot appeared on a drawing of the table, in the location corresponding to the object subjects had to act upon. The circle would shrink 3 times to indicate the duration of the action. The total time that the circle would take to go from size 1 to 3 varied between 3s and 4s, to match the duration of the actions shown in ActionObs. As a control condition (CtrlExe) subjects had to track the same (although red instead of green) dot movements presented during ActionExe with their gaze, but without interacting with the objects. As for the observation task, each condition was repeated 12 times, and blocks were presented in a semi-randomized order. A still frame of the shape of the table with a small dark grey dot in the middle separated the blocks (inter-trial interval = 8-12 s). Subjects practiced and rehearsed the task with the experimenter outside the scanner, and in the scanner, before the beginning of the ~8 min session.

2.2.7 Experimental protocol

The experiment was distributed over three days (Figure 2.1B):

Day1. Localization of the shared circuits and the cluster in SI activated by both action observation and execution

Because the (fMRI) definition of shared circuits requires the involvement of the same voxels during both the observation and execution of goal directed actions, to localize the shared circuits, subjects performed both the observation and execution tasks. To prepare the after scanning neuronavigation (Brain Innovation, Maastricht, The Netherlands), the T1 anatomical scan was acquired prior to the functional tasks and immediately processed. After scanning we first evaluated the individual rMT, and then saved the corresponding optimal scalp position using neuronavigation for further use.

Day2 and Day3. Active and sham cTBS

Day2 and Day3 were equal in everything but the type of cTBS protocol randomly assigned to participants before MR scanning: nine participants received sham cTBS during Day2 and active cTBS on Day3; the opposite was true for the remaining eight. Each day started with the re-assessment of the rMT on the scalp position saved during Day1 and the localization of our target point in SI. Subjects were then taken to the MRI preparation room, seated in the MRI bed (previously moved to the preparation room) for about 5 minutes, and asked to relax trying not to move their right (contra-lateral to stimulation) arm. During cTBS administration subjects were left on the bed, which was then pushed into the scanner to minimize subject

movement just before and after cTBS (Gentner et al., 2008; Iezzi et al., 2008; Todd, Flavel, Ridding 2009). In less than 6 minutes (5.2 minutes±0.41SD) the fMRI scanning sequence was initiated, permitting us to capture the cTBS effect when it reached its maximum level (Huang et al., 2005). The MR data acquisition started with the observation task and was followed by a resting state sequence (analysed in Chapter 3).

2.2.8 Target site selection and neuronavigation

T1 images from Day1 were segmented into white and grey matter using BrainVoyager (BV; Brain Innovation, Maastricht, The Netherlands) to reconstruct a participant's head in 3D for neuronavigation. For each subject the functional data from the observation and execution sessions of Day1 were preprocessed in BV (3D motion correction, FWHM 6mm filter spatial smoothing, temporal filtering) and resulting images were co-registered to the anatomy. For each subject's unnormalized data, we identified the section of the somatosensory cortex that (a) belonged to the cluster resulting from inclusively masking the contrast ActionObs-CtrlObs (visualized for most subjects at $p_{unc}<0.001$, although the threshold was lowered in some cases) with the binary map from ActionExe-CtrlExe (all subjects at $p_{unc}<0.001$, min. cluster size 10, $q_{fdr}<0.05$), and (b) fell within the anterior bank of the post-central sulcus and the adjacent crown of the postcentral gyrus (Geyer, Schleicher, Zilles 1999; Grefkes et al., 2001) as the cTBS target point, using neuronavigation on an EEG cap worn by the participant (Figure 2.2B and 2.2C). Mean Talairach coordinates (±SD) for the activation target site were: -42±5.54, -31±6.56, 55±6.43 (transformed

to MNI coordinates using http://imaging.mrc-cbu.cam.ac.uk/downloads/MNI2tal/ : -42 -35 58).

2.2.9 Data preprocessing and analyses.

Except for neuronavigation, all analyses were carried out with SPM8 (Wellcome Department of Imaging Neuroscience, London, UK). Slice time corrected EPI volumes were aligned to the mean EPI image from all 3 days. The T1 grey matter segment was co-registered to the mean EPI, and used to determine normalization parameters applied to all EPI (2x2x2 mm) and structural (1x1x1mm) images. EPIs were then smoothed with a 8 mm FWHM Gaussian kernel.

At the first (subject) level, for each day separately, ActionObs and CtrlObs were modeled with separate predictors as boxcar functions convolved with the hemodynamic response function. The same was done for ActionExe and CtrlExe. Six movement parameters (translations and rotations), which never exceeded the original voxel size, were included as predictors of no interest. Second level analyses were performed as described in the results. Whole brain results were thresholded at the t-level corresponding to p=0.001 ($t_{p=0.001}$) uncorrected. We then used the FDR-correction option of SPM to determine the t-threshold with a false-positive rate of 5% for each contrast ($t_{q=0.05}$). If $t_{q=0.05} < t_{p=0.001}$ (which was true in most cases), results are presented at $t_{p=0.001,}$ implying that results survive FDR-correction. This procedure was preferred to using $t_{q=0.05}$, because the latter varies substantially across results and makes it impossible to compare maps across contrasts. If $t_{q=0.05} > t_{p=0.001}$ (rarely the case), it means that

thresholding at $t_{p=0.001}$ is inappropriate, and we then show maps at $t_{q=0.05}$ in the main text, and show the other contrasts at the same t-threshold in the supplementary materials.

2.2.10 Definition of the target ROI in SI

For each subject individually, a sphere with 1 cm diameter was built with Marsbar (Brett et al., 2002), centred on the MNI target point individuated from Day1 data. The sphere was intersected with the SI anatomical ROI including BA1 and BA2 (Eickhoff et al., 2005; Eickhoff et al., 2006; Eickhoff et al., 2007), and with the individual grey matter segment. The signal from all the voxels within this ROI was then averaged and analysed using Marsbar.

2.2.11 Definition of the ROIs in dPM, vPM and PF

For the premotor ROIs, we first combined left BA6 with left BA44 (Anatomy toolbox for SPM; (Eickhoff et al., 2005; Eickhoff et al., 2006; Eickhoff et al., 2007) to cover the whole premotor area. Because BA6/44 contains the dorsal, ventral premotor and supplementary motor area (SMA), based on visual inspection of the averaged anatomy of our group, the study of Tomassini and colleagues (2007), and on the Harvard-Oxford cortical atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html), we then split (Marsbar; Brett et al., 2002) BA6/44 in three ROIs: voxels with $-13 \leq x \leq +13$ (in MNI) were combined into the SMA ROI, voxels not belonging to SMA with $z \geq 48$, combined into dPM, and those with $z < 48$ into vPM.

The PF ROI was created from the Anatomy toolbox by combining left PF, PFsm, PFm, PFop and PFt (Caspers et al., 2006; Caspers et al., 2008).

2.3 Results

2.3.1 Shared circuits

Shared circuits were mapped at the group level from the data acquired on Day1: the contrast ActionObs-CtrlObs ($T_{(16)}$≥3.69, p_{unc}≤0.001, min cluster size 10; all results also survive q_{fdr}<0.05) was explicitly masked with the binary image resulting from the contrast ActionExe-CtrlExe thresholded at $T_{(16)}$>3.69 (p_{unc}<0.001, min cluster size 10; also surviving q_{fdr}<0.05). In particular, this identified the shared circuits (Table 1) of the parieto-frontal mirror network (including ventral and dorsal premotor cortex, and posterior parietal regions; Figure 2.2A, warm colors) and primary and secondary somatosensory cortices (SI and SII; Figure 2.2A, cold colors), that was expected based on previous literature.

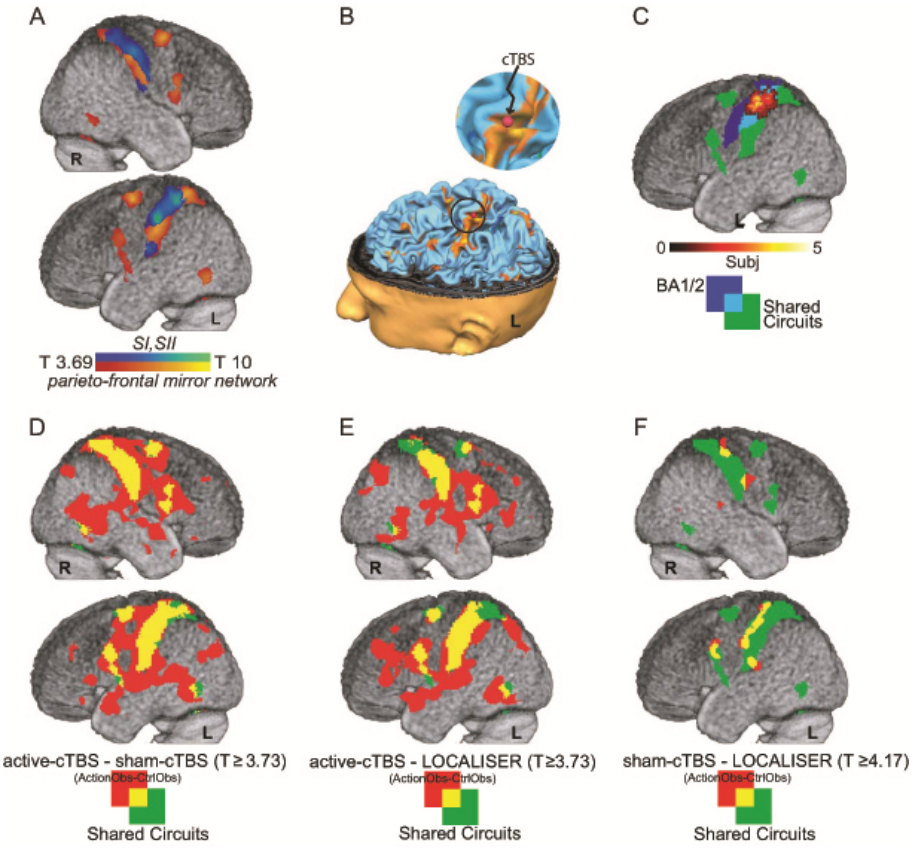

Figure 2.2. (A) Localization of the shared circuits ($p \leq 0.001$, $T_{(16)} \geq 3.69$, $qfdr \leq 0.05$). Warm colors indicate areas classically associated with the parieto-frontal mirror network, cold colors indicate somatosensory regions. (B) Location of the stimulation site (red dot) for one subject as seen on the neuronavigation system. (C) Overlap of the ROIs across subjects, superimposed on the shared circuit map in green (as in A) and the anatomically defined left BA1/2 in blue. Shades of warm colors shows how many subjects' ROIs are included in this voxel. (D-F) Regression analysis results for C, C' ($p \leq 0.001$, $T_{(16)} \geq 3.69$, $qfdr \leq 0.05$) and C'' ($qfdr \leq 0.05$, $T_{(16)} \geq 4.17$), respectively. Green: shared circuits as defined in A. Red: voxels with significant C, C' or C'' regression values. Yellow: overlap between the shared circuits and regression results.

Table 2.1. Group Shared Circuits ($p \leq 0.001$, $T_{(16)} \geq 3.69$, $qfdr \leq 0.05$)**.** From left to right: cluster size in number of voxels; T values, MNI coordinates in mm, hemisphere, anatomical description and, when available, cytoarchitectonic description (as given by the Anatomy toolbox) of the local maxima within the cluster.

Cluster size in n◦ of voxels	T	x	y	z	Hem	Anatomical description	Cytoarchitectonic description
2886	15.18	-50	-26	44	L	Inferior Parietal Lobule	Area 2
	13.49	-30	-42	58	L	Postcentral Gyrus	Area 2
	12.96	-36	-46	60	L	Superior Parietal Lobule	SPL
	10.85	-58	-26	34	L	SupraMarginal Gyrus	IPC
2198	10.22	52	-28	48	R	Postcentral Gyrus	IPC
	9.42	42	-32	40	R	SupraMarginal Gyrus	IPC
	8.12	28	-48	58	R	Superior Parietal Lobule	Area 2
1500	12.65	-26	-6	58	L	Superior Frontal Gyrus	Area 6
	9.04	-6	0	48	L	SMA	Area 6
	6.66	-2	8	30	L	Anterior Cingulate Cortex	

646	15.30	-38	-6	8	L	Insula Lobe	
	6.07	-52	4	28	L	Precentral Gyrus	Area 44
457	10.59	28	-2	62	R	Superior Frontal Gyrus	Area 6
333	7.00	54	8	24	R	Inferior Frontal Gyrus (p. Opercularis)	Area 44
	6.40	40	-4	12	R	Rolandic Operculum	OP 3
	5.83	40	-4	-2	R	Insula Lobe	
217	7.20	32	-58	-26	R	Cerebellum (VI)	
159	4.87	-28	-62	-20	L	Cerebellum (VI)	
99	8.75	-50	-70	-8	L	Inferior Occipital Gyrus	
82	5.03	-12	-20	-2	L	Thalamus	
74	4.54	-24	10	-2	L	Putamen	
69	5.90	-6	-78	-4	L	Lingual Gyrus	Area 17
49	5.36	54	-62	-10	R	Inferior Temporal Gyrus	hOC5

Table 2.2. Remote effect of cTBS. Activations resulting from GLM regression analyses calculated for C, C' and C''. Conventions and abbreviations as in Table 2.1.

Cluster size in n◦ of voxels	T	x	y	z	hem	Anatomical description	Cytoarchitectonic description
Contrast C ($p \leq 0.001$, $T_{(16)} \geq 3.69$, $qfdr \leq 0.05$)**:** $cTBS_{(ActionObs\text{-}CtrlObs)}$-$SHAM_{(ActionObs\text{-}CtrlObs)}$							
22792	17.45	-54	-24	50	L	Inferior Parietal Lobule	Area 1
	15.70	-46	-30	56	L	Postcentral Gyrus	Area 1
	11.73	66	-18	24	R	SupraMarginal Gyrus	IPC
	10.98	40	-34	54	R	Postcentral Gyrus	Area 2
	10.69	-60	-20	38	L	SupraMarginal Gyrus	Aera 2
	9.70	-32	-12	60	L	Precentral Gyrus	Area 6
	9.45	26	2	66	R	Superior Frontal Gyrus	Area 6
	9.29	38	-46	62	R	Superior Parietal Lobule	SPL

5457	10.05	-4	-56	-6	L	Cerebellum (IV – V)	
	9.56	24	-62	-19	R	Cerebellum (VI)	
	7.45	28	-42	-28	R	Cerebellum (IV – V)	
	7.45	-34	-84	30	L	Middle Occipital Gyrus	IPC
	7.14	-22	-58	-26	L	Cerebellum (VI)	
	6.84	-24	-42	-20	L	Fusiform Gyrus	
	6.72	-18	-50	-10	L	Linual Gyrus	
	6.61	6	-62	20	R	Cuneus	SPL
426	5.01	8	-20	-2	R	Thalamus	
121	6.21	-20	10	-16	L	Olfactory cortex	
	5.01	-16	4	-20	L	ParaHippocampal Gyrus	
120	4.64	-34	-22	-28	L	Fusiform Gyrus	Hipp (EC)
102	4.73	-30	36	24	L	Middle Frontal Gyrus	Area 44
96	5.39	42	14	-30	R	Temporal Pole	

	4.65	30	2	-26	R	Amygdala	Amyg
80	4.96	-26	-70	46	L	Superior Parietal Lobule	SPL
74	4.81	-20	-58	12	L	Calcarine Gyrus	Aera 18
74	5.72	-42	22	4	L	Inferior Frontal Gyrus (p. Triangularis)	Area 45/ 45
39	4.43	10	-20	36	R	Middle Cingulate Cortex	
26	4.00	26	50	8	R	Superior Frontal Gyrus	
25	4.02	-6	-18	4	L	Thalamus	
24	4.34	-36	30	-12	L	Inferior Frontal Gyrus (p. Orbitalis)	
22	4.18	26	-20	-20	R	ParaHippocampal Gyrus	Hipp
21	4.08	6	64	20	R	Superior Medial Gyrus	
20	5.45	26	18	2	R	Putamen	
18	4.55	10	56	6	R	Superior Medial Gyrus	
17	4.49	-10	-52	42	L	Precuneus	

17	4.26	8	58	30	R	Superior Medial Gyrus	
12	4.07	-10	56	8	L	Superior Medial Gyrus	
11	4.29	32	-40	-12	R	Fusiform Gyrus	Hipp
11	4.21	36	30	-16	R	Inferior Frontal Gyrus (p. Orbitalis)	
Contrast C' ($p \leq 0.001$, $T_{(16)} \geq 3.69$, $qfdr \leq 0.05$)**:** $cTBS_{(ActionObs-CtrlObs)}$-$LOCALISER_{(ActionObs-CtrlObs)}$							
16107	11.97	-44	-36	61	L	Postcentral Gyrus	Area 2
	9.09	-58	-24	44	L	Inferior Parietal Lobule	Area 2
	8.93	-60	-22	42	L	SupraMarginal Gyrus	Area 2
	8.90	-60	-22	36	L	SupraMarginal Gyrus	IPC
	8.67	60	-14	34	R	Postcentral Gyrus	Area 1
	8.18	-52	4	16	L	Precentral Gyrus	Area 44
	8.14	-46	6	18	L	Inferior Frontal Gyrus (p. Opercularis)	Area 44
1650	7.42	-20	-10	60	L		Area 6

	6.93	-30	-2	50	L	Middle Frontal Gyrus	Area 6
	6.68	-4	-20	44	L	Middle Cingulate Cortex	Area 6
	6.40	-30	-2	44	L	Precentral Gyrus	
	5.11	-4	-4	50	L	SMA	Area 6
	4.85	4	-30	40	R	Middle Cingulate Cortex	SPL
611	6.79	22	36	28	R	Superior Frontal Gyrus	
	5.35	24	16	52	R	Middle Frontal Gyrus	
	4.95	20	10	54	R	Superior Frontal Gyrus	Area 6
520	5.53	28	-78	46	R	Superior Occipital Gyrus	SPL
	5.33	34	-76	46	R	Superior Occipital Gyrus	IPC
	5.17	14	-78	50	R	Superior Parietal Lobule	SPL
	4.68	46	-72	36	R	Angular Gyrus	IPC
	3.92	32	-62	50	R	Superior Parietal Lobule	hIP3
501	7.18	-28	-72	51	L	Inferior Parietal Lobule	IPC

	4.94	-32	-68	28	L	Middle Occipital Gyrus	IPC
	4.84	-24	-66	28	L	Superior Occipital Gyrus	hIP1
494	7.31	-60	-54	-6	L	Inferior Temporal Gyrus	
	5.10	-48	-68	-14	L	Inferior Occipital Gyrus	
	4.26	-42	-76	-14	L	Inferior Occipital Gyrus	hOC4v
385	6.46	60	-54	-6	R	Inferior Temporal Gyrus	
	4.95	44	-68	-14	R	Inferior Occipital Gyrus	hOC4v
	4.92	56	-54	8	R	Middle Temporal Gyrus	
206	7.01	6	6	28	R	Anterior Cingulate Cortex	
	4.62	-4	6	28	L	Anterior Cingulate Cortex	
165	5.84	46	36	0	R	Inferior Frontal Gyrus (p. Triangularis)	Area 45
163	5.06	-10	14	38	L	Middle Cingulate Cortex	
	4.66	-28	24	46	L	Middle Frontal Gyrus	

	4.41	-14	16	46	L	Superior Frontal Gyrus	Area 6
	4.12	-6	24	48	L	SMA	
	4.10	-8	20	52	L	SMA	Area 6
137	4.69	-12	-64	56	L	Precuneus	SPL
61	4.24	0	34	48	L	Superior Medial Gyrus	
51	4.57	26	-88	24	R	Superior Occipital Gyrus	Area 18
	4.12	30	-82	26	R	Middle Occipital Gyrus	
34	5.12	20	-32	8	R	Hippocampus	Hipp
27	4.53	-10	38	20	L	Anterior Cingulate Cortex	
27	4.27	10	24	20	R	Anterior Cingulate Cortex	
20	3.95	-4	8	50	L	SMA	Area 6
	3.86	4	8	50	R	SMA	Area 6
20	4.59	30	52	2	R	Middle Frontal Gyrus	
16	4.52	40	20	-6	R	Insula Lobe	

10	3.95	-10	-54	66	L	Precuneus	SPL
Contrast C'' (qfdr≤0.05, $T_{(16)}$≥4.17)**:** $SHAM_{(ActionObs-CtrlObs)}$-$LOCALISER_{(ActionObs-CtrlObs)}$							
263	6.32	-54	-26	48	L	Inferior Parietal Lobule	Area 2
	6.23	-60	-18	34	L	Postcentral Gyrus	IPC
	5.68	-32	-32	64	L	Postcentral Gyrus	Area 1
	5.47	-44	-30	54	L	Postcentral Gyrus	Area 2
173	6.53	42	-32	64	R	Postcentral Gyrus	Area 1
158	6.25	26	-72	26	R	Superior Occipital Gyrus	
136	5.76	18	-70	-4	R	Lingual Gyrus	hOC3v
	4.40	28	-62	-6	R	Fusiform Gyrus	Area 18
126	6.38	-56	8	34	L	Precentral Gyrus	Area 6
	4.49	-52	6	24	L	Inferior Frontal Gyrus (p. Opercularis)	Area 44
82	6.37	-62	-28	16	L	Superior Temporal Gyrus	OP1

	5.63	-52	-22	24	L	SupraMarginal Gyrus	OP1
59	5.45	60	-12	34	R	Postcentral Gyrus	Area 1
	4.20	62	-14	26	R	Postcentral Gyrus	Area 3b
59	5.06	54	-2	-12	R	Superior Temporal Gyrus	
	4.97	50	4	-22	R	Middle Temporal Gyrus	
54	5.30	-50	-20	-10	L	Middle Temporal Gyrus	
35	4.99	52	-30	12	R	Superior Temporal Gyrus	IPC
26	5.70	-40	-30	41	L	Postcentral Gyrus	IPC
14	5.62	-26	-2	-24	L	Amygdala	Amyg
13	5.08	-22	-36	-26	L	Cerebellum (IV – V)	
10	4.49	-12	-46	-10	L	Lingual Gyrus	
	4.47	-8	-48	-10	L	Cerebellum (IV – V)	

2.3.2 Effect of active cTBS on the targeted ROI in SI

To examine the effect active cTBS had on stimulus processing in the target location, we extracted, from the target ROI in SI for each participant *i* (Experimental procedures and Figure 2.2B and 2.2C), parameter estimates of the contrast *Ci* = active $cTBS_{(ActionObs\text{-}CtrlObs)}$ – sham $cTBS_{(ActionObs\text{-}CtrlObs)}$. *C* showed substantial inter-individual variability, with some participants showing a reduction of the BOLD signal in the somatosensory cortex (*C*<0; parameter estimates in arbitrary units from -0.3 to -0.04; n=8) and some an increase (*C*>0; parameter estimates in arbitrary units from +0.05 to +1.47; n=9). To examine whether this variability was due, at least in part, to the effect of active cTBS, or only to random fluctuations between two scanning sessions, we calculated a similar contrast between the active cTBS and LOCALISER conditions, *Ci'* = active $cTBS_{(ActionObs\text{-}CtrlObs)}$ - $LOCALISER_{(ActionObs\text{-}CtrlObs)}$ and the sham cTBS and LOCALISER conditions, *Ci''* = sham $cTBS_{(ActionObs\text{-}CtrlObs)}$ - $LOCALISER_{(ActionObs\text{-}CtrlObs)}$. If active cTBS indeed acts in a way that can be conceived in analogy to 'injecting noise' in the target location (Ruzzoli, Marzi, Miniussi 2010; Silvanto and Muggleton 2008; Walsh and Pascual-Leone 2005) with different modulatory effects across subjects (Gangitano et al., 2002; Hamada et al., 2012; Hamidi et al., 2009; Ridding and Ziemann 2010), we should see differences between the distributions of *C'* and *C''*, with *C'* showing a larger spread of values than *C''* because only the former includes cTBS effects in addition to spontaneous variance. Comparing the distributions of *C'* and *C''* using the paired-sample Kolmogorov-Smirnov test (a nonparametric test for equality of continuous distributions) confirmed a larger spread for *C'* ($p_{(one\ tailed)}$<0.04, *C'* parameter estimates in arbitrary units from -0.64 to 0.93; *C''* from -0.68 to 0.22). This supports the notion that cTBS injects noise, in the sense of between

participant variance (not within subject variance) into the target ROI in SI, when compared to sham cTBS. Comparing *C* with *C''* using the same method also reveals a significant difference in distributions ($p_{(one\ tailed)}$<0.0015), while comparing *C* with *C'* does not ($p_{(one\ tailed)}$>0.19). This jointly shows that contrasts including an active cTBS session (*C* and *C'*) differ from those only including spontaneous fluctuations (*C''*). Given that active cTBS shaped the distribution of activations in the target ROI in SI, we first confirmed that *C* remained normally distributed (Lilliefors-test; k=0.11, p=0.19). The same test on *C'* and *C''* also did not reject normality (both p>0.48).

