Transcranial alternating current stimulation to areas associated with the human mirror neuron system reveals modulation to mu-suppression and corresponding behaviour

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Abstract

This study was carried out in order to validate the use of EEG mu (μ) suppression as an index of human mirror neuron system (hMNS) related activity. The hMNS is characterized by neuronal activity that responds to both action observation and execution of the same movement. This activity has been directly observed in both macaque monkeys and in humans. There is an abundance of studies using indirect measures of neuronal activity to indicate hMNS-related activity such as TMS, fMRI/PET and EEG/MEG. However, relating indirect indices of neuronal activity to a conceptual group of neurons is controversial because the activity observed could also reflect other neuronal processes. Therefore, the current thesis was designed to establish more direct and causal evidence for the use of EEG in indicating hMNS-related activity through the use of transcranial alternating current stimulation (tACS). This was achieved in six experiments; the first three established an efficient protocol to induce μ-suppression during action observation, and the last three demonstrated by means of tACS that activity in hMNS-related areas is directly related to μ-reactivity during observation of motor movements and in relation to imitation of the movement observed. To this extent, μ-suppression was related to both action observation, and the ability to perform the movement observed. This is interpreted as evidence that EEG μ-suppression is a valid indicator of hMNS-related activity.

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Overview of Chapters

The human mirror neuron system (hMNS) is considered by many as a controversial topic because the concept of mirror neurons in humans was initially a concept generalised from single-cell studies in macaque monkeys. Furthermore, the majority of the evidence supporting a hMNS comes from indirect studies of neuronal activation such as: TMS, fMRI/PET, EEG/MEG. To date, only one study has reported direct evidence for neurons in the human cortex that contains mirror neuron properties (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010). The study conducted for this thesis sought to establish more causal and direct evidence for the use of EEG in indicating hMNS-related activity. This can be achieved by inducing changes to mu (μ) using brain stimulation (in this thesis tACS was used) and consequently relating μ changes to performance on corresponding behaviour. This aim was carried out in six experiments. The outline of these experiments is outlined next.

Chapter 1 presents the background to the main topics of concern for this thesis. In this chapter, mirror neurons are defined and described in terms of physiology, location, and assumed purpose. Alternative interpretations of mirror neurons in monkeys, particularly focusing on their putative purpose is also offered. Next, the proposal of a similar system in humans (hMNS) is addressed, and the literature of direct and indirect evidence supporting this contention is described. It is then explained how mirror neurons in humans may be indicated, and discussed what function the hMNS-related activity may have.

Chapter 2 investigates protocols that have already been associated with the hMNS, and in order to establish a protocol that induces μ-suppression efficiently, three of

these protocols were tested. The main aim of these experiments was to establish one experimental protocol that induces μ efficiently. The three experiments conducted for this purpose were as follows: Experiment 1 investigated μ-reactivity to a basic motor movement; Experiment 2 investigated μ in relation to a social-perceptive task; while Experiment 3 investigated μ in relation to a social-cognitive task. One of these protocols was selected as the most efficient protocol and used for the next chapter, which tested whether μ-suppression during action observation is a valid indicator of hMNS-related activity.

Chapter 3 presents three experiments that investigated the putative relationship between activity in hMNS core areas and μ-rhythms, and the relationship between μ-reactivity during observation of hands movements and the ability to imitate them. This was investigated by stimulating the different brain regions associated with the hMNS in three different experiments. Experiment 4 investigated the inferior parietal lobule (IPL); Experiment 5 investigated the inferior frontal gyrus (IFG); while Experiment 6 investigated the primary motor cortex (M1).

The last chapter (4) presents interpretations and implications for the findings of the study, and offers methodological limitations and future directions.

CHAPTER 1: The Mirror Neuron System

Mirror Neurons in Primates

Mirror neurons are neurons that control execution of motor actions and respond to observation of the same motor act performed by someone else (Di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992). These neurons were initially found in macaque monkeys' inferior premotor area (F5) (Di Pellegrino et al., 1992; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996) and later in area PF/PFG of the inferior parietal lobule (IPL) (Fogassi, Gallese, Fadiga, & Rizzolatti, 1998; Gallese, Fogassi, Fadiga, & Rizzolatti, 2002; Fogassi et al., 2005). These neurons were called *mirror neurons* (Rizzolatti et al., 1996) because individual cells fired in the observer's brain as if the observer performed the movement observed. The function of these neurons was originally interpreted to understand others' actions (e.g. Jeannerod, 1994; Gallese et al., 1996). However, this interpretation is still debated (e.g. Hickok, 2008) as will be discussed on page 6.

Mirror neurons were discovered accidentally during a study investigating motor neurons in macaque monkey area F5. This area was known to be a motor area controlling hand and mouth movements and its cells typically coded size, shape and orientation of objects in addition to specific types of grip necessary to grasp a particular object (Murata, Gallese, Luppino, Kaseda, & Sakata, 2000). Furthermore, activation was related to execution of goal-directed hand movements rather than single hand movements. Two types of neurons had been documented including motor neurons and visuo-motor neurons (also called canonical neurons). Motor neurons

activated during execution of motor actions corresponding to the action coded, such as grasping and holding. Often activation depended on configuration of the hand during the motor act such as a precision grip rather than a whole-hand grip (Rizzolatti et al., 1988). On the other hand, canonical neurons were activated during presentation of objects whose shape and size were congruent with the type of grasp coded motorically by the same neuron (Murata et al., 1997).

The discovery of mirror neurons happened as a macaque monkey performed hand movements such as grasping and placing while single cells were being recorded. As expected, individual cells discharged when the monkey performed the motor act that the cell coded, but unexpectedly also when the monkey observed the experimenter perform the same motor act (Di Pellegrino et al., 1992). Later studies demonstrated that approximately 20% of neurons recorded in F5 included neurons with mirror properties (Rizzolatti et al., 1996; Gallese et al., 1996). These studies also described parameters that triggered mirror neuron activation. More mirror neuron activity was reported during hand movements compared to mouth movements, and the most effective hand movement triggering a mirroring effect included grasping, placing, holding and manipulating objects. However, movements that did not include interaction with an object (intransitive) did not trigger mirroring activity. The majority of mirror neurons responded to the observation of only one action, and only if the observation involved hand movement interacting with an object. Presentations of objects or actions alone (i.e. miming) did not trigger activation (Rizzolatti et al., 1996; Gallese et al., 1996).

Individual cells demonstrated consistent patterns of firing dependent on the congruency between the action observed and the action for which individual cells

coded. However, some cells required more congruency with the observed action than other cells. Gallese and colleagues (1996) described different types of mirror neurons based on levels of congruency: 31% mirror neurons only discharged if the observed movement matched the movement that the neuron coded both in terms of goal and in terms of how the goal was achieved (strictly congruent), 61% did not require observation of exactly the same movement (broadly congruent), and lastly 8% did not demonstrate a clear relationship between the observed movement and the monkey's own movement (non-congruent).

Mirror neurons were later reported in area PF/PFG of the IPL, consisting of similar proportions of mirroring neurons as F5 (approximately 20%) (Fogassi et al., 1998; Gallese et al., 2002; Fogassi et al., 2005). The posterior parietal cortex had traditionally been considered an association cortex that assembled different sensory modalities, but there had also been indications that this region coded motor actions (Murata et al., 2000; Sakata & Taira, 1994). Mirror neurons were investigated in the IPL because area F5 did not have direct anatomical connections with superior temporal sulcus (STS), but it was known that IPL does (Petrides & Pandya, 1984; Matelli et al., 1986). The connection with STS was important because this area was known to receive visual information from the visual cortex providing F5 with a link to visual information through the IPL (Rizzolatti, Fogassi, & Gallese, 1997; Keysers & Perret, 2004). Neurons in the STS demonstrated some properties similar to mirror neurons as they responded to observation of movement (Perret et al., 1989) and a subset to goal-directed hand movements (Perret et al., 1990). However, STS neurons did not fire during motor execution and were therefore not considered as mirror neurons. Instead, it was proposed that the STS supplies visual information representing an action to IPL mirror neurons, coordinating their interaction with the

motor system (F5) (Keysers & Perret, 2004; Rizzolatti, Fogassi, & Gallese, 1997). Recently, the link between STS and F5 has been confirmed (Nelissen et al., 2011).

Additionally, evidence is accumulating that neurons in the primary motor cortex also exhibit mirror properties (Ganesh, Phillip, Lemin, & Kraskow, 2013; Dushanova & Donoghue, 2010; Tkach, Reimer, & Hatsopoulos, 2007). These findings can be viewed as problematic because the lack of mirror neurons in the motor cortex was taken as evidence that the mirroring effect could not be explained by the possibility that the monkey made covert movements while observing actions (Gallese et al., 1996). However, mirror neurons found in the motor cortex may also be interpreted as evidence that mirror neurons are more widespread than has been assumed (Casile, 2013).

The functional property of mirror neurons was early on suggested to facilitate understanding of motor events (Jeannerod, 1994). But it was also a possibility that mirror neuron activity reflected simple motor facilitation. Therefore, Gallese and colleagues (1996) investigated neurons in the primary motor cortex for mirroring properties in order to control for the possibility that monkeys' made covert movements while observing actions. No mirroring neurons in the primary motor cortex were reported and consequently mirror neurons were attributed to higher cognitive functions. It was proposed that mirror neurons match the observed action with the observer's own motor repertoire (Gallese et al., 1996; Rizzolatti et al., 1996) and this observation/execution matching enables the observer to infer the actor's intention (goal) rather than simply recognise it (Gallese & Goldman, 1998).

In order to test the involvement of mirror neurons in understanding the goal of an observed action, Umilta and colleagues (2001) presented two monkeys with goal-directed hand actions in which the final part of the movement was occluded. It was reported that a subset of mirror neurons discharged when the final part of the action presented was missing, suggesting that mirror neurons are still active even when the goal of the action observed is missing. The finding was interpreted as evidence for mirror neurons' involvement in inferring meaning from observation of movement. The rationale for this interpretation was that because the physical feature of the hand-object interaction was missing, it could not be driving the neuronal response. Instead it was suggested that stored knowledge about the actions' meaning was driving the activity. In line with this notion, Fogassi and colleagues (2005) demonstrated that neurons coding grasping movements were selective for the act following the grasp. In this study, 165 neurons (all coding grasping movements) were studied under two conditions: grasping to place, or grasping to eat. The first condition led to the monkey eating the food it brought to its mouth, and in the second condition the monkey was rewarded with food for successfully completing the task (grasping to place). The result of this study revealed that the majority of the neurons studied (N = 46) strongly activated when grasping was followed by bringing food to the mouth, and substantially fewer neurons activated when grasping was followed by placing. Similarly, the opposite was reported for 16 cells in which discharged strongly when the goal of grasping was placing, and less discharge was recorded when grasping was followed by eating. This finding has been interpreted as evidence that mirror neurons code the goal of the action observed as the neuronal response of an action was influenced by its intention.

Alternative Interpretations

Although the literature described above suggests that mirror neurons in monkeys may be involved in action understanding and inferring other's intentions and goals, the interpretation that mirror neurons are an adaptation for action understanding (e.g. Rizzolatti & Arbib, 1998) is still debated. For example, Hickok (2008; 2010; 2013) has repeatedly argued that action understanding in mirror neuron studies are not supported. Hickok argues that in order to make this claim, evidence must be presented that demonstrates deficits in action perception as a result of disruption to motor areas. This evidence has yet to be provided. Instead Hickok and Hauser (2010) suggest that mirror neurons may be understood more simply as *sensorimotor association cells* that function to select appropriate action. Another related view to this is the *associative hypothesis* that proposes that mirror neurons are the consequence of sensory-motor pairing (e.g. Catmur, Welsh, & Heyes, 2007; Heyes, 2010; Mahon & Caramzza, 2008). This view assumes that mirror neurons are motor neurons that have been paired by experience to associate observation and execution of the same act. A different view to the associative hypothesis is that mirror neurons do not facilitate action understanding - they reflect action understanding (Csibra, 2007). Csibra proposed that the primary function of mirror neurons is not action understanding in terms of goals, but predictive action monitoring. Csibra proposed that the mirror neuron mechanism does not match observed actions with existing motor repertoires because action understanding may precede action mirroring. Instead, it was suggested that mirror neurons function to reconstruct the observed action. Several other theories have been proposed that are more related to literature on human mirror neuron system and these will be discussed on page 26.

Human Mirror Neuron System (hMNS)

The hMNS refers to an observation/execution matching system described in humans that is at least conceptually similar to mirror neurons reported in monkeys. The term *mirror system* has often been used instead of *mirror neurons* because human research is largely based on indirect evidence at a systems level and not the behaviour of individual cells. The existence of mirror neurons in humans was contemplated already in the first reports of mirror neurons in monkeys (Di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996b). The possibility of mirroring neurons in humans was initially based on two observations: i) motor facilitation during action observation was indicated by enhanced motor-evoked potentials (MEP's) induced by transcranial magnetic stimulation (TMS) (Fadiga et al., 1995); ii) Increased cerebral blood flow was indicated using positron emission tomography (PET) during grasp observation in areas including Broca's area (BA[1] 44, 45) (Rizzolatti et al., 1996b). The first finding suggest that a similar observation/execution matching mechanism exists in humans as in monkeys because observation of a movement activated the corresponding cortical system recruited for execution. The second finding suggested that the location of a possible observation/execution matching system in humans is in Broca's area. This area had typically been considered an area devoted to speech production before it was reported active during hand and arm movements (Bonda et al., 1994; Schlaug, Knorr & Seitz, 1994) and mental imagery of hand grasping movements (Decety et al., 1994). These studies linked Broca's area with motor representations functionally similar to monkey area F5. Additionally, anatomical similarities between area F5 in monkeys and Broca's area in humans had been reported (Galaburda & Pandya, 1982; Petrides & Pandya, 1994). Consequently, it was proposed that the human homolog for monkey area F5 was Broca's area (Rizzolatti &

[1] BA stands for Brodmann area

Arbib, 1998; Grèzes & Decety, 2002). Later, a similar line of thinking proposed the human rostral IPL as the homolog for monkey area PF/PFG (Rizzolatti et al., 2001; Rizzolatti & Craighero, 2004).

Evidence for mirror neurons in monkeys was gathered using single cell recordings, which is a direct measure of neuronal activation. Because this method is invasive and the risks associated are not usually justified for human research, it is rarely used. Only one study to date has recorded individual cells directly in humans (Mukamel et al., 2010). In this study, patients with epilepsy were implanted with intracranial depth electrodes in order to identify seizure foci for potential surgical treatment. Therefore, electrode placements were determined by clinical considerations and not for the purpose of research. Activity from 1117 cells in the medial frontal cortex and temporal cortices were recorded while patients performed and observed hand grasping actions and facial emotional expressions. In accordance with monkey studies, action execution triggered more neurons than did action observation. The majority of the cells recorded, responded to either observation or execution, but some cells responded to both. These cells were found in supplementary motor area and in temporal areas. Additionally, cells responding with excitation during action execution and inhibition during action observation were found. These cells were proposed to function as a mechanism preventing automatic imitation during observation, and for maintaining self-other differentiation. This report is an important piece of evidence as it demonstrates directly that there are neurons in the human cortex that have mirroring properties like mirror neurons reported in monkeys.

Despite the lack of other direct evidence, the literature has an abundance of indirect evidence for a hMNS stemming from various neuroscientific methods including

neuroimaging (PET and fMRI), brain stimulation (TMS), and neurophysiology (EEG and MEG). The function of hMNS has been much debated, and is based on the functional properties attributed to mirror neurons in monkeys, that is, comprehension (Rizzolatti & Sinigaglia, 2010) and prediction of other's actions (Kilner, 2011; Wilson & Knoblich, 2005). Theories about mirror neurons have been applied to explain a variety of social cognitive abilities including imitation (Iacoboni et al., 1999; Iacoboni, 2005; Liepepelt, Prinz, & Brass, 2010), empathy (Leslie, Johnson-Frey, & Grafton, 2004; Rizzolatti & Craighero, 2004), theory of mind (Gallese & Goldman, 1998), and psychiatric disorders such as autism (Dapretto et al., 2006; Oberman, Pineda, & Ramachandran, 2007), schizophrenia (Enticott et al., 2008) and psychopathy (Fecteau, Pascual-Leone & Theoret, 2008). The content of this thesis will investigate imitation and to a certain extent, empathy, as its focus is indexing the hMNS and not its functional properties per se. The following section will address the indirect evidence supporting hMNS.

TMS Evidence

A significant proportion of the evidence for hMNS comes from TMS studies demonstrating motor system involvement in humans during action observation. TMS is a non-invasive technique based on principles of electromagnetic induction. Stimulation is produced by rapid oscillations of changing magnetic fields that are produced by passing an electrical current in the stimulator coil (Hallett, 2007). In these studies, electrical currents are applied in pulses to the motor cortex (in appropriate intensity) producing motor evoked potentials (MEPs) that can be recorded using electromyogram (EMG) from the corresponding contralateral peripheral muscle (e.g. Enticott et al., 2010; Strafella & Paus, 2000). This effect is considered an index of corticospinal excitability, and when paired with observation of

the activated muscle (e.g. hand movement) there is typically a stronger muscle response to TMS pulses (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995).

The finding that MEPs can be recorded from the muscle corresponding to the muscle used to execute the observed action (Fadiga et al., 1995) has been interpreted as evidence that changes in MEP amplitude reflect changes in excitability of the primary motor cortex. Strafella and Paus (2000) reported evidence suggesting that MEP facilitation during action observation is cortical in origin. The study used a paired-pulse TMS technique, which is the use of a sub-threshold conditioning TMS pulse, followed at various delays by a supra-threshold TMS test pulse. This technique is considered an indirect method of investigating intracortical mechanisms of facilitation and inhibition (Zieman et al., 1996). The results showed that action observation induced a facilitation of MEP amplitude evoked by the single test stimulus and led to a decreased intracortical inhibition at 3 ms interstimulus interval. They concluded that motor facilitation during action observation, was compatible with cortico-cortical facilitating connections.

The first study demonstrating enhanced MEP's during action observation was Fadiga and colleagues in 1995. In this study, participants observed the experimenter grasping objects or performing meaningless arm gestures. These observations were compared with presentation of objects only, and with a dimming light detection task in which participants verbally indicated detection of light changes. Single pulse TMS was delivered to the hand representation area and MEPs were recorded from four targeted hand muscles. The results demonstrated an increase in MEP amplitude during action observation, but not during the control conditions. Importantly, the increase in MEPs was selective for the muscles used for producing the observed movement. This

finding demonstrated a functional link between perceiving and executing similar actions. Therefore, this effect is now commonly considered an index of hMNS activity and has been reported by many (e.g. Fadiga et al., 1995; Strafella & Paus, 2000; Gangitano, Mottaghy, & Pascual-Leone, 2001; Maeda, Kleiner-Fisman & Pascual-Leone, 2002; Borroni et al., 2005). However, this effect is not without controversy as several studies demonstrating this effect used intransitive movements to trigger motor facilitation (Fadiga et al., 1995; Strafella & Paus, 2000; Borroni & Baldissera, 2008; Maeda et al., 2002), and that is in contrast to single-cell recordings in monkeys that showed that mirror neurons do not respond to intransitive movements (see page 2). Another issue with this effect is that actual movement during observation has not been controlled for in many of the studies listed above. That is a problem because the effect reported could be related to actual movement rather than responding to observation of it alone.

Not only is there an increase in MEP during action observation, Gangitano, Mottaghy, and Pascual-Leone (2001) demonstrated that the time course of cortical facilitation during action observation follows that of movement execution. In this study, participants observed grasping movements while MEPs were recorded from target hand muscles at different intervals following the movement onset. MEP amplitude was enhanced progressively as the hand opened, and decreased as the hand was closing. An equivalent pattern of modulation was reported by Baldissera and colleagues (2001) investigating the amplitude of the H-reflex (an electromyographic indication of motor neuron excitability). Gangitano and colleagues (2001) proposed that premotor mirror neurons not only match the observed action with the internal correspondent, they are also sensitive to the sequence of the observed movement (i.e. they are phase-specific). This finding suggests that premotor mirror neurons may

code the entire action in a predictive manner related with the action goal. In a second study, Gangitano and colleagues (2004) investigated the effect of such phase-specific modulation further. This study investigated whether motor facilitation during action observation is triggered in accordance with an expected motor plan as has been demonstrated in monkeys (e.g. Umilta et al., 2001). They demonstrated that while observing a reaching and grasping act that is suddenly modified by an unpredictable movement, MEP facilitation mirrors the time course of the predicted motor act rather than adjusting to its incongruent variant in real time. This finding was used to link hMNS with inferring the goal of the observed action.

More recently, it has been shown that cortical areas code movement and goals differently (Cattaneo et al., 2009). Participants in this study observed an experimenter either opening or closing normal (opened by the extension of the fingers and closed by their flexion) and reverse pliers (opposite to normal pliers) or using them to grasp objects. MEPs in response to TMS were recorded from the hand associated with the action observed. The result showed that observation of pliers simply opening and closing activated cortical representation of the hand movement involved in the observed movement. But when the pliers were grasping an object, cortical representation of the movement necessary to reach the goal was activated; specifically, during observation of grasping with the reverse pliers, MEP in the muscle recorded was enhanced during thumb extension rather than thumb flexion. The authors hypothesised that different types of actions with the same goal are mapped on to the same cortical motor neuron (conceptually) allowing generalization of goal comprehension regardless of the type of movement actually used to achieve it.

The TMS studies described above support the notion that a hMNS exists that is similar to mirror neurons reported in monkeys. However, these studies also imply that the hMNS might be different to that reported in monkeys; Monkey mirror neurons do not respond to intransitive movements (interactions without object), but it appears that the hMNS does. Several studies report TMS indices of hMNS activity during intransitive movements (e.g. Fadiga et al., 1995; Strafella & Paus, 2000; Borroni & Baldissera, 2008; Maeda et al., 2002), although some studies report mirroring only during transitive movements (e.g. Enticott et al., 2010; Donne et al., 2011). Also, macaque monkeys do not imitate, but there are indications that hMNS may facilitate imitation. For example, Heiser and colleagues (2003) demonstrated that TMS applied to Broca's area impairs individuals' ability to imitate finger key presses. Similarly, a study by Catmur, Walsh, and Heyes, (2009) demonstrated that disruptive theta burst[2] to the IFG selectively impaired imitation of index and little finger abduction.

Neuroimaging Evidence

The studies mentioned to this point have discussed the relationship between action observation and excitation of motor cortical areas. But this evidence does not reveal where in the brain the putative hMNS may be located. The neuroimaging literature has investigated hMNS localization by measuring cortical activation during action observation and execution using PET and fMRI. The activity patterns reported have been conceptually related to mirror neurons in monkeys' area F5 and PF/PFG. Rizzolatti and colleagues provided the first report in this line of evidence in 1996. In this study, participants' cortical activity was recorded using PET under three

[2] Theta burst stimulation is a pattern of rTMS stimulation that involves delivering bursts of theta frequencies (~ 5 Hz). Stimulation of the motor cortex commonly employs bursts of three at high frequency (50 Hz) every 200ms (5 Hz) during a short period (20 sec) that produces long-lasting (20 min) reduced cortical excitability (Huang et al., 2005).

conditions: (a) Observing a hand grasping for common objects performed by the experimenter; (b) reaching and grasping the same object; and (c) observation of objects. Grasp observation significantly activated areas including STS (BA 21) and the caudal part of the inferior frontal gyrus (BA 45). The same year, Grafton and colleagues (1996) demonstrated the same activation pattern under similar conditions, but reported in addition activity in the parietal area (BA 40). Both areas BA 45 (Broca's area) and BA 40 (IPL) are considered human homologues of monkey area F5 and IPL respectively. These early studies demonstrated that brain regions activated during execution or imagining hand grasping movements overlapped with observation of the same movement.

Brain activity during observation of hand movements was later demonstrated dependent on the meaning of the action. Decety and colleagues (1997) presented participants with meaningful and meaningless pantomimed hand movements with either the intention to recognize or the intention to imitate. They reported that meaningful actions activated areas including IFG (BA 44, 45) and STS (BA 21) whereas meaningless actions activated mainly occipito-parietal areas. Brain regions associated with strategy (intention to recognize or imitate) irrespective of meaning activated frontal areas but not IFG. This finding was replicated without focusing on aim (intention to imitate vs. imitation to recognize) (Grèzes, Costes, & Decety, 1998) suggesting that the action observed is coded depending on meaning.

Several recent meta-analyses demonstrate the vast scale of neuroimaging studies including PET and functional magnetic resonance imaging (fMRI) studies published since the early PET studies. These meta-analyses reveal a number of areas associated with action observation, but confirm that the most consistent areas reported includes

the rostral IPL (BA 40), ventral premotor cortex and pars opercularis of IFG (BA 44, 45), and STS (BA 21) (Caspers et al., 2010; Grosbras, Beaten & Eikhoff, 2011; Molenbergs, Cunnington, & Mattingley, 2012). Consequently, the proposed core areas of hMNS consist of the IPL, IFG and STS[3] (Rizzolatti & Craighero, 2004; Iacoboni & Dapretto, 2006). It has been postulated that these areas function as a system, similar to the system proposed in monkeys (Keysers & Perret, 2004; Rizzolatti & Craighero, 2004) in which STS sends visual information about the observed movement to the IPL where somatosensory and kinematics information are added and then sent to the IFG where the goal of the action is coded (Iacoboni et al., 2006).

Despite the areas consistently reported, a number of other areas have been associated with action observation. But these areas are not conceptually related to mirror neurons, for example the primary visual cortex. The studies used to trigger hMNS-related activity have used a variety of paradigms and procedures including different effectors (hand, mouth, foot) across different modalities (affect, somatosensory, auditory, visual). Therefore, some of the variation in results may have been the consequence of differing methodologies. Some researchers (e.g. Keysers & Gazzola, 2009) have interpreted the variation as evidence that the hMNS is not limited to the proposed core areas, but for others (e.g. Turella et al., 2009; Dinstein et al., 2008) this is a point of debate that challenges the weight of this line of evidence. Moreover, the nature of fMRI studies does not enable differentiation of conceptual neuronal populations such that the signal can be related to mirroring or facilitation of other motor systems. Additionally, mirror neurons are defined as neurons that respond to both action execution and observation of the same action (Rizzolatti et al., 1996;

[3] STS has not been shown to contain mirror neurons. It is often included as part of an extended hMNS due to its supportive role.

Gallese et al., 1996) – but the majority of the studies mentioned above report cortical activation during action observation only. Only a few report activity during both (Dinstein et al., 2007; Filimon et al., 2007; Gazzola et al., 2007). It is possible that the activity reported during action observation is related to other systems and types of neurons that are also involved with action and execution of movements (i.e. canonical neurons). Therefore, it remains controversial whether the activity pattern described above is due to a hMNS similar to that in monkeys, or whether it reflects something else, for example motor preparation (Rizzolatti et al., 2014).

In order to try to get around these issues, neuronal adaptation studies have been conducted using fMRI. Neuronal habituation/adaptation assumes that sensory neurons habituate (adapt) and become less active when the stimuli they code are presented repeatedly (Grill-Spector & Malach, 2001). Studies using this method report that hMNS core areas do contain neurons selective for both observed and executed movements that can be attributed to the goal of the observed movement (Chong et al., 2008; Dinstein et al., 2007; Hamilton & Grafton, 2006). In particular, two repetition suppression studies using fMRI demonstrated that IPL (Chong et al., 2008) and IFG (Kilner et al., 2009) respond independently to specific actions regardless of whether they are observed or executed. These studies are important because they demonstrate both the defining features of mirror neurons (respond to both action execution and action observation) in core areas of the hMNS. However, habituation studies have also been used to argue against the existence of mirror neurons as executed and observed movements have been related to different areas rather than the same (Dinstein et al., 2008; Lingnau et al., 2009).

Investigation into the functional properties of the hMNS has not only been directly

related to functions ascribed to mirror neurons in macaque monkeys (action understanding and goal prediction), but also to functions that macaque monkeys do not have such as imitation. In humans (like mirror neurons in monkeys), it has been proposed that the hMNS facilitates action understanding and understanding of other's intention by mapping observed actions onto correspondent internal motor representation (Rizzolatti et al., 2001; Rizzolatti & Sinigaglia, 2010). This notion has been asserted in several reports linking cortical activity in hMNS core areas during action observation with the observer's understanding of the observation presented. For example, in a study by Fadiga and colleagues (2006) participants were presented with: (a) hand shadows representing animals opening their mouths; (b) real animals; and (c) meaningless finger movements. The animal hand shadows were created by finger movements that when combined revealed the configuration of an animal. The authors reported Broca's area activation during observation of hand shadows, but not during any other condition. They interpreted this finding as evidence that Broca's area constructed meaning from the presentation of meaningless finger movements. In a study by Gazzola, Rizzolatti, Wicker, and Keysers (2007), participants observed video clips of either a human or a robot grasping objects. The rationale for this study was that if the hMNS codes the goal of the action observed it should not matter whether a human or a robot performed it. The results demonstrated that the sight of both a human and a robot performing the action activated hMNS core areas, and no significant difference in activity was observed. In another study by Gazzola and colleagues (2007b) it was shown that individuals with aplasia born without arms and hands responded to the observation of hand movements with the same area recruited during observation of feet and mouth actions. The authors reasoned that because these individuals have never used their hands before, they responded with areas that can execute the same motor goal using feet or mouth.