2.3.3 The Remote effects of active cTBS

Harnessing the variability in the active cTBS effect across individuals, to explore the relation between the targeted ROI in SI and the parieto-frontal mirror network during action perception, we used a regression analysis to examine if the effect of cTBS on SI induced changes in remote regions of this network. We reasoned that if a given voxel j receives excitatory input from the target ROI in SI, remote effects should mirror the local effects, with participants for whom active cTBS increased activity in the target ROI in SI (contrast $C_{SI\ (i)}$>0) showing increased activity in this voxel j ($C_{i,j}$>0), and participants for whom active cTBS reduced activity in the target ROI in SI ($C_{SI\ (i)}$<0) showing reduced activity in voxel j ($C_{i,j}$<0). Hence, we computed a general linear model (GLM) of the form $C_{j,i}=a*C_{SI,i}+error_i$, and tested H_0:a≤0 against the alternative hypothesis H_1:a>0, taking into account all 17 participants. This regression analysis revealed a large bilateral network encompassing the dorsal and ventral premotor cortex, and the rostral

inferior parietal lobule, SI, primary motor cortex and regions of the middle temporal gyrus (Table 2.2, Figure 2.2D). Amongst the 8792 voxels localized to belong to shared circuits on Day 1 (Figure 2.2A, and green in 2.2C and 2.2F), 6373 (72.5%, Figure 2.2D yellow) were found to have activation changes (C_j) significantly predicted by changes associated with cTBS on SI (C_{SI}). Importantly, although the remote effects predicted by C_{SI} were not restricted (red in Figure 2.2D) to the shared circuits identified, there was a topographic similarity between the spatial maps retrieved by this regression analysis and the shared circuits.

To verify that the results found in the above regression analysis depend on the effect of active cTBS on the target ROI in SI, rather than on unspecific fluctuations across days, we repeated the analysis using the contrasts *C'* and *C''* as defined above, and the models $C'_{j,i}=a'*C'_{SI,i}+error'_i$; $C''_{j,i}=a''*C''_{SI,i}+error''_i$ (Figure 2.2E and 2.2F). Results confirmed that regression analyses including the cTBS data (*C* or *C'*, Figure 2.2D and 2.2E) resulted in a larger network of regions (29768 voxels for *C*, 21310 voxels for *C'*) influenced by SI than that restricted to spontaneous fluctuations (1269 voxels for *C''*, Figure 2.2F). A chi-square test comparing the number of voxels confirms that regressions using spontaneous fluctuations alone (*C''*) result in fewer significant voxels than regressions which include the cTBS session (*C'* and *C''*, both $p_{(one\ tailed)}<0.0001$, chi-square test). The same result is obtained if only significant voxels within shared circuits are compared (yellow in Figure 2.2D-F; *C*:6373, *C'*:4139, *C''*:434; *C*>*C''* $p_{(one\ tailed)}<0.0001$, *C'*>*C''* $p_{(one\ tailed)}<0.0001$).

In the regression analyses, the FDR-correction imposed a higher T-threshold on the results for *C''* (T≥4.17) than for *C* and *C'* (both T≥3.73).

However, even if imposing the stricter threshold (T≥4.17; Figure S2.1) on all regressions, the regressions for *C* and *C*′ still result in significantly larger networks than the regression for *C*′′ (in shared circuits, *C*:5339, *C*′:3238, *C*′′:434; Chi-Square, $p_{(one\ tailed)}<0.0001$). This suggests that by increasing the spread of *C* values, cTBS provides more power to detect regions receiving action specific input from SI.

That the brain networks identified by regressions based on *C* or *C*′ look different from that based on *C*′′ could, as mentioned above, be explained by the increase in inter-individual variability in C_{SI} caused by cTBS. Additionally, or alternatively, active cTBS might have changed the efficacy of the connections between the target ROI in SI and the rest of the brain. The efficacy of the connection can be operationalised as the slope or parameter estimate *a* in the abovementioned GLMs, which represents how much of a change in activation in any given voxel j is explained by a unit of change in activation in the target ROI in SI. If active cTBS changed the efficacy of these connections, *a*′ should differ from *a*′′. A whole brain analysis comparing *a*′ and *a*′′ using a multiple regression analysis did not reveal any significant difference ($q_{fdr}>0.05$). Accordingly, the increase of significant voxels in the whole brain regression analysis, when including active cTBS, does not seem to be due to a change in the strength and pattern of connectivity. Instead, active cTBS, by actively changing the distribution of *C*, seems to increase the number of voxels in the parieto-frontal mirror network whose activity correlates with the activity in the stimulated area of SI. This signal can then be detected more effectively in remote locations than without active cTBS, and reveals pathways that the

limited power of traditional group analysis fails to reveal otherwise (see Figure S2.2 for a more graphic illustration of this effect).

Because the inferior parietal cortex (area PF in particular) is classically considered to be the main source of information to ventral and dorsal premotor regions (vPM, dPM) during action observation (Rozzi et al., 2008), we explored if the predictive power of C_{SI} on C_{vPM} and C_{dPM} might be entirely mediated by $C_{PF.}$ Accordingly, we localized the region of the shared circuits network obtained from the LOCALISER task that overlapped with PF, and calculated C_{PF} from the mean activation in this ROI (see Experimental procedures). Table 3 shows that although *C* values in the target ROI in SI and PF both significantly correlate with vPM and dPM (top two rows), the correlation between *C* values in the target ROI in SI and dPM remains significant after removing the variance explained by PF signals (lowest row). The correlation between the target ROI in SI and vPM, on the other hand, does not.

Table 2.3. Correlation values (r) and their level of significance (p) between C_{SI} and C_{PF} on the one hand, and C_{vPM} and C_{dPM} on the other. C_{SI}*C_{PF} shows the partial correlations results, i.e. r(C_{SI}, C_{vPM}) and r(C_{SI}, C_{dPM}) after taking out the variance explained by C_{PF} using the function 'partialcorr' in Matlab with one-tailed testing.

	C_{vPM}		**C_{dPM}**	
	r	P	r	p
C_{SI}	r=0.7914	p<0.001	r=0.8581	p<0.001
C_{PF}	r=0.8485	p<0.001	r=0.8627	p<0.001
C_{SI}*C_{PF}	r=0.2236	p<0.2026	r=0.4413	p<0.0435

Finally, we performed a number of control analyses to exclude confounds. First, we replicated the analyses shown in Figure 2.2D to 2.2F using non-parametric tests (SnPM; Figure S2.1). As expected, given that non-parametric analyses have less statistical power, SnPM revealed more restricted networks, but confirmed that regressions involving active cTBS (*C* and *C'*) predict premotor activation changes in the ipsilateral hemisphere, while those not involving active cTBS (*C''*), did not. Similar to the parametric analysis, the activation changes in the shared voxels that were predicted by the activation changes in the target ROI in SI using *C* and *C'* significantly outnumbered those predicted using *C''* (Chi-Square test, both $p_{(one\ tailed)}$<0.0001). Second, the global parameters from the active cTBS and sham cTBS sessions (i.e. the time-constant parameters in the GLM) were compared with a T-test to examine if active cTBS systematically altered baseline activity. No significant differences were found between these

sessions (whole brain, $q_{fdr}>0.05$). Third, to investigate if active cTBS affected the goodness of fit of the GLM we compared the residual errors from first-level GLMs fitted to the sham cTBS and active cTBS data using non-parametric permutation tests across subjects (SnPM, because the sums or squares of errors were not normally distributed), and found no significant differences ($q_{fdr}>0.05$). Fourth, to investigate whether the effect revealed by the regression analyses is specific for the contrast ActionObs-CtrlObs, we repeated the main regression analyses exploring the difference between active cTBS and sham cTBS using ActionObs only and ActionCtrl only (Figure S2.1). For both ActionObs and CtrlObs, changes in premotor shared voxels were predicted better by the changes in the target ROI in SI when including active cTBS sessions compared to not including active cTBS (Chi-Square, $p_{(one\ tailed)}<0.001$) and the networks revealed by analyzing ActionObs and CtrlObs alone were similar to those that resulted when analyzing the contrast ActionObs-CtrlObs. Fifth, we explored if the effect of cTBS changed over the time of our fMRI session. We modelled each ActionObs and CtrlObs block separately to generate a single parameter estimate for each occurrence of ActionObs and for each occurrence of CtrlObs. We then calculated *C* in each voxel j separately for each occurrence (i.e. contrasting the first occurrence of ActionObs with CtrlObs, then doing the same for the second occurrence etc.), and used an ANOVA with 36 occurrences to see if *C* changed as a function of occurrence. We did not find evidence for such an effect at $q_{fdr}<0.05$. Sixth, the notion that the connectivity between the targeted ROI in SI and the rest of the brain was changed by active cTBS was further tested by performing PPI (see Supplemental Experimental Procedure 2.2.1). No voxels showed significant

alterations of functional connectivity with the targeted ROI in SI between the active cTBS and sham cTBS conditions in this analysis (q_{fdr}>0.05).

2.4 Discussion

Most models of action observation emphasize the role played by visual cortices and regions of the parieto-frontal mirror network associated with motor functions. We have claimed that the posterior aspects of the primary somatosensory cortices (BA1 and BA2, but not BA3a and BA3b) may also play an important role in processing the actions of others (Keysers, Kaas, Gazzola 2010). This notion finds support from the following facts: lesions in the somatosensory system can impair the capacity to recognize facial actions (Adolphs et al., 2000), SI is the region most consistently activated across viewers of goal directed actions (Gazzola and Keysers 2009), and quantitative meta-analyses have confirmed that SI is consistently recruited during action observation across studies (Caspers et al., 2010).

The aim of this experiment was to investigate whether information processing in SI is related to information processing in the parieto-frontal mirror network. We identified the region of SI involved in action observation and action execution in each subject, used active cTBS to perturb brain activity in this region and then measured the effects of this perturbation elsewhere in the brain while subjects viewed the actions of other people. In this way we evaluated the functional connectivity of SI during action observation.

As outlined in the introduction, we expected the local effect of active cTBS on BOLD activity in the targeted ROI in SI to vary across individuals (Hamada et al., 2012; Ridding and Ziemann 2010; Teo et al., 2011). Such variance across individuals would also explain why previous

studies failed to consistently find a reduction of local activity following 'inhibitory' TMS (either standard low-frequency repetitive TMS or cTBS): some studies failed to find any consistent effect (Arfeller et al., 2012; Conchou et al., 2009; O'Shea et al., 2007; Ott et al., 2011; van Nuenen et al., 2012) others found increased local activity (Chouinard et al., 2003; Havrankova et al., 2010; Lee et al., 2003; Rounis et al., 2005; Stagg et al., 2009), and some found the expected BOLD decrease (Hubl et al., 2008; Volman et al., 2011; Ward et al., 2010). To our knowledge this is the first study of off-line cTBS over SI in combination with fMRI and based on the literature (Hamada et al., 2012; Ridding and Ziemann 2010) we expected our results, as expressed by the change in BOLD, to vary across subjects. Extracting brain activation from the targeted ROI in SI confirmed our expectation: comparing the active cTBS and sham cTBS sessions revealed that some participants showed a decrease and some an increase in the contrast ActionObs-CtrlObs. Comparing changes in brain activity induced by active cTBS with spontaneous fluctuations across two days revealed that active cTBS had broadened the distribution of action observation related brain activity across participants. We then used the increase in spread to explore the connectivity of SI, by identifying voxels elsewhere in the brain, where brain activity changes were predicted by those experimentally induced in the targeted ROI in SI. This revealed a network of regions that encompassed 70% of shared circuit voxels identified using our LOCALISER task, including the dorsal and ventral premotor cortex, and the inferior parietal lobe classically associated with the parieto-frontal mirror network.

In previous TMS/fMRI studies, it was found that local changes induced by active cTBS on the frontal eye-fields go hand in hand with brain

activation changes in the visual cortex, and these remote effects have been interpreted as strong evidence that the frontal eye-fields have a causal backward influence on brain activation in the visual cortex (Driver et al., 2009; Hubl et al., 2008; Reithler, Peters, Sack 2011; Ruff et al., 2006). Although a word of caution is needed when interpreting our results, our stimulation over SI seems to have selectively affected the functional connectivity of this area with the premotor cortices. However, we do not have a direct measurement of the effect of active cTBS over SI, since our subjects performed a passive task (and therefore a change in performance could not be observed) and the BOLD signal in the stimulated area did not change significantly and uniformly across subjects.

The presence of anatomical connections between SI and the ipsilateral premotor and inferior parietal nodes of the mirror neuron system in monkeys (Keysers, Kaas, Gazzola 2010) suggests that humans have the anatomical routes for the influence of SI on the premotor and inferior parietal nodes of the parieto-frontal mirror network. Additionally, strong connections exist between the left and right SI (Keysers, Kaas, Gazzola 2010), providing an anatomical basis for the strong effects we also measured in the right, unstimulated hemisphere. It is generally believed that during the execution of goal directed actions, regions involved in somatosensation and motor programming engage in intensive cross-talk (Franklin and Wolpert 2011; Pearson, Budzilovich, Finegold 1971). Disrupting somatosensory processing is indeed known to impair motor performance (Aschersleben, Gehrke, Prinz 2001; Gordon, Ghilardi, Ghez 1995; Pavlides, Miyashita, Asanuma 1993; Schabrun, Ridding, Miles 2008), showing a clear causal influence of the somatosensory system on the

motor system during action execution. In contrast, some of the most authoritative reviews on the neural basis of action observation, either do not mention the somatosensory system at all (Cattaneo and Rizzolatti 2009) or see it as an 'additional' system merely receiving information from the parieto-frontal mirror network (Rizzolatti and Sinigaglia 2010). This point of view implicitly suggests that the anatomical connections from SI to premotor and posterior parietal regions are dormant during action observation. Our data suggest otherwise. First, that a manipulation of activation in SI seems to carry over to premotor and posterior parietal locations shows that information flows between the targeted ROI in SI and the parieto-frontal mirror network. Second, by using the contrast between ActionObs and CtrlObs we analyse brain activations caused by the observation of meaningful hand-object interactions. Our analyses show that information is exchanged between the targeted region in SI and the premotor areas specifically during observation of meaningful actions. However, how crucial and relevant this information is for the task at hand remains to be shown. If we assume that the functional connection that we found between SI and the premotor regions during action observation is crucial, this would suggest that when we watch somebody act our brain is engaged in computing not only the kinematic consequences of the observed behaviour but also the somatosensory ones.

Before accepting this conclusion, it is important to contemplate alternative explanations of our results. First, because we employ a regression analysis to identify regions receiving information from SI, a possible alternative explanation is that we are not exploring the contribution of SI to the parieto-frontal mirror network, but merely the

correlation between spontaneous fluctuations across time in both regions. Such correlational approaches based on spontaneous fluctuations alone are often used in the analysis of resting state data (Fox and Raichle 2007). That active cTBS changed the distribution of brain activity in the targeted ROI in SI and that the regression analyses evidenced a network of meaningful connections that was much wider when including the active cTBS day (compared to the analysis including only the LOCALISER and sham cTBS day) jointly suggests that the results of our study reflect a possible influence of active cTBS on SI to distal regions of the parieto-frontal mirror network. However, we cannot rule out the possibility that our regression results reflect compensatory mechanisms in the brain after active cTBS has been delivered over SI. A more direct approach to show a causal connection between SI and the premotor regions during action observation could employ combined on-line TMS and fMRI. Second, we might not actually have measured the direct influence on the premotor regions of active cTBS on SI, but the indirect influence of active cTBS onto the nearby rostral inferior parietal lobule (area PF), well known to influence the premotor areas (Caspers et al., 2010; Cattaneo et al., 2009). Two observations speak against this. First, the direct electro-magnetic effects of focal sub-threshold TMS are spatially confined, and PF was too far from the point of active cTBS application to have received the direct effects of magnetic stimulation. Second, for dPM, the explanatory power of SI activity changes remained significant after removing the variance that can be explained by changes in PF, showing that PF did not entirely mediate the distal neuronal effects of our active cTBS as applied to the target ROI in SI on the premotor cortices — the distal effects on vPM, on the other hand,

could have been mediated by the connections between the PF complex and SI, and the PF complex and vPM (Rozzi et al., 2006), as suggested by a loss of significance in the mediation analysis in Table 3. Accordingly, the premotor connections we found seem to include a pathway from SI partially, but not entirely mediated by PF.

An important finding of our study is that the actual connectivity between brain regions was not significantly changed by our active cTBS manipulation. In particular, comparing the 'gain' between the stimulated area in SI and the rest of the brain, as measured at the second level of analysis using the coefficient a in our regression GLM, did not reveal differences between conditions where cTBS was or was not delivered. A PPI analysis also failed to detect changes in connectivity strength. Therefore, active cTBS in our experiment seems to have left the connectivity from SI relatively unchanged. Instead it seems to have perturbed the brain activity specifically related to observing meaningful hand actions. stimulus specific brain activity in SI, as shown by limiting our analysis to the contrast ActionObs – CtrlObs and this perturbation can be traced along an unaltered connectivity towards the distal brain regions of the parieto-frontal mirror network that normally receive stimulus specific input from that region.

While in this study, we focus on investigating the information flow between SI and the parieto-frontal mirror network during action observation, in a separate experiment, we tested, using active cTBS, how crucial is activity in SI for the optimal performance in a task involving action observation. We used a task in which participants see a box being lifted and have to judge the weight of the box from the action kinematics alone, a task that has previously been used to show that the ventral premotor

cortex is necessary for action perception (Pobric and Hamilton 2006). We found that active cTBS over SI (but not over nearby control sites) indeed impaired the accuracy with which participants judged the actions of others (see Chapter 4). In our view, this shows that SI in not only activated by action observation and functionally connected to the parieto-frontal mirror network, but that the information transferred during this task is essential for the optimal performance.

In conclusion, by using cTBS to alter brain activity in remote interconnected regions we found evidence that the premotor cortices, part of the parieto-frontal mirror network, are functionally connected to SI specifically during action observation. This suggests that when watching another person act upon an object the brain activates a network of areas which includes both the parieto-frontal mirror network and the somatosensory cortex. The brain may thus use the tight connections of the somatosensory and motor cortex evolved for motor control to generate dynamic representations of other people's actions in a sensori-motor format — rather than generating separate somatosensory and motor representations. In addition, our study confirms the utility of off-line active cTBS in fMRI connectivity analyses, by showing that this type of active cTBS, without disturbing the connectivity it probes, allows to identify a network of distal influences that cannot be found using the spontaneous fluctuations of brain activity across different days.

Acknowledgements

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2.5 Supplementary materials

2.5.1 Experimental stimuli list.

Table S2.1, related to Figure 2.1A and text: Experimental video-stimuli. List of actions used in the ActionObs task and their durations.

N◦	Description of the recorded actions	Movie duration
1	Stirring coffee with a spoon.	3s
2	Putting a cube of sugar in a cup of coffee.	3s
3	Closing a box with a key.	3s
4	Lighting a candle with a lighter.	3s
5	Putting a flower in a vase	3s
6	Putting a battery in a remote control.	3s
7	Putting a CD on a CDs stack.	4s
8	Hammering a nail.	4s
9	Putting whipped cream on strawberries.	4s
10	Cutting a deck of cards.	3s
11	Placing jewellery in a box.	3s
12	Crumpling a paper sheet.	3s
13	Closing a box of chewing gums.	3s
14	Putting a pin on a foam base.	3s
15	Taking hand cream from a tin.	3s
16	Taking some tape and placing it on a box.	4s
17	Pouring wine in a glass	4s

18	Watering a plant.	4s
19	Stirring eggs.	3s
20	Closing a water bottle.	3s
21	Flipping through a block note.	3s
22	Taking an olive from a jar.	3s
23	Putting a candle in a candleholder.	3s
24	Closing a folder.	3s
25	Cracking walnuts.	4s
26	Placing a wine bottle in a box.	4s
27	Opening a suitcase.	4s
28	Spreading jam on a piece of bread.	3s
29	Cutting a ribbon on a package.	3s
30	Stirring soup with a spoon.	3s
31	Putting business cards in a box.	3s
32	Putting a hair clip in a purse.	3s
33	Disconnecting headphones from an MP3 player.	3s
34	Breaking an egg on the edge of a bowl.	4s
35	Stirring a painting brush in a cup of water.	4s
36	Taking a walnut with chopsticks and placing it in a box.	4s

2.5.2 Additional regression analyses

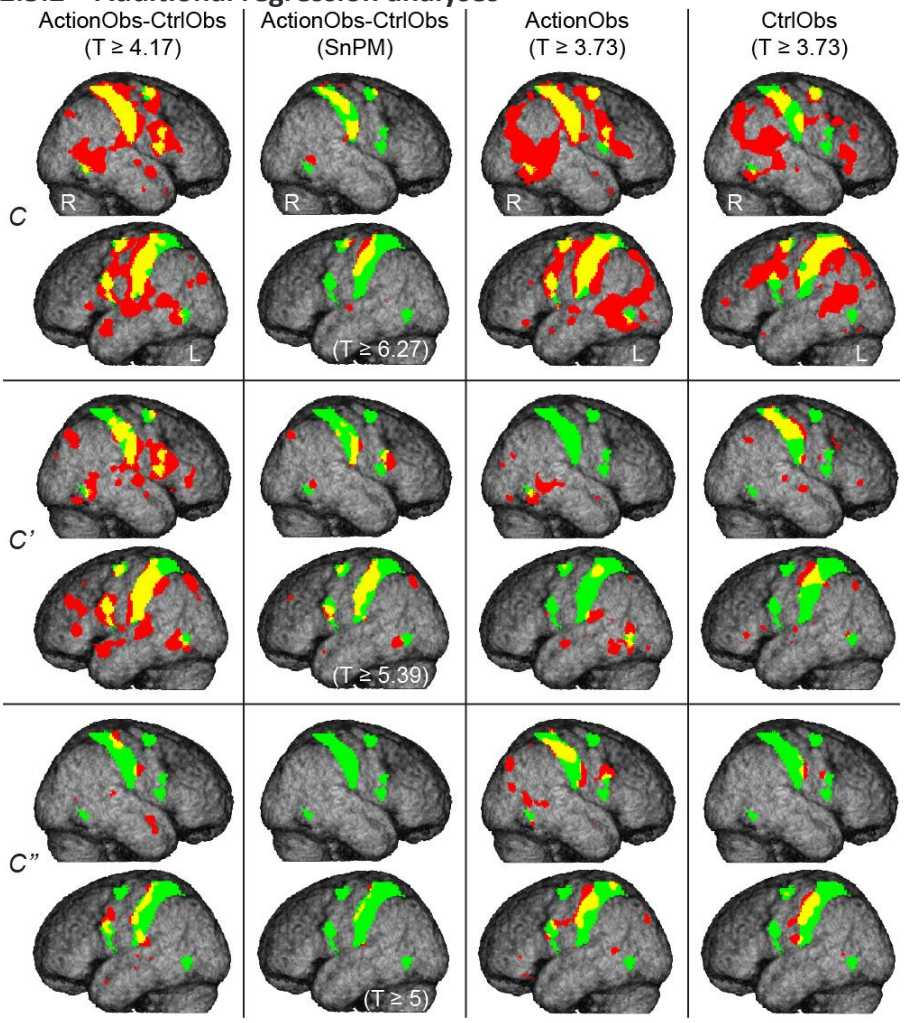

Figure S2.1, related to Figure 2.2D to 2.2F and text: Additional regression analyses results for *C*, *C'* and *C''*. *First column on the left*: same as in Figure 2.2D-F, but all at T≥4.17. *Second column*: same but using non-parametric statistics (SnPM). For each contrast (*C*, *C'* and *C''*) the pseudo T-maps for the negative and positive effects were computed at p_{FWE} <0.05 (5000

permutations). *Third and fourth columns*: regression analyses exploring the difference between active cTBS and sham cTBS using ActionObs and ActionCtrl separately. The contrasts active $cTBS_{(ActionObs)}$ – sham $cTBS_{(ActionObs)}$ and active $cTBS_{(CtrlObs)}$ – sham $cTBS_{(CtrlObs)}$ were calculated, in analogy to *C*, *C*' and *C*''. For *C*, comparing the number of shared circuit voxels predicted in each case (yellow voxels) showed that the regression based on ActionObs-ActionCtrl (6373 voxels) identified more significant voxels than that based on ActionObs (5828, Chi-Square, $p<0.0001$), which in turn identified more significant voxels than that based on ActionCtrl (2951, Chi-Square, $p<0.0001$). A direct comparison of the three regressions did not reveal differences in slope ($q_{fdr}>0.05$). We additionally calculated the contrasts analogous to *C'* and *C''*. For both ActionObs and CtrlObs, changes in premotor shared voxels were predicted better by changes in the target point ROI in SI when including the active cTBS session (*C* and *C'* analogous) compared to not including cTBS (Chi-Square, $p<0.001$) and the networks revealed by analyzing ActionObs and CtrlObs alone were similar to those when analyzing the contrast of ActionObs-CtrlObs.

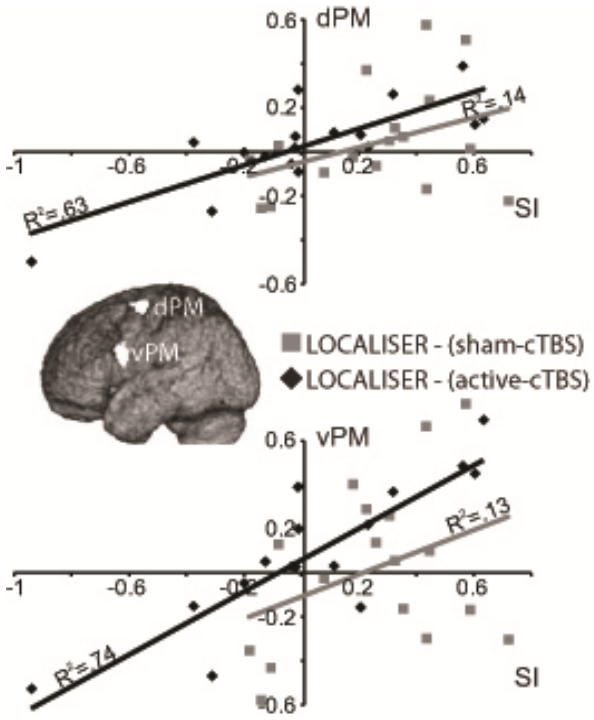

Figure S2.2, related to text: Graphic illustration of the distal effect of cTBS on ventral (vPM) and dorsal (dPM) premotor cortices. Black lines: mean *C'* signal in left (ipsilateral to the cTBS) dorsal and ventral premotor (dPM, vPM) nodes of the parieto-frontal mirror network plotted against $C'_{SI.}$ Grey lines: same but for *C''*. The actual slope of the black and grey regression lines look similar, and comparing them using a multiple regression did not yield significant differences for the dPM ($F \geq 2.02$, $p \leq 0.17$) or vPM ($F \geq 0.24$, $p \leq 0.63$) ROI. However, the larger spread of values along the x-axis in the case involving cTBS (black) probes the relation between the target ROI in SI and dPM/vPM more effectively. This becomes apparent from the t values generated by a simple regression model based on *C'* (dPM, $T \geq 4.85$, vPM, $T \geq 6.71$), which are higher than those on *C''* (dPM, $T \geq 1.03$; vPM, $T \geq 3.95$).

2.5.3 PPI analysis

For each subject, we created a new first level model only including the sham cTBS and active cTBS sessions. We then extracted, for each participant, the first eigen-value of the activation in the target ROI in SI. Two separate psycho-physiological interactions (PPI; Friston et al., 1997) were then performed. In one case, a weight of 1 was given for blocks of active $cTBS_{ActionObs}$ and a weight of -1 to sham $cTBS_{ActionObs}$, to examine how connectivity from the target ROI in SI changed during action observation as a function of active cTBS. In the other case a weight of 1 was given to active $cTBS_{CtrlObs}$ and -1 to sham $cTBS_{CtrlObs}$ to examine connectivity changes during control observation. The interaction parameter estimates for each of the two PPI were then taken to the second level. One-sample t-tests comparing the interaction parameter estimates against zero for ActionObs or for CtrlObs did not reveal significant changes in connectivity anywhere in the grey matter (even at $p<0.005$, $T_{(16)}>2.9$, minimum cluster-size 10). A two-sample t-test was also used to compare the interaction parameters of ActionObs and CtrlObs to examine whether changes in connectivity might have been different across the two conditions, and no significant differences were found in the gray matter even at $p_{unc}<0.005$ ($T_{(16)}>2.9$, min cluster size 10 voxels).

Chapter 3

3 Continuous theta burst TMS delivered to the left somatosensory cortex changes its connectivity with the left dorsal premotor region during rest.

Adapted from the manuscript submitted as:

Continuous theta burst TMS delivered to the left somatosensory cortex changes its connectivity with the left dorsal premotor region during rest. A combined resting state fMRI and cTBS study.

Nikola Valchev, Branislava Ćurčić-Blake, Valeria Gazzola, Alessio Avenanti, Christian Keysers and Natasha Maurits

Abstract
Using a combination of neuroscience techniques we explored the connectivity of the left primary somatosensory cortex (SI) during rest. In a randomized order on two different days we administered active TMS or sham TMS over the left SI. TMS was delivered off-line before scanning by means of continuous theta burst stimulation (cTBS). The target area was selected previously and individually for each subject as the part of SI activated both when the participant executes and observes actions. In this way we could investigate the connectivity of SI during rest with the whole brain and within the previously identified parieto-frontal network activated during action observation (Rizzolatti and Sinigaglia, 2010, Nat Rev Neurosci). Three analytical approaches - both theory driven (partial correlations and seed based whole brain regression) and data driven (Independent Component Analysis) – all indicated a change in connectivity between the targeted area in SI and the left dorsal premotor cortex (dPM). Our results thus show that during rest SI is functionally connected to dPM and that cTBS disrupts this connection.

3.1 Introduction

At the core of healthy motor skills lie years of execution of motor actions and observation of others performing motor actions. Although our own action execution differs from action observation, these two processes seem to share a common network in the brain (Rizzolatti and Sinigaglia 2010; Friston et al., 2011). Several cortical areas are known to co-activate during both the observation and the execution of actions. This phenomenon has been taken as evidence for the existence of mirror neurons in the human brain (Rizzolatti and Craighero 2004). These special neurons that fire when a person is observing an action or executing a similar one were first discovered in the monkey brain (di Pellegrino et al., 1992). Using functional magnetic resonance (fMRI), researchers have identified the brain regions activated by both action observation and action execution in humans. This network of areas includes the premotor and parietal cortices as well as the somatosensory cortex. We here study the connectivity of the primary somatosensory cortex (SI) within this network of areas. SI has also been shown to be engaged in the experience and observation of pain (Bufalari et al., 2007) and touch (Keysers et al., 2004) which suggests that it plays an active role both during the perception of actions performed by others and the execution of actions. We here focus on the connectivity of SI during rest to study the connectivity of SI independent of behavioural task execution. There is a growing body of literature suggesting that the networks of cortical areas that can be identified when the brain is at rest are related to functional networks (Cordes et al., 2000; De Luca et al., 2005). We therefore speculate that if SI

has strong connectivity with another brain area during rest, this same connection might play a role during a behavioural task that activates these two regions.

The system of brain areas activated both when subjects observe, and execute actions, which has been designated shared circuits (Gazzola and Keysers, 2009), includes the parieto-frontal mirror network (Rizzolatti and Sinigaglia 2010), SI and the secondary somatosensory cortex (SII) (Gazzola and Keysers, 2009). Here, we try to directly address the issue of SI connectivity, taking into consideration the whole system of shared circuits. To address this problem, rather than just measuring the activity and connectivity of SI in a given task, we here interfered with its activity by applying Transcranial Magnetic Stimulation (TMS) and derived the change in connectivity due to this interference. This approach which is referred to as "perturb and measure" allows going one step further than simply calculating the correlation between the activity in two areas. If a perturbation in SI caused by our stimulation can be measured in a distant area which is part of the parieto-frontal system, we can infer that there is a strong functional and possibly "causal" connection between them (Fox et al., 2012). Here we define causal not in the sense of temporal causality but as a directional effect of TMS in the shared circuits system of interconnected areas. To avoid the technical difficulties of combining TMS and fMRI online in the scanner, we delivered inhibitory TMS outside of the scanner and then acquired the scans while the brain is still under the effect of the stimulation. In order to do so, the shared circuits were first localized by scanning participants using fMRI while they observed actions and while

they executed actions. We selected the part of SI from each individual shared circuits map and on a second and third day, in a randomized order, we targeted that region with continuous theta burst stimulation (cTBS) or sham stimulation (hereafter designated as active cTBS and sham cTBS to denote that the pulse sequence is the same, but the sham coil delivers no effective stimulation). By contrasting the connectivity patterns identified in the active cTBS and sham cTBS sessions and thereby tracking the effect of our stimulation we can explore the connectivity of SI in general and within the parieto-frontal mirror network.

3.2 Methods

3.2.1 Experimental Procedures

The experiment was divided into three sessions distributed over three days. The data collected in this experiment was collected together with the data reported in Chapter 2. Both reports make use of the action observation and execution data recorded on the first experimental day (LOCALISER), but here we focus on the resting state sequence collected on the second and third days while Chapter 2 focuses on the action observation data also collected on the same days.

The data collected on the first day consisted of a high resolution anatomical scan which was immediately prepared for neuronavigation. Observation and execution runs followed the anatomical scan (see Chapter 2 for more details). Right after scanning, the individual resting motor threshold (rMT) was determined (see section 3.2.3) and the corresponding optimal scalp position (OSP) was saved using neuronavigation software (Brain Innovation, Maastricht, The Netherlands) for further use.

From the participant's point of view the second and third experimental sessions were identical. However, the difference between the two days was that (in a randomized fashion) active or sham cTBS was delivered. Nine subjects received active cTBS on the second day and eight on the third day. Each day started with the identification of the target point for TMS, which was checked for consistency at each experimental session (see section 3.2.4). After marking the target point with a pen on a cap placed over the participants' head, subjects were taken to the MRI preparation room and seated in the MRI bed. Stimulation was delivered in

the MRI bed after 5 minutes of rest during which participants were required to remain as relaxed as possible (see section 3.2.3). Stimulation was delivered in the preparation room using the mark on the cap instead of online neuronavigation to have the participant as close as possible to the scanner and be able to transport him/her as fast as possible into the scanner. The cap used was an EEG cap with a chin stripe which fixated the cap enough to ensure that the marked target point corresponded to the navigated one. After the stimulation (sham or active cTBS) the bed with the participant was transported to the scanner and about 5 minutes (mean 5.2 minutes, std=0.4) after the end of TMS the scanning sequence was initiated. Scanning included (in this order) an observation (~8 min duration), resting state (~12 min duration). Thus, the RS sequence was acquired about 13 min after TMS was completed.

3.2.2 Participants

A total of 24 participants took part in the study of which 18 completed all three sessions. One subject was excluded because his stimulation point was too posterior due to a lack of activation in SI. The final data set analysed here was thus composed of 17 subjects (6 female, age 18-25 years, mean 20.9 years, all right handed, (Oldfield 1971). Of the 6 participants who did not complete all three sessions of the experiment, two had excessive resting motor threshold (>64% of maximum stimulator output)[2], two decided by themselves to quit after the second session, and

[2] This limitation is due to the technical characteristics of the TMS machine used in this experiment. The frequency of the cTBS stimulation (50Hz) requires the capacitors of the machine to recharge at a rapid rate, which is not possible for

two reported light headaches after the second session (involving sham cTBS stimulation for both of them) and were advised to discontinue participation. All subjects had normal or corrected-to-normal visual acuity in both eyes and were naïve to the purpose of the experiment. Full debriefing was provided only at the end of the third session. Participants gave written informed consent and received monetary compensation. Procedures were approved by the Medical Ethical Committee of the University Medical Center Groningen. None of the participants had any neurological, psychiatric or other medical problems or contraindications to TMS or fMRI.

3.2.3 Transcranial Magnetic Stimualtion

The cTBS protocol lasted 40 s and consisted of bursts of 3 TMS pulses delivered at 50 Hz, with bursts being repeated every 200 ms (at 5 Hz) for a total of 600 pulses (Bertini et al., 2010; Franca et al., 2006; Huang et al., 2005). Stimulation was administered with a 70 mm figure-eight stimulation coil connected to a Magstim Rapid2 (The Magstim Company, Carmarthenshire, Wales, UK). Sham cTBS was delivered with the same parameters but through a special placebo coil (The Magstim Company, Carmarthenshire, Wales, UK) which produces a comparable noise and sensation in the subject but produces no effective stimulation. Subjects were all naïve to TMS and upon questioning after the experiment were unable to reliably differentiate between sham and active cTBS.

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stimulations of intensities of more than 51% (corresponding to 80% of the rMT of 64%).

Previous studies have suggested that motor experience before or after the administration of active cTBS may alter its effect on cortical excitability (Iezzi et al., 2008; Todd, Flavel, Ridding 2009; Iezzi et al., 2011). Therefore, participants rested for 5 minutes before stimulation. After cTBS, it took no more than 5 minutes to start scanning which permitted us to capture the effect of the stimulation when it reached its maximum level (Huang et al., 2005).

Pulse intensity was set at 80% rMT (mean 47.35% (SD 5.06) of the maximum output). The rMT evaluation was performed by recording motor-evoked potentials (MEPs) induced by single-pulse TMS of the left motor cortex. MEPs were recorded from the right first dorsal interosseous (FDI) using a Refa amplifier (TMSi, Enschede, The Netherlands). Pairs of silver/silver chloride surface electrodes were placed over the muscle belly (active electrode) and over the associated joint of the FDI muscle (reference electrode). A ground electrode was placed on the ventral surface of the right wrist. EMG signals were sampled at 5 kHz, band-pass filtered (20 Hz-1.0 kHz), digitized and displayed on a computer screen. The optimum scalp position (OSP) was chosen so as to produce maximum amplitude MEPs in the FDI muscle. The rMT was defined as the lowest level of stimulation able to induce MEPs of at least 50 μV with 50% probability.

3.2.4 Target point selection and neuronavigation

The target point in SI was derived from the functional map obtained from the conjunction of the observation run and the execution run from the first session for each individual subject using Brain Voyager (Brain Innovation,

Maastricht, The Netherlands). First, a binary mask was created from the contrast (Action Execution) – (Execution Control), thresholded at p_{unc}=0.001, min. cluster size 10. The mask was then used to limit the contrast (Action Observation) – (Observation Control), thus selecting only voxels which were activated by both tasks. The threshold for the observation contrast was first set to p_{unc}=0.001, min. cluster size 10 and then if needed lowered to better identify the cluster of activation in SI. Each individual subject map was overlaid on the anatomical 3D reconstruction of the individual grey-white matter boundary for use during online neuronavigation. We navigated to the target point and its projection on the scalp was then marked on an EEG cap fixated with a chin strap to the participant's head, so that it could be easily identified without the navigation device. Mean Talairach coordinates for the activation target point were -42 ±5.54, -31 ±6.56, 55 ±6.43 (MNI: -42 -35 58; corresponding to the Left Postcentral Gyrus, as defined in the Anatomy toolbox (Eickhoff et al., 2005; Eickhoff et al., 2006; Eickhoff et al., 2007)).

3.2.5 Data acquisition

Imaging was performed with a Philips Intera 3T Quasar with a synergy SENSE eight channel head coil and maximum gradient strength of 30 mT/m. The resting state sequence employed standard single shot EPI with TE = 35 ms, TA= 1.95 s, TR= 2 s. For each volume, 37 AC-PC aligned axial slices of 3.5 mm thickness, without slice gap and a 3.5 x 3.5 mm in plane resolution were acquired to cover the entire brain using interleaved slice acquisition. A T1-weighted structural scan was acquired with TR = 7.657 ms, TE = 3.54 ms, flip angle= 8 deg, 1x1x1 mm voxel size.

3.2.6 Connectivity analysis methods

We applied three analytical methods to the data set to determine the connectivity of SI. This approach was motivated by the fact that there are few publications using the same RS fMRI and TMS combination, no prior knowledge exists on the functional connectivity of SI during rest and such an approach has been used by other researchers (Doria et al., 2010). By comparing the results of different analysis methods we can evaluate the robustness of the reported results. We performed a partial correlations analysis to evaluate if active cTBS delivered over SI changed the connectivity in the shared circuits network. To evaluate if active cTBS changed the functional connectivity between the targeted region and *any* other region in the brain we performed a seed based whole brain regression analysis. This analysis provides a voxel-wise localization of any change in connectivity in the shared circuits as should also be detected by the partial correlation analysis, as well as the localization of changes in connectivity elsewhere in the brain. The third analysis method applied to the data was Independent Component Analysis (ICA) which was chosen as a data driven method which does not require any previous assumptions and can be used to confirm the results of the correlational approaches.

3.2.6.1 General preprocessing steps for functional connectivity analyses

For each subject a sphere with a diameter of 1cm was built around the MNI coordinates of the target point using Marsbar (Brett et al., 2002). Subsequently the sphere was intersected with the anatomical region of interest (ROI) consisting of BA1 and BA2 (as defined in the anatomy toolbox

in SPM8 (Eickhoff et al., 2005; Eickhoff et al., 2006; Eickhoff et al., 2007), and with the corresponding subject's grey matter segment to obtain the target point ROI. We restricted the target point ROI to BA1 and BA2 because they represent the integration area of the primary somatosensory cortex, receiving input from ipsilateral BA3a and BA3b, the contralateral BA2, and projecting connections to these areas (Jones 1986; Shanks, Pearson, Powell 1985). This ROI is hereafter referred to as the target ROI in SI.

To define the left parietal ROI, usually referred to as IPL (inferior parietal lobe), the group level shared circuits map from the first experimental session was intersected with an anatomical ROI of left area PF (as defined in the anatomy toolbox in SPM). For details on the creation of the group level shared circuits map see Chapter 2.

Left premotor ROIs were created by first combining the anatomical ROIs in left BA6 and left BA44 (as defined in the Anatomy toolbox for SPM). Because these regions contain the ventral premotor (vPM), dorsal premotor (dPM) and the supplementary motor area (SMA) we first excluded all voxels between sagittal "x" coordinates -13 and 13 (SMA). The remaining region was split along the coronal "z" coordinate 48 into vPM ($z<48$) and dPM ($z\geq48$) (Tomassini et al., 2007)

3.2.6.2 Partial correlation analysis

For each subject, and each ROI (target ROI in SI, IPL, vPM, dPM) we calculated the first eigenvector of the activations during the sham and active cTBS sessions, separately. We also calculated for each subject and

session the signal averaged over the whole brain, the average white matter and CSF signal as well as the first temporal derivatives of the movement parameters calculated during realignment. The average white matter and CSF signals were computed using the probability maps included in SPM8. By applying a threshold of 95% for the white matter and 75% for the CSF, we created binary maps and used those to extract the corresponding average signals (Geerligs et al., 2012; Van Dijk et al., 2010). In this way for each subject and session we obtained four first eigenvectors from the four ROIs, the average white matter, cerebrospinal fluid (CSF) signal, 6 movement parameters and their 6 temporal derivatives. Partial correlations were calculated between each pair of first eigenvectors, controlling for the other pairs of eigenvectors and all regressors of no interest. In this way 6 partial correlations were calculated, for each subject and each session, and subsequently normalized using a Fisher–Z transformation. Normalized values were then compared using paired samples T-tests to evaluate if TMS over the target ROI in SI induced a change in connectivity between any of the other ROIs. Results of the six paired T-tests were corrected for multiple comparisons using Bonferroni correction. This approach was adopted as opposed to repeated measures ANOVA since comparing the partial correlations across connections is not relevant for the problem we are investigating and would have resulted in too many comparisons.

3.2.6.3 Whole brain regression analysis

The images that were preprocessed in SPM8 applying the same steps as for the EPI images from the observation task (for details see Chapter 2). The

resting state images were further temporally detrended and band-pass filtered from 0.01Hz to 0.08Hz using the Resting State fMRI Data Analysis toolbox (Song et al., 2011). For each subject a design matrix for the first level SPM analysis was created. We included as a regressor of interest the first eigenvector from the target ROI in SI and as regressors of no interest, to remove sources of regionally nonspecific variance: 6 movement parameters resulting from the realignment procedure and their temporal derivatives, the average signal from the whole brain, the average signal from the white matter and the average signal from the CSF. The parameter estimates associated with the first eigenvector regressor represent the voxelwise functional connectivity with the targeted region. Contrast images were then take to the second level of analysis.

3.2.6.4 Independent Component Analysis

Independent component analysis (ICA) was performed on the preprocessed, temporally detrended and band-pass filtered images. GIFT v. 1.3 (Calhoun et al., 2001) as implemented in MatLab was used to perform the analysis. The toolbox first applies ICA to the concatenated preprocessed data and then computes the session and subject specific components and time courses (Calhoun et al., 2001; Schmithorst and Holland 2004). ICA data analysis is done in three stages: 1) data reduction, 2) application of the ICA algorithm, and 3) back reconstruction. At the first stage a principle component analysis is used to reduce the dimensionality of individual subject data. Then the Infomax algorithm (Bell and Sejnowski 1995) is applied to estimate the independent sources. At this stage also the spatially independent functional maps are created. As a last stage, through

back reconstruction, the individual subject and session image maps and time courses are computed and grouped across subjects. A set of 10 Independent Components (ICs) for each subject and session was extracted. Each IC included a spatial map and its corresponding time course. The value within each voxel represents the degree of correlation of its fMRI signal with the time course of the component. The number of components was selected based on the size of the action observation network mask, such that the possible effects of our stimulation would not be separated into two distinct components and on the existing literature on resting state ICA analysis (Van Den Heuvel and Hulshoff Pol 2010). Since we wanted to investigate whether active cTBS induced any change in the connectivity between the targeted area in SI and the rest of the shared circuits network which is relatively large, it is also beneficial for our analysis that a smaller number of components results in the identification of spatially larger ICs. In addition, when a larger number of components would have been extracted from the resting state data it would have been more difficult to detect activation changes induced by active cTBS as the algorithm would most probably result in different components for each of the two active and sham cTBS conditions. Not knowing the extent of the brain area(s) that will show the change of connectivity induced by TMS, we aimed to estimate a sufficiently high number of meaningful independent components while still identifying large networks so that the effects of the stimulation would be identifiable within the same component(s). Ten ICs also permits to identify the main networks reported in the literature. Eight resting state networks have been reported (Van Den Heuvel and Hulshoff Pol 2010): somato-

motor, primary visual, extrastriate visual, insular-temporal/ACC, left parietal-frontal, right parietal-frontal, default mode and frontal networks.

After calculating the spatial maps of 10 ICs from the sham and active cTBS sessions we selected the maps that were spatially most correlated with the mask of the shared circuits network. In this way we evaluate the effect of our stimulation only on the networks which include part of the shared circuits network. To compare the spatial maps of the identified ICs we used paired samples T-tests in SPM, because the values in each voxel of the spatial map for each IC represent the degree to which the time course of this particular voxel is correlated with the time course of the corresponding component, and there is thus no relationship between the values in a given voxel from different spatial maps.

3.3 Results

3.3.1 Partial correlation analysis

Partial correlations were calculated between each pair of ROIs from the shared circuits network (target point, IPL, vPM, dPM). Paired samples T-tests showed changes in the connectivity after active cTBS only between the target point in SI and dPM ROIs active ($T_{(16)}$=-3.26; p=0.005; Bonferroni correction for applying six T-tests showed that results are significant at p≤0.008 (See Table 3.1)).

Table 3.1. Mean partial correlations and standard deviations for each connection between ROIs as derived from the sham and active cTBS sessions, and T statistics and (uncorrected) p-values resulting from the paired T-tests.