Understanding an action and its intention has been linked with imitation (Rizzolatti & Craighero, 2004). The mirror neuron mechanism has been used to explain the ability to imitate others because it offered a solution to the *correspondence problem*, that is, how visual information about body movements of others translates into matching motor output (Heyes, 2001). The notion that mirror neurons facilitate imitation has been supported by neuroimaging studies demonstrating activity in hMNS core areas during imitation processes (Buccino et al., 2004; Grèzes et al., 2003; Decety et al., 2002). One of the first studies investigating this connection was Iacoboni and colleagues (1999). In this study, participants imitated a simple finger movement immediately after observation or performed the same movement after being presented with either a spatial or symbolic cue. More activity was reported in the IFG (BA 44) when participants imitated finger movements cued by a video, than when cued with a static or symbolic cue. In another study by Buccino and colleagues (2004), participants imitated guitar chords. Results demonstrated activity in the rostral part of IPL and ventral premotor cortex and IFG for both observation and execution (imitation). Not only has activity in the hMNS core areas been recorded whilst individuals imitate, but the ability to imitate has been related to activity in hMNS core areas. Frey and Gerry (2006) presented participants with complex action sequences during fMRI, and asked participants to perform the sequences. While several areas were activated during observation, only activation of the right anterior intraparietal sulcus (BA 40) predicted imitation accuracy. This evidence is important because it demonstrates a functional relationship between cortical activity in the IPL with the ability to imitate rather than mere cortical overlap during observation and execution.

Neurophysiological Evidence

This line of evidence focuses on electroencephalogram (EEG) oscillations associated with motor processing. These studies typically report oscillatory changes in sensorimotor areas during action observation that indicate motor facilitation during observation. Before describing this literature, it is important to describe the EEG as a tool to investigate brain activity because this thesis will focus on the use of this tool. EEG is a non-invasive method that records electrical potentials from the scalp thought to be produced by excitatory/inhibitory post-synaptic potentials in the brain (Dickter & Kieffaber, 2014). These post-synaptic potentials (either excitatory or inhibitory) induce an electrical dipole (a separation of positive and negative charges) that results in voltage. The voltage produced by activity at a single synapse is miniscule, but propagation of potentials at "neighbouring" synapses (that is at a scale of hundreds or thousands of neurons) enables summation of activity that leads to a signal measurable at the scalp (Kirschstein & Köhling, 2009). EEG is as such an indirect tool for indicating neuronal activity.

The voltages expressed at the scalp depend on underlying cell geometry, dipole orientation, and spatial and temporal contiguity of neural activity (Rall, 1962; 1969). Furthermore, the most common cell type in the human cortex are pyramidal cells, in which axons are arranged roughly in parallel (Tombol, 1974; Winfield, Gatter, & Powell, 1980). It is assumed that the EEG signal recorded at the scalp reflects the sum of the activities in populations of cortical pyramidal neurons. These types of cells are ideal for EEG recording because their geometry is such that dipoles of positive and negative charge are produced at opposite ends. This is in contrast to other cells such as stellate cells in which measurable voltage is cancelled out by dipoles of positive

and negative charge at a variety of orientations (McCormick, Connors, Lighthall, & Prince, 1985; Gray & McCormick, 1996).

An issue with EEG is that surface electrodes are only sensitive to pyramidal cells that are oriented perpendicular to the scalp. Therefore, the folding of cortical tissue (sulci and gyri) is problematic because of the possible cancellation of voltage (Nunez & Srinivasan, 2006). However, with another related technique: magnetoencephalogram (MEG), cells that are oriented horizontally to the scalp generate the signal recorded (Cohen, 1972). MEG is based on the superconductive quantum interference device, which is a sensitive detector of magnetic fields created by electrical activity. MEG is superior to EEG in terms of spatial resolution because magnetic fields are less affected by the poor electrical conductivity of the skull (Hämäläinen, Hari, Ilmoniemi, Knuutila, & Lounasmaa, 1993). In contrast to EEG, it is possible with MEG to pick up signals from pyramidal cells that are not perpendicular to the scalp (Baillet, Mosher, & Leahy, 2001). The section that follows describes studies using EEG and MEG as a tool to indicate sensorimotor frequencies during observation and execution of motor actions.

EEG and MEG bandwidths have been identified depending on the number of oscillations per second and are measured in hertz (Hz). A number of bandwidths are associated with one or more cognitive functions (e.g. Klimesch et al., 2004; Başar et al., 1999). These bandwidths include delta (0.1 ~ 4Hz), theta (4 ~ 8Hz), alpha (8 ~ 12Hz), beta (12 ~ 30Hz), and gamma (30 ~ 80Hz), however, note that in the literature, there are some inconsistencies in terms of frequency windows. Additionally, at times rhythms overlap (Nunez & Srinivasan, 2006). An example of this is the mu (μ) rhythm, which is composed of alpha and lower beta frequency

components (Hari & Salmelin, 1997). Although the μ-rhythm includes oscillations in alpha (α), it should be noted that sensorimotor α is different from α localized to the parieto-occipital area: μ is associated with sensorimotor areas (Hari et al., 1998). Additionally, in contrast to α, μ-rhythms are not modulated primarily by visual stimulation, but rather, respond to the onset of motor activity (Gastaut, 1952). Although μ-rhythms are recorded in both α (8 ~ 12) and low beta (β: 13 ~ 20) (e.g. Hari & Salmelin, 1997) over sensorimotor areas, it has become common to narrow the sensorimotor frequency bandwidth to 8 – 13 Hz (e.g. Pineda, 2005). For clarity, this thesis will refer to "μ-rhythms" as including α and low β components rather than one component that is 8 – 13 Hz.

The μ-rhythm is of particular interest for this thesis as it is generated in and recorded over the primary sensorimotor cortex (Cheyne et al., 2003; Hari et al., 1998; Rossi et al., 2002) and has long been associated with motor processing. Suppression, or desynchronization in amplitude in sensorimotor rhythms is a known indicator of cortical excitation in structures mediated by the thalamo-cortical system (Goldman et al., 2002; Steriade & Llinas, 1988) while synchronization reflects deactivation or inhibition (Neuper & Pfurtscheller, 2001; Pfurtscheller, Stancak, & Neuper, 1996). μ-rhythms are observed in the absence of movement (Gastaut, 1952), and during movement, the power is attenuated and the rhythm desynchronised (Cochin et al., 1999; Hari et al., 1998; Altschuler et al., 1997). Therefore, it is assumed that suppression in the μ-rhythm reflects cortical activation in primary sensorimotor areas.

Not only has μ-suppression been observed during movement, suppression also occurs during observation of movement. The first demonstration of μ-suppression during action execution came from Gastaut and Bert in 1954. Anecdotally, the authors

reasoned that μ is suppressed as a result of an individual's identification with the person represented on the screen. This finding and its interpretation did not receive much attention until the existence of mirror neurons was investigated in humans. Cochin, Barthelemy, Roux, and Martineau conducted the first study conceptually linking μ-suppression during action observation with mirror neurons in 1999. In this study, participants' EEG was recorded under three conditions: resting; observing the experimenter perform different pincer movements with the thumb and index finger; and while performing the same actions. The results indicated that observation and execution of finger movements activated the same cortical area, and was interpreted as evidence supporting mirror neurons in humans.

Since Cochin and colleagues' (1999) study, a number of studies have demonstrated μ-suppression[4] during action observation (Oberman et al., 2005; Perry & Bentin, 2009; Puzzo et al., 2010) and conceptually relate this finding to activity of mirror neurons. This connection was initially made because the ventral premotor cortex in monkeys (where mirror neurons are reported) is connected to the primary sensorimotor cortex (where μ-rhythms are generated) by cortico-cortical connections (Dum & Strick, 2005; Matelli et al., 1986). It was also apparent that the μ-rhythm shared several features associated with mirror neuron properties: μ responds to both action observation and execution (Gastaut & Bert, 1954; Cochin et al., 1999); imagined action execution (Pineda, Allison, & Vankov, 2000); it is sensitive to the meaning of an observed action (Muthukumaraswamy & Johnson, 2004); and, it is sensitive to object interaction (Muthukumaraswamy, Johnson, & McNair, 2004). Thus, it was proposed that the μ-rhythm may reflect downstream modulation of primary sensorimotor areas by premotor mirror neuron activity (Muthukumaraswamy &

[4] When the study in question used ERD as a suppression index, it is referred to as such in the text. Other suppression indices are referred to as "suppression"

Johnson, 2004; Pineda, 2005). It is now assumed by many (e.g. Oberman et al., 2005; Perry & Bentin, 2009; Puzzo et al., 2010) that μ-suppression during action observation is an index of hMNS activity. This notion has recently been supported by several recent studies (Arnstein et al., 2011; Babiloni et al., 2016; Braadbaart, Williams, & Waiter, 2013) demonstrating that μ-suppression during action observation and execution correlate with cortical activation in hMNS core areas.

The majority of studies investigating EEG as an index of hMNS-related activity have investigated the bandwidth 8 – 13 Hz exclusively (e.g. Oberman et al., 2005; Pineda & Hecht, 2009). However, as mentioned on page 21, it is known that the μ-rhythm consists of two frequency components (α and low β) and that the generators for these components differ: changes in α is suggested to reflect activation of primary somatosensory cortex, whereas β changes are suggested to indicate motor cortex activity (Salmelin et al., 1995; Hari, 2006; Avanzini et al., 2012). This is important because the 8 - 13 Hz approach that is now commonly used to index hMNS may be too narrow to capture all hMNS-related processes. The reason is the following: β frequencies behave similarly to μ-rhythms during observation of movement (Puzzo et al., 2010; Crone et al., 1998; Hari et al., 1998; Caetano, Jousmaki, & Hari, 2007; Babiloni et al., 2002), mirror-like activity occurs in the motor cortex (Hari et al., 1998; Montagna, Cerri, Borroni, & Baldissera, 2005; Press, Cook, Blakemore, & Kilner, 2011; Szameitat, Shen, Conforto, & Sterr, 2012), lastly, the anatomical connection between IFG and motor cortex (Dum & Strick, 2005; Matelli et al., 1986) enables activation of the motor cortex post-synaptically during action observation. For these reasons, some have investigated α and low β components separately in relation to action observation (Puzzo et al., 2010; Cooper et al., 2013; Babiloni et al., 2002). This approach will be used in this thesis rather than 8 – 13 Hz.

Discussion of Interpretation and Rationale for Thesis

The discovery of mirror neurons in monkeys initiated a great deal of interest for a similar system in humans. The evidence supporting the hMNS as described above is subject to several controversies as will be reviewed next. The proposed functional property of mirror neurons in humans is to facilitate action understanding in terms of goals and intentions (see page 17). The idea that mirror neurons retrieve the meaning of observed motor movements is not straightforward because the meaning of an action may be different depending on each individual's experience. For example, it is known that some common social hand gestures in the Western hemisphere differ from those in other parts of the world. Furthermore, the logic behind the argument that mirror neurons facilitate action understanding is based on the assumption that mirror neurons in monkeys facilitate action understanding. This logic has been criticised for several reasons, but most importantly because of the circularity in the argument (e.g. Hickok, 2008; 2013): the assumption that hMNS facilitate action understanding is dependent on the assumption that mirror neurons in monkeys support action understanding, which is a claim that has been supported from indirect evidence in humans. Unfortunately, evidence is lacking to support the notion that mirror neurons in monkeys facilitate action understanding (see page 6) and therefore the rationale for hMNS role in action understanding is challenged. Secondly, hMNS is demonstrably different to mirror neurons recorded in monkeys. Mirror neurons in monkeys do not respond to either intransitive movements (movement without object interaction) or miming (see page 2) as hMNS has been shown to – at least indirectly (see page 13). Therefore, humans and monkeys appears to code the meaning of an action differently. In humans, actions that are considered meaningful are actions that can be interpreted on a social relevant level such as gesturing, and actions that are transitive are interpreted as symbolic in nature and thus represents a meaning.

However, monkeys also use motor movements for social communication (e.g. Laidre, 2011) yet mirror neurons are not responding to gestures. Intransitive actions do not convey meaning (i.e. there is no involvement of an object that conveys additional information regarding the purpose of the movement), but that may not mean it is meaningless. There may be some parameters of intransitive movements that are relevant for humans that are not relevant for monkeys. Furthermore, hMNS has been shown to respond to imitation processes (see page 9), but whether or not monkeys imitate remains largely controversial (e.g. Hickok, 2013). The literature then suggests that the hMNS is different to mirror neurons in monkeys, and as such the conclusion that these systems support the same function is arguably illogical. An alternative interpretation to the differences between hMNS and monkey mirror neurons is that hMNS evolved beyond that of monkeys (e.g. Oztop, Kawato, & Arbib, 2013; Gazzola, Rizzolatti, Wicker, & Keysers, 2007).

In addition to the theoretical generalisation from monkeys to humans, the human literature demonstrates that the hMNS dissociates from action understanding in several studies. Firstly, in a study by Catmur, Welsh, and Heyes (2007) classic mirror effects were demonstrated using TMS induced MEPs during observation of a moving hand with either the pinky or index finger moving. The participants were then trained to move the pinky finger to observation of the index finger and vice versa. After training, MEPs were greater during observation of the incongruent finger, suggesting that mirror effects had been "learned" and did not depend on understanding the action observed. This finding challenges the action understanding principle because the participants presumably did not "misunderstand" that when presented with the pinky finger it was really the index finger and vice versa. The sensorimotor training had not changed participants' perception, only the motor response triggered by the

observation. The authors of the study suggested that the function of mirror neurons is not to understand actions, but that it is a product and a process of social interaction. If it is possible to alter a motor response without affecting its perception, then the motor response cannot be the basis of perception. In a related study (Venezia, Matchin, & Hickok, 2012), it was shown that mirror-effects could be seen after pairing the movement of an index finger with the observation of a cloud. This study suggests that the hMNS is not driven by action observation, and in this context the action understanding principle becomes nonsensical: presumably the observer did not "understand" the observed cloud by simulating its action in the observers own motor repertoire. Additionally, Buccino and colleagues (2004b) demonstrated mirroring effects during observation of communicative gestures performed by a human and a monkey, but not when performed by a dog (barking) because humans do not bark (Buccino et al., 2004b). However, it has been demonstrated that humans do understand different types of barks in dogs (e.g. Hare & Woods, 2013) and therefore, it cannot be a requirement to be able to perform the action in order to understand it. Similarly, Bogart and Matsumoto (2010) demonstrated that individuals who lack the ability to perform facial expressions (Moebius syndrome) performed no different on an emotion recognition task expressed in faces compared to control participants. These studies suggest that while hMNS may be involved in understanding observed actions, it cannot be the only system that is involved.

Several alternative interpretations have been proposed including sensory-motor pairing (Catmur, Welsh, & Heyes, 2007; Mahon & Caramazza, 2008), motor preparation (Rizzolatti et al., 2014; Crammond & Kalaska, 2000), and social responding (Hamilton, 2013). The latest account for mirror neurons proposes a synergy between the mirror system and the motor system (D'Ausilio, Bartoli, &

Maffongelli, 2014). In this framework it is proposed that the motor system retrieves low-level kinematic information about a movement observed such as joint angle while the mirror system combines the information with the associated stored action goal. The authors argue that it is not yet clear whether mirror neurons encode kinematic aspects of movements or goal representations because studies to date have not yet distinguished them. The lack of evidence demonstrating such distinction, and the presence of evidence suggesting that the hMNS responds to both intransitive and transitive movements, lends support to D'Ausilio and colleagues' suggestion that mirror neurons are in a system in which motor function and goal representation overlaps or interacts rather than facilitating one or the other. In support of their synergy theory is the finding that mirror-like activity is found in the primary motor cortex of both monkeys (Tkach et al., 2007; Dushanova et al., 2010) and humans (Montagna, Cerri, Borroni, & Baldissera, 2005; Press, Cook, Blakemore, & Kilner, 2011; Szameitat, Shen, Conforto, & Sterr, 2012).

The literature reviewed above expresses some of the controversies regarding the hMNS. The content of this thesis will consist of an investigation of the hMNS using EEG. Currently, there is an abundance of EEG evidence supporting the hMNS, but these studies are primarily considered in relation to fMRI findings due to their similarity (in terms of indicating cortical activation) and correlative nature (they are both indirect measures). Consequently, the extent to which EEG is a valid indicator of hMNS-related activity still remains controversial. Therefore, the purpose of this thesis is to validate the use of EEG to indicate hMNS-related activity. To this end, six experiments were conducted with the aim of providing more direct and causal evidence. The first three experiments investigated μ-reactivity to three different experimental protocols that have previously been used to indicate hMNS-related

activity. The purpose of these experiments was primarily to establish an efficient experimental protocol to induce μ-suppression, but also to investigate μ-suppression in relation to behavioural performance on corresponding tasks. The most efficient experimental protocol was established, modified, and used in the last three experiments to investigate μ-reactivity in relation to cortical activity in hMNS core areas, and performance on an imitation task. This was done by stimulating core areas of the hMNS, and assessing the consequential effects on μ-reactivity and to corresponding behaviour.

CHAPTER 2: Establishing an Efficient EEG Protocol

Introduction

A variety of EEG protocols have been used to induce mu (μ) suppression during action observation. Such suppression has conceptually been considered as related to hMNS-related activity for the following reasons: μ is suppressed during both movement and action observation (e.g. Oberman et al., 2005; Perry & Bentin, 2009; Puzzo et al., 2010); cortical activity is observed in hMNS core areas during action observation (e.g. Caspers et al., 2010; Grosbras, Beaten & Eikhoff, 2011; Molenbergs, Cunnington, & Mattingley, 2012); suppression in μ indicates cortical excitation (e.g. Goldman et al., 2002; Steriade & Llinas, 1988); suppression in μ coincides with cortical activation in hMNS core areas (e.g. Arnstein et al., 2011; Babiloni et al., 2016; Braadbaart, Williams, & Waiter, 2013). The variety of such protocols will be reviewed below and demonstrates the wide range of sensory and perceptual implications associated with μ-suppression to action observation as an indication of hMNS activity. It is therefore in the interest of this thesis to establish a protocol that is efficient in order to ensure a systematic investigation of μ as an index of hMNS-related activity.

The range of protocols devised with the aim to induce μ-suppression has typically involved observation of a movement performed by various effectors such as a hand (Oberman et al., 2005; Perry et al., 2009; Puzzo et al., 2011), fingers (Cochin, Barthelemy, Roux, & Martineau, 1999), and legs (Cochin, Barthelemy, Lejeune, Roux, & Martineau, 1998). The most commonly presented effector is that of a hand. Hand movement protocols broadly differ in relation to inclusion of a goal and

inclusion of an object. These will be investigated more thoroughly in Experiment 1 (page 38). Additionally, μ-suppression in relation to various modalities has also been investigated. These protocols have included action sounds (Bangert & Altenmüller, 2003), perception of touch (Perry, Bentin, Bartal, Lamm, & Decety, 2010), social perception (Ulloa & Pineda, 2007; Perry, Troje & Bentin, 2010; Oberman, Pineda & Ramachandran, 2007), robot actions (Oberman, McCleery, Ramachandran, & Pineda, 2007), and emotion perception (Pineda & Hecht, 2009; Moore, Gorodnitsky & Pineda, 2012). Some of these protocols will be investigated in this chapter.

In addition to the wide variety of methodologies applied, the μ-rhythm consists of two components (α and β1) as discussed on page 21. Both of these components have been associated with indexing hMNS-related activity (page 23). However, it is becoming apparent that α and β1 components are sensitive to different parameters of action observation. Despite this, many previous studies have focused on reactivity of a narrow bandwidth (i.e. 8 – 13 Hz; see page 21). The problem with investigating a narrow bandwidth is that it may unintentionally exclude some processes of action observation. Therefore, the following section will discuss studies that investigated α and β1 components separately in relation to action observation.

A number of studies have reported α/μ-suppression during observation of object-directed (transitive) hand movements (e,g. Johnson-Frey et al., 2003; Muthukumaraswamy, Johnson, & McNair, 2004) and during goal-directed movements (e.g. Rizzolatti et al., 1996; Iacoboni et al., 2005; Muthukumaraswamy & Johnson, 2004). However, the majority of these studies focused on C-channels only because this area is directly overlying the hand representation area. However, the μ-rhythm is generated in areas underlying FC-channels as well as it is known that β-

rhythms are generated more anterior than α-rhythms (Salmelin et al., 1995; Hari, 2006; Avanzini et al., 2012). In addition, most studies to date have used a narrow frequency band (8 – 13 Hz), and therefore it is possible that a wider range of bandwidths and channels are beneficial to enhance current understanding of the relationship between μ-rhythms and hMNS-related activity. Puzzo and colleagues (2010) investigated both C and FC-channels and reported α-ERD[5] in C-channels while β1-ERD was observed in both C-channels and FC-channels. In this case, it should be expected to observe β-ERD in FC channels given that β is generated in region overlying FC channels. The lack of α-ERD observed in FC could be related to the generators of α and β, or indicative of selective processing for transitive movements (interactions including object) as has been reported before (e.g. Pfurtscheller & Lopes da Silva, 1999).

Intransitive movements (interactions without object) also appears to trigger selective α and β1 reactivity. For example, in a study by Babiloni and colleagues (2002), participants observed and executed aimless finger movements. The results demonstrated both α and β-ERD from both C and FC-channels. However, differences in ERS[6] were reported suggesting that the α-bandwidth was slower to recover compared to β. Additionally, the peak β-ERS distribution revealed maximum values in the contralateral central area during both observation and execution, while following movement execution, α peaked in the contralateral central-parietal area. Furthermore, following movement observation, α peaked in the parietal-occipital areas. This finding suggests that α and β processes observation and execution of intransitive movements differently. Furthermore, Puzzo and colleagues (2011)

[5] ERD refers to event-related desynchronization (suppression) using Pfurtscheller and colleagues' formula (Pfurtscheller & Aranibar, 1977; Pfurtscheller & Lopes da Silva, 1999).
[6] ERS refers to event-related synchronization using Pfurtscheller and colleagues' formula

demonstrated β1-ERD during observation of a simple hand opening and closing in both C and FC-channels, but no α-ERD in any channels. In a more recent study, Puzzo and colleagues (2013) reported that ERD in α occurred in C-channels, while β1-ERD occurred in FC-channels during observation of the same stimuli. During observation of a moving hand in front of an actor portraying facial expressions, Cooper and colleagues (2013) demonstrated β1-ERD in C-channels (FC channels were not investigated). Although EEG lacks precision due to spatial smearing of signal (see page 19), these studies are revealing distinct roles for α and β1 in action observation.

Empathy

Moving on to the functional properties associated with hMNS-related activity, a number of studies have proposed that the hMNS is associated with various abilities including: social skills (Oberman, Pineda, & Ramachandran, 2007); imitation (Iacoboni et al., 1999); theory of mind (Gallese & Goldman, 1998; Schulte-Rüther, Markowitsch, Fink, & Piefke, 2007); language (Fogassi & Ferrari, 2007); and empathy (Rizzolatti & Craighero, 2004; Baird, Scheffer, & Wilson, 2011). Additionally, some have implicated the hMNS in disorders such as autism (Dapretto et al., 2006; Oberman et al., 2005), schizophrenia (Enticott et al., 2008) and psychopathy (Fecteau, Pascual-Leone, & Theoret, 2008). The most explored proposed function attributed to the hMNS is empathy. The reasons why that might be will now be explored. Empathy is broadly defined as the ability to detect and understand other people's mental states (Blair, 2005; Decety & Jackson, 2004). It has been suggested that an individual gathers information about other people's mental states by simulating others' motor expressions of emotions (Liberman & Wahlen,

2000; Iacoboni & Mazziotta, 2007). This theory of empathy suggests that the motor system is involved in processing empathy, possibly by recruitment of hMNS.

When considering other functions associated with hMNS, it is noticeable that the majority are in some way related to empathy. For example, social skills are positively correlated with empathy (Riggio, Tucker, & Coffaro, 1988; Galinsky, Ku, & Wang, 2005) which facilitates and enables positive social interactions (Björkqvist, Österman, & Kaukiainen, 2000). Additionally, social skills have been used as a factor to define and understand empathy (Friedman, 1979). Another example is imitation. Several electromyographic (EMG) studies suggest that individuals who are more empathic are better at imitating others (Chartrand & Bargh, 1999; Sonnby-Borgström, 2002). It has also been suggested that imitation may facilitate empathy (see Iacoboni, 2009). It is reasoned that people are able to feel what other people feel (empathy) through imitation and mimicry, much like the proposed motor theory of empathy. Additionally, it has been reported that individuals with autism, who are thought to lack empathy, demonstrate impaired ability to imitate (Rogers & Pennignton, 1991; Smith & Bryson, 1994; Rogers, 1999), although this is not without controversy (see Bird, Leighton, Press & Heyes, 2007; Charman & Baron-Cohen, 1997). Given this apparent interrelatedness between empathy and other abilities associated with hMNS, it is perhaps not surprising that the diagnostic criterion for the disorders that has been associated with impairments of the hMNS, includes deficits in empathy (American Psychiatric Association, 2013). The proposed relationship between empathy and hMNS is perhaps the most researched combination because empathy could be construed as the most obviously related ability.

The literature investigating empathy in relation to hMNS however, is not

straightforward. This is because empathy is a multi-faceted concept that is thought to encompass the following sub-components: *cognitive, motor,* and *affective* (e.g. Blair, 2005; Baird, Scheffer, & Wilson, 2011; Preston & de Waal, 2002). Cognitive empathy refers to the ability to recognize mental states in others, while affective empathy refers to the ability to experience mental states vicariously (Davis, 1980; Blair, 2005). Motor empathy was included in the definition of empathy somewhat later, and refers to automatic imitation of motor responses observed in others (Blair, 2005). These sub-components sometimes overlap, but evidence of a double-dissociation between affective and cognitive types has been reported (Shamay-Tsoory, Aharon-Peretz, & Perry, 2009) suggesting that these subtypes are dependent on separate cortical substrates. Additionally, amalgamating sub-components of empathy often confounds the interpretation, because the scales do not all positively correlate with each other (Davis, 1982; 1983). Therefore, empathy inventories tend to indicate one or several aspects of empathy but not scores on empathy as a global construct. This becomes an issue when empathy is investigated in relation to hMNS, because some studies investigate one sub-component while others investigate another. Consequently, comparisons between studies become difficult. In an attempt to untangle this issue, studies reporting a relationship between hMNS and individual scores of empathy will be described next.

Neuroimaging studies have repeatedly demonstrated a correlation between increased cortical activation in core areas of the hMNS with individual scores on empathy (Gazzola, Aziz-Zadeh, & Keysers, 2006; Jabbi, Swart, & Keysers, 2007; Kaplan & Iacoboni, 2006; Pfeifer, Iacoboni, Mazziotta, & Dapretto, 2008). However, it is problematic that some of these studies report a positive correlation[7] (Jabbi et al.,

[7] the higher the score on empathy – the greater cortical activation is recorded

2007; Pfeifer et al., 2008; Gazzola, Aziz-Zadeh, & Keysers, 2006) while others report a negative correlation (Kaplan & Iacoboni, 2006). Additionally, some of the studies mentioned above indicated affective empathy (Jabbi et al., 2007; Pfeifer et al., 2008; Kaplan et al., 2006), while others (e.g. Gazzola et al., 2006) indicated cognitive empathy. Likewise, EEG studies demonstrate a correlation between suppression in μ during action observation and individual scores of empathy. The same issues are present in this literature. For example, Cooper and colleagues (2012) and Woodruff, Martin and Bilyk (2011) demonstrated a positive correlation[8] while Perry, Troje, and Bentin (2010) and Milston, Vanman and Cunnington (2013) reported a negative relationship. Additionally, Cooper and colleagues (2012) indicated affective empathy, while Woodruff, Martin & Bilyk (2011) and Milston, Vanman and Cunnington (2013) indicated cognitive empathy. In addition to these inconsistencies, empathy has been shown to be context dependent (Hein & Singer, 2008), which may have affected some of the results mentioned above. Furthermore, it has been suggested that the function of the hMNS is to contribute to social responding by retrieving the appropriate response to the stimuli observed (Hamilton, 2013). The studies investigating empathy in relation to hMNS varied in terms of social relevance (context). For example, observation of a yawn (Cooper et al, 2012), infliction of pain (Perry, Bartal, Lamm, & Decety, 2010), and face imitation (Bernier, Dawson, Webb, & Murias, 2007). These studies are more socially relevant than observing a finger-thumb tapping action (Woodruff, Martin & Bilyk, 2011) and may therefore have confounded the relevance of empathy with relation to the hMNS, that is: The studies that were more socially relevant were perhaps more ideal for activating responses requiring empathic processes. In addition, different empathic processes could be activated for different contexts. Context then, adds to pre-existing inconsistencies in

[8] the higher the score on empathy – the greater the suppression value

the literature, and for the reasons stated above, the contended relationship between hMNS and empathy remains controversial.

Summary and Aims

Many different protocols have been devised to trigger suppression in μ in order to index hMNS activity. These protocols have varied across modalities, and utilised different effectors. Additionally, despite that both components of μ (α and β1) are involved in processing observed actions, it is becoming apparent that they might be sensitive to different aspects of it. Although many studies report a relationship between empathy and hMNS both using fMRI and EEG, this relationship is inconsistent and is consequently considered as controversial. It is apparent that social context is an important factor to consider. The main aim of the present chapter is to test μ-reactivity to different protocols in order to establish an efficient protocol to use in subsequent experiments. The first protocol (Experiment 1) involved observation of a hand opening and closing (intransitive hand action). This was chosen for two reasons: firstly, investigating rudimentary motor mirroring (i.e. lacking goal-directed movement) enables a more stringent analysis of kinematic parameters of the motor act (see Jeannerod, 1995). As such, the two μ-components can be investigated more clearly, or at least with less ambiguity relating to other processes not exclusively motor in nature. Secondly, several prior studies indicate the efficacy of μ-suppression during observation of an intransitive hand movement (e.g. Oberman et al., 2005; Puzzo et al., 2011; Bernier et al., 2007). The second and third protocols were selected to investigate μ-rhythms in relation to context (social relevance) of the action observed. Experiment 2 investigated μ in relation to a social-cognitive task that involves mental state recognition. This was chosen because mental states have been

suggested to be processed using the motor system (e.g. Pineda & Hecht, 2009). According to simulation theory (see page 4; Gallese & Goldman, 1998), mental states in others can be understood by simulating the motor actions involved in expressing mental states observed. This experimental protocol also tested the ability to understand mental states, and as such, performance on the task can be related to μ-reactivity. Experiment 3 investigated a social-perception task that involved inferring meaning from point-light biological motion videos depicting social interactions. This protocol was chosen because the hMNS has been implicated in inferring meaning under conditions in which visual information is sparse or is abstract (e.g. Fadiga et al., 2006). This protocol also included a measure of the ability to interpret the displays, and therefore, μ-reactivity can be related to performance also in this experiment.

In summary, this chapter will present three different protocols investigating μ-reactivity during: (1) simple motor observation (intransitive hand movement); (2) a social-perception task (mental state recognition); and (3) a social-cognitive task (social interactions depicted by point-light biological motion videos). Individual scores on affective empathy were investigated in relation to μ-reactivity recorded in all of these experiments in order to investigate the proposed relationship between empathy and hMNS.

Experiment 1: Intransitive Hand Movement Observation

Introduction

A moving hand is the most commonly employed effector used to induce μ-suppression during action observation. Presentation of a moving hand has involved a live actor (e.g. Rizzolatti et al., 1996; Grafton et al., 1996) but is more commonly presented in the form of videos (e.g. Oberman et al., 2005; Puzzo et al., 2011). Observation of a moving hand has involved: object-directed movements (e.g. Johnson-Frey et al., 2003; Muthukumaraswamy, Johnson, & McNair, 2004), goal of the action, such as grasping (Rizzolatti et al., 1996; Iacoboni et al., 2005) and precision grip (Muthukumaraswamy & Johnson, 2004), hand interactions without object interaction (intransitive) such as a simple hand opening and closing (e.g. Oberman et al., 2005; Puzzo et al., 2011), and pantomimed goal-directed hand actions such as gesturing to open a bottle without any object-interaction (Decety et al., 1997; Grèzes, Costes, & Decety, 1998). This literature has demonstrated several tendencies: biological movement is more efficient in triggering μ-suppression than are static images (Cochin et al., 1998); non-biological but directional movement (i.e. bouncing balls) does not trigger μ-suppression (Oberman et al., 2005); a static image of a hand triggers significantly less μ-suppression compared to a moving hand (Puzzo et al., 2010; Puzzo et al., 2011) even when the static hand image indicates object-interaction (Perry & Bentin, 2009); meaningful movements trigger greater μ-suppression compared to meaningless movements (Muthukumaraswamy & Johnson, 2004); and hand-object interactions trigger more μ-suppression compared to non-object interactions (Muthukumaraswamy, Johnson, & McNair, 2004).

This experiment focused on μ-suppression (ERD) during observation of a simple motor observation, and therefore addresses the literature primarily on intransitive hand movements. The presentation time and the number of repetitions in studies focusing on μ-suppression during observation of an intransitive hand movement have varied. These parameters are of interest for this experiment in order to optimize efficiency of the protocol. Some presented participants with relatively long stimulus presentation time (80 seconds) repeated twice (Oberman et al., 2005; Raymaekers, Wiersema, & Roeyers, 2009) while others presented multiple (20) but short presentations (3 seconds) (e.g. Bernier et al., 2007). Puzzo and colleagues (2011) compared these two protocols and reported that multiple short presentations were more efficient and had the advantage of averaging trials, which in turn results in higher signal-to-noise ratio.

The current experiment employed the protocol suggested by Puzzo and colleagues (2010), in which video presentations lasted 3 seconds presented 20 times. In the current experiment, μ-reactivity was recorded during observation of a hand opening and closing, a static hand, and two bouncing balls. It was predicted that observation of the moving hand would induce significantly greater μ-ERD compared to observation of the static hand. This prediction was based on the observation that biological movement triggers greater μ-suppression than static images (e.g. Cochin et al., 1998; Puzzo et al., 2010; Puzzo et al., 2011). The moving hand was predicted to trigger greater μ-ERD compared to the bouncing balls because hMNS is thought to be selective for biological movement (Rizzolatti & Fadiga, 1998), and because the μ-rhythm is not sensitive to non-biological directional movements (Oberman et al., 2005). Lastly, it was hypothesized that μ-ERD during observation of the moving hand would be modulated by individual scores on empathy.

General Method

Participant selection

Two-hundred and fifty individuals completed Davis' (1980; 1983) Interpersonal Reactivity Index (IRI: See appendix 1). Individuals reporting the following scores on the *empathic concern* (EC) subscale were invited to participate in the study: (a) low EC scorers, who scored below the 10th percentile; (b) moderate EC scorers, who scored between 45th and 55th percentile; (c) high EC scorers, who scored above the 90th percentile. In total, 38 participants (20 females) participated in the study, mean age = 23.71 SD = 6.83. All participants were right handed, signed informed consent, and were paid GB £6 for their time. The local ethical committee (Department of Psychology, University of Essex) granted ethical approval. See Table 1 for demographics in each empathy group.

Table 1: Overview of empathy groups by mean IRI scores and standard deviation in brackets

Level of Empathy	N	M (SD)
Low	14	12.64 (3.09)
Moderate	10	20.60 (.50)
High	14	25.57 (.91)

Empathy index

The IRI (Davis, 1983; Davis, 1980) is a self-report empathy measure that includes 28 descriptions of subjective empathy. Answers are recorded on a five-point Likert scale ranging from "describes me very well" to "does not describe me well". The instrument includes four subscales: empathic concern, perspective taking, fantasy,

and personal distress. Empathic concern and personal distress are considered a scale indicating affective empathy, while perspective taking and fantasy are considered indicators of cognitive empathy. Participants completed all subscales of the IRI although they were selected based on their scores on the EC subscale. Previous literature linking empathy with hMNS-related activity has demonstrated a connection with both cognitive and affective types of empathy (see page 35). However, as shown in a lesion study, each type has been associated with a different cortical system suggesting that affective empathy is associated with a core area of the hMNS (IFG) while cognitive empathy with ventromedial prefrontal cortex (Shamay-Tsoory, Aharon-Peretz, & Perry, 2009). Consequently, the EC subscale was used as the measure of empathy in the current study.

Stimuli

Participants observed three video clips taken from Puzzo and colleagues (2011). Each clip lasted 3-seconds and depicted either a moving hand, a static hand, or two bouncing balls. The moving hand depicted a right hand opening and closing against a black background. A static image of the same hand (in an open position) was included to control for sensorimotor reactivity to biological movement. Lastly, two balls in Caucasian skin colour moving vertically at the same pace as the moving hand was included to control for sensorimotor reactivity to directional non-biological movement. The moving hands and balls moved at a rate of 1Hz. Pictures of the visual display can be seen in Figure 1.

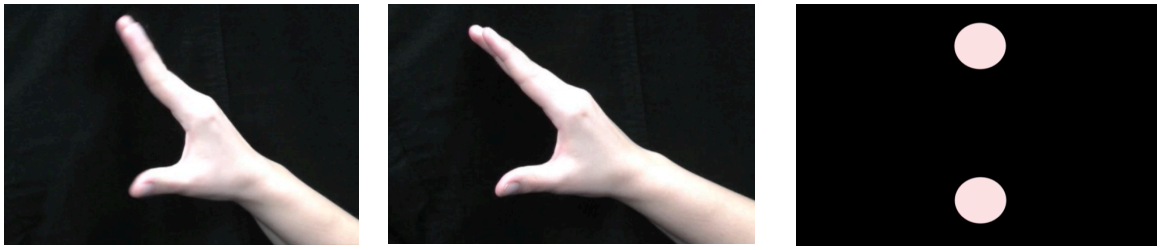

Figure 1: Pictures representing the videos used in Experiment 1. Moving hand (left), static hand (middle), bouncing balls (right).

Procedure

Participants completed an informed consent form and were fitted with a Quick-cap (Compumedics, Neuroscan) for the EEG. Subsequently, participants were shown their live EEG recording to demonstrate noise associated with physical movement in an attempt to reduce movement artifacts. Subsequently, participants' resting EEG was recorded for 2 minutes with eyes-closed, before completing Croft & Barry (2000)'s eye-movement calibration protocol. Lastly, the participant attended one block of 20 x moving hand, 20 x still hand, and 20 x bouncing ball videos, presented in a computer randomized order. Each experimental trial started with 1000ms fixation cross, followed by a 3000ms video clip. Participants were told to remain as calm as possible while observing video clips presented on the screen. See Figure 2 for graphical representation of procedure.

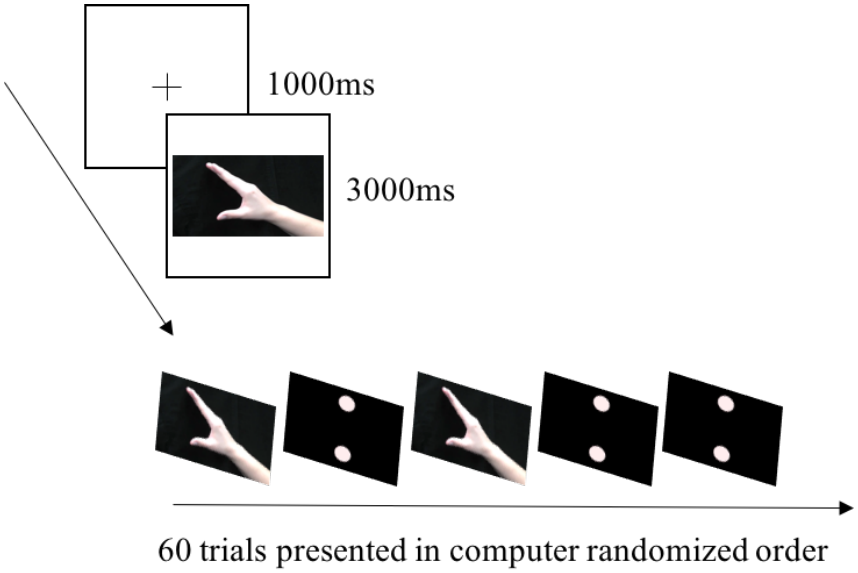

Figure 2: Graphical representation of procedure.

EEG data acquisition

EEG data were recorded using Synamps II amplifiers and SCAN 4.5 acquisition software (Compumedics, Melbourne, Australia) using 64 electrodes mounted on a Quick-Cap with electrodes arranged according to the extended 10-20 system. Electrodes were referenced online to an electrode midway between Cz and CPz and grounded midway between Fz and FPz. Eye movements were recorded using four electrodes: one above and one below the left eye, and on the outer canthi of each eye. Impedances for all of the electrodes were lowered to at least 10 kΩ in all electrodes before data acquisition. EEG data were sampled continuously at 1000 Hz with a band-pass filter of .05 - 200 Hz and a 50 Hz notch filter.

EEG data preparation

Once acquired, data were visually inspected and noisy data blocks and bad electrodes were rejected on a participant-by-participant basis. Bad electrodes detected for each participant differed between 0 and 4 electrodes. Eye-movement artifacts were rejected

according to methods described by Croft & Barry (2000). All data were re-referenced to a common average reference, before undergoing demodulation and concurrent filtering (zero phase-shift, 24 dB roll-off, envelope computed). Remaining artifacts exceeding ± 100 mV were automatically rejected in an automatic rejection sweep (between 0 and 3 epochs were rejected after this final sweep) before event-related desynchronization/synchronization (ERD/S) was computed using event-related band-power transform in Neuroscan Edit 4.4 (Compumedics, Melbourne, Australia). EEG bandwidths of interest were prepared in alpha and low beta (β1: 13 – 20 Hz). Alpha (α) was further split into two sub-bands: low (8 - 10 Hz) and upper (10 - 12 Hz) because functions associated with each end of the α spectrum differ (Klimesch et al., 2007; Petsche, Kaplan, von Stein, & Filz, 1997; Aftanas & Golocheikine, 2001). Electrodes of interest included those overlying the premotor cortex and supplementary motor area (FC5, FC3, FC1, FCz, FC2, FC4, FC6) and those overlying the motor cortex (C5, C3, C1, Cz, C2, C4, C4, C6). Control electrodes included those overlying occipital cortex (O1, Oz, O2) in order to ensure that the sensorimotor rhythms reflect sensorimotor activation and not occipital activation. α-rhythms originating in the occipital region are associated with visual attention processes (e.g. Foxe, Simpson & Ahlfors, 1998) while α-rhythms generated by the sensorimotor cortex is related to motor processes (e.g. Hari et al., 1998).

The data were epoched from –3000 to 4000ms, and trimmed 1000ms from each end to remove filter warm-up artifacts, and then averaged. Note that 0ms in the epoch refers to the beginning of the stimuli presentation. Percentage change between the reference/baseline period was the period in which a blank screen was present (-2000ms to -1000ms) and one active period in which the stimulus was presented (500 to 2500ms). Event-related desynchronization/synchronization (ERD/S) was computed

using event-related band-power transform in Neuroscan Edit 4.4 (Compumedics, Melbourne, Australia). Note that ERD is expressed as positive values and ERS as negative.

Data analysis

All EEG data were included for analysis and examined for heterogeneity using Kolmogorov-Smirnov statistics. This test revealed that the assumption of normality was violated ($p < .05$). To correct this issue, the data were log transformed and as such, re-expressed on a normally distributed scale (note that for clarity, graphical representations of the data are not log-transformed). A repeated measures ANOVA was then conducted to investigate the signal from sensorimotor areas and occipital regions in an attempt to differentiate α-rhythms relating to visual processes and α-rhythms relating to motor processes. This differentiation is important in order to ascertain that reactivity relates to motor and possibly mirror processes as opposed to mere visual attention (see page 21). For this analysis, signal from sensorimotor areas were recorded from central electrodes (C6, C4, C2, Cz, C1, C3, C5) and fronto-central electrodes (FC6, FC4, FC2, FCz, FC1, FC3, FC5), and signal from occipital area from occipital electrodes (O2, Oz, O1). Note that electrodes were collapsed in order to keep the number of comparisons to a minimum, and the bandwidths of interest were dependent variables. One ANOVA was conducted initially, which included two factors: "channels" with three levels (C, FC, O), and "video type" with three levels (bouncing balls, moving hand, still hand). A main effect for the factor channels, or an interaction between the factors channels and video type was expected given that signal recorded from C and FC channels are functionally similar and considered as sensorimotor areas (e.g. Szurhaj et al., 2003), while signal from the

occipital region is functionally different and generated in the occipital region. In the event of such interaction, investigations of μ-reactivity during observation of hands movements were conducted separately for channels FC, C, and O. Three ANOVAs were conducted to investigate μ-reactivity during observation of a moving hand compared with a static hand and bouncing balls. These ANOVAs all included the following factors: "video type" (bouncing balls, moving hand, still hand), "electrode" (C5, C3, C1, Cz, C2, C4, C6), and one between-subjects factor: "empathy" (low, moderate, high), however, for the FC-channels the factor electrode included: FC5, FC3, FC1, FCz, FC2, FC4, FC6; and likewise for O-channels the factor electrode included: O1, Oz, O2. For the O-channels analysis, note that β1 was investigated as a control for bandwidth, as β1 is not thought to be recorded over the occipital region. It was expected to find a significant main effect for the factor video type, and in the event of such finding, the following pairs were compared: (a) moving hand vs. still hand; (b) moving hand vs. bouncing balls; (c) still hand vs. bouncing balls. These comparisons were Bonferroni corrected to control for multiple comparisons. Finally, all effects were compared against zero (indicating no change) in one-samples t-tests. Degrees of freedom were corrected using the Greenhouse-Geisser epsilon values (G – GE) when violation of sphericity was indicated.

Results

Electrophysiological reactivity

Sensorimotor vs. occipital channels

Results of the repeated measures ANOVA indicated no significant interaction, but a significant main effect for the factor channels were indicated in α2: *F*s (2, 74) > 29.22, *p*s < .001, η_p^2s > 0.408, but not α1 (*p* > .330) indicating that ERD differed between regions. Therefore, investigations of μ-reactivity were investigated separately in FC, C, and O-channels.

Central channels

Results of the repeated measures ANOVA indicated no main effect or interaction with the factor empathy (*p*s > .837) suggesting that sensorimotor reactivity during observation of an intransitive hand movement is not modulated by individual scores of empathy. A significant main effect for the factor video type was observed in the α2 bandwidth: $F(2, 70) = 3.59$, $p = .033$, $\eta_p^2 = 0.093$, and in β1: $F(1.67, 70) = 4.99$, $p = .014$, $\eta_p^2 = 0.125$, but not in α1 (*p* =.151). Planned comparisons indicated that the moving hand triggered significantly greater ERD compared to the static hand (*p* = .019) and to the bouncing balls (*p* = .024) in β1, and in α2, the moving hand elicited significantly greater ERD compared to the bouncing balls (*p* = .017). The result of this test is presented in Figure 3 below and suggests that sensorimotor frequencies are more responsive to observation of a moving hand than to a static hand and to bouncing balls observations.

Results of the one-sample t-tests indicated that the change in α2-power differed significantly from zero during observation of the moving hand only: $t(37) = 4.55$, $p < .001$ (static hand and bouncing balls: *p*s > .08). However, in β1 all video types differed from zero (*p*s < .001).

Fronto-central channels

Results of the repeated measures ANOVA indicated no main effect or interaction with the factor empathy (*p*s > .167), suggesting that sensorimotor reactivity during observation of an intransitive hand movement is not modulated by individual scores of empathy. A main effect for the factor video type was observed in the β1 bandwidth only: $F(2, 70) = 4.02$, $p = .022$, $\eta_p^2 = 0.103$ (α1 and α2: *p*s > .133). Planned comparisons indicated that the moving hand triggered significantly greater ERD compared to the static hand ($p = .021$), but not to the bouncing balls ($p > .05$). The results are presented in Figure 3 below and suggests that sensorimotor frequencies are more responsive to observation of a moving hand than to a static hand but not significantly different to the bouncing balls observations.

Results of the one-sample t-tests indicated that all video types induced significant change in power (*p*s < .001).

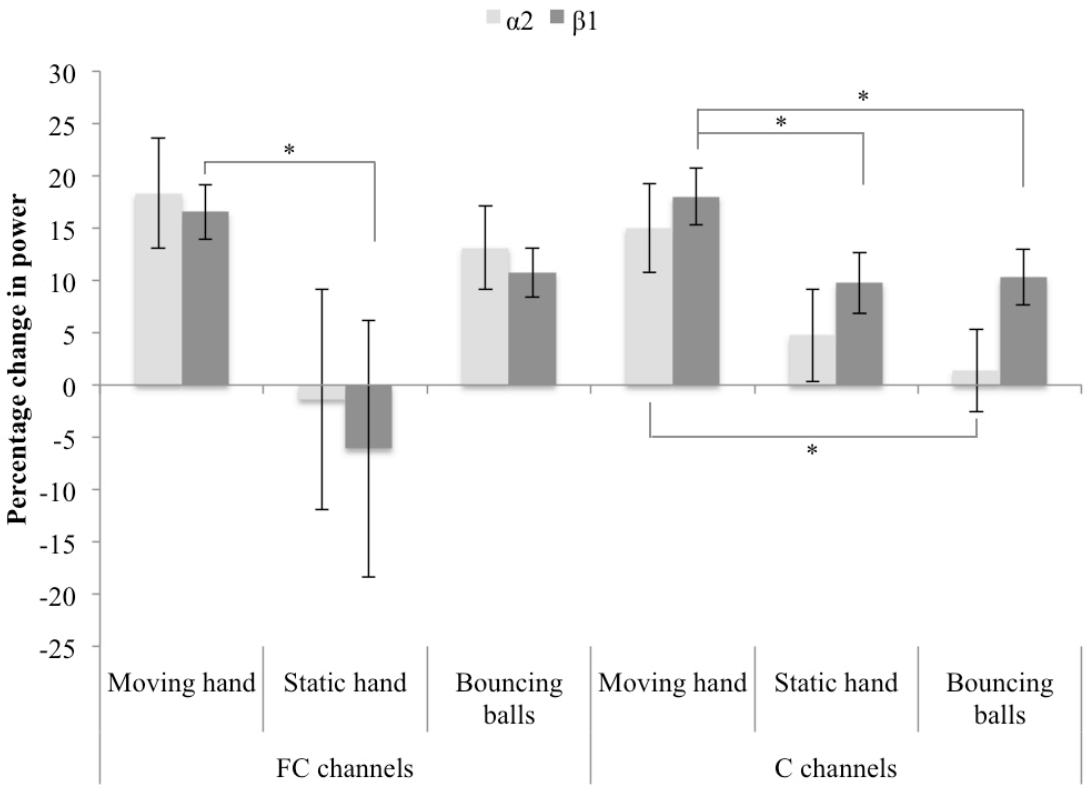

Figure 3: Results of planned comparisons. Bars represent percentage change in α2 and β1 power during observation of moving hand, static hand, and bouncing balls relative to the reference period. Error bars indicate standard error. Note: negative values represent ERS while positive values represent ERD.

Occipital channels

Results of the repeated measures ANOVA indicated no main effects for the factor video type (*p*s > .396), in any bandwidth, suggesting that the signal recorded over the occipital region did not respond differently to video types. It is therefore more certain that the suppression pattern observed in sensorimotor α reflected recruitment of motor systems rather than visual reactivity.

Interim Discussion

The current study employed Puzzo and colleagues' (2011) protocol to induce μ-ERD. Participants observed a hand opening and closing, a static hand, and two bouncing balls. The main finding of this study suggested selective μ-ERD (both α2 and β1) during observation of the moving hand compared to the static hand and the bouncing balls. This finding reflects Puzzo and colleagues' (2011) findings except that Puzzo and colleagues reported significant β1-ERD only. However, two main differences in method are relevant to discuss here. Firstly, Puzzo and colleagues used resting EEG as the reference interval, whilst in the current study the reference interval was 1000 ms preceding the video observation. Secondly, the current study included all FC and C-channels whereas Puzzo and colleagues only included electrodes closer to the midline. Inclusion of more lateral electrodes could have included more generators of α2.

In contrast to many previous studies investigating μ as exclusively 8 – 13 Hz (e.g. Oberman et al., 2005; Muthukumaraswamy, Johnson, & McNair, 2004; Perry & Bentin, 2009), the current study investigated μ using both α and β1 bandwidths. Investigating both α and β1 allows for a more comprehensive investigation of the oscillations involved in motor processing. Previous studies investigating both α and β1-suppression during action observation suggest that these frequencies may process different aspects of the action observed. In the current study, it was noted that in the α2 bandwidth, only the moving hand induced significant change in power, while in the β1 bandwidth, all the video types induced significant change in power. This finding is not surprising given that generators for α and β are known to differ (Hari, 2006; Avanzini et al., 2012) and the behavioural pattern of α and β1 during action observation also differs depending on action content observed: intransitive hand

actions trigger β1 more reliably than α (e.g. Puzzo et al., 2013), while transitive and goal-directed hand actions trigger 8 – 13 Hz (e.g. Muthukumaraswamy & Johnson, 2004; Muthukumaraswamy, Johnson, & McNair, 2004). In line with this interpretation, the results of the current experiment revealed selective ERD in both α2 (10 – 12 Hz) and β1 (13 – 20 Hz) during observation of an intransitive hand movement compared to control videos, but not in α1 (8 – 10 Hz). This result (in light of Muthukumaraswamy & colleagues' findings) suggests that α1 in the current study was not triggered because the hand movement observed was intransitive. In this case, observation of intransitive hand movements may be related to the higher end of the μ spectrum while goal-directed and transitive hand movements are related to lower end of the spectrum.

The finding that significantly less α and β1-ERD was recorded during observation of control videos (bouncing balls and static hand) compared to the moving hand, supports the notion that sensorimotor activity is selective for observation of biological movement. This selectivity has been attributed to activity of the hMNS (e.g. Pineda, 2005; Muthukumaraswamy & Johnson, 2004). Furthermore, no effect of video type was detected in occipital electrodes suggesting that the content coded by sensorimotor α differed from that in posterior α. This is important because α-rhythms originating in the occipital region are associated with visual attention processes rather than motor processes (e.g. Foxe, Simpson & Ahlfors, 1998). Therefore, the μ-ERD observed during the moving hand reflects processing of a biological movement rather than rudimental visual attention. Furthermore, it is likely that the μ-ERD observed, reflects observation/execution matching as observation of a moving hand triggered activity in an area associated with executing hand movements. This pattern corresponds with mirror neuron activity as it has been suggested that the μ-rhythm is modulated by

activity in hMNS core areas during action execution and observation (Muthukumaraswamy & Johnson, 2004; Oberman et al., 2005; Pineda, 2005).

With regard to the suggested relationship between hMNS-related activity and empathy (e.g. Rizzolatti & Craighero, 2004; Iacoboni & Mazziotta, 2007; Iacoboni, 2009), the results of the current experiment demonstrated no indication that individual scores of empathy modulated μ-ERD during observation of a moving hand (or any of the control videos). This finding is not consistent with the majority of other EEG studies demonstrating a relationship with empathy and μ-ERD during action observation (e.g. Perry, Troje, & Bentin, 2010; Cooper et al., 2011). In these studies, μ-ERD was induced by socially relevant stimuli such as observation of a yawn (Cooper et al, 2011), infliction of pain (Perry, Bartal, Lamm, & Decety, 2010), action with intention (Perry, Troje, & Bentin, 2010), and imitating faces (Bernier, Dawson, Webb, & Murias, 2007); while in the current study participants observed an intransitive hand movement that does not convey any socially relevant information. However, Woodruff, Martin and Bilyk (2011) did report a positive correlation with μ-suppression and cognitive empathy. In that study, participants observed and executed finger tapping (intransitive). However, Woodruff, Martin and Bilyk (2011) investigated cognitive empathy (using the IRI perspective taking scale), not affective as in the current study. It may be that cognitive empathy is involved in processing intransitive movements, but that affective empathy is not. It has been shown that cognitive and affective empathy are dependent on different cortical substrates (Shamay-Tsoory, Aharon-Peretz, & Perry, 2009) and it is therefore likely that they are involved in different processes. Furthermore, it has been suggested that the hMNS contributes to social responding and as such, functions to retrieve the appropriate response (Hamilton, 2013). It may therefore be that affective empathy is involved in

socially relevant contexts, and was therefore not triggered in the current study as observation of a hand opening and closing does not require any socially relevant response.

In summary, the current study demonstrated μ (α2 and β1) ERD during observation of an intransitive hand movement. Significantly less ERD was recorded during observation of a static hand and bouncing balls. This selectivity for biological movement was not indicated in occipital α, suggesting that the ERD recorded in μ may be an indication of hMNS activity. No effect was found for individual scores of empathy in relation with observation of an intransitive hand movement, suggesting that affective empathy was not involved in processing observation of an intransitive hand movement.

Experiment 2: Social-Perception

Introduction

The previous experiment demonstrated selective μ-ERD during observation of a simple hand movement, suggesting hMNS-related activity. The current experiment investigated μ-reactivity in relation to a social-perceptive task as an alternative experimental protocol. This protocol includes both a behavioural aspect and a socially relevant aspect, and therefore may enlighten the role in which context plays in hMNS-related activity. The hMNS has previously been associated with social perception (e.g. Pineda & Hecht, 2009), particularly as indicated by *reading the mind in the eyes test* (RMET: Baron-Cohen et al., 1997; Baron-Cohen et al., 2001). Several studies have demonstrated cortical activity in the IFG (core area of the hMNS) during the RMET (e.g. Baron-Cohen et al., 1999; Moor et al., 2012; Nolte et al., 2013) as well as suppression in μ (e.g. Pineda & Hecht, 2009; Moore, Gorodnitsky, & Pineda, 2012). Performance on the RMET has been related to IFG function (Keuken et al., 2011), and in individuals with lesions to the IFG; performance on the RMET is impaired (Dal Monte et al., 2014; Havet-Thomassin et al., 2006; Henry et al., 2006; Muller et al., 2010). This literature will be reviewed next.

The ability to detect mental states in others is commonly referred to as *theory of mind* (Baron-Cohen, Leslie, & Frith, 1985), *mindreading* (Rizzolatti, Fogassi & Gallese, 2001), and *mentalizing* (van Overwalle & Beatens, 2009). Note that in this thesis, theory of mind is the term used to describe this ability. The hMNS has been suggested to facilitate mental state inferences by simulating the motor movement involved in expressing the mental state in the observer's motor repertoire, and consequently retrieving the associated meaning with the motor movement (Gallese &

Goldman, 1998). In support of this notion, it has been demonstrated that emotions expressed in faces trigger greater cortical activation in hMNS core areas (particularly IFG) compared to neutral faces (Leslie, Johnson-Frey, & Grafton, 2004; Schulte-Ruther et al., 2007; Dapretto et al., 2006) as well as greater μ-suppression (Moore, Gorodnitsky, & Pineda, 2012). Additionally, lesions to IFG have been associated with impaired ability to perceive emotions expressed in faces (Adolphs et al., 2003). These studies have been interpreted as evidence that the hMNS is involved in processing facial expressions.

Various tasks have been created to assess theory of mind including the well-known *false belief test* (Baron-Cohen, Leslie, & Frith, 1985) and the increasingly popular RMET (Baron-Cohen et al., 1997). Whereas the false-belief test assesses the ability to assume another person's perspective, the RMET assesses the ability to infer mental states from the eye and eyebrow region. Although both of these tasks are considered measures of theory of mind, it has been suggested that these tasks measure two different components: social-cognition and social-perception (Tager-Flusberg & Sullivan, 2000). Whereas social-perception was proposed to involve inferring mental states from facial and body expressions, social-cognition was proposed to be representation-based and linked to language and theory building. According to Tager-Flusberg and Sullivan's (2000) model, the RMET is a social-perception task in nature, and the false belief task is a social cognitive task. The hMNS has been associated with both social-perception (e.g. Pineda & Hecht, 2009) and social-cognition (e.g. Oberman, Pineda, & Ramachandran, 2007). While social-perception is investigated in the current experiment, social-cognition is investigated in Experiment 3.