Connection	Sham cTBS session Mean (SD)	Active cTBS session Mean (SD)	paired T-test
target ROI in SI to IPL	0.25 (0.19)	0.28 (0.24)	$T_{(16)}$=-0.72; p=0.48
target ROI in SI to vPM	0.28 (0.3)	0.27 (0.24)	$T_{(16)}$=0.04; p=0.97
target ROI in SI to dPM	-0.5 (0.25)	-0.29 (0.32)	$T_{(16)}$=-3.26; p=0.005
IPL to vPM	0.22 (0.23)	0.26 (0.3)	$T_{(16)}$=-0.56; p=0.58
IPL to dPM	0.12 (0.23)	0.09 (0.27)	$T_{(16)}$=0.38; p=0.71
vPM to dPM	0.02 (0.17)	-0.06 (0.23)	$T_{(16)}$=1.15; p=0.27

3.3.2 Seed based regression analysis

The first eigenvector of the time course in the target point in SI ROI from the sham and active cTBS sessions was used as regressor for each subject in the first level design matrix. Evaluating the contrast (sham cTBS) – (active cTBS) at the second level ($T_{(16)}$ ≥3.69; $p_{(uncor)}$ ≤0.001; min cluster size 10) resulted in a decrease in the connectivity due to active cTBS between the

target point ROI and a cluster in left BA6 (18 voxels), which is also part of the shared circuits network (see Figure 3.1 and Table 3.2). Another cluster that survives this threshold is in the white matter (10 voxels). None of the clusters survives FDR correction. At a lower threshold of $T_{(16)} \geq 2.92$; $p_{(uncor)} \leq 0.005$; min cluster size 20, both clusters identified previously grow in size, the one in the left BA6 increases to 207 voxels, the one in the white matter to 60, and one more cluster in the contralateral BA6 survives (88 voxels). The opposite contrast (active cTBS) – (sham cTBS) shows no clusters surviving FDR correction. At a threshold of $p_{(uncor)} \leq 0.001$ ($T_{(16)} \geq 3.69$; min cluster size 10) several clusters appear in the parietal and prefrontal cortex distributed over the white and grey matter. Lowering the threshold to $p_{(uncor)} \leq 0.005$ ($T_{(16)} \geq 2.92$; min cluster size 20) identifies a number of clusters scattered throughout the white and grey matter.

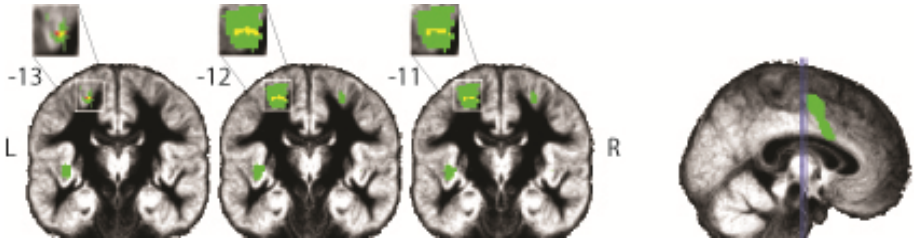

Figure 3.1. Group results from the seed based regression analysis, contrast (sham cTBS) – (active cTBS) at $T_{(16)} \geq 3.69$; $p_{(uncor)} \leq 0.001$; min cluster size 10 (red and yellow), overlaid on the shared circuits mask (green).

Table 3.2. Group results for the seed based regression analysis, contrast (sham cTBS) – (active cTBS) at $T_{(16)} \geq 3.69$; $p_{(uncor)} \leq 0.001$; min cluster size 10, cluster size k in voxels and for the local maxima within each cluster: corresponding T value, MNI coordinates (x, y, z) in mm, hemisphere (R: right, L: left), anatomical localization and, cytoarchitectonic localization when available (as given by the Anatomy toolbox).

k	T	x	y	z	hem	Anatomical description	Cytoarchitectonic description
(sham cTBS) – (active cTBS) masked with shared circuits							
18	4.15	-22	-12	56	L	Superior Frontal Gyrus	BA 6

3.3.3 Independent Component Analysis[3]

To check whether active cTBS over SI induced a change in the ICs related to the shared circuits network we sorted the mean spatial maps from the sham cTBS and active cTBS sessions separately, according to their spatial correlation with the shared circuits mask (see Figure 3.2). Networks A from the sham cTBS session and A' from the active cTBS session showed the highest spatial correlation with the mask (k=0.39 for A and k=0.38 for A'), followed by component B (k=0.11 for both B and B'), component C (k=0.1 for both C and C') and component D (k=0.09 for D and k=0.08 for D'). We chose to compare the spatial maps of these four components because they represent the highest spatial correlation with the shared circuits mask (all other components show a correlation close to zero) and their spatial maps include not only regions of the mask but they represent sensorimotor networks, as well (see Figure 3.2).

The contrast (active cTBS) – (sham cTBS), masked with the shared circuits map, revealed significant differences only in networks B and B' in one cluster of 6 voxels in left BA 6 ($q_{(FDR)} \leq 0.05$; $T_{(16)} \geq 5.37$). When considering the same contrast but at a lower threshold, the same cluster contained 42 voxels ($p_{unc} \leq 0.001$, $T_{(16)} \geq 3.69$, min cluster size 10) and no other clusters survived this threshold. The inverse contrast showed no differences at $q_{(FDR)} \leq 0.05$. When the unmasked contrasts were considered, no results were detected at $q_{(FDR)} \leq 0.05$ in either contrast. When lowering the threshold to $p_{unc} \leq 0.001$, $T_{(16)} \geq 3.69$, min cluster size 10, the contrast (active cTBS) -

[3] The differences between the spatial maps of the selected components from the sham cTBS and active cTBS sessions were also assessed by a permutation test. This approach allowed to apply both voxel-wise and test-wise FDR correction for multiple comparisons. This analysis confirmed the results reported here.

(sham cTBS) resulted in a difference in left BA6 (same cluster as previously detected, increasing in size to 64 voxels) and three more clusters located in the left inferior temporal gyrus, right inferior temporal gyrus and right amygdala (see Figure 3.2 and Table 3.3). The opposite unmasked contrast (sham cTBS) – (active cTBS) at $p_{unc} \leq 0.001$, $T_{(16)} \geq 3.69$, min cluster size 10 identified three clusters, one in left Area 18 (70 voxels), one in the right anterior cingulate cortex (17 voxels) and one in the parietal (Rolandic) operculum (SII) (16 voxels). None of those three clusters includes voxels from the shared circuits mask.

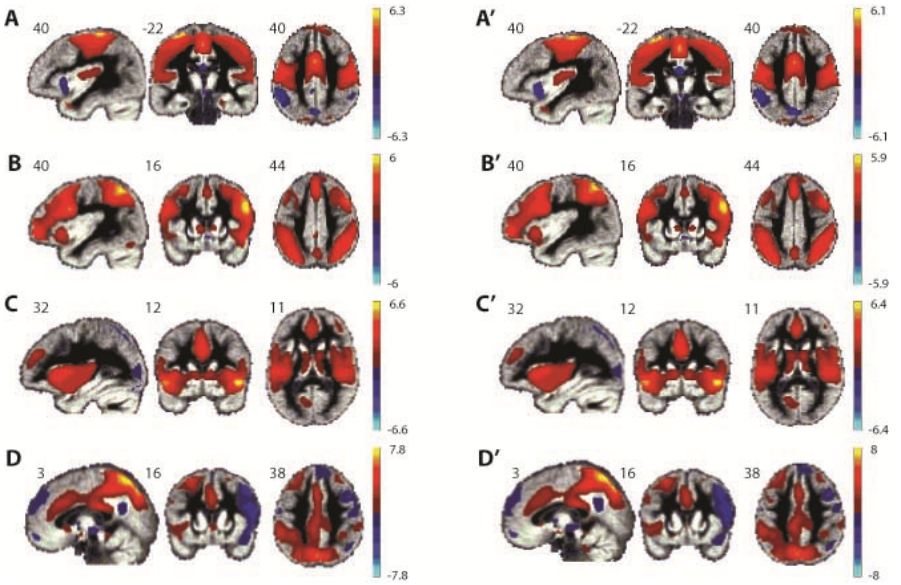

Figure 3.2. Spatial maps of the components resulting from the ICA analysis spatially correlated with the shared circuits mask. Components A, B, C and D were derived from the sham cTBS resting state data and A', B', C' and D' were derived from the active cTBS resting state data. Values in each spatial map represent the normalized correlation of each voxel with the estimated time course of the corresponding component thresholded at -1≥z≥1.

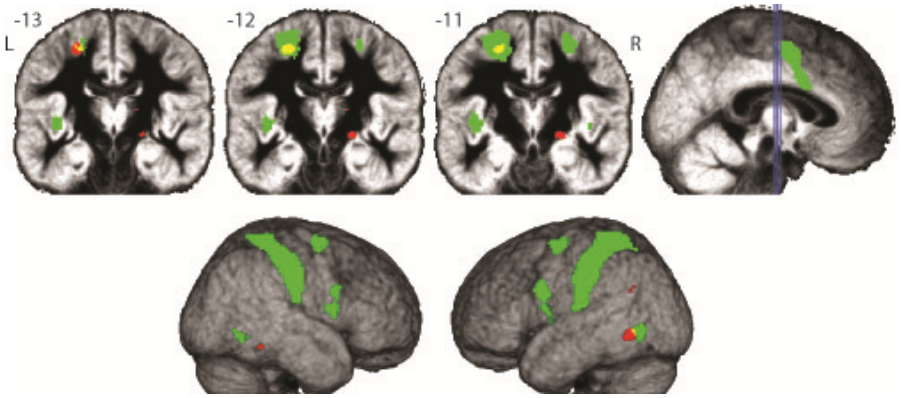

Figure 3.3. Results from the paired samples T-test contrasting the spatial maps of ICs B and B', contrast (active cTBS) – (sham cTBS) $T_{(16)} \geq 3.69$; $p_{(uncor)} \leq 0.001$; min cluster size 10 (in red and yellow), overlaid on the shared circuits mask (in green).

Table 3.3 Group results for the paired samples T-test contrasting the spatial maps of ICs B and B', contrast (active cTBS) – (sham cTBS) at $q_{(FDR)} \leq 0.05$;$T_{(16)} \geq 5.37$ and contrasts (active cTBS) – (sham cTBS) and (sham cTBS) – (active cTBS) unmasked at $T_{(16)} \geq 3.69$; $p_{(uncor)} \leq 0.001$; min cluster size 10; cluster size k in voxels and for the local maxima within each cluster: corresponding T value, MNI coordinates (x, y, z) in mm, hemisphere (R: right, L: left), anatomical localization and, cytoarchitectonic localization when available (as given by the Anatomy toolbox).

k	T	x	y	z	hem	Anatomical description	Cytoarchitectonic description
FDR corrected paired samples T-test (active cTBS) – (sham cTBS) masked							
6	6.01	-24	-12	54	L	Left Precentral Gyrus	Area 6
uncorrected paired samples T-test (active cTBS) – (sham cTBS) unmasked							
64	6.01	-24	-12	54	L	Left Precentral Gyrus	Area 6
59	5.12	-52	-60	-6	L	Inferior Temporal Gyrus	
29	6.68	22	-10	-8	R	Amygdala	

10	4.41	52	-44	-14	R	Inferior Temporal Gyrus	
uncorrected paired samples T-test (sham cTBS) – (active cTBS) unmasked							
70	5.10	-2	-70	20	L	Calcarine Gyrus	Area 18
17	4.78	2	28	-6	R	Anterior Cingulate Cortex	
16	4.91	62	-20	16	R	Rolandic Operculum	OP1

3.4 Discussion

In this study we explored the connectivity of SI during rest using a combination of inhibitory TMS (cTBS) and fMRI. In particular we evaluated the connections of SI within the shared circuits network. We compared the spontaneous activations in the brain during rest after subjects received active cTBS or sham cTBS over the part of SI activated by both action execution and action observation. Our results show that active cTBS delivered over SI affects its connectivity with a cluster in dPM, which is part of the shared circuits network. The result was confirmed by three analytical approaches and suggests a directional influence of SI over dPM since stimulation was delivered over an area of SI and the change in connectivity was detected between the target ROI in SI and dPM. We suggest that the connectivity we measure in this study is directed from SI to dPM since we have stimulated the first area and detected a change in its connectivity with the latter. If any two areas are causally connected then perturbing the functioning of one of them would affect the other one, although the reverse inference is not necessarily true. The relationship between SI and dPM could also be mediated by other brain regions not revealed in our analyses.

To our knowledge, there are only two earlier studies (Eldaief et al., 2011; van der Werf et al., 2010) which combine resting state fMRI and TMS, and the most appropriate method of analysis has not yet been established. We therefore decided to use two theory driven methods for analysis (partial correlation, seed based whole brain regression) as well as a data driven method (ICA). In the present study all three methods detected

a change in connectivity between the stimulated area in the left SI and the left dPM cortex, demonstrating the robustness of the finding, although the slope of the correlation and the polarity of the changes in connectivity between the two areas differed depending on the applied methodology. The seed-based whole brain regression analysis showed a change in connectivity between the targeted area in SI and a cluster in left dPM which did not survive correction for multiple comparisons. The partial correlation analysis involving just the nodes of the shared circuits, indicated a strong negative relationship between activation in the targeted area in SI and the cluster in left dPM after sham cTBS, which increased (became less negative) after active cTBS. Since partial correlation correct for intermediate influence, our results suggest that the relationship between activation in SI and dPM is mediated by one of the other nodes of the shared circuits and that the direct relationship (without intermediary nodes) is negative.

When considering the ICA results it should be noted that the effect of active cTBS is detected in one particular component. The change in connectivity between the targeted ROI in SI and dPM can be interpreted only for that particular component and its estimated time course. Yet, the reported result again shows a significant change in connectivity during rest between SI and dPM due to cTBS delivered over SI.

In our study we used inhibitory cTBS to induce a change in the part of SI that has been activated when the same subjects observe actions performed by others or perform actions themselves. It has been shown in previous studies that the combination of resting state fMRI and low frequency (inhibitory) repetitive TMS (rTMS) can be a valuable tool to

measure the dynamic relationships between regions of an intrinsic brain network. Van der Werf and colleagues (2010) applied low frequency rTMS or sham TMS over the left dorsolateral prefrontal cortex and found a reduction in the connectivity during rest between the default mode network and the lateral temporal cortices in addition to a trend towards a reduction in connectivity with the bilateral hippocampus. Eldaief and colleagues (2011) extended these findings by testing two different stimulation frequencies over the left posterior IPL. They found that low frequency rTMS increased the intrinsic correlations between the stimulated site and the hippocampal formation. In contrast high-frequency rTMS to the same area decreased the correlation between the default network nodes, but did not affect the correlation with the hippocampal formation. Note that, although inhibitory rTMS and cTBS both probe functional connectivity and may expose a directional connection of a targeted region by affecting its brain activity, they are different stimulation techniques and the connectivity of SI identified by one might differ from the one identified by the other. Here, we have shown that active cTBS allows to identify the connectivity of SI during rest. Moreover, the identified connection could be regarded as directional since active cTBS was applied over an area in SI and the effect was measured on its connectivity with a distant brain region (dPM). It should be noted that formally effective connectivity can only be established using TMS if the stimulation is combined online with fMRI and the effects of TMS are measured in the distant cortical areas immediately after they are delivered.

We can relate the results reported here to the results of the action observation task performed immediately before the resting state run to

compare the effects of active cTBS over SI during rest with the connectivity of the same area detected while the region is actively engaged in action observation. We previously analysed the action observation data collected after subjects were stimulated with sham or active cTBS over SI (see Chapter 2). Results showed that there was a positive relationship between activation in SI and the premotor regions during action observation that was not influenced by the type of stimulation subjects received (sham or active cTBS), i.e. the connectivity between the premotor regions and SI was not changed. However, active cTBS caused an increase of the spread of the parameter estimates from the contrast (Action observation) – (Control observation) in the target ROI in SI. In this way when seeding in the target ROI and using a contrast between two sessions (active cTBS and sham cTBS or active cTBS and Localiser) a bigger portion of the parieto-frontal mirror network correlated with the activations in the target ROI in SI. One interpretation of these findings would be that a functional connection between the premotor regions and SI exists and is used during action observation. However, during rest (as reported in the present study), after filtering out the influence of the other nodes of the shared circuits, a negative relationship between the activity in SI and the activity in dPM was observed after sham cTBS (see section 3.3.1 for results from the partial correlation analysis). This negative correlation was increased (became less negative) by active cTBS. These two results suggest that during action observation a positive relationship between activity in SI and the premotor regions is needed which cannot be cancelled by active cTBS. In the absence of a task during rest, however, the relationship between SI and dPM becomes sensitive to the stimulation, possibly exactly because there are no

task requirements. Here, we need to consider the possibility that the action observation task performed by our subjects immediately before the resting state run might have influenced the resting state networks reported in this paper. It has been shown that a task that is performed before resting state data is collected might have an effect on both the default mode network and task–positive networks (Lewis et al., 2009; Evers et al., 2012). However, in our experiment resting state data was collected always after the observation task, regardless whether the stimulation delivered before scanning was active or sham cTBS. In this case both resting state data sets would be equally affected by the observation task, but contrasting the two sessions will still isolate the effect of the stimulation un-confounded by the influence of the preceding task.

The relationship between activation in SI and dPM evidenced here can be interpreted in light of the findings of De Jong and colleagues (2002). They have shown that limb-independent anti-phase movements, as opposed to synchronous in-phase movements, activate a set of brain areas in the right hemisphere located in the dPM and posterior surface of the postcentral gyrus (BA2, posterior part of SI). The authors propose that BA2 is more involved in these anti-phase movements because of the need for inter-hemispheric unification in somatosensory processing. Both conditions involve exactly the same movement patterns but these are more demanding when they have to be performed in anti-phase. In the present study we detected the same connectivity in the left hemisphere. On the one hand we thus probed the known anatomical connection between the somatosensory cortex and the premotor areas (Jones 1986; Shanks, Pearson, Powell 1985), but on the other hand we demonstrated that SI and

dPM are connected in a way analogous to the one detected by De Jong and colleagues (2002), and this connection is possibly directional from SI to dPM. A possible interpretation of the findings presented here would thus be that by cTBS over SI we probe a connection important for sensory-motor integration both during action observation and execution. When subjects are executing actions both areas (dPM and SI) are activated and we already know that the involvement of SI is crucial since patients with a lesion in the parietal lobe show severe impairments in motor control (Freund 2003). Van Nuenen and colleagues (2012) have suggested that the dPM plays an inhibitory role during the preparation of grip force during object lifting. After targeting the area with active cTBS subjects were not able to downscale their grip force correctly when lifting a light object. Combined with our findings it may thus be that cross-communication between SI and dPM during action observation is active and engaged in calculating the predicted outcome of the action (Keysers, Kaas, Gazzola 2010; van Nuenen et al., 2012).

Our results combined with the literature on action perception suggest that there is a network in the brain which includes the parieto-frontal regions classically associated with the mirror neuron network (Rizzolatti and Craighero, 2010) and that SI is functionally connected with dPM within this parieto-frontal network. Since we delivered active cTBS over SI and then measured its effect on the connectivity of the targeted region and a distant brain area we could argue that there is a directional influence of SI on dPM, although this claim needs to be supported by further experiments. To further understand the connectivity of SI we need to know how information flows from SI to the rest of the parieto-frontal

mirror circuit and whether this information is crucial for correct action understanding. Thus, a possible continuation of our study would be to explore the functional connectivity of dPM, by stimulating it with cTBS and then collecting resting state data. To explore the role of SI within the network of areas activated during action perception we need to stimulate the premotor nodes of the system and measure the effect of the stimulation both on a behavioural level and on the connectivity of these regions with SI during a suitable task. Such a task needs to combine the observation of movement and a sensorial judgement. One such task is weight estimation, when subjects observe videos of a hand lifting an object and have to estimate its weight. Pobric and Hamilton (2006) have already demonstrated that inhibitory TMS over the left vPM affects participants' ability to perform this task accurately. We have also shown (see Chapter 4) that inhibitory TMS over SI has a negative effect on task performance in a comparable task. Since the results reported in the present study suggest that SI is connected with dPM in a possibly directional way, it would be interesting to investigate the connectivity of the latter area to understand where in the network of the shared circuits this crosstalk between premotor and somatosensory areas takes place during weight estimation.

In conclusion we have shown that the combination of resting state fMRI and cTBS is a valuable technique for evaluating the functional connectivity in a system of brain areas. This type of inhibitory TMS over the part of the left somatosensory cortex activated by both action observation and execution affects the connectivity between this region and part of the ipsilateral dPM during rest. This suggests that the parieto-frontal mirror

circuit and the somatosensory cortex are functionally connected. Moreover, the “perturb and measure approach” adopted here shows that the connection between these two areas is most probably directed from SI towards dPM.

Acknowledgments

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Chapter 4

4 Primary somatosensory cortex necessary for optimal weight estimation through observation: a continuous theta-burst TMS experiment.

Adapted from the manuscript submitted as:

Primary somatosensory cortex necessary for the perception of other people's action: a continuous theta-burst TMS experiment.

Nikola Valchev, Emmanuele Tidoni, Antonia Hamilton, Valeria Gazzola, and Alessio Avenanti

Abstract
The existence of a network of areas in the parietal and premotor cortices, active both during action execution and observation, suggests that we might understand the actions of other people by simulating what we would do in the same circumstances. Although neurophysiological and imaging studies show an involvement of somatosensory cortices (SI) during action observation and execution, it is not clear whether SI plays an essential role in understanding the observed action. To test if SI is required for action understanding we used (off-line) transcranial magnetic continuous theta-burst stimulation (cTBS) just before a weight judgment task. Participants observed an actor lifting a box and judged the weight of the box. In counterbalanced sessions, we delivered sham and active cTBS over the hand region of SI and, to test anatomical specificity, over the motor cortex (M1) and the superior parietal lobule (SPL). Active cTBS over SI, but not over M1 or SPL, impaired the task performance relative to sham conditions. Moreover, active cTBS delivered over SI just before the participants were asked to evaluate the weight of a bouncing ball did not alter performance compared to the sham condition. These findings indicate that SI plays a causal role in extracting somatosensory features (heavy-light weight) from observed action kinematics.

4.1 Introduction

When we observe somebody lifting a box we can readily judge if the load is heavy or light. Motor simulation, i.e. the recruitment of motor regions in perceiving the actions of others, has been suggested as a possible basis for such understanding (Rizzolatti and Sinigaglia 2010). Transcranial magnetic stimulation (TMS) and lesion studies focusing on the motor system provide evidence that people become less accurate at perceiving certain aspects of the actions of others following a perturbation of inferior frontal cortex (IFC) and inferior parietal lobule (IPL) (Avenanti and Urgesi 2011; Kalénine, Buxbaum, Coslett 2010; Pazzaglia et al., 2008; Urgesi et al., 2007). In particular, Pobric and Hamilton (2006) found that TMS interference with IFC reduces participants' ability to judge the weight of a box when seen lifted.

On the other hand, mounting evidence suggests that the somatosensory cortices may also represent a key node of the action simulation network (Keysers, Kaas, Gazzola 2010) whose activity is strongly increased, for example, when seeing hands grasping objects (Caspers et al., 2010; Gazzola and Keysers 2009; Pierno et al., 2009) or observing extreme joint stretching (Avenanti et al., 2007; Costantini et al., 2005). This suggests that somatosensory cortices may simulate somatosensory consequences of observed actions. In line with these findings, somatosensory regions are active when viewing others' tactile or painful bodily states (Bufalari et al., 2007; Keysers et al., 2004; Lamm, Decety, Singer 2010) and recently, Bolognini and colleagues (2011) have shown that TMS over the primary

somatosensory cortex (SI) makes people less accurate at judging whether a hand was touched or not.

When judging the weight of a box that we observe being lifted, we need not infer the presence or absence of touch, but must judge the motor effort exerted and/or the intensity of the proprioceptive and tactile feedback experienced by that person. Whether SI plays a critical role in this latter process remains poorly understood and is the focus of the present study. We used the paradigm developed by Pobric and Hamilton (2006) in four new experiments. Participants had to estimate the weight of a box, by observing it being lifted. The task was performed in two counterbalanced sessions carried out after active or sham continuous theta-burst stimulation (Huang et al., 2005) over a target area. In the first three experiments we targeted SI to test its critical role in action understanding, and two neighboring regions, the motor and the superior parietal cortex, to test for spatial specificity. In the fourth experiment, we applied cTBS over SI before participants judged the weight of a bouncing ball, to test for SI specificity to action understanding.