The studies investigating hMNS involvement in RMET have revealed several findings, as will be reviewed next. Baron-Cohen and colleagues (1999) reported the first evidence of cortical activity in areas including the IFG (core hMNS area) during the RMET. In that study, cortical activation was investigated using fMRI. Two participant groups were compared: individuals thought to lack theory of mind (autism spectrum disorder) and matched control participants. The results indicated that the IFG was activated in control participants but not in individuals with autism. In another clinical study, Russell and colleagues (2000) demonstrated that RMET triggered significantly less cortical activation in IFG in individuals with schizophrenia (another psychiatric condition that involves an impaired theory of mind) compared to healthy controls. Additionally, in both of these studies, performance on the RMET was significantly lower in the clinical group compared to the control group. These findings indicate that there is a link between IFG and theory of mind. Since then, several others have demonstrated IFG activation during RMET in non-clinical populations. For example, Nolte and colleagues (2013) demonstrated increased activation in IFG during the RMET but not during an age judgment task using the same images. Furthermore, Moor and colleagues (2012) demonstrated IFG activation during the RMET in children and adolescents with some age-related differences. Lastly, Adams and colleagues (2009) demonstrated cortical activation in the IFG during the RMET across cultures, however, performance was best when faces observed matched the participants' cultural membership.

Another line of evidence comes from neuropsychological studies that consistently demonstrate impaired performance on the RMET in individuals who have suffered a traumatic brain injury (TBI). Patients with severe TBI (patient in coma for at least

one day and CT[9] scan indicating lesion) to prefrontal regions often including the IFG, performed significantly worse on the RMET compared to healthy controls (Havet-Thomassin, Etcharry-Bouyx, & Le Gall, 2006; Turkstra, 2008; Muller et al., 2010). Similar patterns have been reported for less severe TBI cases involving injuries to temporal and frontal regions (Henry et al., 2006). Although these studies imply a link between IFG and performance on the RMET, it is not clear the extent to which IFG is involved in task performance because TBI rarely affects one area only. Therefore, a recent study investigated the relationship between TBI patients' performance on the RMET with the associated lesion (Dal Monte et al., 2014). In this study, voxel-based lesion symptom mapping data (VLSM)[10] were analysed, and results confirmed that lesions in the IFG were associated with decreased performance on the RMET as this pattern overlapped for 20 patients.

In two recent studies it has been indicated that the hMNS may be more sensitive to social-perception compared to social-cognition (Pineda & Hecht, 2009; Keuken et al., 2011). In Pineda and Hecht's study (2009), participants completed a social-perception task (RMET) and a social-cognitive task (cartoons task: Brunet, 2000) whilst EEG was recorded. The results indicated selective μ-suppression (8 – 13 Hz) during mental state recognition trials of the RMET compared to gender discrimination trials using the same pictures. Additionally, trials that were correctly identified in the mental state recognition trials induced significantly greater μ-suppression compared to incorrect trials. This pattern was not indicated for the cartoons task (social-cognitive task). Additionally, μ-suppression during the RMET correlated negatively with performance on the RMET; that is, a greater score on the RMET was associated with

[9] Computerized axial tomography scan
[10] VLSM is a neuroimaging method designed to identify lesion-symptom relationships in stroke patients. This method involves investigating a defined lesion in relation to behavioural scores on a voxel-by-voxel basis in one or several TBI patients (Bates et al., 2003).

increased μ-suppression[11]. No correlation was observed for the cartoons task, suggesting that social-perception requires more processes dependent on μ-rhythms (and therefore arguably, hMNS activation) than social-cognition.

Keuken and colleagues (2011) extended Pineda and Hecht's (2009) findings by demonstrating a causal relationship between the IFG and changes in the μ-rhythm on RMET performance, by disrupting the IFG with rTMS. In this study, participants completed the following procedure before and after stimulation: (1) Observation of videos including simple biological movements (e.g. hand picking up objects), non-biological movements (e.g. bouncing balls), and complex biological movements (e.g. social interactions) while sensorimotor frequencies were recorded (8 – 12 Hz, 12 – 15 Hz, and 15 – 25 Hz). Subsequently, (2) participants' performance on the RMET (social-perceptive) and the cartoons task (social-cognitive) was assessed (EEG was not analysed for this period). Results demonstrated selective suppression in μ (8 – 12 Hz and 12 – 15 Hz) during observation of biological movement compared to non-biological movement, but not in β (15 – 25 Hz). Subsequent to rTMS stimulation to IFG, this pattern was abolished in α but not in β, suggesting that β generators were not affected by interference to the IFG. Furthermore, reaction times on the social-perception task (RMET) were increased after stimulation, but not on the social-cognitive task (cartoons task). This finding indicates that there is a relation between μ-rhythms and activity in the IFG as previously suggested (e.g. Pineda, 2005), and that the hMNS is more involved in social-perception compared to social-cognition as suggested by Pineda and Hecht (2009).

The link between IFG and social-perception (as opposed to social-cognition) was also

[11] Because suppression in this instance was indicated by negative values, that is, greater μ-ERD was indicated by greater negative values, consequently resulting in a negative correlation that may appear counterintuitive.

reported by Turkstra (2008), showing that patients with moderate-severe TBI affecting frontal areas including IFG, performed better on a social-cognitive (social inference test) task than on a social-perception task (RMET). However, Muller and colleagues (2010) demonstrated the opposite. In this study it was shown that patients with severe-moderate TBI to the IFG performed worse than control participants on both the cartoon task (social-cognitive) and on the RMET (social-perception), but performance ratios suggested that performance was more impaired on the RMET than on the cartoon task. Additionally, TBI patients did not differ in performance compared to control participants on a false-belief task (social-cognitive task), suggesting that in these participants, processing involving social-perception was more impaired than social-cognitive.

Summary and Aims

The hMNS have been implicated in the ability to recognize mental states in others by simulating the muscles used to express the mental state (see page 4; Gallese & Goldman, 1998). The RMET involves recognising mental states from faces, and is a measure of theory of mind. During the RMET, several studies have demonstrated increased cortical activation in a core area of the hMNS (IFG), and some have reported suppression in μ. Impaired performance on the RMET has been demonstrated subsequent to disrupting the IFG. Additionally, individuals with lesions to the IFG perform worse on the RMET compared to healthy controls.

The aim of the current study was to investigate μ-reactivity in relation to social perception as an alternative protocol to simple motor processing (Experiment 1) and to investigate μ-reactivity in relation to context (social relevance). In this experiment,

participants performed the RMET whilst μ-reactivity was recorded. Performance on the RMET was measured in reaction times and accuracy. Additionally, based on Pineda and Hecht's (2009) study, μ-reactivity was assessed for correct and incorrect trials. It was predicted that the correct trials would elicit greater ERD in μ than the incorrect trials based on the notion that hMNS facilitates mental state recognition, and as such, should modulate μ more for trials that were recognised correctly. It was also predicted that μ-ERD during correct trials would correlate with performance on RMET but not for incorrect trials. This prediction was based on Pineda and Hecht's (2009) findings, and other studies demonstrating a causal link between performance on RMET and activity in IFG (e.g. Keuken et al., 2011; Dal Monte et al., 2014). Because empathy has been related to the hMNS (e.g. Gallese & Goldman, 1998) and the evidence suggesting the involvement of IFG in RMET (measure of theory of mind), it was assumed that individual levels of empathy would modulate μ-ERD during RMET. Lastly, based on the notion that people who are more emphatic are better at social perception (Szalavitz & Perry, 2010; Bjorkqvist, Osterman, & Kaukiainen, 2000), it was predicted that levels of empathy would modulate performance.

Method

Participant selection

See general method section (page 40).

Empathy index

See general method section (page 40).

Stimuli

Pictures used for the RMET were taken from Baron-Cohen and colleagues (1997) and included pictures of the eye and eyebrow region in women and men performing facial emotions. However, the current experiment did not include all of Baron-Cohen and colleagues' original stimuli because in the original version, four emotion pictures were duplicated, therefore in the present study, 32 out of 36 were included. The duplicated four were excluded to limit number of stimuli presentations. Emotions depicted included both positive and negative expressions (for instance, concerned, serious, friendly, and dominant are some examples of the emotions depicted). Examples of the pictures and the answer screen can be seen in figures 4 and 5, the full test can be seen in Appendix 2.

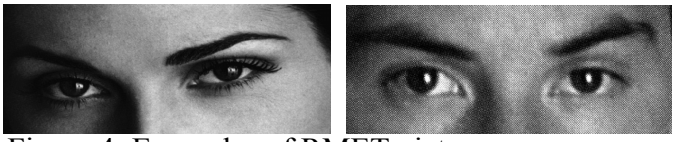

Figure 4: Examples of RMET pictures

Figure 5 Examples of RMET answer screens

Procedure

Participants attended one block containing 32 mental state recognition trials were presented in a random order. Each trial started with a blank screen presented for

1000ms, followed by a 1000ms fixation cross, then a RMET picture appeared for 3000ms, and finally the word-selection task in which four words (one emotion word matching the emotion depicted, one emotion word related to the target, and two emotions that were incorrect) were presented. The words were present on the screen until the participant made a response by clicking the mouse. Participants were instructed to be certain about selecting rather than making a rapid decision. Response time was measured from stimulus onset to when the response was given, but there was no time limit. The words were presented on a grid in size 36 white Calibri font, and located in the middle of the screen. The position of the word types in the grid was randomly allocated. See Figure 6 for graphical representation of procedure.

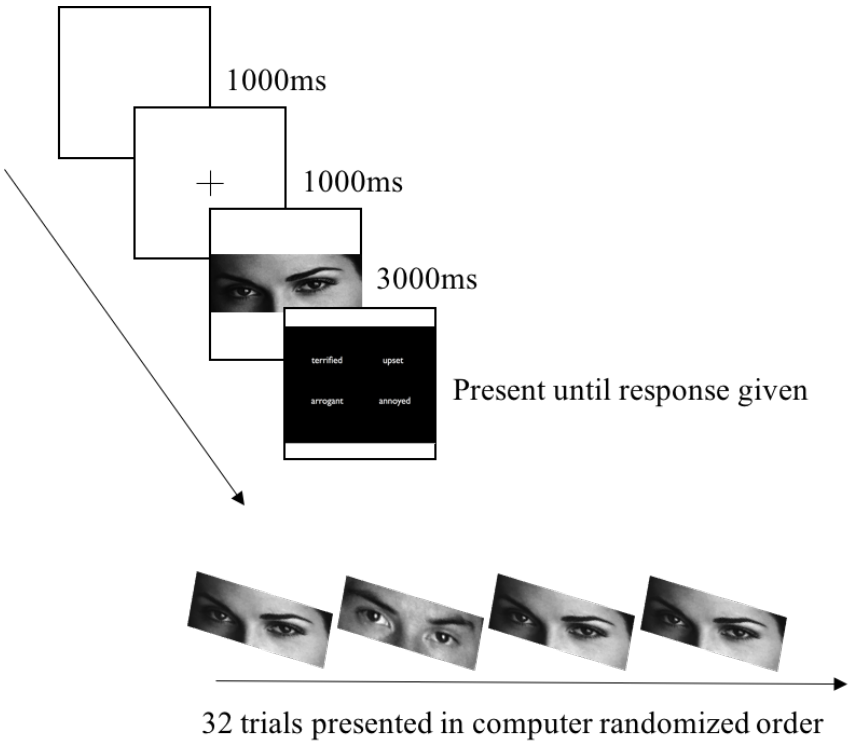

Figure 6: Graphical representation of procedure.

EEG data acquisition

Data were acquired as in Experiment 1 (see page 43).

EEG data preparation

The data were prepared as in Experiment 1 (page 43), except for the nature of the control condition. The current experiment did not include an actual control condition, instead, the current experiment investigated correct and incorrect trials of the RMET. Correct trials were trials that participants correctly identified the emotions displayed, whereas incorrect trials were not recognised. These trials were epoched as in Experiment 1. This procedure was employed by Pineda and Hecht (2009) and demonstrated selective μ-ERD during correct trials. It is assumed that hMNS facilitates processes involved in the RMET, therefore correct trials should elicit greater suppression in μ.

Data analysis

All data were included for analysis and treated like in Experiment 1 (page 45), except for some details as described. Performance on the RMET was calculated for accuracy (percentage correct) and reaction time (stimulus onset to response given) on the word-matching task. The behavioural data were normally distributed ($p > .05$), but the EEG data were not ($p < .05$) and therefore the EEG data were log transformed. The behavioural data were investigated with a one-way ANOVA with two dependent variables, "accuracy" (percentage correct) and "reaction time" and one between subjects factor "empathy" (low, moderate, high) in order to investigate whether performance on the word-matching task was modulated by empathy. A difference in performance (accuracy or reaction time) between groups was expected, and it was

planned to compare performance between groups on accuracy and reaction times. These comparisons were Bonferroni corrected.

In order to differentiate signal from sensorimotor areas and occipital areas, the same step as in Experiment 1 was conducted, the expectation was the same as in Experiment 1. In the event of an interaction or main effect for the factor channels and response, investigations of μ-reactivity during observation of hands movements were conducted separately for channels FC, C, and O. Subsequently, μ-reactivity during the RMET was investigated in three repeated measures ANOVAs (one for each: FC, C, O) in order to investigate μ-reactivity during the RMET. Bandwidths were dependent variables in these analyses. These ANOVAs all included the following factors: "response" with two levels (correct, incorrect), "electrodes" with seven levels (C5, C3, C1, Cz, C2, C4, C6), and one between-subjects factor: "empathy" with three levels (low, moderate, high). For FC-channels, the factor electrode included: FC5, FC3, FC1, FCz, FC2, FC4, FC6, similarly, for the O-channels, the factor electrode included: O1, Oz, O2. It was expected to observe a main effect for the factor response, and in this event, ERD for correct trials were compared with ERD for incorrect trials. For each cluster of channels (FC, C), one comparison was conducted in the bandwidth(s) of interest.

Results

Word-matching performance

The results of the one-way ANOVA revealed no significant differences between groups on either accuracy: $F(2, 35) = .015$, $p < .985$, or reaction time: $F(2, 35) = 3.02$,

$p < .062$ suggesting that empathy did not modulate performance on the RMET.

Electrophysiological reactivity

Sensorimotor vs. occipital channels

Results of the repeated measures ANOVA indicated no significant interaction (*p*s >. 291), however a significant main effect for the factor channels were found in all bandwidths: *F*s (2, 74) > 4.46, *p*s < .017, η_p^2s > 0.164, indicating that ERD differed between regions. Therefore, investigations of μ-reactivity were investigated separately in FC, C, and O-channels.

Central channels

Results of the repeated measures ANOVA indicated no significant main effect for the factor response in any bandwidth: *F*s(1, 35) < 1.55, *p*s > .221, η_p^2s < .043, suggesting that signal from the C-channels was not selective for correct trials. This result is presented in Figure 7 below (note that only β1 demonstrated ERD in response to RMET). No main effect or interaction was indicated for the factor empathy (*p*s > .310) suggesting that empathy did not modulate μ-reactivity during the RMET.

Results of the one-sample t-test indicated significant change in power, however only in the β1 bandwidth: $t(37) = 4.75$, $p < .001$ (α1 and α2: *p*s > .079).

Fronto-central channels

Results of the repeated measures ANOVA indicated no significant main effect for factor response in any bandwidth: Fs(1, 35) < 2.04, ps > .162, η_p^2s < .055, suggesting that the signal from the FC-channels was not selective for correct trials. This result is presented in Figure 7 below. No main effect or interaction was indicated for the factor empathy (ps > .167) suggesting that empathy did not modulate μ-reactivity during the RMET.

Results of the one-sample t-test indicated a significant change in α1: $t(37) = 2.31$, $p = .026$, and in β1: $t(37) = 6.71$, $p < .001$ (α2: $p = .785$).

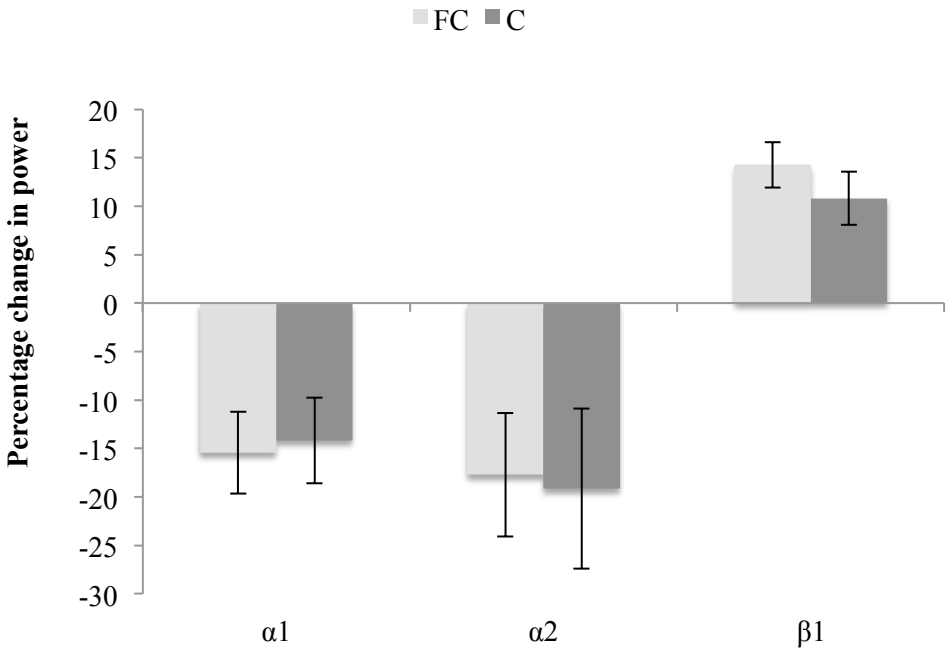

Figure 7: Graphical representation of ERD/ERS. Bars represent percentage change in all bandwidths during RMET relative to the reference period. Error bars indicate standard error. Note: negative values represent ERS while positive values represent ERD.

Occipital channels

Results of the repeated measures ANOVA indicated no main effects for the factor response (*p*s > .317) in any bandwidth, suggesting that signal from neither occipital nor sensorimotor region were selective for correct trials.

μ-reactivity and word-matching performance

Pearson's correlation was used to assess whether performance on the RMET was related to sensorimotor reactivity during the RMET. Results demonstrated no relationship between μ-reactivity and RMET score: *r*s < .438, *p*s > .206 in either FC or C-channels in any bandwidth. Likewise, no relationship was found with reaction time: *r*s < .343, *p*s > .230 in either FC or C-channels, in any bandwidth.

Interim Discussion

The current study investigated μ-reactivity in relation to social-perception as an alternative to observation of an intransitive hand movement as investigated in Experiment 1. The benefit of this experimental protocol compared to Experiment 1, was that it included a behavioural measure. In this experiment, participants completed the RMET while μ-reactivity was recorded. μ-reactivity was assessed for trials that participants correctly identified and trials that participants got wrong. The purpose of this was to investigate whether understanding mental states modulates μ-reactivity during observation. Based on the notion that the RMET is facilitated by the hMNS, and with Pineda and Hecht's (2009) findings, it was predicted that the trials that participants correctly identified would trigger greater ERD in μ as an indication of greater hMNS-related activity. The results of the current experiment did not support this prediction, as μ-reactivity was not modulated by whether or not the response was

correct or incorrect. However, the RMET did induce significant changes in power in α1 and β1 (relative to the reference period) from zero when collapsed across response (incorrect/correct). The results indicated that ERD only occurred in the β1 bandwidth while ERS was indicated in the α bandwidths. The finding that ERS rather than ERD was found in α bandwidth is problematic because ERS in α has traditionally been associated with a reduced state of active information processing in the underlying neuronal network (e.g. Pfurtscheller & Lopes da Silva, 2005). Therefore, the main finding could be interpreted as an indication that the RMET involves β1 but not α processes.

Alternatively, ERS in α has been suggested to reflect active cognitive task performance involving cognitive inhibition processes (e.g. Klimesch et al., 2007; Cooper et al., 2002), and internal information processing involving top-down control on internally represented information (Sauseng et al., 2005; Von Stein & Sarnthein, 2000); Therefore, ERS could reflect inhibition of task irrelevant processes involved with performing the RMET, for example inhibition of automatic imitation. Several studies indicate that during observation of faces, observers automatically imitate the expression observed (Dimberg, Thunberg, & Elmehead, 2000; Neumann, Schulz, Lozo, & Alpers, 2014; Heyes, 2011). The divergence between α and β1 processes could then be interpreted as complimentary processes rather than independent processes. In support of this interpretation, generators for α and β1 are known to differ (Hari, 2006; Avanzini et al., 2012) and each has been associated with different aspects of processing motor events (see page 50). For example, β-suppression has been assumed to reflect response preparation and inhibition (Zhang, Chen, Bressler, & Ding, 2008) and maintenance of the current sensorimotor or cognitive state (Engel & Fries, 2010), while α-synchronization has been associated with inhibition of task

irrelevant processes (e.g. Klimesch et al., 2007). It may be that β1 was involved in maintaining the cognitive task at hand, while α inhibited task irrelevant processes such as automatic imitation of facial expression during the RMET. This is beyond the scope of the present thesis but further, carefully designed experiments are needed to explore this possibility in more depth. However, it is also possible that the lack of α-ERD observed during the RMET reflects visual attention rather than motor processing as the occipital electrodes did demonstrate ERD, although no selectivity to correct vs. incorrect trials was observed.

Another possible explanation for the lack of α-ERD is that RMET images were static rather than moving. Several studies have reported that biological movement is more efficient in triggering μ-suppression compared to static images (Cochin et al., 1998; Puzzo et al., 2010; Puzzo et al., 2011). However, using the same static images, Pineda and Hecht (2009) reported suppression in μ (8 – 13Hz), and therefore this does not in itself explain the finding of the current experiment. Pineda and Hecht's (2009) methodology however differed to that in the current experiment. Firstly, Pineda and Hecht presented RMET images for 5 seconds, while the current study, images were presented for 3 seconds only. It may be that involvement of α-processes require longer to exposure to RMET images, however this is unlikely to fully account for the results of the current experiment. Secondly, the period between stimuli could have been too short (4 seconds) in the current experiment as Pineda and Hecht's time period between trials ranged from at least 20 to 34 seconds. The time in between presentations in the current experiment may have been too short for neuronal networks to "settle" before the next trial, this too is unlikely to fully account for the findings of the current experiment. Thus, it is not clear why the current experiment failed to replicate Pineda and Hecht's (2009) results. There are some other

considerations to be made as will be discussed next.

It is assumed by many that suppression in μ during action observation reflects recruitment of hMNS (e.g. Muthukumaraswamy & Johnson, 2004; Pineda, 2005), but in the current study this was only indicated in the β1-bandwidth. Although β1 did not demonstrate selectivity for correct responses, significant change in power compared to zero was demonstrated suggesting that β1 was involved in the RMET. It may however be that a different comparison to correct vs. incorrect could be more optimal in illuminating the effect in β1. Several studies used age or gender matching as a control task for the mental state recognition aspect of RMET (Moor et al., 2012; Pineda & Hecth, 2009; Keuken et al., 2011). However, Pineda and Hecht (2009) reported no difference in μ-suppression between gender matching trials and mental state matching trials. Therefore, another control condition for the mental state recognition task should be devised. This is beyond the scope of the present thesis but further, carefully designed experiments are needed to explore this possibility in more depth.

No significant correlation was detected between reactivity in either α or β1, with performance on the RMET (either score or reaction times). This finding is in contrast with Pineda and Hecht (2009) whom reported a correlation between suppression in μ (8 – 13 Hz) during RMET and reaction time on the RMET. The lack of relationship between performance on the RMET with ERD in μ in the current experiment suggest that the RMET requires involvement of additional neuronal systems other than sensorimotor.

Lastly, empathy was investigated due to the proposed relationship between empathy

and hMNS (e.g. Leslie, Johnson-Frey, & Grafton, 2004; Rizzolatti & Craighero, 2004). However, in the current study, level of empathy did not modulate μ-reactivity, suggesting that affective empathy was unrelated to sensorimotor processes during the RMET. This finding is not surprising given that Experiment 1 also failed to demonstrate a relationship between empathy and μ-reactivity. Furthermore, individual scores of empathy did not modulate performance either on accuracy or reaction times. This result was surprising given reports suggesting that individuals who have higher levels of empathy tend to be better at social interactions (e.g. Björkqvist, Österman, & Kaukiainen, 2000). A methodological limitation may be partially responsible for the failure of demonstrating such relationship; the current experiment did not investigate correlations between individual scores on empathy with performance on the RMET, rather, the current experiment investigated whether levels of empathy modulated performance. It may that empathy was involved in the RMET, but that there were no differences on RMET performance between groups based on empathy levels. Alternatively, RMET is a measure of theory of mind and as such of cognitive empathy (Tager-Flusberg & Sullivan, 2000). It has been suggested that cognitive and affective empathy are dependent on separate cortical substrates (Shamay-Tsoory, Aharon-Peretz, & Perry, 2009). It may be that that because our group empathy measure was derived from one of the IRI's affective empathy subscales (i.e. empathic concern), performance on RMET and sensorimotor reactivity during the RMET was not modulated because affective empathy is not related to these processes. In the current study, participants completed all the subscales of the IRI, and therefore it could have been possible to investigate the link with cognitive empathy rather than affective empathy. However, as participants were selected based on their scores on the IRI empathic concern subscale, it was considered inappropriate to investigate the relation with cognitive empathy because scores on cognitive

empathy would have been confounded by the selection procedure leading to high, moderate, low levels of affective empathy. In future, one of the cognitive empathy subscales might be a more fruitful variable to investigate.

In summary, the current experiment failed to demonstrate ERD in α during RMET. Although ERD was indicated in β1, reactivity was not modulated by whether trials were correct or not. The finding that the RMET induced ERS in sensorimotor α, and ERD in occipital α suggest that the α-reactivity may reflect visual attention rather than motor processes per se as performance on the RMET was not related to μ-reactivity, and does not appear to be modulated by empathic concern.

Experiment 3: Social-Cognition

Introduction

Results of Experiment 1 indicated hMNS activation during observation of a simple hand movement, but evidence was not indicative of such activation during the social perception task (Experiment 2). The current experiment investigated μ-reactivity in relation to a social-cognitive process (social interactions) as an alternative protocol to previous experiments. It has been reported that observation of social interactions trigger cortical activity in core areas of the hMNS (e.g. Iacoboni et al., 2004) as well as μ-suppression (e.g. Oberman, Pineda, & Ramachandran, 2007) suggesting that the hMNS is involved in social-cognition. Although the hMNS has been suggested to be more sensitive to social-perception than social-cognition (see page 57; Pineda & Hecht, 2009), evidence presented in Experiment 2 does not support this notion. Additionally, a neuropsychological study has also suggested that social-cognition is more relevant than social-perception (Turkstra, 2008) see page 59.

Iacoboni and colleagues (2004) provided the first evidence suggesting that core areas of the hMNS are involved in processing social interactions. In that study, cortical activation was recorded using fMRI while participants observed video clips of everyday events performed by one person or by two individuals interacting. The video clips contained "communal sharing" (perceived as more positive) or "authority ranking" (perceived as more negative). The results of this study suggested that observation of both a single individual and two individuals interacting triggered activation in areas including IFG compared to rest. However, observation of two individuals induced stronger activation than a single individual, suggesting that the interaction component of the interaction was processed differently than that of a

single individual. The pattern recorded in this study could however simply reflect the presence of two individuals compared to one. The same year another fMRI study was conducted, which challenged this possibility. Walter and colleagues (2004) demonstrated that two individuals acting in isolation did not induce cortical activation in areas including the IFG, but significant activation was recorded when two individuals interacted. It is therefore more likely that the social interaction content and not the number of people present drove cortical activation. These findings have been interpreted as evidence suggesting hMNS involvement in social interaction processing.

Suppression in μ has also been recorded during observation of individuals engaging in a social interaction (Oberman, Pineda, & Ramachandran, 2007). In this study, participants were presented with video clips depicting a group of people playing a ball game. In one condition, the participant was merely a spectator while in another condition the participant was virtually included in the interaction. The results suggested that the participants' perceived degree of involvement, modulated μ-suppression (8 – 13 Hz) during observation. That is, when the observer was virtually interacting with the observed group, greater μ-suppression was recorded compared to when the participant was a spectator. This finding supports the notion that the hMNS is involved in processing social interactions, but additionally that the perception of inclusion modulates the reactivity pattern. In another EEG study, it was demonstrated that social interaction modulates suppression in α (8 – 10 Hz) depending on the context of social coordination (Naem, Prasad, Watson, & Kelso, 2012). In this study, participants were interacting with another participant on a rhythmic finger movement task under three conditions: maintaining own rhythm (intrinsic), synchronize rhythm (in-phase), and syncopate rhythms (anti-phase). Suppression in α was significantly

greater for conditions in which the participant was required to coordinate movements to the partner's movements (in-phase and anti-phase) compared to non-coordinated movements. These studies suggest that μ-suppression is involved in processing social interactions, and that conditions that facilitate perception of inclusion or that require social coordination can modulate μ-reactivity further.

Studies have demonstrated that social interactions are even understood when only motion cues are available (Manera, Schouten, Becchio, Bara, & Verfaillie, 2010). For example, in a recent study by Thurman and Lu (2014) participants observed spatially scrambled point-light biological motion (PLBM) videos depicting various social interactions such as two individuals playing tug of war. The spatial scrambling of the PLBM videos eliminated participants' ability to explicitly recognise human body shape, yet the results indicated that participants recognised the social interaction depicted with ease. Children also recognise social interactions from PLBM videos (Centelles, Assaiante, Etchegoyhen, Bouvard, & Schmitz, 2013). Furthermore, PLBM displays have been shown to trigger cortical activation in areas including IFG (Saygin, Wilson, Hagler, Bates, & Sereno, 2004) suggesting that the hMNS may be involved. In Saygin and colleagues' study (2004), participants observed PLBM videos depicting human actions such as walking and throwing, and reported significantly enhanced cortical activity in areas including IFG. In contrast, scrambled displays elicited activation in the occipital region. The authors suggested that the hMNS might be involved in integrating fragments of information in order to infer meaning. An example of Saygin and colleagues' (2004) stimuli is presented in Figure 8.