4.2 Methods

4.2.1 Participants

A total of 71 students from the University of Bologna took part in one of four TMS experiments (see Table 4.1 for the details) or in a psychophysical pilot study. All participants received course credit for their participation and provided written informed consent. All of them were right-handed with normal or corrected to normal vision. None of them had neurological, psychiatric, or other medical problems, or had any contraindication to TMS (Rossi et al., 2009). The protocol was approved by the local ethics committee at the University of Bologna and was carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki. No discomfort or adverse effects during TMS were reported or noticed.

Table 4.1. Task, stimulation site, and sample characteristics in the four cTBS experiments.

	Stimulation Site				Task: weight estimation of:	Nr of participants (nr of female)	Mean participant's age in y (±SD)	rMT as % of max stimulation output (±SD)
	Anatomical description	Mean MNI coordinates in mm (± SD)						
		x	y	z				
Exp. 1	SI	-42.2 (±6.3)	-38.4 (±3.7)	60.6 (±3.5)	Box	14 (9)	23.1 (±1.6)	54.4 (±7.2)
Exp. 2	M1	-42.7 (±4.1)	-20.6 (±4.0)	60.3 (±3.4)	Box	14 (9)	23.5 (±1.8)	55.6 (±10.7)
Exp. 3	SPL	-41.1 (±3.2)	-61.9 (±3.7)	51.5 (±4.9)	Box	14 (8)	24.1 (±2.1)	55.4 (±10.7)
Exp. 4	SI	41.6 (±5.5)	-37.7 (±3.3)	60.4 (±4.9)	Ball	14 (9)	22.4 (±2.0)	54.0 (±7.6)

4.2.2 Experimental design, tasks and procedure

All four experiments were composed of three parts: preparatory, active cTBS, and sham cTBS sessions.

During the preparatory session the optimal scalp position (OSP) and the resting motor threshold (rMT) were evaluated by means of motor-evoked potentials (MEPs) recording (see section 4.2.4 for more details). Once the target site was individuated, it was marked on the scalp and Talairach coordinates were estimated using neuro-navigation. The participant was then familiarized with the experimental task by performing a practice block of 60 trials. At the end of the practice, the participant rested for 10 minutes in front of the computer before continuing with the other two sessions.

During the active cTBS session the experimenter administered 40s of off-line cTBS over the target site, by placing the intersection of the loops of the figure of eight coil tangentially to the scalp with the handle pointing backward and laterally at a 45˚ angle away from the midline. Two blocks of 30 trials (~5 min duration each) were performed at five and twelve minutes after the stimulation (Figure 4.1A). Between blocks and trials, participants were asked to rest. Active cTBS is known to suppress the excitability and disrupt functions related to the target area for about 30-60 minutes (Bertini et al., 2010; Franca et al., 2006; Huang et al., 2005). Since the task was completed within 20 minutes after active cTBS, performance should reflect the inhibitory influence of active cTBS over the stimulated site. The sham cTBS session was exactly the same as the active cTBS session except that the coil was positioned, over the target site, perpendicular to the scalp.

The order of the active and sham cTBS sessions was counterbalanced across participants inside each experiment. Additionally, active and sham cTBS sessions were separated by 90 minutes to ensure that any inhibitory effects were not carried over from one session to the other. During these 90 minutes participants were asked to remain relaxed and seated on a comfortable chair. Participants were randomly assigned to the different experiments.

In experiments 1-3, participants watched 4.4s video-clips showing a hand lifting a small box and placing it on a shelf after receiving stimulation over the left SI, left M1 and left SPL, respectively (see also Figure 4.1A). After each video, participants had to estimate the weight of the lifted box by answering the question "How heavy is the box?" by means of a 5 points scale, with 1 corresponding to the lightest and 5 to the heaviest weight estimation (Figure 4.1A). Five different movies, representing 5 different box weights were shown to the participants in a randomized order. Each movie was presented 12 times, 6 for each block (total number of movies per block = 30). In experiment 4, stimulation was delivered over the left SI and the movies of the box were replaced with movies of a ball falling from the top of the screen to then bounce at the bottom until stop (no hand throwing the ball was visible; Figure 4.1A). The task consisted in judging the weight of the ball ("How heavy was the ball?"). As for the box there were 5 different movies representing 5 different ball weights. The number of trials was the same as in experiments 1-3.

In both tasks, each video was preceded by a 1 s fixation cross, and participants answered by pressing one of 5 keys with the left hand (ipsilateral to the stimulation site) to indicate a number from one to five.

They were instructed to answer as quickly and accurately as possible. Participants wore headphones providing white noise thereby eliminating auditory information during task performance.

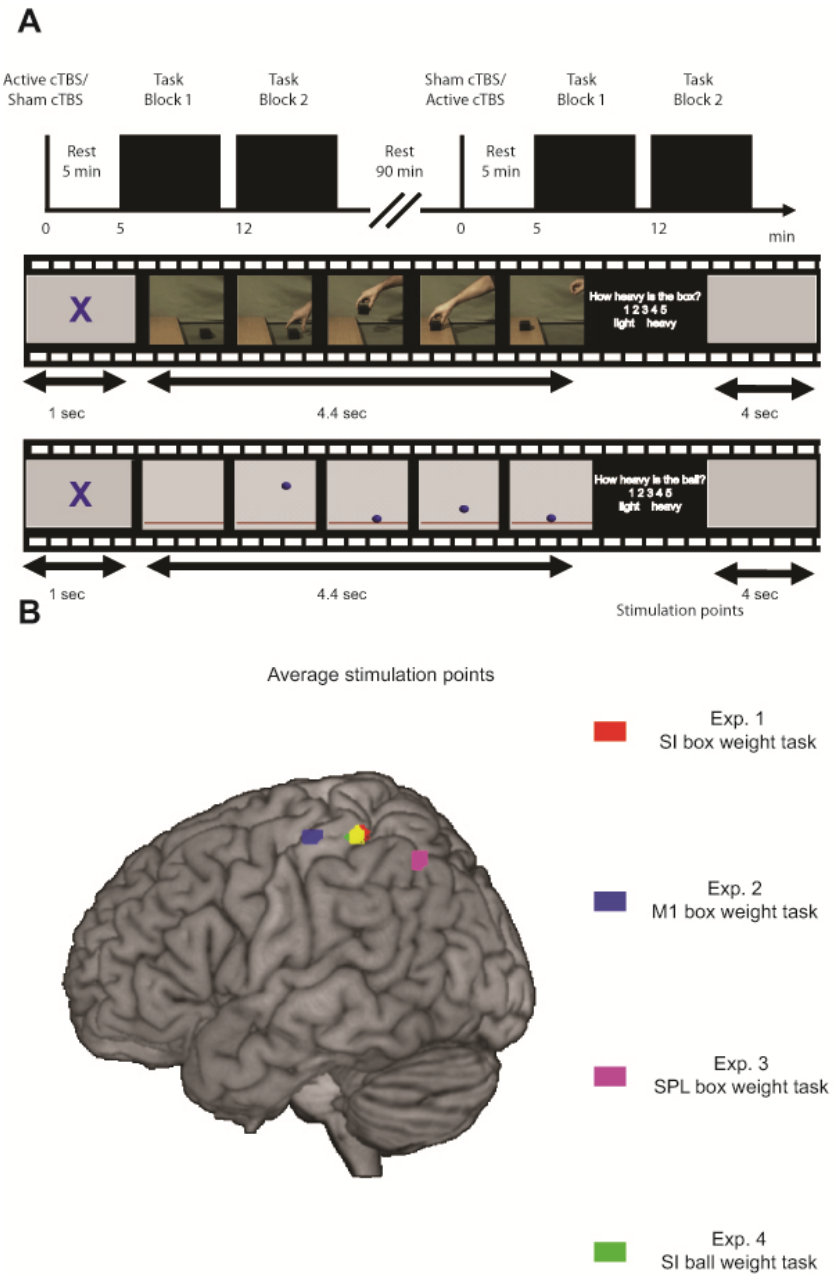

Figure 4.1. (A) Experimental design. (B) Average stimulation sites for experiments 1 to 4 (MNI coordinates), SI box weight task in red, SI ball weight task in green (overlap in yellow), M1 box weight task in blue and SPL box weight task in purple.

4.2.3 Visual stimuli and pilot study

All video stimuli were based on previous experiments (Hamilton, Brindley, Frith 2007; Pobric and Hamilton 2006). Briefly, the five different videos of the hand lifting a box (experiment 1-3) were generated by manipulating a single high-speed clip of a lifting hand to create the perception of 5 different box weights, ranging from approximately 50g to 850g. Since they all derive from the same video, they are very well controlled for visual differences not relevant for the task. The videos of the bouncing balls (experiment 4) were generated using Matlab (www.mathworks.com) (Pobric and Hamilton 2006). The perception of 5 different weights was created by modifying two parameters which affect the elasticity of the ball thereby creating the perception of observing balls of different weights. All video clips were presented using custom-made software written in Matlab (www.mathworks.com) at a resolution of 512x480 pixels and 30 frames per s on a 17 inch monitor.

A pilot study conducted on 12 participants (8 females, mean age 22.8 y ± 2.0) not participating in the TMS experiments was performed to check that accuracy in judging the weight of the ball was comparable to that of the box. Two participants presented very low performance ($R^2 < 0.2$; same procedure used in the TMS experiments, see section 4.2.6) in both tests and were discarded. A t-test in the remaining sample confirmed that the performance was indeed comparable in the box (mean R^2 ± s.e.m. = 0.46 ± 0.04) and ball (0.47 ± 0.04) weight estimation tasks ($t_9 < 1$, $p = 0.93$).

4.2.4 Transcranial magnetic stimulation protocol

The cTBS protocol lasted 40 s and consisted of bursts of 3 TMS pulses delivered at 50 Hz, with each train burst repeated every 200 ms (5 Hz) for a total of 600 pulses (Huang et al., 2005). Stimulation was administered with a 70 mm figure-eight stimulation coil connected to a Magstim Rapid2 (The Magstim Company, Carmarthenshire, Wales, UK).

Previous studies have suggested that motor experience before or after the administration of cTBS may alter its effect on cortical excitability (Iezzi et al., 2011; Iezzi et al., 2008; Todd, Flavel, Ridding 2009). Therefore, in all experiments, before active cTBS participants rested for at least 10 minutes. After active cTBS, they rested for 5 minutes before running the task to allow the active cTBS effect to reach its maximum level (Huang et al., 2005). To maintain blindness for the subjects, the same rest periods were included in the sham cTBS sessions.

Pulse intensity was set at 80% of the resting motor threshold (rMT) and was comparable in the four experiments ($F_{3,52}$ = 0.10, P = 0.96; Table 4.1). In those participants with rMT > 64% of maximum stimulator output (2 participants in experiments 1 and 4, and 3 participants in experiments 2 and 3) the intensity was set at the maximum allowed by the stimulator (51%; on average this intensity corresponded to 76% ± 3 of rMT; Bertini et al., 2010). The rMT evaluation was performed by recording motor-evoked potentials (MEPs) induced by single-pulse TMS of the left motor cortex. MEPs were recorded from the right first dorsal interosseus (FDI) by means of a Biopac MP-150 electromyograph (Biopac Corp, Goletta, CA.). EMG signals were band-pass filtered (20 Hz-1.0 kHz, sampled at 5 kHz), digitized and displayed on a computer screen. Pairs of silver/silver chloride surface electrodes were placed over the muscle belly (active electrode) and over

the associated joint of the FDI muscle (reference electrode). A ground electrode was placed on the ventral surface of the right wrist. The OSP was chosen so as to produce maximum amplitude MEPs in the FDI muscle. The rMT was defined as the lowest level of stimulation able to induce MEPs of at least 50 μV with 50% probability (Rossini et al., 1994).

4.2.5 Target site determination and neuro-navigation

The target site on the scalp was identified based on functional-anatomical methods and then the Talairach coordinates corresponding to the projection of the target site on the brain surface were estimated by neuronavigation (SofTaxic Navigator). Figure 4.1B illustrates the stimulation sites on a brain model.

In experiments 1 and 4 the scalp location corresponding to the left SI was targeted by moving the coil 2.5 cm back with respect to the OSP (corresponding to the M1 hand area). TMS studies that successfully targeted the somatosensory hand area positioned the coil 1-4 cm posterior to the motor hotspot (Avenanti et al., 2007; Balslev et al., 2004; Fiorio and Haggard 2005; Harris et al., 2002; Merabet et al., 2004). We therefore assumed that positioning the coil 2.5 cm from the previously marked optimal scalp position (OSP) for activation of the right FDI muscle would reduce the activity of SI with minimum effects on M1. To test this assumption directly, we verified that TMS pulses at 105% rMT with the coil in the above position did not elicit any detectable MEPs. Neurophysiological studies indicate that cTBS over SI reduces the amplitude of somatosensory evoked potentials, confirming the inhibitory disrupting effect of cTBS-SI on the somatosensory system (Ishikawa et al., 2007; Poreisz et al., 2008).

TMS may modulate activity in remote interconnected regions but can also reveal local functional properties of the underlying target brain region (Avenanti et al., 2012; O'Shea et al., 2007), which also holds true for TBS protocols (Stefan et al., 2008). For example, stimulation of SI induced changes not only in SI but also in nearby regions such as the motor cortex (M1) (Ishikawa et al., 2007; Mochizuki et al., 2007), while excitatory intermittent TBS (iTBS) over SI but not over M1 modulated tactile perception (Ragert et al., 2008) and the same iTBS sequence over M1 but not over SI altered motor performance (Schabrun, Ridding, Miles 2008).

To test anatomical specificity directly, in experiments 2 and 3, we applied cTBS over two sites adjacent to SI: the primary motor cortex (M1) and the superior parietal lobule (SPL). In experiment 2, left M1 was stimulated by placing the coil over the OSP, corresponding to the scalp projection of the motor cortex hand area (Rossini et al., 1994). In experiment 3, left SPL was stimulated by moving the coil 5 cm back with respect to the OSP (Balslev et al., 2004). Thus stimulation of M1 and SPL occurred 2.5 cm forward and backward to SI, respectively.

Brain surface Talairach coordinates corresponding to the stimulated sites in SI (experiments 1 and 4), M1 (experiment 2) or SPL (experiment 3) were identified on each participant's scalp with the SofTaxic Navigator system (Electro Medical Systems, Bologna, Italy) in line with previous studies (Avenanti et al., 2007; Bertini et al., 2010; Serino, Canzoneri, Avenanti 2011). As part of the neuronavigation procedure, skull landmarks (nasion, inion, and two preauricular points) and about 100 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra digitizer (Northern Digital Inc, Ontario, Canada).

Coordinates in Talairach space were automatically estimated by the SofTaxic Navigator from an MRI-constructed stereotaxic template and later transformed to the MNI space for better visualisation. For illustrative purposes, spherical ROIs of 4 mm diameter around the mean target point from each TMS experiment were created using Marsbar (Brett et al., 2002) running under MATLAB 7.5 (Mathworks Inc., Sherborn, MA, USA) and then overlaid on the MNI brain template from MRIcron (http://www.cabiatl.com/mricro/mricron/index.html; Table 4.1 and Figure 4.1B).

4.2.6 Data analysis

Data were processed off-line. Performance for each participant in each session (active cTBS, sham cTBS) was summarized by the R^2 of the linear regression between the correct responses and the participant's judgments, which gives a single measure incorporating both accuracy and variability. Moreover, mean response times (RTs) for each session were computed. Responses with RTs that deviated by more than two standard deviations from the individual mean RT in the particular session were excluded from the analysis. Participants with inaccurate performance ($R^2<0.2$) were removed from data analysis. In experiments 1, 3 and 4 we tested a total of 15 participants, however in each of these experiments one participant was excluded due to inaccurate performance. R^2 and mean RT of the remaining participants (N = 14 in each experiment) were submitted to mixed-model ANOVAs with Experiment (1-4) as between subject factor and Session (active cTBS, sham cTBS) as within subject factor. An additional one-way repeated measure ANOVA was carried out on performance contrasts computed as the R^2 difference between sham cTBS and active cTBS session.

Post-hoc analysis was carried out using the Duncan test to correct for multiple comparisons.

4.3 Results

The Experiment x Session ANOVAs on the R^2 data revealed a significant interaction ($F_{3,52}$ = 3.50, p = 0.02) but no main effect of Experiment ($F_{3,52}$ = 0.09, p = 0.97) or Session ($F_{1,52}$ < 0.15, p = 0.70; Figure 4.2A). Post-hoc analysis showed that in experiment 1 (box weight judgment, SI stimulation) R^2 was lower in the active cTBS than in the sham cTBS session (p = 0.02), indicating a reduction in participant performance to estimate the weight of the box seen lifted only after suppression of SI. No difference between sessions was found in experiments 2-4 (all p > 0.2). The results were also compared using the Intraclass correlation coefficient (ICC) calculated for each session and each subject. Mean, and SD values of the ICC per group and per session were: SI box weight task: active cTBS: 0.63 (±0.14), sham cTBS 0.70 (±0.09); M1 box weight task: active cTBS: 0.69 (±0.14), sham cTBS 0.69 (±0.1); SPL box weight task: active cTBS: 0.73 (±0.15), sham cTBS 0.71 (±0.16); SI ball weight task: active cTBS: 0.72 (±0.13), sham cTBS 0.68 (±0.13) . A repeated measures ANOVA Experiment x Session showed a significant interaction ($F_{3,52}$=3.4, p = 0.02) and no significant effect of the factor Experiment ($F_{3,52}$=0.5, p = 0.69) For Session ($F_{1,52}$= 0.001, p = 0.98).

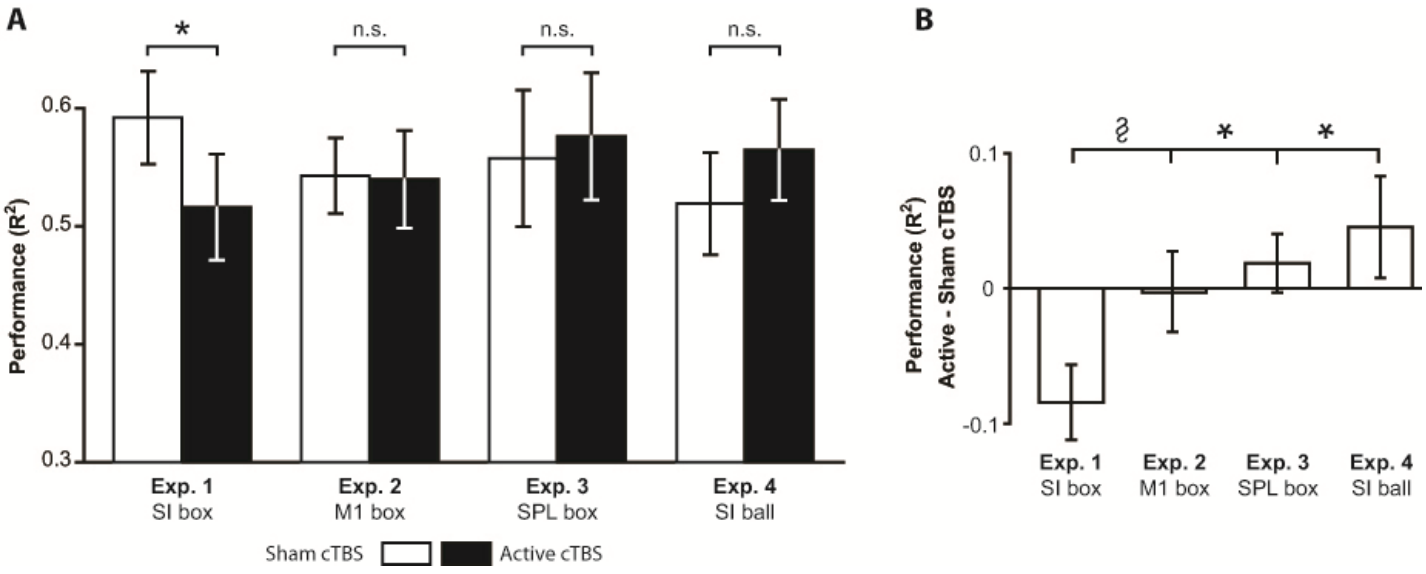

Figure 4.2. (A) Mean R^2 scores for the active cTBS and sham cTBS sessions in experiments 1 (SI box weight task), 2 (M1 box weight task), 3 (SPL box weight task) and 4(SI ball weight task). (B) Difference in R^2 scores between the active cTBS and sham cTBS sessions in experiments 1 (SI box weight task), 2 (M1 box weight task), 3 (SPL box weight task) and 4(SI ball weight task).

The analysis of the R^2 difference (active cTBS minus sham cTBS; Figure 4.2B) computed for each experiment showed a lower index (worse performance after active cTBS) for experiment 1 (box weight estimation, cTBS over left SI), than for experiment 3 (box weight estimation, cTBS over left SPL; $p = 0.02$) and experiment 4 (ball weight estimation, cTBS over left SI; $p = 0.006$). Moreover, the R^2 difference for experiment 1 was marginally lower than for experiment 2 (box weight estimation, cTBS over left M1; $p = 0.06$).

A comparison of the mean RTs between sham and active cTBS in Experiment 1 revealed that responses after active cTBS were on average 68 ms slower (Table 4.2), ruling out that lower accuracy in the box weight estimation after SI disruption was due to a speed-accuracy trade off. The Experiment x Session ANOVAs on mean RTs, however, did not show any main effect of Experiment ($F_{3,52} = 0.05$, $p = 0.65$) or Session ($F_{1,52} = 0.78$, $p = 0.38$; Table 2), nor an interaction ($F_{3,52} = 0.63$, $p = 0.60$). Thus, active cTBS over SI selectively impaired accuracy in weight estimation of the observed lifted box, but did not affect response speed.

Table 4.2. Mean RTs (±SE) in ms for the four TMS experiments

	Sham cTBS	**Active cTBS**
Exp. 1	564 (±68)	615 (±90)
Exp. 2	495 (±47)	515 (±53)
Exp. 3	557 (±48)	549 (±62)
Exp. 4	495 (±43)	492 (±45)

4.4 Discussion

Our results show that, compared to sham stimulation, cTBS perturbation of SI selectively worsened participant's accuracy at estimating the weight of a box when seen lifted. In contrast, participants' performance remained comparable to sham stimulation when (i) participants judged the weight of a bouncing ball, and (ii) the stimulation was applied over the adjacent M1 and (iii) SPL. This suggests that SI is necessary for optimal weight estimation when a human agent is involved, and supports the idea that SI may enrich action understanding by providing vicarious representations of the proprioceptive consequences of the observed actions (Keysers, Kaas, Gazzola 2010).

So far only IFC and IPL have been shown to be necessary for action perception. TMS-disruption of IFC worsens participants' performance at judging the weight of a box when seen lifted (Pobric and Hamilton 2006), impairs visual discrimination of static images of actions with different kinematics (Urgesi et al., 2007) and correct recognition of deceptive movements (Tidoni et al. unpublished observations). Evidence for the role of the IFC in perceptual judgments of seen actions also comes from the TMS-adaptation (Cattaneo et al., 2011; Cattaneo, Sandrini, Schwarzbach 2010) and TMS-priming paradigms developed by Cattaneo and colleagues (2010). Additionally, patients with IFC lesions showed reduced performance in re-ordering pictures of human actions compared to physical events (Fazio et al., 2009), and were impaired in gesture comprehension (Pazzaglia et al., 2008) and recognition of biological motion (Saygin, 2007). With regard to the IPL, lesions of this region impair

recognition of transitive gestures (Buxbaum et al., 2005; Kalénine et al., 2010; Weiss et al., 2008) and of biological motion (Battelli, Cavanagh, Thornton 2003). Finally, Tranel and colleagues (2003) showed that patients with lesions in both IFC and IPL were impaired in tasks involving action recognition from pictures. Although TBS may modulate activity in remote interconnected regions, TBS also reveals local functional properties of the stimulated areas (Stefan et al., 2008). If the effect of cTBS over SI were not the results of a perturbation of neurons in SI but, instead, of a spread of the effect of cTBS onto nearby premotor or parietal regions, known to be involved in action perception, one would expect that moving the coil forward or backward would increase rather than decrease the detrimental effect on perception. This was not the case and can be interpreted as supporting our claim that the effect was mediated by SI and that SI itself contributes to action perception. However, we do not rule out that other regions, interconnected to SI (other than M1 or SPL), may have partially contributed to the observed effects. Many imaging and neurophysiological studies show that an entire network composed of ventral and dorsal premotor, anterior and posterior parietal cortices are activated in both action observation and execution (Avikainen, Forss, Hari 2002; Caetano, Jousmäki, Hari 2007; Caspers et al., 2010; Gazzola et al., 2007a; Gazzola et al., 2007b; Hasson et al., 2004; Kilner, Marchant, Frith 2009; Pierno et al., 2009; Raos, Evangeliou, Savaki 2007; Rossi et al., 2002). Of all these areas, the posterior sector of SI (BA1/BA2) that we stimulated in the current study is the region showing vicarious representation most consistently across participants (Gazzola and Keysers 2009).

Given the importance of both IFC (Pobric and Hamilton 2006) and SI (this paper) to action observation, as well as the exchange of information between these regions during action observation (Kokal and Keysers 2010; Schippers and Keysers 2011), it is relevant to consider what aspect of perception each region conveys. TMS studies show that seeing biomechanically possible and extremely overstretching movements facilitates the corticospinal representation of the muscles involved in the observed movements (Romani et al., 2005). Notably, rTMS over IFC disrupted motor facilitation during the observation of possible actions, while rTMS over SI disrupted the facilitation during observation of overstretching movements (Avenanti and Urgesi 2011). The IFC could therefore provide vicarious motor representations derived from the kinematics that would enable the observer to produce a similar action, if the movement is biomechanically possible. SI, on the other side, could contribute to vicarious somatosensory (tactile and/or proprioceptive) action components, that emerge for instance during observation of overstretching finger movements. The contribution of SI to mapping somatosensory consequences of observed actions is supported by the findings that SI activity is increased when seeing other people grasping or manipulating objects (Keysers, Kaas, Gazzola 2010) or when seeing extreme joint stretching movements (Costantini et al., 2005). Evidence that somatosensory cortices are recruited both when sensing the body and during perception of others being touched or painfully stimulated (Keysers, Kaas, Gazzola 2010; Lamm, Decety, Singer 2010; Valeriani et al., 2008), and that rTMS over SI impairs the ability to detect touch in others (Bolognini and Maravita 2011) further supports this interpretation.

While manipulation of biomechanical plausibility may dissociate somatosensory and motor components of action simulation, typically these two components are tightly interlinked. This is particularly evident when observing somebody else lifting objects. Recently, Alaerts and colleagues (2010) found that when participants observe an actor lifting objects of different weights, motor-evoked potentials are facilitated mainly by two factors: the kinematics of the movement and the degree of contraction of the hand. This facilitation could be the result of the integration in M1 of the observed kinematic information from IFC with proprioceptive/tactile information about hand-contraction from SI. The contribution of IFC, SI and other sensorimotor regions to perceiving the weight of objects seen to be lifted was suggested by previous studies showing that: i) lifting a box influences participant's perceptual judgments of the weight of a box lifted by others (Hamilton, Wolpert, Frith 2004); and, ii) the strength of this perceptual bias correlated with neural activity in a network of cortical regions including IFC, SI, M1 and SPL (Hamilton et al., 2006). However, these methods could not establish whether activity in SI was necessary for action perception. While previous evidence showed that IFC is necessary for correct performance in the box weight estimation task (Pobric and Hamilton 2006), the present study provides further causative evidence that also SI, but not M1 or SPL, is critical for the perception of weight.

The lack of significant effects on weight judgement performance with M1 stimulation is not surprising. Although neural activity in this region may be modulated by action observation (Fadiga, Craighero, Olivier 2005; Gazzola and Keysers 2009; Schütz-Bosbach et al., 2009), it is likely that such activity plays no functional role for action perception. The activity may be a

simple consequence of the strong reciprocal cortico-cortical connections, for example with IFC and/or SI (Geyer et al., 2000; Rizzolatti and Luppino 2001). Similarly, previous TMS studies reported that M1 stimulation did not influence mirror-like motor facilitation (Avenanti et al., 2007) or perceptual judgments of seen actions (Cattaneo et al., 2011).

The absence of effects after rTMS over SPL may be less expected. The SPL is a high-order multisensory region integrating visual and somatosensory information about limb position (Lloyd et al., 2002). Similarly to SI stimulation, direct stimulation of SPL (area 7) in awake neurosurgery patients produces sensations on the body but not motor output (Desmurget et al., 2009). Moreover, rTMS over this region may impair performance in proprioceptive tasks, although to a slightly less extent than rTMS over SI (Balslev et al., 2004). Although SPL is not classically considered as part of the mirror neuron system, studies show activation in SPL both during action execution and observation (Gazzola and Keysers 2009; Raos, Evangeliou, Savaki 2007). However, this region is less consistently activated relative to other sectors of the parietal cortex, such as the anterior intraparietal cortex or IPL (Van Overwalle and Baetens 2009). It may thus be possible that SPL (and in particular area 7, the target of our study), plays a minor role in action perception, relative to nearby parietal regions, including SI and IPL that appear more critical for action perception.

In conclusion, mounting evidence supports the claim that somatosensory cortices are activated not only during action execution, but also during perception of others' actions. Whether activation of SI is necessary to judge the actions of others remained unclear until now.

Indirect evidence came from sensory neuropathy patients that lack a sense of touch on their own body. These patients showed impaired performance in a task requiring inference of another's expectation of a weight when seeing him lifting a box (Bosbach et al., 2005). Our findings, that cTBS over SI negatively influences the capacity to judge the weight of a box by observing the action (lifting) of other people, now provides direct evidence that SI is necessary for the optimal perception of at least certain aspects of other people's hand actions. Together with evidence that SI is also necessary for recognizing the facial expressions of others (Adolphs et al., 2000; Banissy et al., 2010; Pitcher et al., 2008), this suggests that SI seems to play a more important role in action perception than previously thought.

Acknowledgments.

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Chapter 5

5 Evidence for top-down modulation of motor resonance during weight estimation through observation: a motor evoked potential study

Adapted from the manuscript submitted as:

Evidence for top-down modulation of motor resonance during weight estimation through observation: a motor evoked potential study

Nikola Valchev, Inge Zijdewind, Valeria Gazzola, Christian Keysers and Natasha Maurits

Abstract
We presented participants with videos of a right hand lifting a box of three different weights and asked them to estimate its weight. During each trial we delivered one transcranial magnetic stimulation pulse (TMS) over the left primary motor cortex of the observer and recorded the motor evoked potentials (MEPs) from three muscles of the right hand (first dorsal interosseous (FDI), abductor digiti minimi (ADM) and brachioradialis (BR)). In the videos participants could observe a hand lifting a box while the hand itself was hidden behind a screen. The actor's BR muscle was thus visible during the entire videos, while the actor's FDI and ADM muscles were hidden during the actual lift. Results from the FDI muscle ($p=0.007$) showed an increase in the amplitudes of the recorded MEPs corresponding to the perceived weight of the box; a weaker similar effect was observed in the BR muscle ($p=0.06$). These effects suggest that the excitability of the primary motor cortex of the observers is influenced by top-down processes engaged in predicting the outcome and effort of the observed behaviour.

5.1 Introduction

When observing a box being lifted by somebody, most of the time, people can easily estimate its weight. Many studies have already shown that the brain areas activated when a person is observing actions are similar to the ones activated when the same person is executing actions (Rizzolatti and Sinigaglia 2010). In this way our brain "resonates" with the observed action and might help us to correctly estimate the weight of the object the other person is lifting.

To study how our brain responds to the observation of actions Fadiga and colleagues (1995) compared the excitability of the motor cortex when a person is observing a grasping action versus observing an object alone, observing a moving arm or detecting a change of light conditions. The motor evoked potentials (MEPs) recorded from the hand muscles of the participants after a single transcranial magnetic stimulation (TMS) pulse over the contralateral primary motor cortex were largest when observing grasping actions. This result implies that mere action observation increased the excitability of the observer's motor system. Moreover, the resonant activation of the motor cortex of the observer, as measured by the increase of the amplitude of the MEPs, was specific to the muscles that would be used to perform the actual grasping action. The same somatotopical activation was found in the premotor and parietal brain regions using functional magnetic resonance (fMRI): Buccino and colleagues (2001) found that the activations in the premotor and parietal regions corresponded to the effector used in the observed actions, i.e. when subjects observed actions performed with the mouth, hand or foot,

cortical areas associated with the effector were activated. In sum, previous studies have shown that our brain is activated during the observation of actions and that this activation is somatotopically organized in certain brain areas.

It has also been shown that during the task of weight estimation the activation of brain areas classically involved in action observation is crucial. Pobric and Hamilton (2006) used inhibitory TMS to investigate whether the inferior frontal gyrus (IFG) plays a crucial role in weight estimation. The region was selected because it has repeatedly been shown to be activated by both tasks of action observation and execution. They found that when subjects watched a hand lifting a box and had to estimate its weight, inhibitory, repetitive TMS (rTMS), delivered to the IFG disrupted their performance. In contrast, rTMS to the occipital cortex did not affect performance, and rTMS over the IFG or the occipital cortex did not affect performance when people had to judge the weight of a bouncing ball. This observation implies that IFG activation is crucial for weight estimation when a human hand is lifting the object and not for weight estimation of objects as such.

Going one step further, Alaerts and colleagues (2010) have shown that when watching somebody lift an object the observers' brain is not only activated but that this activation is proportional to the weight of the object being lifted. They recorded MEPs from the first dorsal interosseous (FDI) muscle when participants observed an actor lifting two objects of different weight using a precision grip. Results showed that the amplitude of the MEPs was modulated by the weight of the objects being lifted. In a complementary experiment, Alaerts and colleagues (2010) compared the

amplitudes of the MEPs measured from the opponens pollicis (thumb), flexor carpi radialis (wrist) and extensor carpi radialis (ECR, wrist) muscles when participants observed videos of a hand lifting an empty, half-full or full bottle. The amplitude of the MEPs measured from the opponens pollicis and ECR muscles was higher when the observed videos showed a hand lifting a half-full or full bottle as compared with an empty one. These results suggest that during weight estimation the excitability of the primary motor cortex of the observers is proportional to the force requirements of the action. The question remains whether the activation of the primary motor cortex during action observation is solely based on cues available in the environment (i.e. visible muscle contractions during the lift of heavy objects, bottom-up influences) or whether they are triggered by cues in the environment but represent a predictive mental computation influenced by top-down processes.

In the present study we therefore asked participants to estimate the relative weight of a box being lifted, while the box and the hand grasping the box were hidden from view. In the videos, participants could only observe the hand and arm during the approaching phase, while the grasp and lift of the object were hidden behind a screen and only the forearm proximal to the wrist was visible. This approach allowed us to determine whether modulation of the MEPs according to the weight of the box being lifted can be found in the muscles directly visible to the subjects (arm muscles) or also in the muscles involved in the action but not directly observable (hand muscles). The distinction between observable and not-observable muscles allow to explore whether motor resonance is triggered only by the observation (detectable only in the observable muscles) or

whether it can be influenced by top-down processes (detectable also in the non-observable muscles).

5.2 Materials and methods

5.2.1 Participants

A total of 28 subjects participated in the study. Of these 7 were excluded because their resting motor threshold (rMT) was too high (above 80% of the stimulation output of the machine[4]), 6 because they failed to learn to discriminate between the videos during the practice run of the experiment, and 3 because the total amount of valid MEPs was less than 65%. The data of 12 participants was analysed (5 males, 7 females, mean age = 25 years, SD = 7.27). All participants were right-handed (Edinburgh handedness inventory mean=79.85, SD=27.38) with normal or corrected to normal vision. None of them had neurological, psychiatric, or other medical problems, or had any contraindication to TMS (Rossi et al., 2009). The protocol was approved by the local ethics committee of the University Medical Center Groningen and was carried out in accordance with the ethical standards of the 2008 Declaration of Helsinki. No discomfort or adverse effects during TMS were reported or noticed.

5.2.2 Experimental stimuli

Video stimuli consisted of short movies (3 to 5 sec) of a right hand entering from the right side of the screen and lifting an object hidden behind a screen (see Figure 5.1B). Participants viewed the medial side of the arm and could observe the contraction of the brachioradialis (BR) muscle of the right forearm but not the FDI or abductor digiti minimi (ADM) muscles, during the actual weight lifting. Three different weights were used: 185 g, 900 g and 3500 g. For each weight 20 different videos were

[4] MEPs are recorded after a single TMS pulse is delivered to the motor cortex at an intensity of 120% of the individual rMT. Participants who have a rMT above 80% would need to receive single pulse TMS of intensity close to the maximum output of the machine.

presented once (practice run) or twice (MEPs recording run) for a total of 60 (practice run) or 120 trials (MEPs recording run). Movies were presented in a semi-randomized order, such that no more than two videos of the same weight were presented consecutively. Videos were made as similar as possible, and after cutting, it was verified that the speed of the lifting movement and height of the maximum lift point were not correlated with the weight being lifted.

5.2.3 Experimental design, task and procedures

Each participant participated in one experimental session composed of three parts: preparation, practice run and MEPs recording run (see Figure 5.1A).

During the preparation part of the experiment the optimal scalp position (OSP) and the rMT were determined by recording MEPs (see section 5.2.4 for details). Once the OSP was identified, it was marked on an EEG cap placed on the participant's head and secured with a chin strip so that the mark did not move.

During the practice run participants watched and evaluated 60 trials of the experimental task to become familiar with it. Each trial was composed of a one second fixation cross, the presentation of the video and question "How heavy is the box?". Participants were required to answer as accurately and quickly as possible and to pay attention to the effort of the lifting action. To deliver the answer subjects used their left hand and one of three keys on the computer keyboard. The interval between trials was set to 2 seconds. If the performance on the practice run was close to chance level the participant was asked to perform the training trials again and if

the score didn't improve after the additional block, the subject was excused from further participation in the experiment.

MEPs were recorded after the practice run while participants were executing the same task. Participants responded in 120 trials and one TMS pulse was delivered to the OSP in the interval between the grasp and the highest point of the lifting movement. Trials were composed in an identical way to the practice run, but the interval between the trials was randomly varied between 8 to 12 seconds to avoid any influence of one TMS pulse to the next one. The TMS pulse was triggered by a voltage change in a photo cell placed on the screen on which the movies were displayed. A white square in the top left corner of the video (not visible to the subject) activated the photo cell. The frame in which the white square was embedded was chosen at random for each video and varied between the second frame after the grasp and two frames before the end of the video.

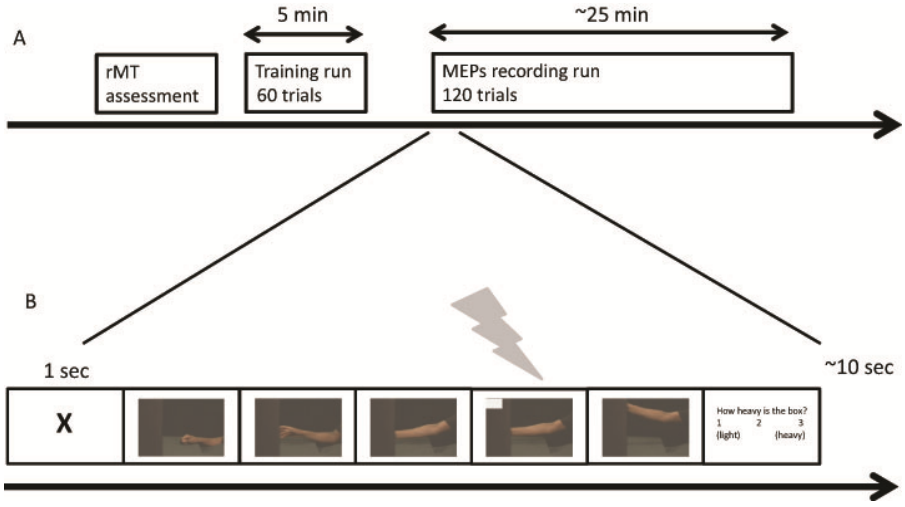

Figure 5.1. Experimental design (A) and single trial procedure (B).

5.2.4 TMS

TMS during the experiment was delivered with a 70 mm figure-eight stimulation coil connected to a Magstim Rapid2 (The Magstim Company, Carmarthenshire, Wales, UK).

The rMT was determined by recording MEPs induced by single-pulse TMS over the left motor cortex. MEPs were recorded from the right FDI, right ADM and right BR muscles by means of a Biopac MP-150 electromyograph (Biopac Corp, Goletta, CA.). Pairs of silver/silver chloride surface electrodes (active and reference) were placed over the muscle belly and over the associated joint of the muscle (on the first metacarpophalangeal joint for the FDI, on the fourth metacarpophalangeal joint for the ADM and at the end of the proximal tendon for the BR muscle). Since each differential channel of the EMG machine used in this experiment requires a separate ground electrode, two were placed on the ventral surface of the right wrist (for the FDI and ADM muscles) and another one at the olecranon (for the BR muscle) (see Figure 5.2A for electrode placement). EMG signals were band-pass filtered (20 Hz-1.0 kHz, sampled at 5 kHz), digitized and displayed on a computer screen. The OSP was determined such that reliable MEPs were produced in all three muscles. The rMT was defined as the lowest level of stimulation able to induce MEPs of at least 50 μV in at least 5 out of 10 TMS pulses in all three muscles (Rossini et al., 1994). The coil was positioned in such a way that the intersection of the figure of eight was tangential to the scalp with the handle towards the back of the head at an angle of 45° relative to the mid sagittal line. In this way we were able to induce a current in the neural tissue approximately directed perpendicular to the direction of the central

sulcus which has been shown to be optimal for activating the corticospinal pathways (Brasil-Neto et al., 1992; Mills, Boniface, Schubert 1992).

During the experimental task MEPs were recorded while stimulating the OSP for each individual subject. The intensity was set at 120% of the individual rMT.

5.2.5 EMG activity validation

To determine the extent to which the muscles we are measuring MEPs from, were activated during the lifting of a box of 185, 900 or 3500 g, we recorded EMG activity from the FDI, ADM and BR muscles in four subjects. Each subject lifted each of the three boxes five times and the average absolute EMG signal was calculated during four phases of the movement: 500 ms before the start of the action (start), approach phase (approach), lifting period (lift) and retraction of the hand (retraction). Two mechanical sensors sending a square wave to the EMG amplifier detected the moment when the hand was lifted from the table (start of action) or returned to the table (end of retraction) and the moment when the object was lifted from the table (end of approach, start of lifting) and was placed back on the table (end of lifting, start of retraction). The EMG during a maximum voluntary contraction (MVC) was determined for each individual muscle by calculating the average absolute signal during 500 ms of maximal contraction. We computed the activation of each muscle as %MVC during the four phases of the action. Results show that all three muscles increase their activation with increasing weight of the lifted box (see Figure 5.2B).

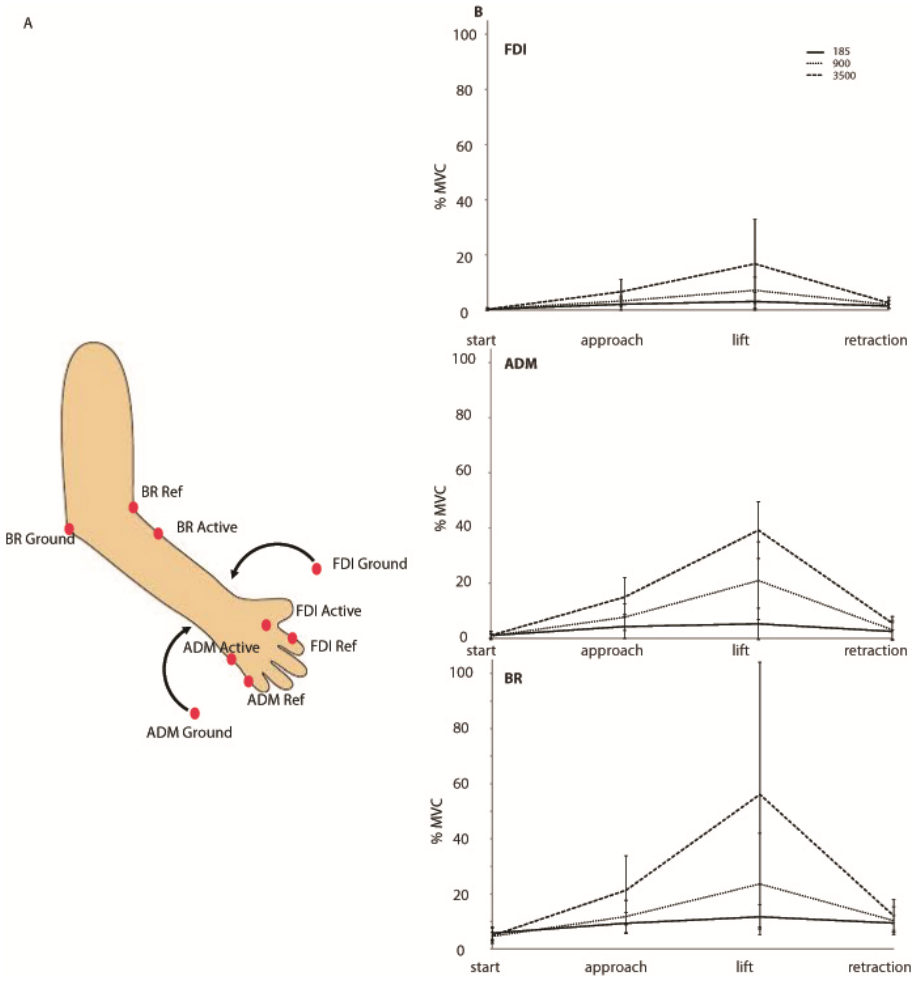

Figure 5.2. (A)Electrodes setup and (B) Median and Interquartile range of the activation of the FDI, ADM and BR muscles expressed in %MVC of the corresponding muscle during lifting of three weights for each of the four phases of the movement (start; approach; lift; retraction).

5.2.6 Data analysis

Performance for each participant was calculated during both the practice run and MEPs recording run. Subject performance was summarized by the ICC. We chose to use the ICC index as opposed to a Pearson correlation index, since the latter only evaluates the degree to which there is a linear relationship between two variables. In our case we aim at evaluating how well one variable (*y* vector of responses given by the subject) can be equated to another variable (*x* vector of correct responses), i.e. $y \_ x$ or $y \_ x \_ b$, depending on the ICC form (McGraw and Wong 1996). We used the ICC(A,1), where A stands for absolute agreement, and 1 denotes that the number of observations per subject are fixed.

EMG data was analyzed offline. For all 120 MEPs per subject we determined the peak to peak (P-P) amplitude. We analyzed only the MEPs for which there was no background muscle activity in the 100 ms before the trigger, as assessed visually. All MEPs associated with an incorrect estimation of the observed weight were excluded from the analysis.

5.3 Results

5.3.1 Performance

We calculated the performance score of all subjects, as measured by the ICC, to verify that participants were able to discriminate between the videos of different weights (for analysed participants: mean ICC(A,1)= 0.68, SD = 0.09). On average analysed participants had 73 correct responses out of 120 (SD = 8).

5.3.2 Effect of the perceived weight on MEP amplitude

The valid MEPs that were obtained when a correct response was given were analysed by means of a multilevel linear model. Data from each muscle was analysed separately, including the P-P amplitude of the MEPs as a dependent variable and the weight of the box being lifted fixed effects covariate (185, 900 and 3500 g). All individual MEPs were included in the model separately. This model takes into account that there is individual variability in the increase in P-P amplitudes of the MEPs recorded from the observer's muscles with the weight of the observed box (See Figure 5.3).

Results show that the P-P amplitude of the MEPs measured from the FDI muscle were modulated by the weight of the observed box ($F(1,723)=7.42$, $p=0.007$). For the BR muscle the linear increase of the amplitude of the MEPs with the weight of the observed box showed a trend towards significance ($F(1,723)=3.6$; $p=0.06$). No significant effect was observed for the ADM muscle.