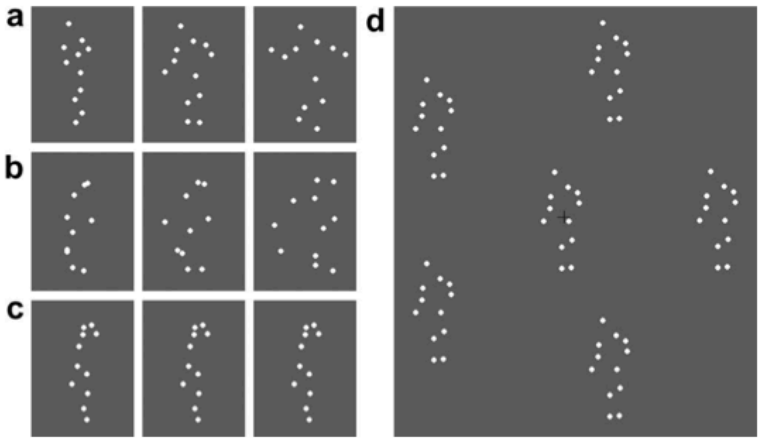

Figure 8: Examples of PLBM displays taken from Saygin and colleagues (2004). Pictures represents three of the 20 frames shown on one animation of each biological motion: a) scrambled biological motion, b) static point point-lights, c) baseline, d) screenshot from the actual experiment.

Using EEG, Ulloa and Pineda (2007) reported greater μ (8 – 13 Hz) suppression during observation of PLBM displays depicting an individual performing jumping jacks and kicks, but not to scrambled versions of the same stimuli. Additionally, in another EEG study, it was demonstrated that suppression in both α and β (8 – 13 Hz & 15 – 25 Hz) during observation of PLBM displays of a human walking was modulated more greatly by expression of intention rather than gender and emotion (Perry, Troje & Bentin, 2010). These studies can be interpreted as evidence that the hMNS is involved in constructing meaning from biological motion cues lacking any other explicit visual details.

The studies mentioned above suggest that the hMNS may be sensitive to both PLBM and social interaction information. Social interactions however can be perceived as positive or negative (valence) depending on the observer's own desires, beliefs and intentions (Forgas, Bower, & Krantz, 1984; Berry & Hansen, 1996). Therefore,

valence may be an important factor to consider in the investigation of hMNS relation to social interactions. Indeed, perception of valence has been show to modulate cortical activation in areas including hMNS core areas. For example, Leslie, Johnson-Frey, and Grafton (2004) demonstrated enhanced cortical activation (using fMRI) in areas including IFG during observation of both smiling and frowning. This study did not disentangle cortical activation patterns for positive and negative valence, but several other studies have reported greater hMNS-related activity in response to happy faces compared to angry or neutral (Niedenthal et al., 2010; O'Doherty et al., 2003). Moreover, a recent study by Rochas and colleagues (2012) demonstrated a direct relationship between pre-SMA[12] activation and recognition of happiness. In this study, participants observed happy, angry, and fearful facial expressions before pre-SMA or vertex was stimulated with TMS using an interference (or 'virtual lesion' technique). Subsequently, participants performed a facial emotion recognition task. Results revealed that disruption to pre-SMA with TMS impaired the ability to recognise happy faces without affecting recognition of angry or fearful faces. This effect was not seen when the control area (vertex) was stimulated.

Similarly, an effect of positive valence has also been demonstrated using EEG. For example, Cooper and colleagues (2013) demonstrated that α and β1-ERD during observation of an intransitive hand movement is modulated by the facial expression of the actor performing the hand movement. In this case, ERD in μ was greater during observation of happy faces compared to angry faces, but this pattern was dependent on individual traits of autism: While individuals with higher scores on an autism demonstrated β1-ERD to angry and neutral facial expressions, individuals with lower scores on autism demonstrated β1-ERD to happy and neutral faces. In a recent study

[12] Pre-SMA is the supplementary motor area and is one of the areas in the human brain where mirror neurons have been located (Mukamel et al., 2010)

it was demonstrated that happy faces trigger ERD in α and β1 (7 – 12.5 Hz & 12.5 – 25 Hz) but even more ERD was recorded when the faces were pre-conditioned with reward (Gros, Panasiti, & Chakrabarti, 2015). These studies suggest that the hMNS-related activity in neurotypical individuals may be more sensitive to positive expressions of emotion and positive associations.

Lastly, the ability to detect mental states in others (empathy) has been suggested to influence perception of social interactions: Individuals who are more empathic are better at detecting mental states in others and use this information to adjust to others (Szalavitz & Perry, 2010; Björkqvist, Österman, & Kaukiainen, 2000). Being an empathic individual is therefore a benefit in social interactions because it enables the individual to predict the actions of other members and respond to them appropriately (Coll, Grégoire, Latimer, Eugéne, Jackson, 2011; Decety & Jackson, 2004). Empathy therefore appears to be an important component for successful social interactions, and has also been related to the hMNS as was discussed on page 32.

Summary and Aims

The aim of this experiment was to investigate the μ-rhythm during a social-cognitive task as an alternative to simple motor processing (Experiment 1) or a social-perception task (Experiment 2). As described above, it has been demonstrated that μ is suppressed during observation of social interactions (see page 73) and during PLBM videos (see page **Error! Bookmark not defined.**). However, no study has investigated μ-ERD during observation of social interactions depicted by PLBM videos. Investigating social interactions in such visually fragmented displays enables investigation of the proposed action understanding principle attributed to hMNS (see

page 17). It has been proposed that hMNS facilitate action understanding when visual details are meagre as mentioned above (page **Error! Bookmark not defined.**). For these reasons, in the present study, participants were presented with social interactions depicted by PLBM videos, and scrambled versions of the same videos. The social interaction displays reflected positive and negative connotations in order to investigate the notion that hMNS is sensitive to valence. Directly after presentation, the participants were required to match the meaning of the interaction observed in a forced choice word-matching task in order to assess individuals' ability to comprehend the interactions observed.

Based on the findings suggesting that hMNS is involved in processing social interactions and comprehending PLBM videos (see page 73), it was predicted that the PLBM videos depicting social interactions would trigger greater μ-ERD compared to scrambled PLBM videos. Because the meaning of PLBM displays unfolds with time (Johansson, 1973), it was predicted that greater ERD in μ would be observed after longer exposure to the stimuli compared with earlier in the trial. Next, based on the notion that hMNS is involved in action understanding (e.g. Fadiga et al., 2006; Gazzola et al., 2007), it was hypothesized that μ-ERD during observation of the social interaction displays would correlate with the scores on the word-matching task, but not for the scrambled videos. It was also hypothesized that the positive social interaction displays would induce greater μ-ERD compared to negative. This was based on studies suggesting that positive expressions of emotions induced greater hMNS-related activity than negative (Niedenthal et al., 2010; O'Doherty et al., 2003; Rochas et al., 2012). Lastly, because it has been suggested that people who are more empathic are better at social perception (page 78; Björkqvist, Österman, & Kaukiainen, 2000), it was predicted that individual scores of empathy would

modulate μ-ERD during observation of social interaction videos but not the scrambled versions.

Method

Participant selection

See general method section (page 40).

Empathy index

See general method section (page 40).

Stimuli

Participants viewed modifications of Johansson's (1973) point-light biological motion videos. However, the videos in the current experiment included dyads of people rather than a single individual, and the actions depicted were complex human interactions rather than simple actions. These videos were created in-house using two actors: a male and a female of similar physique; both dressed in black morph-suits with 12 circular reflex patches (2.5cm in diameter) attached to each major joint (e.g. shoulder, elbow, wrist, neck, hip, knee, ankle). The actors were directed to act out different positive and negative human interactions, which were recorded by a Panasonic HDC-SD5 camcorder (1920 x 1080 Pixels) placed on a tripod one metre above the floor and five metres away from the actors. The recording took place in a dark room, and against a black surface background and floor. The actors were illuminated with two spotlights located behind the video camera. Once acquired, brightness, exposure and contrast were manipulated on a video-by-video basis in

iMovie version 9.0.8 (Apple Inc.) excluding any visible feature except for the reflective material visible only as moving white dots. Each video was edited to three seconds and depicted nine positive (welcoming, playful, flirtatious, friendly, congratulatory, comforting, cheerful, greeting, affectionate) and nine negative (dismissive, disrespectful, threatening, indifferent, reckless, defiant, provoked, dominant, embarrassed) social interactions. A graphical representation of stimuli is presented in Figure 9 below.

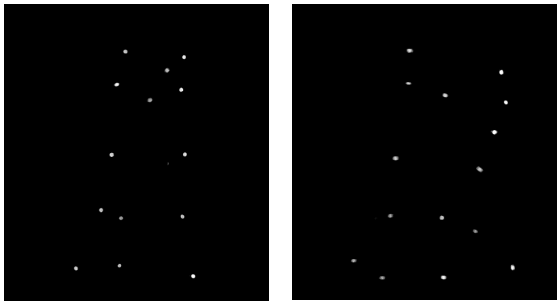

Figure 9: Examples of the PLBM videos presented. Positive "affectionate" (left), negative "assaulting" (right)

Scrambled versions of these videos were used as control videos for the social interaction content in the videos. Two positive (affectionate, playful) and two negative (assaulting, dominant) were used as control videos based on participants' responses in a pilot study. These were modified to eliminate participants' ability to interpret the meaning whilst keeping the trajectory and the velocity of the original videos. To do this, the videos were segmented into at least 3 but - no more than 4 horizontal parts (depending on specific movement vector to avoid segments cutting across movement paths). These segments were then re-ordered in order to make the original interaction hard to interpret. See Figure 10 below.

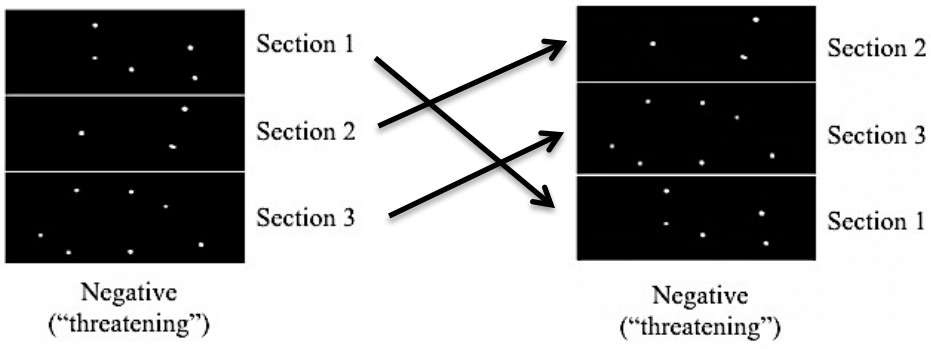

Figure 10: Graphical representation of how the original stimuli (left) were sectioned for the scrambled version (right).

All stimuli contained 14 or 15 white dots (depending on the action depicted) moving on black background and measured 18 cm horizontally x 20 cm vertically, occupying 20° degrees of horizontal angle and 24° degrees of vertical angle. Each dot measured 7 pixels, and played at 24 frames a second. For the word selection task, 4 white words were presented on a black background, directly after video presentation. Words were arranged on a grid in white Calibri font and size 36. These words included a target, synonym of the target, unrelated action word and an opposite action word. The position of the word types in the grid was randomly allocated. For the word selection task, 4 white words were presented on a black background, directly after video presentation. Words were arranged on a grid (random allocation) and printed in white Calibri font and size 36.

Procedure

For this experiment, participants were tested in two sessions: one for recording EEG and the other for measuring behavioural performance. For the EEG part, one block

was presented in which included video observation only. For this session, the number of stimuli presented was limited to two different videos from each valence type in order to limit the number of trials. Therefore, 20 positive (10 x playful and 10 x affectionate), 20 negative (10 x assaulting and 10 x dominant), and 40 matched controls were presented to participants in a computer randomized order. Each experimental trial started with a blank screen presented for 1000ms, followed by a 1000ms fixation cross, and finally a 3000ms video clip. See Figure 11 for graphical representation of the EEG part of this experiment.

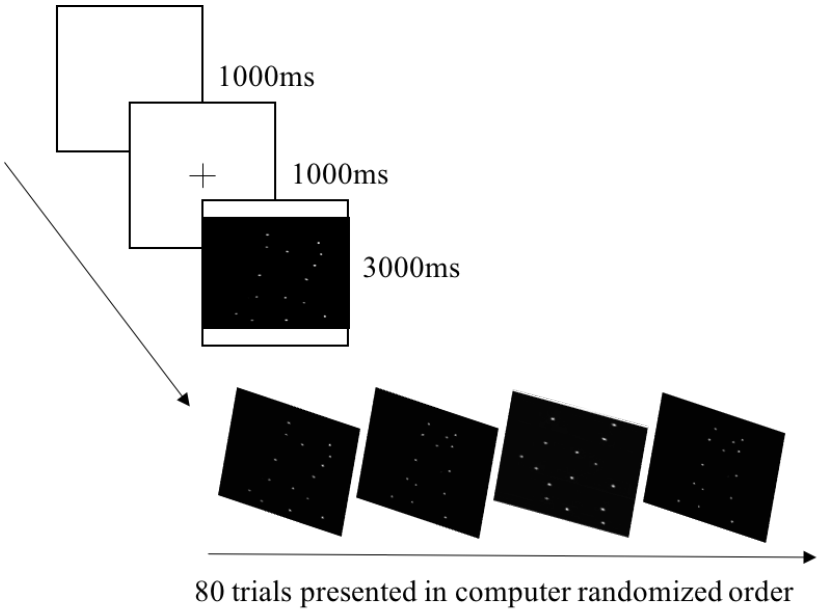

Figure 11: Graphical representation of the procedure for EEG part of the experiment.

Subsequent to EEG recording, participants performed the behavioural task. In this phase, the whole range of videos (9 different positive, 9 different negative, and same 4 matched control videos) was presented once, also presented in a computer randomized order. Each experimental trial started with a blank screen presented for 1000ms, followed by a fixation cross visible for 1000ms, then a 3000ms video clip,

and then asked to complete the forced-choice selection task and respond with the mouse cursor. See Figure 12 for graphical representation of procedure.

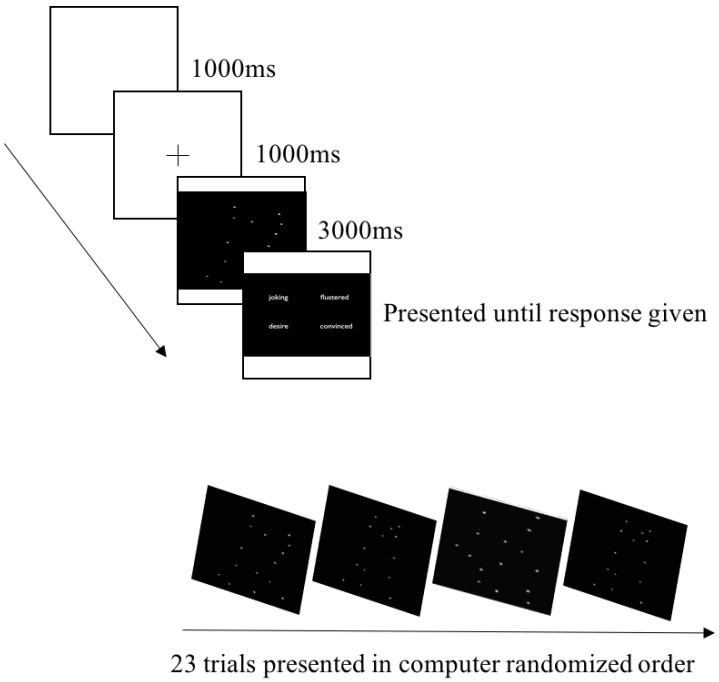

Figure 12: Graphical representation of the procedure for the behavioural part of the experiment.

EEG data acquisition

Data were acquired as in Experiment 1 (see page 43).

EEG data preparation

Data were prepared as in Experiment 1 (see page 43). Baseline period included the period in which a blank screen was presented (-2000 to -1000), and two active periods were included rather than one: early (500 to 1500ms) and late (1500 to 2500ms). Two active periods were included because the meaning of PLBM is revealed as the sequence unfolds (Johansson, 1973), and is therefore likely to affect sensorimotor reactivity.

Data analysis

All data were included for analysis and treated like in Experiment 1 (page 45), except for some details as described. Performance on the forced-choice word-matching task included reaction times on all trials, and percentage correct responses from all trials. No trials were rejected. These values were turned into inverse efficiency scores (IES = reaction times divided by percentage correct response; see Romei et al., 2011) instead of performing separate analysis for reaction times and accuracy (percentage correct). The behavioural data were normally distributed ($p > .05$), but the EEG data were not ($p < .05$) and therefore the EEG data were log transformed.

In order to investigate individuals' performance on the word-matching task in relation to empathy, a repeated measures ANOVA was conducted. For this analysis the following factors were included: "video type" with two levels (action, control), "valence" (positive, negative) and one between-subjects factor "empathy" with three levels (low, moderate, high). It was expected to find a significant interaction between the factors video type and valence, and in the event of such finding, it was planned to compare performance on the following pairs: (a) positive action (PA) vs. negative action (NA); (b) PA vs. positive control (PC); and (c) negative action (NA) vs. negative control (NC). These comparisons were Bonferroni corrected. Additionally, it was predicted that performance on each video type is related to individual level of empathy, and in the event of such finding, it was intended to investigate between groups performance for each video type.

The EEG data were investigated like in Experiment 1. In order to investigate μ-reactivity to observation of PLBM displays depicting social interactions, three

repeated measures ANOVAs were carried out. Factors included: "time" (with two levels: early, late), "video type" (two levels: action, control), "valence" (two levels: positive, negative), and "electrode" (seven levels: FC5, FC3, FC1, FCz, FC2, FC4, FC6), and one between-subjects factor: "empathy" (three levels: low, moderate, high). Bandwidth was a dependent variable. For C-channels, the electrode included: C5, C3, C1, Cz, C2, C4, C6, and for the O-channels, the factor electrode included: O1, Oz, O2. The same planned comparisons were conducted on the EEG data as in the behavioural data in the event of a significant main effect for the factor video type, and interaction with empathy.

Lastly, Pearson's correlation was used to assess whether performance on the word-matching task was related to μ-ERD during observation of PLBM videos. It was predicted that μ-ERD would correlate with performance on the word matching task given the observation that hMNS is involved in action understanding (e.g. Fadiga et al., 2006; Gazzola et al., 2007). It was reasoned that a significant correlation would indicate that the hMNS was involved in both observation and understanding of PLBM displays depicting social integrations.

Results

Word-matching performance

The results of the repeated measures ANOVA revealed a significant main effect for the factor video type: $F(1, 31) = 9.43$, $p = .004$, $\eta_p^2 = 0.233$, for the factor valence: $F(1, 31) = 18.15$, $p < .001$, $\eta_p^2 = 0.369$, and an interaction between these two factors: $F(1, 31) = 8.73$, $p = .006$, $\eta_p^2 = 0.220$. Planned comparisons revealed that participants performed better on PA compared to NA ($p < .001$), and better on NA compared to

NC (p = .004). No significant difference in performance was detected between PA and PC (p = .540). These results are presented in Figure 13 and suggest that cognitive understanding of the positive social interactions was significantly better than for the negative. Additionally, cognitive understanding of social interaction content was better compared to scrambled versions, however only for the negative displays. No interaction or main effect was detected for the factor empathy (p > .392), suggesting that affective empathy is not involved in the ability to interpret social interactions depicted by PLBM.

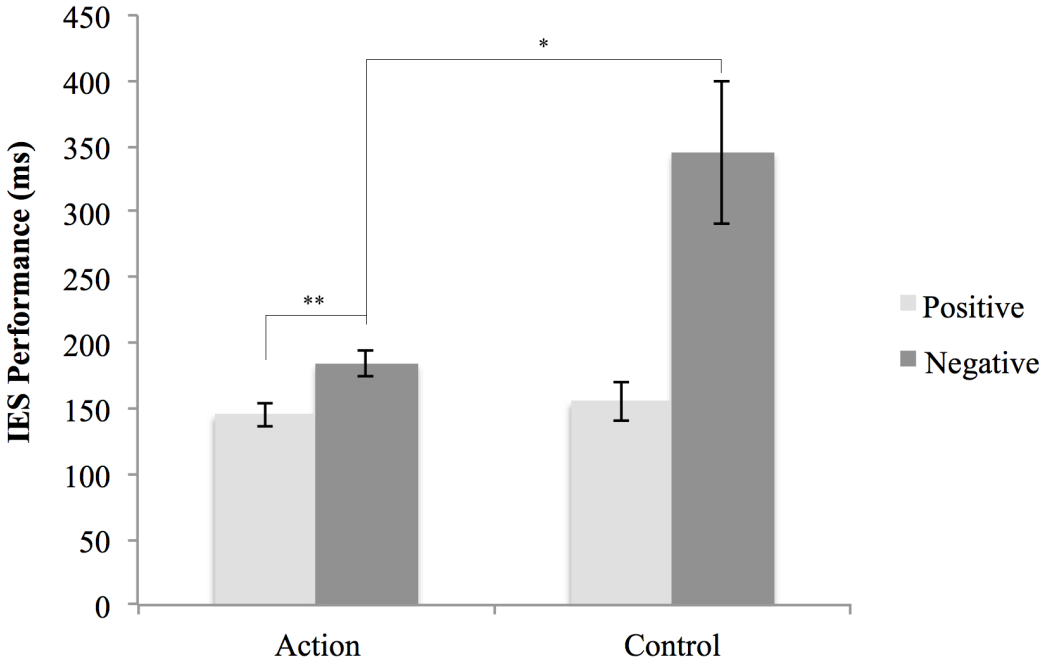

Figure 13: Graphical representation of word-matching performance. Bars represent IES for PA, NA, PC, and NC. Three comparisons are indicated: (a) PA vs. NA; (b) PA vs. PC; and (c) NA vs. NC. Error bars represent standard error. Significant differences are indicated with an asterisk. * p < .05, ** p < .005

Electrophysiological reactivity

Sensorimotor vs. occipital channels

The result of the repeated measures ANOVA indicated no significant interaction between the factors channels and video type (*p*s > .383), however a significant main effect for the factor channels was observed in α2: F (2, 70) = 31.44, $p < .001$, $\eta_p^2 > 0.473$, but not in α1 ($p = .644$) indicating that ERD differed between regions. Therefore, investigations of μ-reactivity were investigated separately in FC, C, and O-channels.

Fronto-central channels

The results of the repeated measures ANOVA demonstrated a significant main effect for the factor time in α1: $F(1, 20) = 15.09$, $p = .001$, $\eta_p^2 = 0.430$, and in β1: F (1, 20) = 37.91, $p < .001$, $\eta_p^2 = 0.655$, but not in α2 ($p = .813$). Time was investigated by a post hoc Bonferroni corrected pairwise comparison, which indicated that ERD was significantly larger in the late period compared to the early period (*ps* < .001). No significant main effect was found for the factor video type or valence in any bandwidths (*p*s > .469), however a significant interaction was found for the factors time and video type in the α2 bandwidth: $F(1, 20) = 6.86$, $p = .016$, $\eta_p^2 = 0.255$ (α and β1: *p*s > .454). Bonferroni corrected planned comparisons revealed no significant differences in ERD between video types or valence dependent on the factor time (*p*s > .089) suggesting that signal from FC-channels were not sensitive to video-type or valence.

Results of the one-sample t-test with the test value zero, demonstrated that the observed change in power differed significantly from zero during observation of PA: $t(37) = 3.78$, $p < .001$, NC: $t(37) = 2.47$ $p = .018$, and a trend for PC: $t(37) = 1.98$ $p = .055$, and NA (*p*s > .062) suggesting that during observation of PA, NC and PC,

significant desynchronisation was observed, but not during NA.

Lastly, empathy as a factor did not reveal a significant main effect or interaction with μ-suppression in any bandwidths (*p*s > .173), suggesting that affective empathy was not related to processing PLBM displays depicting social interactions.

Central channels

The results of the repeated measures ANOVA demonstrated a significant main effect for factor the factor time in α1: $F(1, 23) = 27.34$, $p < .001$, $\eta_p^2 = 0.543$, and in β1: $F(1, 23) = 31.37$, $p < .001$, $\eta_p^2 = 0.577$, but not in α2 ($p = .813$). Time was investigated by a post hoc Bonferroni corrected pairwise comparison, which indicated that ERD was significantly larger in the late period compared to the early period (*ps* < .001). No significant main effect or interaction was observed for the factor video type or valence in any bandwidths (*p*s > .137), however, a significant interaction between the factors time, video type and valence was indicated in β1: $F(1, 23) = 5.17$, $p = .033$, $\eta_p^2 = 0.184$. Bonferroni corrected pairwise comparisons indicated that ERD differences occurred in the late time period only (early: *p*s > .110), PA induced significantly larger β1-ERD compared to PC ($p = .014$), no other differences were observed (*p*s > .200). See Table 2 for results of Bonferroni corrected pairwise comparisons and Figure 14 for graphical representation of the result. Although no main effect was observed for empathy (*p*s > .079), a significant interaction between empathy and the factor time was observed in α1: $F(1, 23) = 5.06$, $p = .015$, $\eta_p^2 = 0.306$. This interaction was not further investigated as it falls outside the scope of the current chapter of the thesis.

Table 2: *t* (and *p*-values) for planned comparisons in the EEG data

	PA vs. NA	PA vs. PC	NA vs. NC
Early	-.053, (.735)	.023, (.838)	2.76, (.110)
Late	.193, (.200)	**2.57, (.014)**	.006, (.964)

Note. Results in bold reached significance at .05 level.

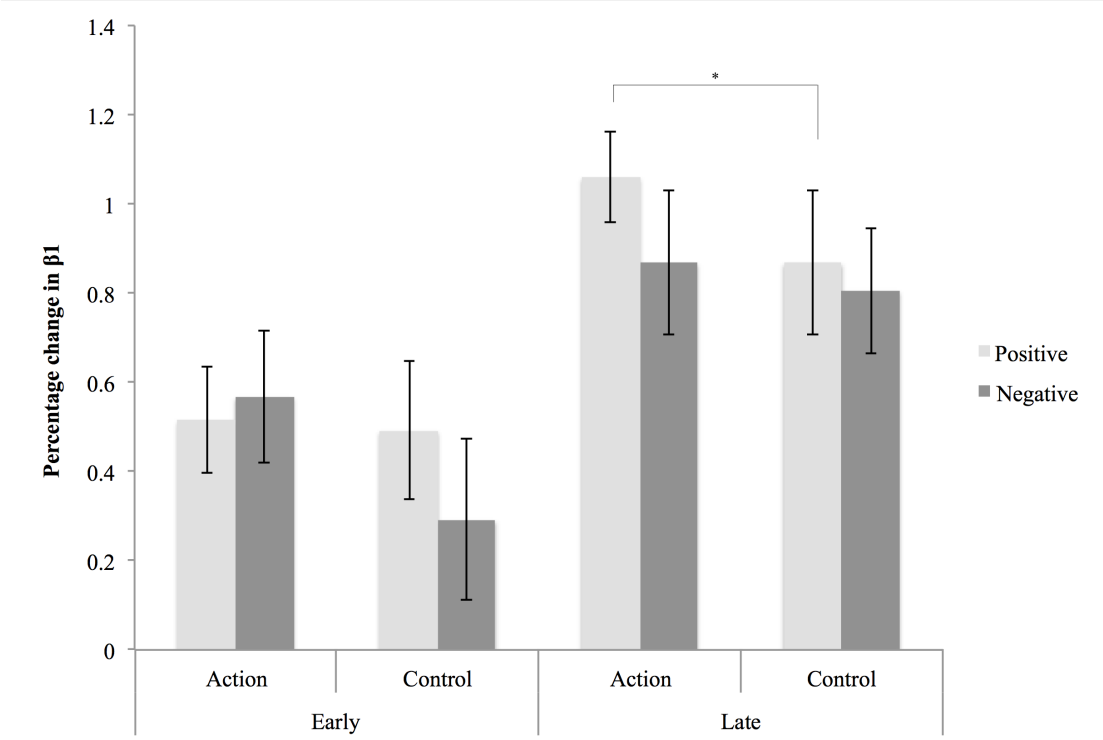

Figure 14: Results of the planned comparisons. Bars represent percentage change in C-channels in β1 during observation of PA, NA, PC and NC in the early and late periods. Error bars indicate standard error. Note: positive values represent ERD.

Results of the one-sample t-test with the test value zero, demonstrated that the observed change in power differed significantly from zero during observation of all video types in α1 and β1: *p*s < .001, but not in α2 (*p*s > .395), suggesting that significant change in power was observed in signal from the C-channels during all video types in α1 and β1.

Occipital channels

The results of the repeated measures ANOVA indicated a significant main effect of

time in both α1 and α2: *F*s (1, 37) > 22.14, *p*s < .001, η_p^2s > 0.374. In line with sensorimotor regions, post hoc comparisons demonstrated that the late period elicited significantly greater ERD compared to the early period in both α1 and α2 (*p*s < .001). Importantly, no main effect or interaction with video type was found in any bandwidth (*p*s > .206), suggesting that signal from the occipital region did not distinguish between observations of video types as sensorimotor (FC) α2 did. Additionally, no main effect or interaction was observed with the factor empathy (*p*s > .106), indicating that empathy did not modulate occipital reactivity.

μ-reactivity and word-matching performance

Because the effect for video type was only found in the late period in C-channels in β1, correlations were only conducted for those conditions. The results are presented in Table 3 and demonstrate that performance does not correlate with μ-ERD in response to any type of PLBM video type with IES performance (*p*s > .232), suggesting that observation and understanding where not functionally related.