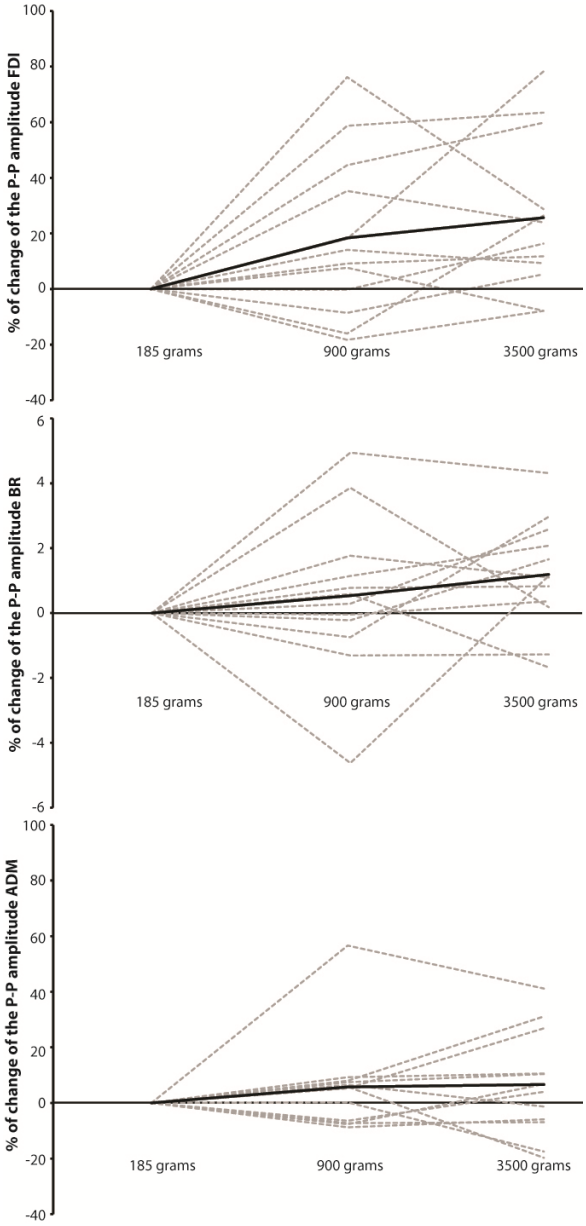

Figure 5.3. Change of the P-P amplitude of the MEPs (in %) relative to the lightest condition (185 grams) for each recorded muscle. Broken lines represent the % change of the P-P amplitude for each subject, solid lines represent the mean % of change of the P-P amplitudes.

5.4 Discussion

In this study we investigated whether motor resonance during weight estimation through observation is only induced by visual cues derived from visibly active muscles or whether it also involves activity in cortical representations of muscles that are implemented in the action but not visible. We compared MEP amplitudes measured from three muscles of the right hand and forearm (FDI, ADM and BR) while participants were watching videos of a hand lifting objects of different weights. Our results showed a significant increase with weight in the amplitudes of the MEPs recorded from the FDI muscle and a marginally significant effect on the amplitudes of the MEPs recorded from the BR muscle. No significant effect was found for the amplitudes of the MEPs recorded from the ADM muscle. The findings reported here give support to the theory that changes in the excitability of the motor cortex during action observation are due to both bottom-up and top-down influences. A mental motor representation can be triggered by external cues (visible muscles) but it is also influenced by the expectations of the observer and can thus influence the excitability of the motor cortex even when MEPs are measured from muscles not directly visible in the environment.

Motor resonance is a well-established phenomenon in the literature. Fadiga and colleagues (1995) were the first to show that watching an action activates the motor cortex of the observer in a somatotopic way. Recently, Alaerts and colleagues (2010) found that when observing another person lift objects of different weights the motor system of the observer is activated proportionally to the weight of the object being lifted. We extended on those results by showing that motor resonance is

not only triggered and modulated by the cues available in the environment but also influenced by top-down processes in the observer's brain. Thus, although the small effect observed in the BR muscle could be induced by environmental cues, the increased excitability of the FDI cannot be explained by a simple internal matching of the visible cues in the observed action. This result can be interpreted in light of the theories which attribute predictive roles to the brain activations during action observation and propose their involvement in action understanding. The fact that results did not show a significant modulation of the amplitudes of the MEPs measured from the ADM muscle, could be due to the experimental setup. Subjects did not know the real weights of the boxes or their size. The FDI muscle would be activated in any grasp (precision or full hand), while the ADM muscle would be activated more during a full hand grasp of a heavy object.

Taking into account the work of Hamilton and Grafton (2007) we can define several levels of "action understanding": 1) long term intention; 2) short term goals necessary to achieve the long term intention; 3) the kinematics that describe the movement; 4) the pattern of muscle activations required by the action. Taking this distinction as a starting point we can place motor resonance in the third and fourth levels of action understanding. The classical effect of increased somatotopically distributed activation in M1 during action observation (Fadiga et al., 1995) demonstrates that the motor system of the observer is primed by the kinematics and pattern of muscle activations in the observable environment. Rizzolati and Craighero (2004) have suggested that automatically matching the observed action into the motor representation

in the observer's brain would be sufficient to infer the intentions and goals, i.e. the actions are "recognized" by the motor system of the observer (Beudel et al., 2011). However, many times in real life and in the present experiment, only part of the actions are visible and still people are able to accurately infer goals and intentions (Iacoboni et al., 2005). In the present study participants watched videos of an object being lifted hidden behind a screen and were still able to accurately estimate its weight. Moreover, the fact that we found differences between the MEPs measured from the FDI of the observer's hand when they were watching videos of objects of different weight being lifted, speaks in favour of a predictive property of the motor resonance. The increase in MEP amplitude of the FDI muscle of the participants could not have been triggered by the visual cues available in the videos, since this particular muscle was hidden during the lift. Participants could have only inferred its involvement in the action. In a similar manner Roosink and Zijdewind (2010) have shown that motor resonance in the observers' brain increases with the complexity of the observed action. Their result can be interpreted as an anticipatory (i.e. predictive) influence of the participant's expectations on brain activity. In our case however, we show that motor resonance can be detected as a result from direct observation and expectations of the participants. We interpret our results as supporting the theory of predictive coding in the human mirror neuron system as proposed by Kilner and colleagues (2007). According to the authors the goal and intention of an action are computed by minimizing prediction error. Predictions are calculated through circular interactions among different levels of processing which correspond to different levels of cortical hierarchy. At each level a generative model is

computed through backward connections, so that it can feed predictions to the level below. Recently Friston and colleagues (2011) have developed the predictive coding model and proposed a mechanism of active inference as a basis of action understanding. The modulation of the MEPs measured from the FDI muscle we have found can be interpreted as the result of a prediction generated at a higher level in the model which influences the excitability of the motor cortex.

If we assume the perspective of the "motor matching" mechanisms proposed by Rizzolati and Craighero (2004), our results would need to show clear differences in the activation of the BR muscle in the participant's hand, during the observation of objects of different weights and a weaker effect of the observed weight in the FDI muscle. Environmental cues would trigger directly the motor representations and the match between these and the observed action would lead to action understanding, but the modulation of the MEPs in muscles that are not directly observable can only come from a higher order process involved in estimating the weight of the observed box.

A possible explanation for the lower significance of the effect of the observed weight on the amplitudes of the MEPs measured from the BR muscle could be that the scalp position used to induce the MEPs was not optimal for the stimulation of the area where this muscle is cortically represented. This possibility, although plausible, would not be in line with how the rMT was determined during the preparation phase of the experiment, when valid MEPs were detected in all three muscles with at least 50% probability before the stimulation site was defined. On the other hand, taking into account that the size of the cortical area stimulated by

the TMS beam is approximately 2 cm^2 and that the intensity of the TMS pulses used to evoke the MEPs is 120% of the individual rMT, we would expect to be able to detect a possible effect of the observation task in that muscle, as well.

In conclusion, we have shown that the excitability of the motor cortex induced during the observation of a hand lifting an object influences the amplitude of the MEPs measured from the FDI muscle, which is involved in the action but not directly visible to the subject. Moreover, the amplitude of the MEPs measured from the non-visible muscle is proportional to the estimated weight of the observed box. The same effect but of lower significance is present in the BR muscle, which is also involved in the action and directly visible to the subject. We therefore propose that these findings support the notion that motor resonance in the human brain is not only triggered by the direct observation of actions but also by top-down predictive processes.

Acknowledgements

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Chapter 6

6 General Discussion

The research presented in this thesis aimed at investigating the functional connectivity of SI and its role during action perception in general and weight estimation through observation in particular. We demonstrated that SI is functionally connected to the premotor regions during action observation (Chapter 2) and to the dPM during rest (Chapter 3). This suggests that the somatosensory cortex is involved in the extraction of information from perceived behaviours. If we adopt the perspective that mirror neurons in the human brain aid in the computation of mental simulations during action observation, our results would thus suggest that these simulations are not limited to the motor (kinematic) aspects of the observed behaviour (for a review on the classical mirror neuron system theories see Rizzolatti and Craighero 2004), but also involve the somatosensory aspects (as suggested in Keysers and colleagues, 2010). In Chapter 4 we reported results supporting the crucial role of SI during weight estimation through observation. After delivering inhibitory cTBS over SI, subjects were more inaccurate at estimating the weight (on a scale from one to five) of a box when observing a hand lifting it and placing it on a table. The same stimulation over the primary motor cortex or the posterior parietal cortex did not have an effect on task performance.

Moreover, when SI was stimulated with the same cTBS sequence but subjects were asked to estimate the weight of a bouncing ball, their judgments were not affected (compared to sham stimulation). The results suggest that SI contributes to optimal task performance possibly by internally computing ("simulating" in the perspective of mirror neuron system theories) the effort observed in the hand lifting the box. Finally, in Chapter 5 we provided more evidence for this interpretation by demonstrating that the resonant activity in the primary motor cortex of an observer, as measured by the amplitude of MEPs derived from muscles in the subject's hand and forearm, is proportional to the weight of the box that is observed while being lifted. In this last study we even showed that the perceived weight of the lifted box modulates MEP amplitude for muscles that are not directly observable in the action but are involved in it. This result supports the view that, during action perception, human brain activations are triggered by external stimuli but are also influenced by top-down processes. Such a mechanism would fit the theory that during action perception predictive models are computed in the brain and that such models are matched and updated against the information available in the external environment.

6.1 SI connectivity during action observation

In the studies reported in Chapters 2 and 3, we scanned participants on three different days. On Day 1 subjects performed two tasks; action observation and action execution. This allowed us to determine the part of SI active in both conditions in each individual subject

by identifying overlapping activations in SI from each of the tasks. We then proceeded on Days 2 and 3 to stimulate this area with either active cTBS or sham cTBS, randomizing the order of these two stimulations between subjects. After stimulation participants were again scanned while performing the same action observation task. In this way we could evaluate the effect of cTBS delivered over SI on brain activity in the whole brain and in particular in the parieto-frontal mirror system (Rizzolatti and Sinigaglia 2010). Results showed no uniform direct change of the activity in the stimulated region in SI. We also did not find a change in the baseline activity (global parameter estimates) during the observation run or in the goodness of fit of the SPM model between sessions (residual errors from the first level GLMs). PPI analyses showed no change in the functional connectivity of SI during the observation of meaningful actions (ActionObs) or control videos (CtrlObs) either. Our stimulation over the part of SI activated during action observation and action execution only resulted in an increase of the between subjects variability of the parameter estimates associated with the contrast (action movies) – (control movies). We interpreted this effect as "noise" induced by our stimulation in SI during the perception of meaningful hand actions. Because our stimulation had different effects in different subjects (8 subjects showed a decrease in the mentioned parameter estimate, and 9 showed an increase), we could not directly compare brain activations obtained after sham cTBS and active cTBS. Instead we adopted a regression analysis approach, which permits to include the values of the parameter estimates extracted from the stimulated region for each subject as a covariate and then search for brain regions in the whole brain where activations correlated with the change in

activations in the stimulated area of SI. In this way we took into account the between subjects variability of the effect of our stimulation and identified the cortical regions where activations became more synchronized with the activations in SI after stimulation, regardless of the direction of the effect of the stimulation. This procedure identified regions where activity decreased when cTBS induced a decrease of the parameter estimates in the stimulated area, and vice-versa. Since directly comparing the sham and active cTBS sessions would identify both changes in synchronisation induced by our stimulation but also changes induced by the fact that participants were presented with the action observation task for a second and third time, we had to perform additional analyses comparing the sham cTBS and Localiser sessions, the active cTBS and Localiser sessions and also the sham cTBS and active cTBS sessions. Results showed that a regression between two sessions that includes the active cTBS day identified larger brain areas in the premotor cortices in particular, and inside the parieto-frontal mirror network in general compared to the regression between two sessions which do not include active cTBS. A multilinear regression analysis showed that there is no significant change in the slope of the correlation between the stimulated region in SI and the premotor regions after cTBS stimulation. This result shows that our stimulation did not change the connectivity between these regions, in the sense of changing the polarity of the correlation between the stimulated area in SI and the premotor regions, but increased the number of voxels in the premotor whose activations are synchronized with the targeted area in SI. This shows that an active functional connection between SI and the premotor regions exists and plays a role during action observation. One

way of interpreting these findings is that action perception triggers motor and somatic representations of observed behaviours in the human brain. Taking into account that mirror neurons exist in the human brain (Mukamel et al., 2010) and that several cortical regions have been found to have "mirror like" properties (Caspers et al., 2010), it might be that the whole system of shared circuits (Gazzola and Keysers 2009) is engaged in computing these somato-motor mental representations of observed behaviours.

6.2 SI connectivity during rest

On Days 2 and 3 of the experiment described in Chapters 2 and 3 we also collected resting state fMRI data. After sham cTBS or active cTBS, we first collected the action observation data described in Chapter 2 and then a 12 minutes resting state sequence was run. This resting state data was analysed using three different approaches to explore the effects of stimulation on the connectivity of SI with the whole brain and with the parieto-frontal mirror network (Chapter 3). When considering whole brain connectivity, we took the stimulated area in SI as a seed and searched for brain regions for which the correlation between their activation and that in the seed region changed after cTBS. A cluster in dPM, which is part of the parieto-frontal mirror network, changed significantly its correlation with the targeted area in SI when the active cTBS and sham cTBS sessions were compared. A more theory driven approach, partial correlation analysis, was used to explore the effect of cTBS over SI on its connectivity with the rest of the nodes in the parieto-frontal mirror network (dPM, vPM and IPL). Results showed a change in connectivity between SI and dPM. This time

though, the strong negative partial correlation between the activations in SI and dPM increased (became less negative) after cTBS. The fact that partial correlations identify a negative association between SI and dPM, whereas the seed-based regression analysis results in a positive association, suggests that one of the other nodes in the network must mediate this connection. A data driven approach (ICA) was also applied to the same data. ICA showed that activation in brain regions included in one component, which included part of the parieto-frontal mirror network, changed with the delivered stimulation. Moreover, this change was again localized in dPM.

In sum, results from all three analytical methods used in Chapter 3 showed that cTBS, delivered over the area in SI activated both by action observation and execution, changed its connectivity with a cluster of voxels in dPM, a region classically identified as part of the parieto-frontal mirror network. Taking into account the results reported in Chapter 2 and the fact that anatomical connections between SI (the posterior part, BA2 in particular) and dPM have been reported previously (De Jong, Leenders, Paans 2002), it seems that the identified connection might be important for action perception. The results reported in Chapters 2 and 3 suggest that cTBS delivered over SI affects the connectivity of this area during rest but has no significant effect during action observation. During action observation there was an increase in the strength of the correlation between the stimulated area in SI and the premotor regions, but no change of the connectivity *per se* (slope of the correlation). This apparent conflict could be due to the difference between task requirements or to the (fixed) order in which the tasks were executed. Taking into account that TMS can

have different effects on the same neuronal population depending on its state (Cattaneo, Sandrini, Schwarzbach 2010), the fact that SI is activated by action observation (Gazzola and Keysers 2009) might have influenced our results. The second possibility is related to the fact that the resting state sequence was always collected after subjects performed the action observation task. As mentioned in Chapter 3, it has already been shown that a task performed immediately before the collection of the resting state data can affect both the default mode network and task-positive networks (Evers et al., 2012; Lewis et al., 2009). However, the resting state run followed the observation task in both the sham cTBS and active cTBS sessions on Days 2 and 3. Any effects of the task on the resting state networks would thus have been the same for both sessions, which means that when comparing the activations from these two sessions we measure the effect of our stimulation on the resting brain and the possible task effect would be cancelled out.

There are some limitations to the results reported in Chapter 2 and 3. In particular the lack of a localized effect of cTBS on activity in the targeted area in SI during action observation gives room for alternative interpretations of the results which relate the changes in correlation between SI and the premotor regions with spontaneous fluctuations of brain activity rather than an effect of cTBS. The increase in the number of voxels in the parieto-frontal mirror network whose parameter estimates correlate with the parameter estimates in the targeted area in SI can be due to chance rather than to a real effect of cTBS. Note in this respect, that the number of voxels deemed statistically significant is threshold dependent. On the other hand, assuming that cTBS has increased the

variability of the parameter estimates in SI, i.e. has induced noise in the stimulated region, the increase of the correlation between SI and the premotor regions after cTBS can be due to a synchronisation of the noise rather than to synchronisation of signal. Moreover, we need to carefully interpret two results reported in Chapter 2. First, there is no clear significant increase in the correlation or change in the slope of the correlation between activations in the premotor regions and SI due to active cTBS and second, we have delivered our stimulation off-line (which is also the case for the resting-state study) and not simultaneously with the task which limits the certainty with which we can say that the effect observed in the premotor areas is *caused* by cTBS over SI. A first alternative explanation could therefore be that the effects seen in the synchronisation between SI and the premotor regions are due to compensatory processes in the brain, rather than due to a directional effect of cTBS from SI to the premotor regions. However, since there is a positive association between the stimulated area in SI and the premotor regions, regardless of the type of stimulation (active or sham) when we consider the contrast (ActionObs – CtrlObs), our results clearly show a functional connection between SI and the premotor regions, which is active during the observation of meaningful actions. This result also supports the idea that the information transfer between the targeted region in SI and the premotor areas is task relevant. Second, we have not measured the behavioural effect of cTBS over SI in our subjects. We did not include a behavioural task to measure the effect of our stimulation, because it is known that cTBS consequences in the human brain could be cancelled due to such a task (Gentner et al., 2008; Iezzi et al., 2008; Todd, Flavel, Ridding 2009).

We already pointed out that the resting state scanning sequence was collected immediately after subjects performed the action observation task. This might have led to changes in the task relevant brain networks, which could have influenced the results of our resting state data analysis. However, such changes are expected to be equal or very similar for both sham and active cTBS sessions and will therefore cancel out when comparing the two sessions. We did find an effect of cTBS on the connectivity of SI, expressed as a difference in connectivity between the sham and active cTBS sessions. This result thus excludes the possibility that our results are driven by the action observation task performed before the resting state run. A more serious limitation of the study presented in Chapter 3 is that we only delivered sham stimulation and one type of active TBS to one location, before collecting resting state data. Results could be made stronger if resting state data were to be collected in an additional session in which, preferably in the same subjects, we would deliver another type of stimulation (e.g., 1Hz inhibitory rTMS or excitatory iTBS) over SI. Alternatively we could also design two more sessions, just as we did for the weight estimation study reported in Chapter 4, in which sham and active cTBS would be delivered over another brain area (premotor region or control area in the occipital cortex). Without these extra sessions the effects of cTBS on the connectivity of SI are still clear, but need to be confirmed. The same claim of directionality in the connection between SI and dPM could also be tested with more rigour when inhibitory TMS would be delivered online during fMRI scanning. However, the TMS sequence would likely be different, because of the technical issues associated with

delivering cTBS online during scanning, and the connectivity of SI might be differentially affected.

Since we have demonstrated in Chapters 2 and 3 that SI is functionally connected to the premotor regions during action observation and during rest to the dPM, we subsequently addressed the question of the importance of the information that is being transferred. TMS is by excellence the tool in neuroscience that can establish non-invasively how crucial the activation in a targeted region is for optimal performance in a given task. In Chapters 4 and 5 we used TMS to investigate the role of SI during weight estimation (Chapter 4) and to evaluate whether the increase of the excitability in the motor cortex during action observation is triggered by top down or bottom up processes.

6.3 Role of SI during action perception

In the experiment described in Chapter 4 we directly addressed the role of SI in the task of weight estimation through observation. Three groups of subjects received active cTBS and sham cTBS in randomized sessions over one of three cortical locations: SI, M1 and PPC. After stimulation participants watched videos of a hand lifting a box and placing it on a table and had to estimate its weight (on a scale from 1-5). Another group of participants received the same stimulation over SI but watched videos of a bouncing ball and had to estimate its weight. When comparing the accuracy of the answers given after active cTBS and after sham cTBS, only the subjects who received stimulation over SI and estimated the

weight of the box, showed an effect. Stimulating SI with active cTBS caused a decrease in the accuracy of weight estimation.

The task of weight estimation was chosen for this experiment for two reasons. First, it was already shown that a classical part of the parieto-frontal mirror network, the IFG, is essential for optimal performance on this task. Pobric and Hamilton (2006), using the same videos we used, asked participants to estimate the weight of the box and delivered inhibitory TMS over the IFG or the occipital cortex. Results showed that targeting the IFG caused a decrease in subject performance. The second reason for choosing this task was that it combines observation of an action, i.e. kinematic cues available in the videos, with a sensorial judgement (how heavy is the box). From this perspective, our results show that in the task of weight estimation through observation there is an interplay between cortical areas that are responsible for motor planning and control (Pobric and Hamilton 2006), but also purely somatosensory areas (SI). We propose that the same functional connection between SI and the premotor regions identified in Chapter 2 between SI and the premotor regions and in Chapter 3 between SI and dPM might be responsible for the extraction of sensory information from the observed kinematics of the hand lifting the box.

Although the results reported in Chapter 4 are solid and confirm the importance of SI for correct weight estimation, we need to be careful in our conclusions. The involvement of mirror neurons in action simulation has been suggested by many researchers. But the fact that we find impaired weight estimation after stimulating SI does not directly imply that observing someone's actions triggers complex somato-motor simulations in

our brains. The targeted region in the somatosensory cortex might play an independent role in this task. This issue was addressed in our study by including the control task of estimating the weight of a bouncing ball after receiving sham or active cTBS over SI. However, this task is much simpler than estimating the weight of a box being lifted by a human hand. The parameters manipulated in the videos of the hand task are the duration of the lifting phase and the grasp phase (Hamilton et al., 2005). The parameters of the ball stimuli were determined such that balls of different elasticity and weight were displayed. By simply paying attention to the highest point of the first bounce of the ball subjects could discriminate between the different videos. This is a fundamental difference between the two tasks of estimating the weight of a box lifted by a hand or estimating the weight of a bouncing ball, which we could not avoid.

In the last experiment included in this thesis we explored the effects of action observation on the excitability of the primary motor cortex. This method is more direct in the sense that an external stimulus is presented to the participant and at the same time a single TMS pulse is delivered to the primary motor cortex. By comparing the amplitudes of the MEPs recorded when different stimuli have been presented and from different muscles we can directly evaluate how much each condition had an influence on the excitability of the primary motor cortex of the observer. We recorded MEPs from one arm and two hand muscles while participants watched videos of a hand lifting a box and estimated its weight. We chose to record from these three muscles because their visibility varies in the observed videos. The hand muscles - although involved in the action - were not visible since the box being lifted was

hidden behind a screen. Two hand muscles were selected since participants did not know whether the object was grasped with a precision grip or with a full hand grip. The FDI would be used in both conditions but the ADM would only be involved in the full hand grip. In contrast, the arm muscle is visible throughout the whole movie. Results showed that watching somebody lifting an object increases the excitability (as reflected in an increase in MEP amplitude) of the primary motor cortex of the observer. We observed this effect in a hand muscle (FDI) that is involved in the action but not visible in the videos and, although the effect was weaker, it was also detected in the arm muscle involved in the action and visible to the subject. Moreover, the amplitudes of the measured MEPs increased linearly with the perceived weight of the box being lifted in the non-visible muscle (FDI). A similar effect, but weaker, was found in the visible arm muscle. We interpreted these results as evidence for top-down influences on the excitability of the primary motor cortex during action observation. Several theories of action perception postulate that there is an active process that calculates predictions of the outcomes of the observed behaviours (Friston, Mattout, Kilner 2011). One way of integrating the findings reported in Chapters 3 and 4 is by assuming that when estimating the weight of an observed object, subjects actively predict the effort of the action. The dependency of the amplitudes of the MEPs measured from the FDI muscle on the perceived weight of the box shows that the excitability of the motor cortex is not only triggered by the observation of the action but is modulated by the previous knowledge of the observer and the observer's predictions of the characteristics of the action.

An important and logical extension of the findings reported in Chapter 4 would be to repeat the same experimental setup while using videos in which all hand and arm muscles are visible. This would permit to compare the MEP amplitudes recorded from the same muscles when clearly visible or non-visible to the subjects. This would help ruling out an alternative explanation of our results: the fact that we find modulation of the MEPs recorded from muscles that are not visible in the observed actions, might be due to the mental effort subjects made to perform the task. The demands of the task might have led to a more complete mental re-enactment of the observed action and thus to the increased excitability in the part of M1 corresponding to the FDI muscle. Provided that we find the same modulation of the MEPs in a "full vision" condition, as already reported in the literature (Alaerts et al., 2010), it would make our point that people actively predict the behavioural outcome during action observation stronger.

6.4 Future directions

In this thesis we demonstrated that the primary somatosensory cortex is functionally connected with the premotor regions during action observation and rest and that it plays a crucial role during weight estimation through observation. We also used a novel combination of neuroscience tools (fMRI/ RS-fMRI/cTBS/TMS) and successfully demonstrated that new scientific questions can be answered in this manner. In the future, more research can help establish the specific role of

SI during action observation and its importance for other tasks besides weight estimation. These tasks would need to be carefully selected such that they contain enough kinematic cues to permit the participant to make a judgement, but the decision should be sensorial. Other tasks besides weight perception could be the detection of heat or the roughness of a touch. It has already been shown that the parieto-frontal mirror network and SI are activated both during detection of pain (Bufalari et al., 2007) and touch (Bolognini et al., 2011; Keysers et al., 2004). However, showing that motor excitability in the observer's brain is proportional to the amount of pain observed or roughness of the touch experience is a step that has not yet been taken. In general, experiments which target the brain activations during action observation can benefit from a step backwards, where the more fundamental and basic properties of the system are explored, rather than focusing on higher order cognitive functions. What is lacking in the literature at the moment is a way to pinpoint the building blocks by which an accurate representation of the observed behaviour forms in the brain.

The connectivity of SI can be further explored by applying different TMS sequences as well as by combining fMRI and TMS on-line. In this way the temporal and causal connections of the area can be better explored. Another approach may be to analyse the data obtained through the combination of off-line cTBS and task related fMRI or resting state using different methodological tools. Adapting the paradigm to have tasks which engage the whole system (shared circuits), or only part of it (somatosensory cortex or the parieto-frontal mirror network without SI), in a way that tasks are hierarchically related, will permit researchers to apply Dynamic Causal Modelling. This tool permits to explore causal relationships

in the studied networks and may shed more light on the effects of cTBS stimulation over SI. In addition, new analytical tools become available every day and maybe the best one for our purposes has just been discovered today.

7 Curriculum Vitae and publications list

7.1 Personal information

Nikola Stanimirov Valchev was born on January 18th 1980 in Assenovgrad, Bulgaria. Later he moved to Sofia and then to Lisbon, Paris and Groningen. He started education in Sofia at a Russian school. Since between 1994 and 1997 Nikola moved between Portugal and Bulgaria, his high school education was completed between the Spanish Institute Miguel de Cervantes in Sofia, the Spanish Institute Giner de los Rios in Lisbon and the Portuguese Secondary School Rainha D. Amelia in Lisbon. The combination of countries Nikola lived in meant that he has both Bulgarian and Portuguese citizenships and the schools and high-schools he attended made him fluent in Bulgarian, Russian, Spanish, Portuguese, French and English, by the end of 1999.

In 1999 Nikola started his bachelor degree studies in Psychology at the University of Lisbon. During the last two years he specialised in Social Psychology and research in Social Cognition. He completed parts of his studies at the University Paris V, and at the Complutense University of Madrid. Nikola obtained his degree in 2005 but remained working as a research assistant in the lab of Prof. Leonel Garcia Marques. Having spent several periods in foreign countries, he decided to continue his education with a master studies in The Netherlands. A strong research interest in the social aspects of human cognition and attraction to the methodological challenges of Neuroscience methods and data analyses led to the choice of the Behavioural and Cognitive Neuroscience research master; a program that gives the opportunity to explore different research topics and methodologies while also applying your own ideas. The master in Groningen gave him the opportunity to complete two research projects in two different labs. During the first year (2006/07), he worked with Dr. Sander Martens on the Attentional Blink. Besides this project Nikola performed research in computational linguistics. During the second year (2007/08), he worked with Dr. Joset Etzel on an fMRI experiment studying action perception. Also during the second year of his master, he secured a PhD grant from the Calouste Gulbenkian Foundation in Lisbon, Portugal. Immediately after the completion of his master studies, he started working on his PhD in the Social Brain Lab of Prof. Christian Keysers. Shortly after starting his PhD project, he obtained another grant from the Foundation

for Science and Technology in Lisbon, Portugal, which allowed him to perform the research required for his PhD. During the first four years, Nikola worked in Groningen and in Cesena (Italy) at the University of Bologna with Prof. Alessio Avenanti. Besides becoming familiar with two more languages, he became proficient in using TMS for neuroscience research and in collecting and analysing fMRI data. His research included behavioural studies with TMS and also studies which combined off-line TMS and fMRI. During the last year of his PhD studies Nikola worked with Prof. Natasha Maurits and completed all projects initiated earlier. Alongside the scientific work Nikola has managed to set small photography exhibitions in Groningen, actively participate in the Portuguese climbing community and publish socio-cultural opinion articles in the Bulgarian media.

7.1.1 Details

Name: Nikola Stanimirov Valchev

Address: Antonius Deusinglaan 2, 9713AW, Groningen, The Netherlands

Telephone: +31503638794

e-mail: nikola.valtchev@gmail.com

Nationality: Bulgarian/Portuguese

Date of birth: 18/01/1980

7.2 Publications list:

7.2.1 Published:

- Etzel, J., **Valchev, N.**, & Keysers, C. (2011). The Impact of Certain Methodological Choices on Multivariate Analysis of fMRI Data with Support Vector Machines. *NeuroImage*. 54 (2): 1159-1167.
- Martens, S., & **Valchev, N.** (2009). Individual Differences in the Attentional Blink, *Experimental Psychology.* Vol. 56(1), pp. 18-26.
- Dorscheidt, T., **Valchev, N.**, Zoelen T., and Nerbonne J. (2007) Minimal Generalization of Dutch Diminutives In Petya Osenova et al. (ed.) *Proceedings of the RANLP Workshop on Computational Phonology* Workshop at the conference *Recent Advances in Natural Language Phonology* Borovetz, 2007. pp. 21-32.l Language Processing) September 27-29, 2007, Borovets, Bulgaria.
- Garcia-Marques, L. & **Valchev, N.** (2006). "*O sonho (*DREAM*) comanda a memória*: Listas de palavras associadas para estudos de falsas memórias", (The sleep (DREAM) controls the memory. Lists of words to be used in the study of false memories) *Laboratório de Psicologia*.
- Ferreira, M., Morais, A., Ferreira, D. & **Valchev, N.** (2006). "Frases implicativas de traços com continuações situacionais e neutras para o estudo das Inferências Espontâneas de Traços" (Trait implicative sentences with situational and neutral continuations for the study of Spontaneous Trait Inferences), *Laboratório de Psicologia*.

7.2.2 Submitted

- **Valchev, N.**, Tidoni, E., Hamilton, A., Gazzola, V. & Avenanti, A. Primary somatosensory cortex necessary for the perception of other people's action: a continuous theta-burst TMS experiment.
- **Valchev, N**., Gazzola, V., Avenanti, A., & Keysers, Ch. Functional connectivity in the human mirror neuron system. The impact of continuous Theta Burst Stimulation over the primary somatosensory cortex on the BOLD signal during action observation and execution.
- **Valchev,N**., Curcic, B., & Maurits, N. Functional connectivity between the left somatosensory cortex and the putative mirror neuron system.

7.2.3 In preparation

- **Valchev, N**., Zijdewind, I., & Maurits, N., (in prep). Perceived weight of a box modulates the Motor Evoked Potentials in muscles not directly observable in the environment.
- Etzel, J., **Valchev, N.**, Gazzola, V., & Keysers, C. (in prep). Motor, parietal, and somatosensory activations to perceived movement stimuli not modulated by social interactions

7.3 Education and training

01.09.2008 - present	PhD candidate, University of Groningen, School for Behavioural and CognitiveNeurosciences, NeuroImaging Centre, University Medical Centre Groningen, The Netherlands
01.09.2006 -29.08.2008	MSc in Neuroscience, Behavioural and Cognitive Neuroscience research master, C-track (Computational), University of Groningen, The Netherlands
01.09.1999 – 18.10.2005	BSc in Psychology, Specialization in social psychology and experimental research, University of Lisbon, Portugal

7.3.1 Grants

- Doctoral Grant from the Foundation for Science and Technology at the Portuguese Ministry of Science and Higher Education. SFRH / BD / 47576 / 2008; from February 1st 2008 to January 31st 2013.
- Doctoral Grant from the Calouste Gulbenkian Foundation (process number 85430) from 16 September 2007 to 31 January 2008.
- ERASMUS exchange stipend September 2002 – February 2003.

8 Summary in English

In the last decades neuroscientists have focused their attention on the brain's activity when we perceive and interpret other people's actions. We now know that this day-to-day behaviour that we all perform effortlessly and almost without noticing activates a whole network of brain areas and is part of a complex cognitive process, which ultimately permits us to "understand" the goals and intentions of other people. Rather than attempting to answer the question of how we "understand" the actions of the people around us, the research reported in this thesis tries to increase our comprehension of the functioning of the brain when we perceive other people executing simple hand actions, how the brain areas engaged in this process interact and what role each of these regions plays in accomplishing this brain function. With this general goal in mind we centred our attention on one particular brain area – the primary somatosensory cortex (SI) shown to be activated in a "mirror like" fashion. Results show that SI is engaged both when people experience pain and observe pain (Bufalari et al., 2007), and when people experience and observe touch (Keysers et al., 2004). It has also been shown that SI is crucially involved in the detection of touch during observation (Bolognini et al., 2011). Together with the fact that SI is active both during the execution of actions and during the observation of actions (Gazzola and Keysers 2009), these results show that the primary somatosensory cortex might form a crucial part of a network of brain areas important during the observation of other people acting. In four experiments we investigated the role of SI during action perception and the

functional connectivity of this area both during the observation of actions and when the brain is at rest.

In order to address our questions we used several neuroscience methodologies alone or in combination. In our first experiment we delivered inhibitory continuous theta burst (cTBS) – a form of transcranial magnetic stimulation (TMS) - over SI immediately before collecting functional magnetic resonance imaging (fMRI) data, while our participants were observing actions or at rest (resting state – RS). To define the stimulation point we asked subjects to observe actions and execute actions in the scanner and then selected the peak of overlapping activation in SI for each individual subject. We compared brain activations from the same subjects after delivering active-cTBS and after delivering sham-cTBS. During action perception the effect of cTBS over SI resulted in an increase of the between subject variability of the parameter estimates in the stimulated area. This increase of variability meant that a larger number of voxels in the premotor regions displayed activations which strongly correlated with the activations in the stimulated area of SI. During rest, cTBS over SI had an effect on the connectivity between this area and a cluster in the dorsal premotor area. This result was confirmed by three analytical approaches; whole brain regression, partial correlations and independent component analysis. From both studies we concluded that SI is functionally connected to the premotor regions which have classically been included in the areas which might contain mirror neurons reacting in the same way when people observe and execute actions. Since we have adopted a “perturb and measure” approach we could argue that the connectivity we have shown in these two experiments is directed from SI towards the premotor regions

(during action observation) and the dorsal premotor area (during rest). The logic behind this conclusion is that if any two brain areas are causally connected then perturbing the functioning of one of them would result in a change of activation in the other one. However, a degree of caution is needed when making this conclusion. Since we combine cTBS and fMRI off-line, we cannot measure the immediate effect of our stimulation on the targeted region and the "possibly" causally connected areas. Compensatory mechanisms in the brain might lead to the increase (or decrease) of activation in a brain area involved in the task but not necessarily connected to the targeted one. While we cannot reject or accept any of these two interpretations we can speculate that both during rest and during action observation SI is causally connected to the premotor regions.

In another experiment three groups of subjects received active-cTBS or sham-cTBS over SI, the primary motor cortex or the superior parietal lobule and were asked to estimate the weight of a box they observed while being lifted by a human hand. An additional group received the same stimulation over SI but estimated the weight of a bouncing ball. Results showed that only active-cTBS stimulation over SI before estimating the weight of the box, had an effect on the subjects' performance. We interpreted these results as showing that activity in SI is crucial for estimating the weight of an object being lifted by a human agent. Our findings together with the ones of Pobric and Hamilton (Pobric and Hamilton 2006) suggest that during this task there is a set of areas in the premotor and the somatosensory cortices, that is essential for weight perception. These areas play a crucial role in the task possibly by

calculating not only motor but also somatosensory simulations of the observed action.

In an additional experiment we explored whether brain activity during action observation is triggered and entirely driven by the cues in the observed action or can be influenced by the prior knowledge of the observer, and is predictive of the observed behaviour. We evaluated the amount of motor resonance in the observer's motor system while watching videos of a hand lifting a box and estimating its weight. In order to estimate how much subjects activated their motor system we measured the amplitudes of the motor evoked potentials (MEPs) in the arm and hand muscles of their right hand during the task of weight estimation. Since in the video stimuli the box being lifted was hidden behind a screen, the hand muscles (first dorsal interosseous – FDI and abductor digiti minimi – ADM) where visible only until the moment of the actual lift, while the arm muscle (brachioradialis – BR) was visible during the entire clip. Analysis showed that when participants answered correctly the amplitude of the MEPs recorded from the FDI increased according to the weight of the box being lifted. The same effect but weaker was observed in the BR muscle. In our opinion this finding shows that the excitability of the motor cortex during action observation is not only influenced by the cues available in the environment but also by top-down cognitive processes. This conclusion fits with some of the existing theories of action observation, which attribute a predictive nature to the cognitive processes engaged during the task.

To conclude, the research reported in this thesis provides evidence that SI is functionally connected to the premotor regions during action observation and rest. We also showed that activity in SI is crucial for the

correct estimation of the weight of a box being lifted by another person and that the excitability of the motor cortex during this task may be modulated by top-down processes possibly originating in both the premotor and somatosensory regions. These processes are triggered by the cues available in the environment but are also influenced by the prior knowledge of the observer. In this sense we can speculate about the predictive nature of the mental representations of the observed actions.

The scientific questions answered in this thesis also provoke new questions. The connectivity of SI during action observation seems to be established, but how important and crucial the information is that is transferred during the task remains to be investigated. Correct weight estimation depends on the activation in SI, but it is still unclear whether this area or the premotor regions affect the amount of motor resonance.

9 Nederlandse samenvatting (Summary in Dutch)

De afgelopen decennia hebben neurowetenschappers veel aandacht besteed aan de hersenactiviteit die optreedt als we de handelingen van andere mensen waarnemen en interpreteren. We weten nu dat dit gedrag, dat we dagelijks allemaal zonder moeite en bijna zonder het te merken uitvoeren, een heel netwerk van hersengebieden activeert en dat dit gedrag deel uitmaakt van een gecompliceerd cognitief proces, dat er uiteindelijk voor zorgt dat we de doelen en intenties van andere mensen begrijpen.

Het onderzoek in dit proefschrift probeert niet zozeer de vraag te beantwoorden hoe we de daden van de mensen om ons heen 'begrijpen'. Wel probeert het ons begrip te vergroten van het functioneren van onze hersenen op het moment dat we andere mensen waarnemen die simpele handelingen uitvoeren. Ook hebben we onderzocht hoe de hersengebieden die betrokken zijn bij dit proces met elkaar samenwerken en welke rol ieder gebied dat betrokken is bij dit proces speelt om deze complexe hersenfunctie mogelijk te maken.

Met dit algemene doel in gedachten hebben we onze aandacht gericht op één specifiek hersengebied, de primaire somatosensorische cortex (SI). Eerdere resultaten van anderen hebben aangetoond dat SI zowel actief is als mensen pijn ervaren als wanneer ze pijn waarnemen (Bufalari et al., 2007) en ook als mensen aanraking voelen én waarnemen. (Keysers et al., 2004). Ook is aangetoond dat SI betrokken is bij de detectie

van waargenomen aanraking (Bolognini et al., 2011). Samen met het feit dat SI zowel actief is tijdens het uitvoeren van handelingen als tijdens de observatie van handelingen (Gazzola and Keysers, 2009), laten deze uitkomsten zien dat de primaire somatosensorische cortex een cruciaal onderdeel van een netwerk van hersengebieden – belangrijk tijdens het observeren van de handelingen van andere mensen - zou kunnen zijn. In dit proefschrift onderzochten we in vier experimenten de rol van SI gedurende de waarneming van handelingen en de functionele connectiviteit van dit gebied, zowel tijdens de waarneming van handelingen als wanneer de hersenen in 'rust' zijn.

Om onze vragen te beantwoorden, hebben we verschillende methoden uit de neurowetenschappen gebruikt, zowel afzonderlijk als gecombineerd. In ons eerste experiment stelden we SI bloot aan directe transcraniële magnetische stimulatie (TMS), in dit geval aan inhiberende continue theta burst stimulatie (cTBS). Daarna verzamelden we functionele magnetische resonantie beelden (fMRI), terwijl onze deelnemers handelingen observeerden of terwijl zij in staat van rust (Resting State – RS) verkeerden.

Om de stimulatie lokatie voor elke afzonderlijke proefpersoon vast te stellen, vroegen we onze proefpersonen eerst om handelingen te observeren en ook om handelingen uit te voeren in de scanner. Vervolgens bepaalden we welk deel van S1 overlapte met zowel de activaties tijdens observatie als de activaties tijdens uitvoeren van handelingen: dat werd ons stimulatiepunt. We vergeleken de hersenactiviteit van dezelfde

proefpersonen na toediening van actieve-TBS en na toediening van sham-cTBS. Bij sham-cTBS klinkt de stimulatie voor de proefpersoon hetzelfde, maar wordt niet actief gestimuleerd.

Tijdens het waarnemen van handelingen resulteerde het effect van cTBS op SI in een verhoging van de 'between-subject' variabiliteit van de geschatte parameters in het gestimuleerde gebied. De verhoging van deze variabiliteit betekende dat een groter aantal voxels in the premotor gebieden activatie vertoonde die sterk correleerde met de activatie in het gestimuleerde gebied van SI.

Tijdens rust had cTBS een effect op de connectiviteit tussen SI en een cluster in het dorsale premotor gebied. Dit resultaat hebben we bevestigd aan de hand van drie verschillende analyse methoden: regressie op activaties in het hele brein ('whole-brain regression'), partiële correlaties en onafhankelijke componenten analyse ('independent component analysis'). Uit beide onderzoeken (actie observatie en rust) concludeerden we dat SI functioneel is verbonden met de premotor gebieden die traditioneel worden gezien als een deel van de gebieden die mogelijk spiegelneuronen bevatten. Spiegelneuronen zijn zowel actief wanneer mensen handelingen observeren als wanneer zij handelingen uitvoeren. Omdat we met cTBS voor een "perturb and measure" aanpak hebben gekozen, konden we waarschijnlijk maken dat de connectiviteit die we in onze onderzoeken vonden, gericht is vanaf de somatosensorische cortex naar de premotor gebieden (tijdens de observatie van handelingen) en het dorsale premotor gebied (tijdens rust). Hierbij gebruikten we dat als

twee hersengebieden causaal met elkaar verbonden zijn, verstoring van de activiteit in één van deze gebieden zou resulteren in een verandering van activatie in het andere gebied. Toch moeten we bij het trekken van deze conclusie een zekere mate van terughoudendheid in acht nemen. Omdat we cTBS en fMRI off-line hebben gecombineerd, kunnen we niet het onmiddellijke effect meten van onze stimulatie op het doel gebied en de "mogelijk" causaal verbonden gebieden. Compensatiemechanismen in de hersenen zouden kunnen leiden tot toename (of afname) van activatie in een hersengebied dat wel betrokken is bij de taak, maar niet noodzakelijk verbonden is met het gestimuleerde gebied. Hoewel we niet één van deze twee interpretaties zomaar kunnen verwerpen of aannemen, speculeren we wel dat zowel tijdens rust als tijdens actie observatie, de somatosensorische cortex causaal verbonden is met de premotor gebieden.

In een ander experiment kregen drie groepen proefpersonen actieve cTBS of sham cTBS gericht op SI, de primaire motor cortex of de superieure pariëtale kwab. Daarna werden zij gevraagd om het gewicht van een doos in te schatten terwijl ze toekeken hoe deze werd opgetild door een menselijke hand. Een andere groep proefpersonen kreeg dezelfde stimulatie op SI, maar deze groep werd gevraagd het gewicht in te schatten van een stuiterende bal. De resultaten toonden aan dat alleen actieve cTBS gericht op SI, voordat het gewicht van de doos werd geschat, een effect had op de prestaties van de proefpersonen. Wij interpreteerden deze resultaten als bewijs dat activiteit in SI cruciaal is voor het inschatten van het gewicht van een object dat wordt opgetild door een menselijke hand.

Onze bevindingen samen met die van Pobric en Hamilton (2006) suggereren dat een verzameling gebieden in de premotor en de somatosensorische cortices cruciaal is voor het uitvoeren van de taak. Het is mogelijk dat deze gebieden gedurende actie observatie niet alleen de bewegingen, maar ook de sensaties betrokken bij de geobserveerde handeling simuleren.

In een aanvullend experiment hebben we onderzocht of hersenactivatie tijdens de observatie van handelingen geheel wordt aangestuurd door bepaalde signalen die een onderdeel vormen van de geobserveerde handeling, of dat deze activatie wordt beïnvloed door voorkennis van de persoon die de handeling observeert. Deze hersenactivatie zou dan voorspellend kunnen zijn voor het geobserveerde gedrag.

We onderzochten deze ‘ motorresonantie’ in het motorsysteem van de waarnemer tijdens het inschatten van het gewicht van een doos. Deze doos werd weer opgetild door een menselijke hand in een videofilmpje. Om in te kunnen schatten in welke mate de proefpersonen hun motorsysteem activeerden, hebben we de amplitude van de Motor Evoked Potentials (MEPs) in de rechter arm- en handspieren gemeten tijdens de taak van het gewicht schatten. Aangezien in de videofilmpjes de opgetilde doos verborgen was achter een scherm, waren de handspieren (eerste dorsale interosseus spier (FDI) en abductor digiti minimi spier (ADM)) alleen zichtbaar tot het moment van het daadwerkelijke optillen van de doos terwijl de armspier (brachioradialis spier (BR)) tijdens het hele filmpje zichtbaar was.

Analyse toonde aan dat wanneer de proefpersonen correct antwoordden, de MEP amplitudes (van de FDI) toenamen, in proportie met het gewicht van de opgetilde doos. Hetzelfde resultaat, maar zwakker, werd gevonden in de armspier. Naar onze mening toont deze bevinding aan dat de exciteerbaarheid van de motorcortex tijdens de observatie van handelingen niet alleen wordt beïnvloed door signalen uit de omgeving, maar ook door top-down cognitieve processen. Deze conclusie sluit aan bij bestaande theorieën over actie observatie, die een voorspellend karakter toeschrijven aan cognitieve processen, die worden betrokken bij de uitvoering van de taak.

In conclusie geeft het in dit proefschrift beschreven onderzoek aanwijzingen dat SI functioneel verbonden is met de premotor gebieden tijdens de observatie van handelingen en tijdens rust. We hebben eveneens aangetoond dat activiteit in SI cruciaal is voor een correcte schatting van het gewicht van een door een ander opgetilde doos en dat de exciteerbaarheid van de motorcortex tijdens deze taak gemoduleerd zou kunnen worden door top-down processen die mogelijk hun oorsprong hebben in zowel de premotor als de somatosensorische gebieden. Deze processen worden niet alleen opgewekt door in de omgeving aanwezige signalen, maar worden ook beïnvloed door de voorkennis van de waarnemer. In dit kader kunnen we speculeren over het voorspellende karakter van de mentale representaties van de geobserveerde handelingen.

De wetenschappelijke vragen die zijn beantwoord in dit proefschrift geven ook aanleiding tot nieuwe vragen. De connectiviteit van SI tijdens de observatie van handelingen lijkt aannemelijk gemaakt, maar hoe belangrijk en cruciaal is de informatie die tijdens de taak wordt uitgewisseld tussen hersengebieden? Dit aspect zal nog nader onderzocht moeten worden. Correcte gewichtschatting is afhankelijk van de activatie van SI, maar het is ook nog steeds onduidelijk of dit gebied, dan wel de premotor gebieden, de hoeveelheid motorresonantie beïnvloeden.

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