Table 3: *r*-values (and *p*-values) for β1-ERD by IES performance on the word-matching task

	IES
PA	-.015 (.927)
NA	.198 (.232)
PC	.070 (.675)
NC	.052 (.769)

Interim Discussion

The current study investigated μ-reactivity in relation to a social-cognitive task (social interactions) as an alternative experimental protocol to observation of an

intransitive hand movement (Experiment 1) and a social-perceptive task (Experiment 2). The results of the current experiment revealed selective β1-ERD for positive PLBM social interactions compared to scrambled versions. As predicted, this effect was found in the late time period, given that the meaning of PLBM displays unfolds with time (Johansson, 1973). The finding that PLBM social interactions induced significantly larger β1-ERD than scrambled versions supports the study prediction, and previous literature suggesting that the hMNS is sensitive to social interactions (Iacoboni et al., 2004; Walter et al., 2004; Oberman, Pineda, & Ramachandran, 2007; Naem et al., 2007), because the scrambled versions were designed to eliminate the appearance of any social interaction. The finding that β1-ERD was significantly larger for positive expressions compared to negative is also consistent with the study prediction, and supports other observations that suggest that hMNS-related activity favours positive expressions (Leslie, Johnson-Frey, & Grafton, 2004; Niedenthal et al., 2010; O'Doherty et al., 2003; Rochas et al., 2012).

It appears that β1-reactivity was modulated by valence, as positive social interactions induced significantly greater β1-ERD compared to the positive control. This pattern was not observed for the negative interactions. However, because the behavioural results suggested that participants performed better on the positive social interaction videos compared to the negative ones, it is conceivable that the difference in β1-ERD observed reflected level of difficulty rather than valence. If this was the case, one would expect the most challenging task to induce greatest β1-ERD and the least challenging task to induce the least. This is what would be expected based on the observation that oscillatory suppression corresponds to task demands (Stipacek, Grabner, Neuper, Fink, & Neubauer, 2003; Klimesch, 1999; Boiten, Sergeant, & Geuze, 1992) in such a way that greater task demand corresponds to greater

suppression. In the current experiment however, no relationship was detected between the word-matching task and β1-ERD. Additionally, β1-ERD was large for both the least challenging (PA) and the most challenging (NC) task. Therefore, it is very unlikely that the β1-ERD difference between positive and negative expressions reflects mere task difficulty.

Moving on to the lack of correlation between β1-ERD and corresponding behaviour, the results did not support the notion that the hMNS is involved in interpreting social interactions depicted by PLBM, as no correlation was observed between the cognitive understandings of the social interactions with β1-ERD. It has been demonstrated that observation of PLBM displays induces hMNS-related activity (e.g. Saygin et al., 2004; Ulloa & Pineda, 2007), and that individuals' understand the meaning of social interactions depicted by PLBM (e.g. Thurman & Lu, 2014; Manera et al., 2010). Although it has not been demonstrated that hMNS-related activity is actually related to the understanding of PLBM displays, several others have conceptually related hMNS-related activity with the integration of meagre visual details consequently facilitating understanding of the displays (Ulloa & Pineda, 2007; Saygin et al., 2004). However, the results of the current experiment do not support this interpretation, and are consequently inconsistent with previous studies reporting that action understanding is related to hMNS-related activity (e.g. Fadiga et al., 2006; Gazzola et al., 2007). In support of the current results, the notion that action understanding is facilitated by the hMNS has been disputed (e.g. Hickok, 2008; 2013) for lack of direct evidence. Alternatively, it has been suggested that the nodes of the hMNS (IPL and IFG) are responsible for different actions in the process of matching observed actions with execution of the same action (e.g. Iacoboni & Wilson; Rizzolatti & Craighero, 2004). Furthermore, there are studies suggesting that the IFG is more

strongly related to action understanding than the IPL (e.g. Fadiga et al., 2006; Pobric & Hamilton, 2006), and it has been demonstrated that μ-suppression correlates with hMNS-related activity in the IPL but not the IFG. Therefore, the process involved in observation of PLBM videos appears to be different from the process involved in interpreting the PLBM videos. It is plausible that observation of PLBM was related to the IPL, while the cognitive understanding of them was related to the IFG.

The finding that less β1-ERD was recorded during observation of the scrambled versions of the PLBM, suggest that motor processes were involved possibly including the hMNS. This interpretation is in line with the finding that occipital α-reactivity did not demonstrate selective processing for social interaction videos, and therefore the effect observed for the social interaction cannot be better explained by simple visual attention. It is therefore more likely that social interactions recruited the hMNS.

The effect for social interactions was found in β1 in C-channels, but no effect was found for α. This lack of ERD in α suggests that social interactions indicated by PLBM may not recruit generators of α. Although there is no comparative study that investigated social interactions depicted by PLBM videos that also measured μ-reactivity during observation; some comparisons can be drawn from other studies that investigated either social interactions or PLBM videos. The majority of these studies investigated narrow μ frequency ranges (8 – 13 Hz or 8 – 10 Hz) and demonstrated ERD to social interactions (Oberman, Pineda, & Ramachandran, 2007; Naem et al., 2007) and to PLBM (Ulloa & Pineda, 2007; Perry, Troje, & Bentin, 2010). The current finding that β1 is suppressed during observation of PLBM videos depicting social interactions corroborates these findings. Two studies did investigate β1 and β: Cooper and colleagues (2013) demonstrated β1 ERD during observation of emotion

expressed in faces; and Perry, Troje, & Bentin (2010) demonstrated both α and β (15 – 25 Hz) ERD during observation of a single individual depicted by PLBM videos. However, in the current study, valence was expressed in whole-body social interaction videos, and PLBM videos involved dyads rather than a single person. Therefore, these differences may underlie the lack of differences in beta activity in the current study.

Lastly, even though empathy has been suggested to influence social interactions (e.g. Bjorkqvist, Osterman, & Kaukiainen, 2000; Decety & Jackson, 2004), the current study demonstrated no such link: level of empathy did not modulate either performance or α2-ERD during observation of PLBM videos. It was speculated in Experiment 1 whether the lack of relationship between μ-reactivity and empathy was due to a lack social relevance in observing a hand opening and closing. However, neither in the current experiment nor the previous (Experiment 2) was there a relationship between μ-reactivity and empathy despite both experiments including social relevance. Therefore, social relevance in itself is not the reason for a lack of relationship between μ-reactivity and empathy. In a study similar to the current experiment, Perry, Troje, and Bentin (2010) demonstrated a significant negative correlation with suppression in μ during observation of PLBM videos expressing intention. However, Perry, Troje, and Bentin's study used a cognitive empathy measure while the current study used an affective empathy measure (EC). Cognitive and affective empathy are considered different sub-components of empathy, and has been associated with different neuronal substrates (Shamay-Tsoory, Aharon-Peretz, & Perry, 2009). It is possible that the conditions in the current experiment did not require affective empathy processes, and therefore, a different empathy measure could lead to a different result.

In summary, the current study demonstrated selective β1-ERD for positive social interactions depicted by PLBM videos. This selectivity for positive social interactions was not observed in occipital electrodes, suggesting that the ERD recorded in β1 may be an indication of hMNS activity. These findings support the notion that hMNS is involved in social interactions and that it is more sensitive to positive expression of emotion. However, no relation was established between performance on the word-matching task and μ-reactivity during observation, suggesting that the μ-rhythm is not involved in inferring meaning from social interactions depicted by PLBM. Lastly, level of empathy did not modulate either performance nor μ-reactivity, suggesting that affective empathy may not be involved in processes required for the task.

Chapter Discussion

The aim of experiments 1 - 3 was to establish an efficient EEG protocol that induces μ-ERD to be used in future experiments. Experiment 1 investigated a protocol that has previously been shown to be an efficient experimental protocol in triggering μ-suppression (e.g. Oberman et al., 2005; Puzzo et al., 2010; 2011). The result of the current experiment demonstrated selective ERD in α2 and β1 during observation of a moving hand compared to a static hand and bouncing balls. The μ-reactivity pattern observed implies recruitment of hMNS activity. Experiment 2 investigated μ-reactivity during a social-perception task (RMET). The results of this experiment demonstrated ERD in β1 and ERS in α-bandwidths, but the β1-ERD was not modulated by trials that were correct. ERD was also demonstrated in the occipital electrodes, and the lack of α-ERD in sensorimotor areas but presence of α-ERD in occipital electrodes could suggest that α-reactivity observed reflects visual attention rather than motor processing. There is then some evidence that hMNS-related activity

was observed in β1 but not α. Lastly, experiment 3 investigated μ-reactivity during social interactions indicated by PLBM videos. This experiment demonstrated selective ERD in β1 for positive social interactions. No relationship was established between performance on the word-matching task with μ-reactivity suggesting that μ is not related to the ability to infer meaning from social interactions depicted by PLBM.

The aim of Chapter 2 was to select one EEG protocol that efficiently induced μ-suppression. Given that there is an abundance of different experimental protocols reported in the literature, it was decided to test three different protocols. Although all of the experiments demonstrated hMNS-related activity to some extent, comparisons between the results are difficult. The selection was therefore based on the protocol that performed the best regardless of the other protocols. Starting with the results considered the weakest; Experiment 2 resulted in ERD in β1-bandwidth but was not sensitive to trials that were correct. β1 has typically been associated with motor preparation, and therefore may be more involved in pure motor tasks rather than implied motor involvement. The results were also difficult to interpret given the observed lack of α-ERD in sensorimotor areas but a presence of occipital α-ERD. A fundamental problem with this protocol is that the images observed were static, and it has been reported that μ-suppression is not responsive or is less responsive to static images. Another problem was that there was no control condition for this protocol. Furthermore, the results were not consistent with previous studies (e.g. Pineda & Hecht, 2009). For these reasons, Experiment 2 was not included in the following discussion regarding selection of experimental protocol to use in future experiments.

Experiment 1 investigated rudimentary motor mirroring, and as the action presented

did not include a goal, the reactivity recorded is less likely to be confounded by other systems not exclusively motor in nature. It was reasoned that the two μ-components could be investigated more clearly as a result. The results of this experiment were as predicted, and in line with previous studies. However, Experiment 1 did not include a measure of behavioural performance that could be related to μ-ERD. The lack of such measure is a problem in attempting to differentiating motor function from mirror function, as mirror neurons by definition responds to both action observation and execution. Although Experiment 3 included such measure, the results were less clear than Experiment 1. This experiment investigated μ in relation to socially relevant actions and in relation with corresponding behaviour. Although this protocol indicated recruitment of hMNS-related activity, there were no relationship between performance on the word-matching task and μ-reactivity. Additionally, the negative social interaction videos elicited no difference in μ-reactivity compared to the control condition. This is problematic because conceptually, the negative social interactions should have included more hMNS-related activity than the control condition. Therefore, the clearest results were indicated for observation of a simple hand movement (Experiment 1). In support of this selection, the most basic principles of mirror neurons (in monkeys and humans) addresses simple motor processes (see Chapter 1). This literature also contains the most convincing evidence because all other functions associated with hMNS has been generalized from the basic principles. Furthermore, the rational for such generalization has been criticized for using a circular argument (see page 24). For these reasons, and in light of the results from experiments 1 – 3, the current thesis will focus on simple motor processing.

The contended relationship between empathy and μ-ERD was not supported by any of the experiments presented in this chapter. The first experiment investigated μ in

relation with an intransitive hand movement. Although the lack of relationship in Experiment 1 can be attributed to lack of social relevance, the lack of such relationship in experiments 2 and 3 is harder to explain because these included socially relevant stimuli. Neither of these experiments revealed any indication that level of empathy modulated μ-reactivity or performance. Several considerations in regards to this lack of relationship was discussed on page 95 and involves the possibility that affective empathy was not related to the tasks, but that cognitive empathy might have been. The current sets of experiments add to the empathy-hMNS controversy, and because the functions associated with hMNS are not the main objective of this thesis, empathy will not be investigated further.

Studies mentioned on page 50 suggest that α and β bandwidths are sensitive to different parameters of action observation: Whereas intransitive hand actions seem to trigger β1 more reliably than α (e.g. Puzzo et al., 2013), transitive and goal-directed hand actions trigger α (e.g. Muthukumaraswamy & Johnson, 2004; Muthukumaraswamy, Johnson, & McNair, 2004). The results of experiment 1 and 2 are in line with this notion, but in addition suggested that the upper end of the μ-spectrum (α2 and β1) was more relevant in processing action observation than α1. These findings suggest that the popular tendency to investigate μ exclusively as 8 – 13 Hz is too narrow to investigate hMNS-related processes. Future investigations of motor processes and hMNS in this thesis will therefore continue to investigate μ as comprised of both α and β1.

In summary, the clearest protocol out of the three tested, was the intransitive hand movement protocol (Experiment 1). Inopportunely, this protocol was the only protocol of the three that did not incorporate a behavioural measure. However,

adaptations can be made to incorporate such a behavioural component. The moderations to the protocol included observation of two hands rather than one, and imitating the movements observed. The imitation was in terms of number of correct reproduced movement sequences rather than other movement related parameters such as kinematics. The following chapter will describe this further, and how the moderated protocol was developed, as well as explore more directly the relationship between μ-power changes and the hMNS.

CHAPTER 3: Investigating Causal Evidence for hMNS

Introduction

Chapter 2 investigated three experimental protocols from different domains that have all been associated with the hMNS. These protocols were tested in order to establish the most efficient one inducing μ-suppression (ERD) as an indication of hMNS-related activity. Although electrophysiological results in all three protocols demonstrated μ-reactivity in support of hMNS-related activity, it was reasoned that the moving hand observation (Experiment 1) was the most efficient protocol for the following reasons: the most convincing evidence supporting hMNS comes from mirror properties demonstrated in basic motor processes (e.g. Di Pellegrino et al., 1992; Rizzolatti et al., 1996), other functions associated with hMNS has been generalized from basic motor mirror principles (see Hickok, 2008 for a review), and the result of Experiment 1 was more robust in terms of clarity and predictability. The drawback with that protocol is that it did not incorporate a behavioural measure. The development of a modified version of Experiment 1 that includes a behavioural component will be described in this chapter.

Relating Mu with hMNS activity

The EEG literature contains a wealth of studies demonstrating μ-suppression during action observation (see Chapter 1 and 2), but the extent to which these findings reflect hMNS involvement remains controversial. This controversy is largely due to the correlative nature of EEG, as it is an indirect measure of neuronal activation (see page 19), and it is often considered in relation to fMRI findings because BOLD signal

positively correlates with μ-suppression (e.g. Arnstein et al., 2011; Braadbaart, Williams, & Waiter, 2013). However, fMRI is also an indirect measure of neuronal activation, and fMRI cannot readily distinguish whether the signal for execution and observation originates from the same individual cells (e.g. Kilner et al., 2009). Brain stimulation studies however can provide causal evidence, and are therefore considered more direct evidence for observation and execution matching. For example, such studies have demonstrated that observation of a motor act triggers activation in corresponding cortical system recruited for execution of the same motor act (See page 10; Fadiga et al., 1995). The gap between correlative EEG studies and more direct brain stimulation studies can however be bridged by applying these techniques together, and consequently the extent to which EEG indicates hMNS-related activity can be investigated. Studies of this nature are lacking in the literature and are indispensable to validate the use of EEG as a tool to indicate hMNS-related activity. The use of brain stimulation in this context will be described next.

Modulating Brain Oscillations

Several brain stimulation methods exist to influence excitability of the brain. The two techniques most commonly used in modern times are transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (TES). Both techniques are non-invasive and considered safe and useful tools in investigating various aspects of human neurophysiology granted that relevant guidelines are followed (Guleyupoglu et al., 2013; Rossi, Hallet, Rossini & Pascual-Leone, 2011; Veniero, Vossen, Gross & Thut, 2015; Wasserman, 1997). As the names suggest, the mechanism of action are different for these tools. TMS (as was described on page 9) induces electric currents in the brain using principles of electromagnetic induction. This induction prompts

action potential of cells influenced by the magnetic field generated by the stimulation (Hallet, 2007). When applied rhythmically/repetitively (rTMS) the method becomes a more powerful and potentially a more dangerous tool (Wassermann, 1998). On the other hand, TES delivers low power electrical currents to the brain (Paulus, 2011), which affects resting membrane potentials rather than action potentials (Nitsche et al., 2008). These currents can be delivered in varying waveforms such as direct current stimulation (tDCS), alternating current stimulation (tACS), and random noise stimulation (tRNS). When tACS is applied with a DC-offset it is known as oscillating transcranial direct current stimulation (otDCS; Veniero, Vossen, Gross & Thut, 2015).

The effect of TES is milder than TMS because the electrical currents passing through the scalp and skull become more dispersed before reaching brain tissue. Magnetic induction (TMS) on the other hand is less affected by the poor electrical conductivity of the skull (Rossi et al., 2009). There are however advantages in employing TES techniques over TMS, such as participants experiencing less pain, cost efficiency, ease of online application, and disguising sham conditions (Paulus, 2011; Antal & Paulus, 2013). Further differences between these techniques are outside the scope of this thesis. Nevertheless, stimulation techniques that enable *frequency tuning*[13] are of particular interest to this thesis because it enables the selective targeting of brain oscillations (Veniero et al., 2015). Thus this technique can be applied in order to selectively modulate oscillations associated with hMNS (i.e. 8 ~ 20 Hz; see page 23). This will be explored more in the following section.

Rhythmical brain stimulation techniques enable stimulation in a given frequency.

[13] Frequency tuning refers to matching of the externally applied electromagnetic field to the intrinsic frequency of oscillatory neuronal population (Veniero et al., 2015).

These include rTMS, tACS and otDCS (See review by Veniero et al., 2015). However, the current thesis will focus on tACS, which involves the induction of a weak sinusoidal electric current between two (or more) scalp electrodes (Antal & Paulus, 2013). It is known that when sinusoidal alternating electric fields are applied extra-cellularly across pyramidal neurons, the transmembrane potential is altered sinusoidally (Chan & Nicholson, 1986). This effect has been called *entrainment* and has been demonstrated in numerous in-vivo and in-vitro in animal studies (Frolich & McCormick, 2010; Ozen et al., 2010; Deans, Powell, & Jefferys, 2007; Reato et al., 2010). The effect of entrainment is particularly robust when stimulating at the frequency of the networks' own rhythm (See Reato et al., 2013 for review). In humans, the effect of tACS has predominantly been studied offline (after stimulation) until recently, as the artefacts associated with stimulation online (during stimulation) compromises analysis (Neuling et al., 2012; Zaehle et al., 2010). However, several recent studies have reported electrophysiological (using MEG and EEG) evidence of tACS-induced entrainment effects (online) after separating stimulation artefacts from on-going and event-related cortical activity (Helfrich et al., 2014; Neuling et al., 2015; Witkowski et al., 2015). These studies are evidence that entrainment effects can occur in human neuronal networks during tACS. Additionally, such entrainment effects are stronger when the stimulation frequency is at or close to the neuronal networks' dominant frequency (Halbleib et al., 2012; Herrmann, 2001). These studies suggest that frequency tuned alternating current stimulation can be used to interact with intrinsic neuronal networks with some specificity.

The effects of tACS has also been shown to affect performance on behaviour that corresponds to the neuronal network or specific oscillation targeted (e.g. Miniussi et al., 2012). This effect has been reported for a variety of fields including perception

(Feurra et al., 2011; Helfrich et al., 2014; Neuling et al., 2012), multisensory processing (Cecere et al., 2015), motor control (e.g. Pogosyan et al., 2009; Joundi et al., 2012), and memory (Marshall et al., 2006; Polania et al., 2012). The rational for inducing behavioural changes with frequency-tuned stimulation comes from the observation that cortical oscillations are associated with cognitive performance (e.g. Klimesch, 1999; Basar et al., 1999; Knyazev, 2007; Basar & Guntekin, 2008), and that modulating cortical oscillations alters corresponding cognitive performance (Klimesch, Sauseng, & Gerloff, 2003; Sauseng et al., 2009; Romei, Gross, & Thut, 2010). However, lack of frequency-specific change in behaviour subsequent to tACS has also been reported (e.g. Neuling et al., 2013, but see review by Veniero et al., 2015).

In contrast to the documented entrainment effects of online tACS, prolonged stimulation has been reported to result in oscillatory changes that persist after the end of the stimulation (after-effects). These after-effects have been shown to last longer than entrainment effects (See Veniero et al., 2015 for a review), which are known to only last a few cycles after stimulation terminates (Marshall et al., 2006; Reato et al., 2013). Little is known about the mechanism responsible for after-effects, but some have asserted that they cannot be explained by mere continuation of entrainment, due to the observation that entrainment effects ceases after a few cycles after the stimulation terminates (Veniero et al., 2015; Vossen et al., 2015). However, it has been reported that tACS-induced entrainment (online) is positively correlated with after-effects (Helfrich et al., 2014) suggesting that entrainment may at least influence after-effects. Another theory regarding the mechanism for which tACS procures after-effects is related to spike-timing dependent plasticity (STDP; e.g. Polania et al., 2012; Zaehle, Rach, & Herrmann, 2010; Vossen et al., 2015). In this model, the order and

timing of pre- and post-synaptic potentials determine the magnitude, and direction of changes in synaptic strength (Feldman, 2012; Dan & Poo, 2006; Caporale & Dan, 2008). Zaehle and colleagues (2010) incorporated principles of STDP in a neural network model and demonstrated how 10Hz periodic stimulation (using tACS) can strengthen or weaken synaptic weights of neuronal circuits depending on their reverberation frequency. In this model, online entrainment is the window into longer-lasting synaptic plasticity effects that translate into frequency-specific changes in oscillatory activity (Vossen et al., 2015). There is however no further evidence for this view except from the computational model provided by Zaehle and colleagues (2010).

Although tACS after-effects are frequently reported (e.g. Vossen et al., 2015; Zaehle et al., 2010; Wach et al., 2013), there is also evidence for a failure to produce such effects under some circumstances (e.g. Antal et al., 2008; Brignani et al., 2013; Struber et al., 2015). Furthermore, the direction of modulation and length of after-effects reported varies greatly (from one minute to at least half an hour), and while some studies report enhancement (e.g. Marshall et al., 2006; Kirov et al., 2009; Antonenko et al., 2013; Sahlem et al., 2015) others report suppression (e.g. Eggert et al., 2013; Garside et al., 2015). After-effects have also been found to rebound (i.e. initial power suppression turning into power enhancement; Marshall et al., 2011). The stimulation outcome has also been demonstrated to depend on the concurrent brain state or the task being executed (Herrmann, Rach, Neuling and Struber, 2013; Neuling, Rach, & Herrmann, 2013; Feurra et al., 2013) potentially leading to further inconsistencies. These inconsistencies can be related to the large variation in stimulation parameters applied such as stimulation intensity, electrode montage, stimulation length, and stimulation frequency (See Veniero et al. 2015 for review).

Some of these parameters will be reviewed next.

Moliadze, Atalay, Antal and Paulus (2012) demonstrated that tACS over the primary motor cortex at 140 Hz at 1 mA intensity, significantly increased motor cortex excitability, while 0.4 mA significantly decreased cortical excitation. No other intensity (0.2, 0.6, and 0.8 mA) induced significant oscillatory change. Vossen and colleagues (2015) also reported a power increase, however at a significantly lower frequency (10Hz). Additionally, the stimulation intensity was comparatively higher (ranging from 1.35 mA to 2 mA) than Moliadze and colleagues (2012). Another study (Garside et al., 2015) reported suppression in power subsequent to tACS applied with a stimulation intensity of 0.55 mA, which is comparative to Moliadze and colleagues' (2012) finding, however, the stimulation frequency was significantly lower (0.75 Hz: EEG delta frequency). These studies suggest that stimulation intensity can be used to control the direction of modulation. However, all of the studies above also differed in terms of stimulation frequency and length of stimulation. These factors also affect the modulatory effect. For example, Brignani, Ruzzoli, Mauri and Miniussi (2013) applied tACS at 1 mA intensity in different frequencies (6 Hz, 10 Hz, 25 Hz), for 5 minutes, and reported no frequency-specific modulation. Comparatively, Antal and colleagues (2008) applied tACS at 10 Hz at intensity 0.4 mA for 5 minutes and demonstrated no effect on oscillatory power. However, Zaehle and colleagues (2010) applied tACS for 10 minutes at individual alpha frequency (IAF) in individually adjusted stimulation intensities, and reported enhanced power in alpha. While Zaehle and colleagues (2010) and Brignani and colleagues (2013) stimulated occipital regions, Antal and colleagues (2008) stimulated the motor cortex. Different electrode montage obviously affects the stimulation outcome. Thus, some parameters appear to enable some specificity in terms of modulation effect, however inconsistencies

highlighted above suggest that predictability of modulation effect is meagre.

Frequency tuned brain stimulation techniques such as tACS can interact with intrinsic neuronal networks and modulate its corresponding behaviour with some specificity (e.g. Veniero, Vossen, Gross & Thut, 2015). However, it is apparent that tACS online effects are better understood than offline or after-effects. After-effects are further complicated by the use of a number of different combinations of stimulation parameters, and the predictability of the stimulation effect suffers as a consequence. The next section will move on to the behavioural component that will be included in the modified version of Experiment 1 (moving hand observation).

Imitation

For the modified behavioural version of the experimental protocol used in Experiment 1, it was decided to use an imitative task due to the large amount of evidence supporting the notion that hMNS is involved in imitation. Note that in this thesis, the term imitation will be used to refer to voluntary reproduction of movements. There are several other interpretations and definitions of imitation as will be addressed next. Imitation, at the most basic level refers to the reproduction of an observed behaviour (Fridland & Moore, 2014; Heyes, 2001). To varying degrees, this involves the imitator recognising that the behaviour to be imitated is goal-directed, and has some interest or importance to the imitator. Earlier definitions of imitation involved reproducing behaviours in terms of the behaviour, but also its intended goal (Boesch & Tomasello, 1998). It was assumed that an individual who imitates an observed action must first understand the goal or the meaning of the action (Csibra, 2007). However, in recent years, the goal requirement has been disputed because

behaviours can be imitated without prior knowledge regarding the meaning of the action (see review by Fridland & Moore, 2015).

It is also important to point out that imitation has been distinguished from other related concepts such as *emulation, motor mimicry* and *automatic imitation.* These will be addressed briefly in turn. Emulation refers to copying of goals of an action, but not the specific movements used to achieve the goal. Thus, the imitator imitates the goal perceived of the observed movement, but may achieve the perceived goal by using a different effector (e.g. foot instead of a hand), or a different sequence of movements (Hamilton & Grafton, 2008; Subiaul, 2010). Furthermore, whereas imitation is considered voluntary, automatic imitation and motor mimicry are considered unconscious and automatic (Heyes, 2011). It has however been proposed that motor mimicry is the same psychological phenomenon as automatic imitation except that it is detected under more naturalistic conditions (Van Baaren et al., 2009). For clarity, automatic imitation refers to the *stimulus-response compatibility effect* that is frequently observed during motor imitation tasks (Proctor & Vu, 2006). For example, in a study by Stürmer and colleagues (2000), participants were asked to open and close their hands in response to a colour cue superimposed on a video of a hand opening (compatible) or closing (incompatible). When the colour cue was compatible with the movement presented, participants were faster compared to when the colour cue was incompatible with the movement. In contrast, an example of motor mimicry is the unconscious imitating of social partner's movements. For example, Chartrand and Bargh (1999) demonstrated that interacting with a confederate whom repeatedly touched his or her face increased the likelihood of participants also touching their faces. This kind of imitation has been related to prosocial attitudes (See

Van Baaren et al., 2009 for a review). Note that in this thesis, it is voluntary imitation of movements that is investigated in the modified experimental protocol.

Moving on to the relation between imitation and hMNS, it was mentioned on page 9 that mirror neuron theories have been used to explain a variety of social cognitive abilities including imitation (Iacoboni et al., 1999; Iacoboni, 2005; Liepepelt, Prinz, & Brass, 2010), and the amount of evidence supporting this contention is encouraging. This literature will be addressed next. The first studies investigating hMNS (though not investigating imitation per se) employed study protocols that inevitably involved imitation. In these studies (e.g. Rizzolatti et al., 1996), observation of a specific movement was presented before the participant was asked to execute (imitate) the same movement. These studies typically implied that there is an overlap between cortical activity triggered during observation and execution of the same movement. Later, Mukamel and colleagues (2010) confirmed that (some) cells that are active during observation of a specific movement are also active during execution (imitation) of the same action. It is thus possible that imitation is a direct product of the matching between action observation and execution. A growing literature supports this interpretation as: increased cortical activity is continuously reported in core areas of the hMNS (IPL and IFG) during imitative processes (e.g. Buccino et al., 2004; Grezes et al., 2003; Decety et al., 2002; Iacoboni et al., 1999, see review by Caspers et al., 2010); cortical activity is greater during action observation when the intention is to imitate rather than simply observe (e.g. Decety et al., 1997; Jackson et al., 2006); imitation performance correlates with μ-suppression during observation of movement (Bernier et al., 2007; Bernier, Aaronson, & McPartland, 2013); and being imitated modulates μ-suppression during observation of movement (Hogeveen, Chartrand, & Obhi, 2015); disrupting core areas of the

hMNS (IPL and IFG) results in impaired performance on imitation tasks (Heiser et al., 2003; Catmur, Walsh, & Heyes, 2009); and lesion to areas overlapping with IFG (Heilman et al., 1982) and IPL (Goldenberg, 1995) is associated with poor imitation performance compared to healthy controls and excessive imitation, indicating inappropriate imitation (De Renzi et al., 1996; Lhermitte et al., 1986). Moreover, it is well documented that individuals with autism perform poorly on a variety of imitation tasks (Williams et al., 2006). This observation in relation to the contended relationship between autism and hMNS-related activity, suggest that imitation is related to hMNS function. However, the putative relationship between autism and hMNS is controversial (see page 33; Bird, Leighton, Press, & Heyes, 2007; Charman & Baron-Cohen, 1994).

Although hMNS appears to be involved in imitation, there is considerable evidence suggesting that the hMNS is not the only system involved. It is well established that action observation automatically activates the corresponding motor representation, yet under normal circumstances, observed actions are not overtly imitated unless it is intended to do so (except from motor mimicry which is an unconscious process). This is likely due to an active control system that inhibits unwanted imitation. Mukamel and colleagues (2010) who performed the only single-cell study in humans, confirmed that there are cells with mirror properties in the human brain (see page 8), but in the imitative control context, it is more interesting that they also reported that there are cells in which responds with excitation during action execution and inhibition during action observation. These cells were proposed to function as a mechanism preventing automatic imitation during observation, and for maintaining self-other differentiation. Others have related this self-other differentiation to imitation control as well (e.g. Brass et al., 2009). Imitative control has been suggested

to involve several areas including the temporoparietal junction (TPJ), the medial prefrontal cortex (mPFC) and the posterior superior temporal sulcus (pSTS) (Spengler et al., 2009; Wang et al., 2011). These regions are also often associated with the *mentalizing system* (MS; Overwalle and Beatens, 2009), which refers to the ability to understand and predict other people's behaviours by attributing mental states to them (Baron-Cohen, 1995; Premack & Woodruff, 1978). Furthermore, Mainieri and colleagues (2013) demonstrated evidence suggesting that both hMNS and the MS are involved in imitation, however, the hMNS demonstrated selectivity to the social relevance of movements imitated while the MS demonstrated selectivity to observation rather than execution. The authors reasoned that the MS' selectivity to observation rather than execution reflects engagement of processes related to self-other differentiation. It is therefore likely that the hMNS is not the only system involved in processing imitation. The next section describes the development of the modified experimental paradigm where imitation has been incorporated as a behavioural dependent variable.

Development of Experimental Protocol

For the purpose of investigating μ-reactivity in relation to behavioural changes, the moving hand observation protocol was modified to include a behavioural component (imitation). The original protocol involved observing a right hand open and close. By adding execution (imitation) of the movements presented, this protocol was turned from an observational protocol to an observation/execution (imitation) protocol. Imitation was a suitable task conceptually due to the proposed relationship with the hMNS (see page 9; e.g. Iacoboni, 1999) and for its relative ease of inclusion. In the modified protocol, participants observed hands movements, and imitated them

subsequently. However, imitating one hand opening and closing is likely to result in limited performance variance because it is too easy. Therefore, it was decided to include both hands (left and right) in the modified protocol with the aim to increase task difficulty. The modified protocol then, involves observing a sequence of two hands opening and closing (one at the time), and subsequently imitating the sequence presented (left vs. right hand movement order). The nature of this task involves a memory component, and therefore a pilot study was conducted to assess the approximate number of hands movements individuals can re-produce in a single sequence. The pilot study is described underneath.

Pilot study

Ten participants (4 males) age ranging from 18 – 39 (mean age = 25.5, SD = 7.37) were recruited from social media (Facebook, Inc.) and completed the experiment on a Macintosh laptop (15-inch screen). Ten videos depicting two hands opening and closing were shown from an egocentric point of view in sequences ranging from 1 to 10 movements. A graphical representation of the videos are presented in Figure 15 below.

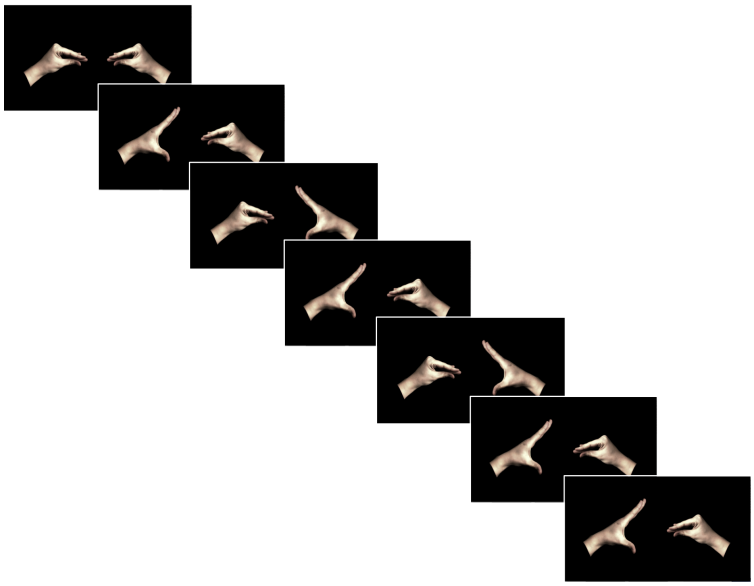

Figure 15: Graphical representation of videos used in the pilot study.

Each trial started with a sequence of one movement and progressed to ten hands movements in one sequence. Each sequence lasted one second, and therefore, a sequence with one movement lasted one second while a sequence of 10 movements lasted 10 seconds. Left versus right hand movement in the sequence had been selected randomly in advance of presentation, but participants viewed the same ten movements in the same sequences. Participants reproduced the sequence directly after presentation, and performance was recorded using the built-in video camera on the laptop. The number of correct responses was turned into percentage correct responses. The results are presented in table 4.

Table 4: Mean percentage correct hands movements reproduced per trial

	Percentage correct per trial									
Participant	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	100	100	100	100	100	71	33	0	0	0
2	100	100	100	100	60	43	17	100	33	20
3	100	100	100	100	100	57	67	0	0	0
4	100	100	100	100	100	100	67	100	44	70
5	100	100	100	100	40	100	50	37.5	33	40
6	100	100	100	100	100	43	33	37.5	44	40
7	100	100	100	100	100	43	67	25	78	40
8	100	100	100	100	100	100	67	75	44	70
9	100	100	100	100	100	100	67	100	56	50
10	100	100	100	100	100	71	67	37.5	56	30
Group average	**100**	**100**	**100**	**100**	**90**	**73**	**53**	**51**	**39**	**36**

The table above indicates that performance in most participants declined after four movements in a sequence, but significantly so during six movements, suggesting that the first five trials were too easy. Performance declined to under 50% after eight hands movement suggesting these were too hard to re-produce correctly. A repeated-measures ANOVA confirmed that the length of the sequence significantly reduced performance: F (9, 81) = 20.32, $p < .001$. Bonferroni corrected pairwise comparisons were conducted on the following pars: a) 4 vs. 6, b) 4 vs. 7, and c) 4 vs. 8. These pairs were selected because sequences 6, 7, and 8 demonstrated performance above 50% accuracy but less than perfect. It was assumed that this approach would eliminate ceiling and floor effects. Results confirmed that performance on sequences 6, 7, and 8 were significantly less than perfect (sequence 4): ps $< .001$, and therefore, six, seven, and eight sequences were used for the experiments presented in this chapter. Note that the task used is a serial recall task, and therefore taps into working memory processes. This issue will be further addressed on page 210. Furthermore, the results of the pilot study is consistent with George Miller's (1956) classical theory of

working memory; that the number of items that people can reliably remember are seven plus or minus two.

Summary and Aims

The EEG literature indirectly suggest that μ-suppression during action observation reflects activation of hMNS-related activity, particularly when considering that BOLD signal correlates with μ-reactivity (e.g. Arnstein et al., 2011; Braadbaart, Williams, & Waiter, 2013). However, these studies are correlative in nature and lack causal and more direct evidence, and therefore, the argument that μ-suppression is a valid indicator for hMNS-related activity remains controversial. Causal (and more direct) evidence can be established using brain modulation techniques such as tACS, in which intrinsic neuronal networks can be modulated within a specific frequency. This property enables a systematic investigation of function(s) corresponding to specific oscillations (Veniero et al., 2015). Although the effect of modulation appears to be difficult to predict (see page 106), any demonstration of interference with μ-suppression on corresponding behavioural (imitation) performance will arguably verify the notion that μ-suppression can indicate hMNS-related activity. Imitation is a suitable behavioural component for the modified version of Experiment 1 (hand movement observation) due to its well-documented relation with hMNS-related activity (see page 110; e.g. Buccino et al., 2004; Grezes et al., 2003; Decety et al., 2002; Iacoboni et al., 1999) and relative ease of inclusion. Two hands were included rather than one hand in the modified protocol in an attempt to increase task difficulty. The modified protocol therefore involved observing both hands (left and right) opening and closing (one at the time) in sequences of 6, 7 or 8 movements (as determined by the pilot study on page 113), and subsequently imitating the sequence as accurately as possible. It is assumed that μ-suppression during action observation

reflects activity in hMNS core areas (IFG and IPL) due to cortico-cortical connections (see page 22). In recent years, the primary motor cortex (M1) has also been implicated in the hMNS as several studies have reported mirror-like activity in both monkeys (Tkach et al., 2007; Dushanova et al., 2010) and humans (Montagna et al., 2005; Press et al., 2011; Szameitat et al., 2012). Therefore, stimulating core areas of the hMNS - and presumably M1 - should modulate μ-reactivity to action observation, and consequently affect the ability to imitate. This chapter will present three experiments that used the same method, but investigated the effect of stimulating one core area of hMNS at the time. The experiments are presented in the following order: (4) IPL, (5) IFG, and (6) M1. Performance on the imitation task was investigated in relation to recorded μ-reactivity.

Experiment 4: Stimulating the Parietal Node (IPL)

Introduction

The inferior parietal lobe (IPL) and the inferior frontal gyrus (IFG) are both considered as core areas of the hMNS, both of which are presumed to be involved in the process of matching observed actions with execution of the same action (see page 14). The IFG and IPL have also been proposed to be responsible for different actions in the process of matching observed actions with existing motor representations. This issue will be addressed in more detail in this chapter. Note that this subdivision of the chapter will focus on the IPL while the next subdivision will focus on the IFG.

Following the proposed trajectory of the hMNS, the STS provides a visual description of the observed action to the IPL where somatosensory information and kinematics of the observed action are added. This information is received by the IFG where it is matched with existing motor representations consequently retrieving the goal of the action (Iacoboni & Wilson, 2001; Rizzolatti & Craighero, 2004). This model is not strictly linear, but rather, it has reciprocal connections enabling forward and backward communication between nodes (Kilner, Friston, & Frith, 2007). The forward and backward communication property is evident in the empirical literature suggesting that the IPL codes the goal of observed actions rather than the movement per se; this will be discussed next.

The IPL has been associated with motor sequence learning (Berns et al., 1997), and its cortical activity has been demonstrated to correspond to the goal of the action rather than specific movements (Grafton et al., 1998). It is also known that damage to

parietal regions (including the IPL) is associated with impaired ability to interpret actions (Rothi et al., 1985), suggesting that the IPL is selective for processing goals and meanings of actions observed. In support of this notion, virtual lesion by TMS of the anterior intraparietal cortex (AIC), which is located within the IPL, leads to goal-dependent impaired ability to adapt kinematics required for reach-to-grasp-object (Tunik, Frey, & Grafton, 2005). These deficits are goal-dependent such that aperture-related deficits were produced if adjustment of the grip was the goal, and forearm related deficits were produced if adjustment of the forearm orientation was the goal. These results were interpreted as evidence that the AIC coded the goal of the action observed, and not the motor movement used. Several studies have corroborated this finding. For example, Hamilton and Grafton (2006) who used a neuronal habituation paradigm (see page 16 for an explanation of this approach) to investigate goal-representation in the AIC. Participants observed video clips of a hand reaching and grasping one of two objects during fMRI. The results indicated that repeated observation of an action directed towards the same goal, results in systematic reduction of activation in the AIC, but not in other hMNS-related areas. These findings support the notion that the IPL is responsible for coding goals of observed actions (and not the IFG) rather than the motor movement itself, and are consistent with mirror neurons found in monkeys' parietal cortex, where single cells were found to respond selectively to both the performance and observation of an action within a sequence leading to a specific goal, and not to the same action when it was part of a sequence achieving a different goal (see page 5; Fogassi et al., 2005).

Besides from coding goals of observed actions, the IPL is notably involved in imitation as demonstrated by fMRI (see meta-analysis by Caspers et al., 2010). In a study by Frey and Gerry (2006), participants observed video clips of two hands

assembling an object (always the same) but using different assembly sequences. After presentation of assembling the object, participants were instructed to assemble the object either using the same sequence as observed, or no mention was made of using any particular procedure. The results demonstrated increased cortical activity (using fMRI) in both IFG and IPL during action observation, and that the intention to imitate increased cortical activation further. Note that it is possible that part of this cortical increase is due to increased cognitive demand as increased cognitive demand is known to increase cortical activity (Klimesch, Schimke, & Pfurtscheller, 1993; Klimesch, 1998). Only activation in the AIC predicted accuracy on a sequential object assembly (imitation) task. In another study, Decety and colleagues (2002) investigated cortical activation (using PET) during imitation and being imitated. Results demonstrated that both being imitated and imitating activates the IPL, however, the left IPL responded selectively to producing imitation whereas the right IPL responded selectively to being imitated. This suggests that the IPL may also be involved in self-other agency differentiation. Self-other agency was also briefly discussed on page 111. While lesions to the left IPL has been associated with impaired imitation (Goldenberg, 1995; Goldenberg & Karnath, 2006), hyperactivity in the right IPL has been reported in individuals with schizophrenia who suffer the passivity phenomenon (the belief that one's thoughts or actions are being influenced or replaced by those of an external agent) during performance of freely selected joystick movements (Spence et al., 1997). The authors argued that such abnormal response might prompt the misattribution of internally generated acts to external agents. These studies suggest that the IPL specifically is involved in the ability to imitate under conditions involving motor sequence learning and self-other agency.

Moving on to the contended relationship between hMNS (in this instance IPL) and μ-

rhythms, an effect of stimulating the IPL on μ-rhythms has been documented using rTMS (Puzzo et al., 2013). In this study, participants attended two sessions: in one session the IPL was stimulated in IAF[14] + 1Hz[15], and in the other, sham stimulation was administered. The stimulation was applied directly preceding observation of a simple hand movement (opening and closing) and during observation of a static hand. The results revealed that during sham rTMS, μ-suppression was significantly greater during observation of a moving hand compared to a static hand. However, during active rTMS, this pattern was abolished (i.e. μ-suppression magnitude did not differ between the moving hand and the static hand). This result suggests that μ-reactivity was affected by stimulation over the IPL, which can be interpreted as evidence supporting the notion that changes in mu reflects activity in hMNS (in this instance IPL). The effect of the stimulation however did not affect the observation of the moving hand, but it enhanced μ-reactivity during observation of the static hand. This finding suggests that μ-reactivity during observation of a moving hand cannot be further enhanced by stimulation over the IPL, but it may be enhanced during observation of a still hand. Puzzo and colleagues (2013) interpreted this finding to indicate that mirror mechanisms are already at work during hand movement observation, and that activation in the sensorimotor cortex has reached its potential. As observation of a static hand does not tend to trigger sensorimotor simulation, the effect of the stimulation activated the sensorimotor cortex when this was under-activated.

[14] IAF refers to Individual Alpha frequency, and is the frequency peak in individual alpha power (Klimesch, 1998).
[15] rTMS in IAF + 1Hz has been shown to have excitatory effects on neuronal networks (e.g. Klimesch et al., 2003).

Summary and Predictions

The current experiment was designed to investigate the contended relationship between μ-rhythms and hMNS-related activity (in this instance IPL) (e.g. Muthukumaraswamy & Johnson, 2004; Pineda, 2005). For this purpose, the IPL was stimulated using tACS, and the effect of the stimulation was measured by changes in μ-reactivity to observation of moving hands, and on individual ability to imitate hands movements. Imitation was a natural choice in this context as it is one of the functions frequently associated with the hMNS (e.g. Buccino et al., 2004; Iacoboni et al., 1999), and has been correlated to μ-suppression during action observation (Bernier et al., 2007; 2013). The first prediction was that μ-reactivity to observation of hands movements would be modulated by tACS applied at IAF to the IPL. This prediction is based on the observation that α-power demonstrates large inter-individual differences relating to age and memory performance (Klimesch, 1998), thus IAF provides a tailored and more effective parameter of stimulation. Additionally, IAF stimulation to the occipital region leads to increased power in IAF (Neuling, Rach, & Herrmann, 2013). While it is known that task related changes in power depends on power in the reference period (Klimesch, Sauseng, & Gerloff, 2003), it is not known which direction to expect in the current experiment. According to relevant tACS literature, the effect of the stimulation is likely to be enhancing power (e.g. Moliadze et al., 2012; Vossen et al., 2015; Zaehle et al., 2010), which is likely to lead to greater suppression according to work by Klimesch and colleagues (2003). However, rTMS to the IPL has been shown to decrease suppression (Puzzo et al., 2013). Therefore, the current experiment tested whether tACS to the IPL would lead to increased or decreased suppression. Given the relationship between hMNS and imitation (e.g. Buccino et al., 2004; Iacoboni et al., 1999), and studies reporting a relationship between increased suppression during the active period with improved

performance on corresponding behaviour (e.g. Klimesch, Sauseng, & Gerloff, 2003; Kirov et al., 2009), the next prediction was that tACS would modulate performance on the imitation task. However, the direction of change in performance will depend on the effect of the stimulation. That is, if the effect of the stimulation is increased suppression during observation of the moving hands, then performance subsequent to tACS is expected to be improved. A decrease in performance is expected if the effect of the stimulation is decreased suppression. Based on Bernier and colleague's (2007; 2013) work suggesting that suppression in μ correlates positively with imitation performance, it was predicted that μ-suppression would correlate positively with performance on the imitation task also in the current experiment. Lastly, suppression in sensorimotor frequencies are related to task demand and cognitive performance (Klimesch, Schimke, & Pfurtscheller, 1993; Klimesch, 1998), and therefore, it was predicted that μ-reactivity would be dependent on sequence length.

General Method

Participant selection

Participants were recruited via an online system for managing research participation (SONA-system), and the psychology department's email list for research participation. Eighty-four individuals were screened in relation to their suitability for tACS using the TMS safety screen (TASS: see Appendix 5) questionnaire (Keel et al., 2001). In total, 60 (28 males) participants completed the study, age ranging from 18 – 38 (mean age = 24.15, SD = 4.38). Participants were randomly allocated to the following conditions: sham stimulation, or active tACS to one of the following: IPL, IFG, or M1. Fifteen participants were included in each stimulation condition. All

participants were right handed, reported no neurological or psychological disorder, signed informed consent, and were paid GB £10 for their time. The local ethical committee (Department of Psychology, University of Essex) granted ethical approval.

Stimuli

Participants observed video presentations of a female actor opening and closing her left or right hand (one at the time) at a rate of 1 Hz. These videos were based on videos used in Experiment 1. The hands were Caucasian skin coloured, and shown from an egocentric viewpoint. An egocentric perspective was chosen given the literature suggesting that movements observed from a self-related perspective induces larger neurophysiological responses than movements observed from the perspective of another person (e.g. Jackson, Meltzoff, & Decety, 2006; Maeda, Kleiner-Fisman, & Pascual-Leone, 2002). These were presented against a black background. Hand movement sequences were constructed using Motion 5 (Apple Inc. version 5.1.2) video editing program. Videos were edited to include 6, 7, and 8 movements in a single sequences, each lasted 1 second per number of movements (i.e. 6 sequences lasting 6 seconds). The sequence of right versus left movement was computer randomised, but participants observed the same sequences. Twenty of each sequence length was created for the pre-stimulation period, and 20 of each sequence length was created for the post stimulation period. All of these sequences can be viewed in appendix 3. A schematic example of a movement sequence is presented in Figure 15 (page 112).

Procedure

Participants completed an informed consent form and were fitted with electrodes to record eye movements and reference signal. Skin surface underlying electrodes for

recording eye movements and reference signal were lightly abraded to reduce impedance of electrode-to-skin contact. Next, a Quick-cap (Compumedics, Neuroscan) was fitted for the EEG. Resting EEG was recorded for two minutes with eyes-open, before completing Croft & Barry (2000)'s eye-movement calibration protocol. Subsequently, Individual Alpha frequency (IAF) was defined based on individual peaks in alpha. In order to establish IAF, the resting period was epoched to 1024 data points and subsequently the time domain data were transferred into power values in the frequency domain using fast Fourier transformation (FFT). IAF was calculated with the participant in the lab. The calculation was conducted using Neuroscan Edit 4.4 (Compumedics, Melbourne, Australia), and lasted roughly 3 minutes. The calculation was based on individuals' most commonly occurring peak frequency between 8 and 12Hz over parietal and occipital electrodes (P3, P1, Pz, P2, P4, O1, Oz, O2). The occipital and parietal sites were chosen based on the rationale that alpha oscillations are strongest over these areas, and due to numerous previous studies also using these electrodes to define IAF (e.g. Klimesch, 1999; Puzzo et al., 2013; Grandy et al., 2013; Gutman et al., 2015; Haegens et al., 2014). Some studies define IAF based on resting period gathered with eyes-closed (e.g. Puzzo et al., 2013; Klimesch, 1999) but the current study recorded resting state with eyes-open because endogenous alpha power is known to peak whilst eyes are closed (Nunez et al., 2001) and as a consequence, power may not be further enhanced in that bandwidth (Neuling, Rach, & Herrmann, 2013).

Participants attended 60 hands movement videos (20 x 6-movements, 20 x 7-movements, and 20 x 8-movements) before, and 60 different hands movement videos (20 x 6-movements, 20 x 7-movements, and 20 x 8-movements) after stimulation. Trials were presented in a computerized random order. Each experimental trial started

with 1000ms fixation cross, followed by a video clip lasting between 6000ms – 8000ms depending on number of movements in the sequence (i.e. 6000ms for 6 movements, 7000ms for 7 movements, or 8000ms for 8 movements). Immediately after presentation, participants were instructed to re-produce the hands movement sequence observed. Lastly, each trial ended with a message on the screen "wait for next" lasting 2000ms. See Figure 16 for a graphical representation of procedure.

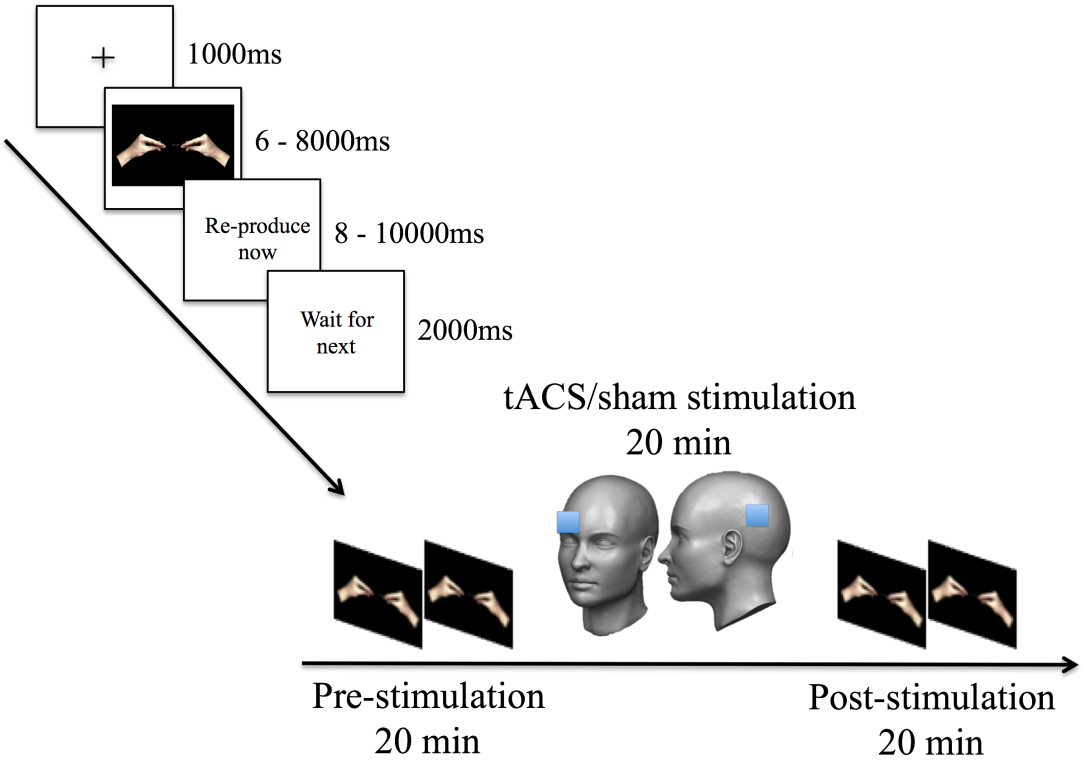

Figure 16: Graphical representation of procedure. Note that the behavioural paradigm was performed in the pre-stimulation period and in the post-stimulation period only.

Allowed response time was 2000ms in addition to the stimulus presentation time. This time was chosen based on a pilot trial of the experiment that suggested that the observation time alone was too short for re-producing the entire sequence. Hand movement responses were recorded using a Panasonic HDC-SD5 camcorder (1920 x 1080 Pixels) placed on a tripod that was adjusted to each participant in order to cover individuals' hands only.

tACS procedure

Fifteen participants received received sham stimulation while 45 received active tACS. Participants were randomly allocated to conditions. Active tACS was delivered via two surface conductive-rubber electrodes (3 x 3 cm) enclosed in saline-soaked sponges sown to the inside of the EEG cap. For the following experiment (4), one stimulation electrode was positioned over the IPL (P3 on the 10/20 system), while the other was always positioned over the contralateral frontal polar (FP2 on the 10/20 system) in line with several previous studies targeting this area (e.g. Wach et al., 2013; Moliadze, Antal, & Paulus, 2010; Nitsche & Paulus, 2000; Moliadze et al., 2012). A graphical representation of the electrode montage is presented in Figure 17 below. An alternating sinusoidal current individually adjusted (IAF) was delivered by a battery-operated stimulator system (DC-Stimulator Plus, NeuroConn GmbH, Ilmenau, Germany). Current intensity was set to 1mA (peak-to-peak) in accordance with numerous previous studies (e.g. Wach et al., 2013; Moliadze et al., 2012) and safety protocols regarding DC and AC stimulation (Iyer et al., 2005; Nitsche et al., 2003). Impedance was kept below 10 kΩ. Active tACS was applied for 20 minutes based on work by Neuling, Rach, and Hermann (2013) indicating sustained after-effects lasting at least 30 minutes when applying tACS for 20 minutes at IAF in 1mA. The current intensity was faded-in and faded-out for 10 seconds to avoid retinal phosphenes. The sham group received active stimulation for the first and last 10 seconds in order to elicit the typical tingling sensation under the electrode at the beginning of stimulation. The sham stimulation was delivered under the same parameters as the tACS group. This approach to deliver sham stimulation has been used by several others (e.g. Wach et al., 2013; Polania et al., 2012).

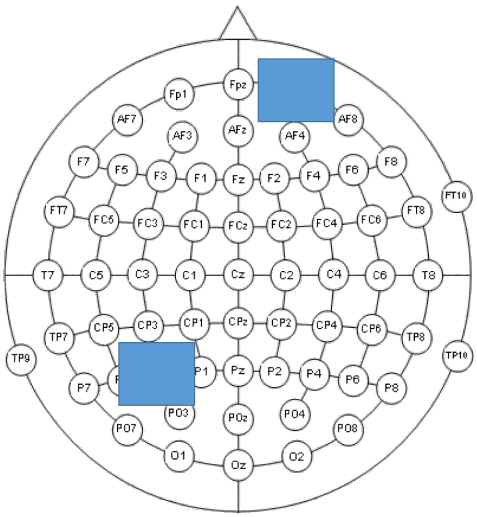

Figure 17: Graphical representation of tACS electrode montage targeting the IPL.

EEG data acquisition

EEG data were recorded using Synamps II amplifiers and SCAN 4.5 acquisition software (Compumedics, Melbourne, Australia) using 64 electrodes mounted on a Quick-Cap with electrodes arranged according to the extended 10-20 system. Electrodes were referenced online to an electrode on the left mastoid and grounded midway between Fz and FPz. Eye movements were recorded using four electrodes, above and below the left eye and on the outer canthi of each eye. Impedances for all of the electrodes were lowered to at least 10 kΩ in all electrodes before data acquisition. EEG data were sampled continuously at 1000Hz with a band-pass filter of .05 - 200Hz and a 50Hz notch filter.

EEG data preparation

The data were prepared as in Experiment 1 (see page 43), except that data were: i) epoched from -3000 to 9000 and trimmed 1000ms from each end to remove filter warm-up artefacts, and ii) three active periods were included; six (5000 to 6000ms),

seven (6000 to 7000ms) and eight (7000 to 8000ms). The active periods were selected to investigate differences in μ-reactivity in relation to the number of movements presented (6, 7 and 8).

Data Analysis

All data were included for analysis. Performance on the imitation task was established by computing percentage correct hands movements (all movements in the sequence reproduced correctly) for each trial in each sequence length, before and after the stimulation period. The data were then examined for heterogeneity using Kolmogorov-Smirnov statistics. The test confirmed that the behavioural data were normally distributed ($p > .05$). A repeated measures ANOVA was conducted in order to investigate the effect of tACS to the IPL on imitation performance. For this analysis the following factors were included: "time" with two levels (pre-stimulation period, post-stimulation period), "sequence length" with three levels (6, 7, 8), and one between-subjects factor "stimulation condition" with two levels (sham and active IPL-tACS). It was expected to find significant main effects for factors time and sequence length. In the event of the former, it was planned to compare performance pre vs. post, and in the case of the latter the following pairs were compared: (a) 6 vs. 7; (b) 6 vs. 8; (c) 7 vs. 8. These comparisons were Bonferroni corrected. Furthermore, the main predicted outcome of the experiment was that the factors time and stimulation condition would interact such that imitation performance changes with time depending on stimulation condition. In the case of such interaction, it was planned to compare performance pre to post for each group and then compare between-group differences for pre and post stimulation values.

For the EEG data, all data were included for analysis and were tested using

Kolmogorov-Smirnov test of normality. The result of this test indicated that the assumption of normality was violated for the majority of variables ($p < .05$). To correct this issue, all EEG data were log transformed and as such, re-expressed on a more normally distributed scale (note that for clarity, graphical representations of the data are not log transformed). The same step as in Experiments 1-3 were carried out, in which a repeated measures ANOVA was conducted to differentiate the signal from sensorimotor areas and occipital regions (see page 21). This differentiation is important in order to reinforce that the activity observed relates to motor activity and not activity that is unrelated to the matter under consideration for this thesis. For this analysis, signal from sensorimotor areas were recorded from central electrodes (C4, C2, Cz, C1, C3), fronto-central electrodes (FC4, FC2, FCz, FC1, FC3), and signal from occipital area from occipital electrodes (O2, Oz, O1). Electrodes were collapsed in order to keep number of comparisons to a minimum, and factors included: "channels" with three levels (C, FC, O), "sequence length" with three levels (6,7,8), and one between-subjects factor "stimulation condition" with two levels (sham, active tACS). It was expected to observe a significant main effect of the factor channels or interaction between the factors channels and sequence length given that signal recorded from C and FC channels are functionally similar and are both considered as sensorimotor areas (e.g. Szurhaj et al., 2003), and that signal from the occipital region is functionally different both to C and FC. Consequently, a main effect of the factor channels was expected. In the event of such main effect, investigations of μ-reactivity during observation of hands movements were conducted separately for channels FC, C, and O.

In order to investigate the effect of tACS on μ-reactivity during observation of hands

movements, three ANOVAs were conducted with the following factors: “time” with two levels (pre-stimulation period, post-stimulation period), “sequence length” with three levels (6, 7, 8), “hemisphere” (left [C3, C1], right [C4, C2]), and one between-subjects factor “stimulation condition” with two levels (sham, active IPL-tACS). For the FC-channels, the factor hemisphere included: left (FC3, FC1) and right (FC4, FC2), and for the O-channels the factor hemisphere was replaced with “electrodes” including levels: O1, Oz, and O2. The planned comparisons conducted here were the same as for the behavioural data.

Consequently, a one-way analysis of covariance (ANCOVA) was conducted to control for the possible confounding influence of pre-stimulation ERD values on post-stimulation ERD values. This analysis was conducted with post-stimulation μ-ERD as the dependent variable, stimulation condition as the fixed factor, and ERD values in the pre-stimulation as the covariate. Finally, all effects were compared against zero (indicating no change) in one-samples t-tests. Degrees of freedom were corrected using the Greenhouse-Geisser epsilon values (G – GE) when violation of sphericity was indicated.

Lastly, Pearson correlations were conducted in order to investigate the relationship between μ-ERD during observation of hands movements with imitation of the hands movements. Pre and post stimulation for each stimulation group (sham, tACS) values were correlated with left and right hemisphere signal in the bandwidth- and in the brain region (C, FC) which demonstrated a significant interaction between factors time and stimulation condition. Four correlations were made, note that these were not Bonferroni corrected.

Results

Imitation performance

Results of the repeated measures ANOVA indicated a significant main effect for the factor sequence length: $F(2, 56) = 37.67$, $p < .001$, $\eta_p^2 = .574$. Planned comparisons indicated that participants performed significantly better on the short length trials compared to both medium and long length trials (ps < .001). Additionally, the medium length trials elicited significantly more correct responses compared to the long length trials (ps < .001). These findings suggest that performance declined progressively with more movements as predicted. A significant interaction was also found between factors time and sequence length: $F(2, 56) = 32.63$, $p < .001$, $\eta_p^2 = .538$. Bonferroni corrected post hoc comparisons were conducted on the following pairs to investigate this interaction further: (a) pre vs. post on short length trials; (b) pre vs. post on medium length trials; (c) pre vs. post on long length trials. The result of these comparisons indicated that participants' performance was not affected by greater exposure or learning on the short length trials post-stimulation ($p = .359$), however, performance improved post-stimulation for the medium length trials ($p < .001$), and declined post-stimulation for the long trials ($p < .001$). The result of these comparisons are presented in Table 5 and Figure 18, and suggest that learning-related changes in performance is dependent on number of movements in a sequence. No main effect or interaction was found for factor stimulation condition (ps > .581) suggesting that performance on the imitation task was not modulated by tACS.

Table 5: t (and p-values) for planned comparisons for: (a) main effect for the factor sequence length, and (b) interaction between factors time and sequence length

(a)	6 vs. 7	7 vs. 8	8 vs. 6
	7.2 (.006)	9.13 (< .001)	16.33 (< .001)

(b)	6	7	8
Pre vs. Post	2.7 (.359)	15.23 (< .001)	19.9 (< .001)

Note. Results in bold reached significance at .05 level or lower.

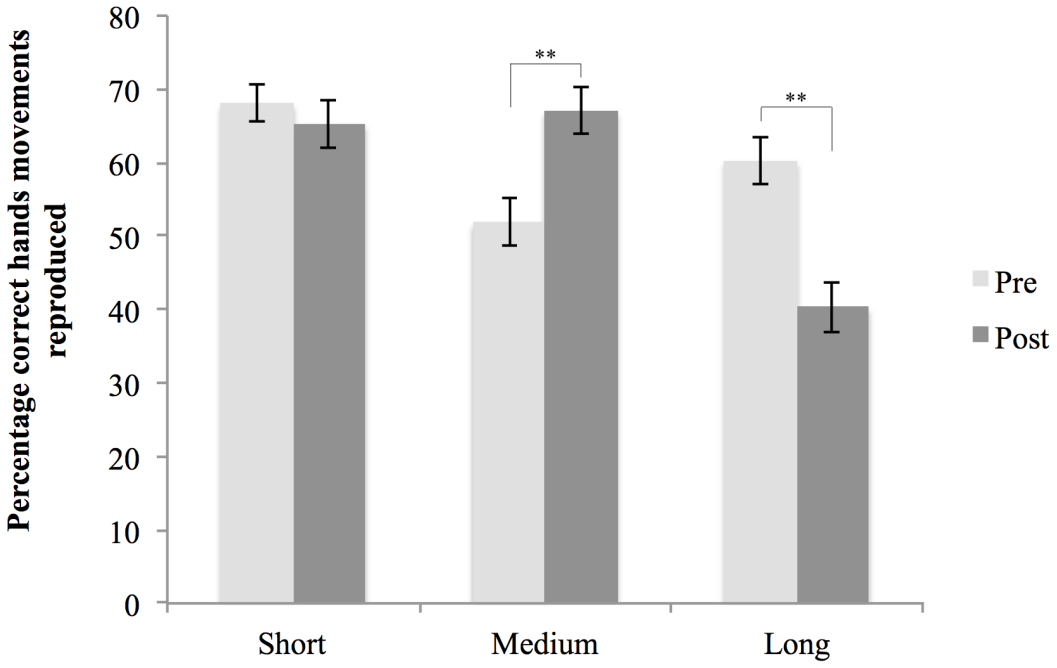

Figure 18: Results of the planned comparisons. Bars represent percentage change correct movements reproduced for short (6), medium (7), and long (8) sequences by time. Error bars indicate standard error. Note: ** indicate significance level < .001.

Electrophysiological reactivity

Sensorimotor vs. occipital channels

Results of the repeated measures ANOVA indicated no significant interaction between factors channels and length (*p*s > .531), however, a significant main effect for the factor channels was observed in all bandwidths: *F*s (2, 56) > 5.49, *p*s < .001,

η_p^2s > 0.164, indicating that ERD differed between regions. Therefore, investigations of μ-reactivity were investigated separately in FC, C, and O-channels.

Central channels

Results of the repeated measures ANOVA indicated a significant main effect for the factor sequence length in α2: $F(2, 56) = 3.21$, $p = .048$, $\eta_p^2 = 0.103$, and β1: $F(2, 56) = 3.65$, $p = .032$, $\eta_p^2 = 0.115$, but not in α1 (*p*s > .098). Planned comparisons indicated that ERD was significantly larger during the long length trials compared to medium length (*p*s < .045), but no difference was detected between the short length trials and the medium length trials (*p*s > .367), or between the short length trials and the long length trials (*p*s > .082), suggesting that ERD was affected by the length of the sequence. Furthermore, a significant interaction was indicated between factors time and stimulation condition in β1: $F(1, 28) = 8.18$, $p = .008$, $\eta_p^2 = 0.226$. Planned comparisons indicated that ERD differed significantly pre- to post-stimulation for the sham condition ($p = .021$) but not for the active tACS condition ($p = .122$), suggesting that tACS moderated μ-reactivity during observation of the moving hands. This result is presented in Figure 19 below.

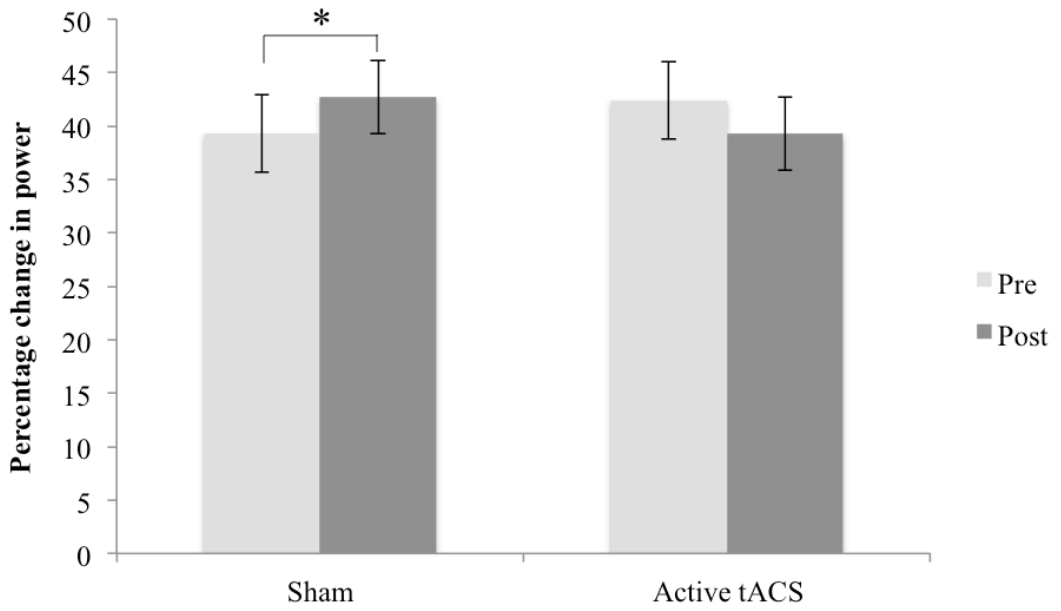

Figure 19: Results of planned comparisons. Bars represent percentage change in β1 in C-channels during observation of hands movements, pre and post stimulation for sham and active tACS. Error bars indicate standard error. Note: positive values represent ERD.

The result of the ANCOVA yielded a significant effect for both the covariate (pre-stimulation ERD values): $F(1, 29) = 30.41$, $p < .001$, $\eta_p^2 = 0.530$, and the stimulation condition: $F(1, 29) = 4.9$, $p = .036$, $\eta_p^2 = 0.154$, suggesting that there was a significant effect of stimulation condition on post-stimulation ERD after controlling for pre-stimulation ERD. Bonferroni corrected pairwise comparisons confirmed that significantly less ERD was observed during observation of moving hands subsequent to active tACS compared to sham ($p = .036$). This result is presented in Figure 20 below.

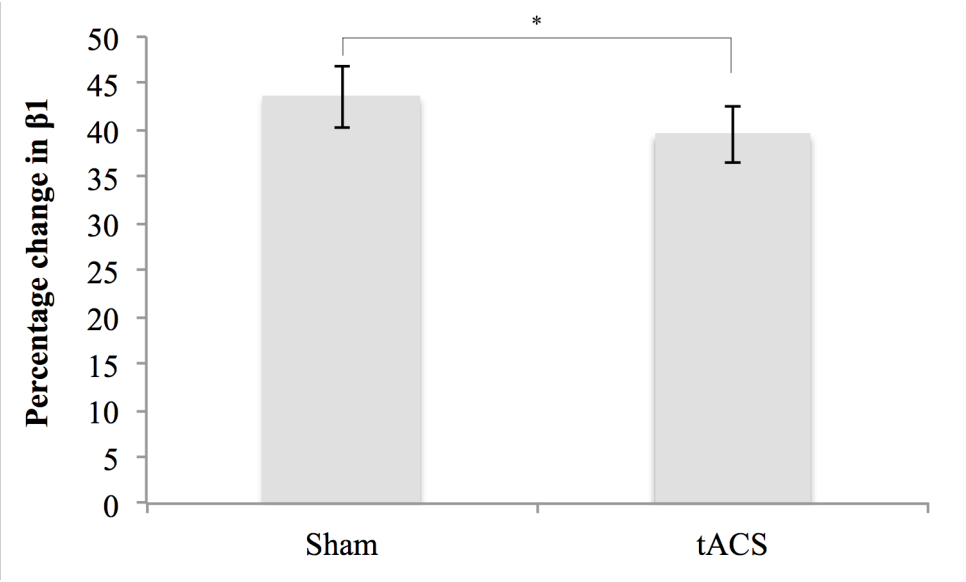

Figure 20: Results of ANCOVA. Bars represent percentage change in β1 in C-channels during observation of hands movements, post stimulation controlling for pre-stimulation values for sham and active tACS. Error bars indicate standard error. Note: positive values represent ERD.

The result of the one-samples t-tests indicated that the ERD observed in both pre and post stimulation differed significantly from zero for both the sham and the active tACS: *t*s(14) > 11.80, *p*s < .001.

Fronto-central channels

Results of the repeated measures ANOVA indicated a significant main effect for the factor stimulation condition in α2: $F(1, 27) = 4.52$, $p = .043$, $\eta_p^2 = 0.143$, but not in α1 or β1 (*p*s > .128). Bonferroni corrected pairwise comparisons suggested that participants in the active tACS condition elicited significantly less ERD during observation of moving hands than participants in the sham condition ($p = .043$). No other main effects or interactions were detected in FC-channels (*p*s > .077).

Occipital channels

Results of the repeated measures ANOVA indicated no main effects (*p*s > .784), and

no interactions were found (*p*s > .295) in any bandwidth, suggesting that signal from the occipital region did not differentiate hand sequences and was not affected by tACS to the IPL. It is therefore more certain that the suppression pattern observed in sensorimotor α reflected recruitment of motor systems rather than visual reactivity.

μ-reactivity and imitation performance

Pearson's correlation was used to assess whether performance on the imitation task was related to μ-ERD during observation of hands movements. Because the effect of time by stimulation condition was found in the β1 only, only β1 was investigated here. The results are presented in Table 6 below and suggest that subsequent to tACS, performance positively correlated with μ-ERD.

Table 6: Pearson's *r* (and *p*-values) for μ-ERD correlated with the percentage correct hands movements reproduced.

	Sham		Active tACS	
	Left	Right	Left	Right
Pre	-.174, .534	.188, 503	.100, .723	-.020, .942
Post	.273, .325	.015, .958	**.553, .032**	.420, .119

Note: ERD was correlated pre-ERD with pre-behavioural performance, and post-ERD with post-behavioural performance. Results in bold reached significance at .05 level. Note that multiple comparisons were not controlled for.

Interim Discussion

The current study investigated the contended relationship between μ-ERD and hMNS-related activity (e.g. Muthukumaraswamy & Johnson, 2004; Pineda, 2005), in this instance the IPL. In order to test this relationship, the IPL was stimulated using tACS, and consequential changes in μ-reactivity and performance on an imitation task were assessed. It was assumed that tACS would modulate μ-reactivity during

observation of hands movements and the ability to imitate them. The results indicated increased β1-ERD subsequent to sham but not to tACS. Additionally, significantly less ERD was observed post-stimulation in the tACS group compared to the sham group, suggesting that tACS lead to a decrease in β1-ERD. Furthermore, performance on the imitation task did not change significantly for either sham or tACS, despite this, a significant and positive correlation between β1-ERD and imitation performance was observed post-tACS suggesting that performance was modulated by stimulation indirectly. The implications for these findings will be discussed next.

The finding that the sham group elicited a significant increase in β1-ERD but not the tACS group, supports Puzzo and colleagues' (2013) finding that rTMS to the IPL leads to a decrease in β1-ERD. This finding appears to be reasonably robust given that the current experiment used a different method and neuromodulation approach: Puzzo and colleagues used rTMS directly preceding observation while the current experiment presented hands movements off-line, before and after a stimulation period. The current results then extend Puzzo and colleagues' finding, and together these results suggest that activity in the IPL is directly involved in observation of hands movements. In itself, mu-suppression during action observation is considered an indication of hMNS activity (e.g. Muthukumaraswamy and Johnson, 2004; Pineda, 2005), and therefore, current results can be interpreted as evidence for hMNS-related activity in the IPL. This interpretation is consistent with several previous studies indicating that the IPL is specifically involved in hMNS-related activity (e.g. Grafton et al., 1998; Tunik, Frey, & Grafton, 2005; Hamilton & Grafton, 2006). Furthermore, some of the studies relating activity in the IPL to the hMNS involved goal-directed movement. Because the movement observed in the current experiment was intransitive, the results of the current experiment extend the current knowledge of IPL

activity to include intransitive movements.

Although it has been demonstrated that stimulation induced changes in the reference power relates to the magnitude of suppression observed in the active period, such that increased reference power leads to increased suppression in the active period (Klimesch, Sauseng, & Gerloff, 2003); the effect of the stimulation was not predicted prior to the current experiment given the discrepancy between comparative tACS literature demonstrating enhanced power in α-power (e.g. Zaehle et al., 2010; Vossen et al., 2015; Neuling, Rach, & Herrmann, 2013), and Puzzo and colleagues' (2013) finding that rTMS leads to decreased β1-ERD. Puzzo and colleagues applied rTMS in IAF + 1Hz in order to increase power, however the results did not reveal increased β1-ERD. Instead, selective β1-ERD was recorded during observation of a moving hand compared to a static hand (as is commonly found see page 38; e.g. Puzzo et al., 2011) subsequent to sham stimulation, but this pattern was abolished after rTMS stimulation. This finding suggests that rTMS disrupted neuronal processes involved in generating β1 rather than increase them. The effect of tACS in the current experiment was similar to Puzzo and colleagues' finding in that the sham group elicited significantly greater β1-ERD pre to post stimulation but not the tACS group. Additionally, when controlling for pre-stimulation differences in β1-ERD, post-stimulation ERD values indicated significantly less β1-ERD for the tACS group compared to the sham group. These results suggest that tACS may have interrupted processes involved in facilitating β1-ERD.

The question is, what does the increase in ERD observed for the sham condition reflect, and therefore what did tACS disrupted? It is likely that the ERD in β1 reflect learning processes, expertise, or increasing familiarity with the moving hands, as

these are known factors associated with increased hMNS-related activity (e.g. Bangert & Altenmüller, 2003; Haslinger et al., 2005; Margulis et al., 2009). These studies typically demonstrate a significant increase in cortical activity subsequent to learning or increased practice or exposure (e.g. Calvo-Merino et al., 2005; 2006). The observation that the tACS group elicited no significant change in power pre to post stimulation may then reflect disruption to hMNS-related activity. However, note that the opposite tendency (i.e. that greater exposure leads to decreased hMNS-related activity) has also been reported (e.g. Babiloni et al., 2010) and therefore it is conceivable that the increase in β1-ERD for the sham group reflects activation of systems other than hMNS. Activity in the IPL has been related to maintaining self-other differentiation (e.g. Brass et al., 2009; Spengler et al., 2009; Wang et al., 2011), and given that the hands movements observed belonged to someone else – it is possible that the ERD observed relates partly to such processes. If however this were the case, one would expect that disruption to the IPL would interfere with the tACS group's perception of the ownership of the hands observed. No participants in any of the groups reported any misperception of this sort.

Given that the IPL has been specifically related to imitation performance (Decety et al., 2002; Frey & Gerry, 2006), particularly imitation of motor sequences (Tunik, Frey, & Grafton, 2005; Hamilton & Grafton, 2006), tACS to the IPL was expected to modulate imitation performance. However, no effect of tACS on imitation performance was observed. The notion that hMNS-related activity facilitates imitation (e.g. Buccino et al., 2004; Iacoboni et al., 1999) was therefore not supported by the current results. Despite this, a significant and positive correlation between β1-ERD and imitation performance was indicated post-tACS in the left hemisphere. This finding suggests activity in the IPL is at least indirectly involved in imitative

processes. This correlation supports the notion that β1-ERD observed in the current experiment reflects hMNS-related activity, because there is some evidence that observation relates to imitation. Furthermore, this correlation supports Bernier and colleagues (2007; 2013) finding that imitation performance correlates positively with μ-suppression during observation of hands movements, but also extends their findings to highlight the role that the IPL specifically might play in this interaction; that observation and imitation are governed by different but interacting neuronal systems, as the relationship between imitation and observation was indicated only after the IPL was disrupted. Given that μ-suppression is thought to reflect cortical activity in both the IPL and IFG (Muthukumaraswamy & Johnson, 2004; Pineda, 2005) and that the effect of tACS to the IPL was decreased μ-ERD, it can be inferred that when the IPL is disrupted, it is activity of the IFG that is reflected in the relationship with imitation performance. This interpretation is consistent with the notion that nodes in the hMNS circuit are not strictly linear, but have reciprocal connections enabling forward and backward communication (Kilner, Friston, & Frith, 2007). This possibility remains a speculation here, but may be enlightened by the results of the next sub-division of the chapter, which investigates the relationship between μ-reactivity and the IFG.

The finding that the effect of tACS applied to the IPL was decreased β1-ERD - suggesting a reduction in power rather than enhancement – is consistent with Puzzo and colleagues' finding but inconsistent with comparative tACS literature demonstrating enhanced power subsequent to tACS (e.g. Zaehle et al., 2010; Vossen et al., 2015; Neuling, Rach, & Herrmann, 2013). However, several issues need to be addressed here. The literature investigating tACS after-effects has revealed inconsistent results in terms of direction of the modulation, and the use of a variety of stimulation parameters such as: electrode montage, stimulation length, and frequency.

The studies in which the current tACS protocol was based on (e.g. Zeahle et al., 2010; Vossen et al., 2015; Neuling, Rach, & Herrmann, 2013) were similar in terms of stimulation frequency (i.e. 10Hz or IAF) used, but differed on other parameters such as stimulation time and electrode montage. It is known that these factors affect the modulation effect (e.g. Veniero et al., 2015), but differences in method make the results difficult to compare and the predictability of tACS suffers as a consequence. Despite the lack of predictability in relation to the direction of the modulation in the current study, Puzzo and colleagues (2013) demonstrated a very similar pattern using rTMS to the IPL. Therefore, the effect observed in the current experiment can be considered more robust. Furthermore, the current experiment involved stimulation during a period of rest rather than directly prior to the behavioural task. This set up involves after-effects rather than online effects. Unfortunately, current understanding of the specific mechanism involved in procuring after-effects remains inadequate in comparison to online effects (Zaehle et al., 2010; Vossen et al., 2015), and it could be that stimulating directly preceding the behavioural task would have lead to a more predictable outcome. There are currently two main theories used to explain tACS after-effects, including continuing entrainment effects (Helfrich et al., 2014) and forms of plasticity such as spike-timing dependent plasticity (STDP; Polania et al., 2012; Zaehle, Rach, & Herrmann, 2010; Vossen et al., 2015). These will be addressed next.

It has been proposed that after-effects are at least partly due to continuing of entrainment effects (Helfrich et al., 2014). However, in relation to the current results, this proposal is not supported, as the effect appears to be neuronal disruption. Neuronal disruption induced by tACS is hard to explain under the assumptions of entrainment in which endogenous rhythms phase-aligns with the stimulation

frequency (Frölich & McCormick, 2010; Ozen et al., 2010; Deans, Powell, & Jefferys, 2007; Reato et al., 2010). Presumably, neuronal disruption would not appear as rhythms phase-aligning to the stimulation frequency. There are however some reasons why entrainment effects were not indicated in the current study. Firstly, entrainment effects are predominantly associated with online tACS as the entrainment effect ceases after a few cycles consequent to termination of stimulation (Marshall et al., 2006; Reato et al., 2013). The current study however investigated offline effects up to 20 minutes after termination of the stimulation, and may therefore have been too long to involve entrainment. Secondly, it is known that entrainment effects are most effective when the stimulation frequency is the same as or close to the neuronal networks' preferred oscillation (Halbleib et al., 2012; Herrmann, 2001). It is possible that the dominant frequency in the IPL is not μ-oscillations in which is assumed to originate in sensorimotor areas (Salmelin et al., 1995; Hari, 2006; Avanzini et al., 2012). This explanation is however unlikely as it was demonstrated that tACS to the IPL modulated β1-ERD. Another explanation for the lack of entrainment is that after-effects are not related to entrainment, but rather forms of plasticity such as STDP (explained on page 105; Polania et al., 2012; Zaehle, Rach, & Herrmann, 2010; Vossen et al., 2015). Under the STDP framework, both enhancement and suppression can be explained by tACS induced periodic hyper- and depolarization of neuronal membranes, which leads to synaptic strengthening or weakening depending on the efficacy of its component synapses (Veniero et al., 2015). This framework is therefore a more likely candidate for the disruption effect observed in the current study.

In addition to the β1-ERD observed during observation of moving hands, the length of the sequences presented affected amount of ERD observed. The greatest ERD was

indicated for the longest sequence, which additionally induced significantly greater ERD compared to the medium length. However, no difference was detected between long and short length and no difference between the medium and short length. In addition to the finding that greatest ERD was observed during the longest sequence is the behavioural finding that the shortest sequences were the easiest to imitate (i.e. these elicited the greatest percentage correct responses). These findings are consistent with the literature indicating that sensorimotor suppression (Klimesch, Schimke, & Pfurtscheller, 1993; Klimesch, 1998; Brinkman et al., 2014; Pfurthscheller & Lopes da Silva, 1999) is enhanced by task demands and cognitive load.

In the current experiment, no effects were observed in α despite that tACS was applied in IAF. This finding is on first glance puzzling given that Experiment 1 used similar stimuli (intransitive moving hand) and demonstrated α2-ERD. The current study used two hands as opposed to one hand, and this may have affected α-reactivity. However, Puzzo and colleagues (2011; 2013) and Cooper and colleagues (2013) also demonstrated lack of α-ERD during observation of single hand movements. Therefore, the lack of α-ERD is not likely to reflect that the observation included two hands as opposed to one. Another consideration is that there is significant inter-individual variability in α frequencies (Klimesch, 1997) and therefore some individuals' α-power may have fallen outside the fixed frequency window (8 – 10 Hz and 10 – 12 Hz) used in the current study. This explanation is unlikely because the same frequency-widow was used in Experiment 1. Furthermore, it is noticeable that the effect of tACS applied in IAF lead to changes in β1-ERD in the current experiment, but also in Puzzo and colleagues' (2013). These findings suggests that α and β1 processes are interrelated as has been reported before (e.g. Carlqvist et al., 2005; de Lange et al., 2008) – but that they serve distinct functions. It

was discussed on page 50 that the behavioural pattern of α and β1 during action observation differs depending on the action content observed, such that intransitive hand actions trigger β1 more reliably than α (e.g. Puzzo et al., 2013), while transitive and goal-directed hand actions trigger α (e.g. Muthukumaraswamy and Johnson, 2004; Muthukumaraswamy, Johnson, & McNair, 2004). It may be that in the current study, no goal-directed movement, or object was included and therefore, α-processes were not required. However, during mental simulation of goal-directed actions, both α and β oscillations have been reported (Brinkman et al., 2014), but the authors proposed distinct functions for each: While α-oscillations mediate allocation of computational resources by disengaging task-irrelevant cortical regions, β oscillations are involved in the computations of movement parameters. The specific roles of α and β oscillations are therefore starting to be revealed.

In summary, the current study demonstrated β1-ERD during observation of moving hands. The sham group demonstrated a significant change in ERD pre to post stimulation in addition to significantly larger ERD post-stimulation compared to the tACS group, suggesting that tACS disrupted neuronal activity in the IPL. Although tACS did not modulate performance on the imitation task, a positive and significant correlation was indicated subsequent to tACS in the left hemisphere, suggesting that when IPL is disrupted, β1-ERD is positively related to imitative performance. This could potentially be facilitated by the IFG as both (IPL and IFG) are thought to modulate μ-reactivity. The results of this experiment can be interpreted as evidence that activity in the IPL modulates μ-reactivity, but that its activity is more strongly related to observation than to preparing to imitate.

Experiment 5: Stimulating the Frontal Node (IFG)

Introduction

The previous experiment confirmed that activity in the IPL modulates μ-reactivity, and suggested that IPL activity is partly related to imitative processes. This observation supports the notion that μ-suppression during action observation is an indication of hMNS-related activity in the IPL. However, it was suggested (see page 141) that activity in the IFG may be responsible for the relationship found between imitation performance and β1-ERD subsequent to tACS to the IPL. The rationale behind this proposal was that μ-rhythms indicate activity in both the IPL and the IFG, and because the correlation was only apparent after the IPL was interrupted with tACS, it is likely that μ indicated activity in the IFG more strongly than activity in the IPL. If this is true, then the IFG is likely to be more important for imitation-related processes than the IPL. This possibility has been suggested by several others (e.g. Iacoboni et al., 2005), and will tested in the current experiment, in which sought to investigate the relationship between μ-reactivity and activity in the IFG, and the effect of modulating this relationship on imitation performance.

The previous subdivision of the chapter, addressed the IPL and its proposed role in the hMNS circuit and imitation performance. The IPL was described as an area selectively associated with imitative abilities (e.g. Frey & Gerry, 2006) but also dedicated to goal interpretation in action observation rather than motor specification (page 118; e.g. Grafton et al., 1998). Several neuroimaging studies (e.g. Hamilton & Grafton, 2006) and brain stimulation studies (e.g. Tunik, Frey, & Grafton, 2005) supports this interpretation. Additionally, mirror neurons in monkeys' PF/PFG of the

IPL respond selectively to goals rather than motor movements (Fogassi et al., 2005). Similar arguments have been made for the IFG as will be discussed next.

In a study by Iacoboni and colleagues (2005), the IFG was shown to selectively code the intention of actions observed. In this study, participants observed three different types of videos (context, action, and intention) during fMRI. In the context condition, two different scenes were depicted: before tea (table and objects ready for tea) and after tea (table and objects after tea). In the action condition, two different hand actions were presented: precision grip and a whole handgrip. In the intention condition, grasping actions (precision or whole hand) were embedded in the before and after tea scenes which consequently can be inferred as two different intentions (drink or clean up). The intention to drink was depicted by a precision grip movement embedded in the before tea scene, while the intention to clean was depicted by a whole handgrip embedded in the after tea scene. The results demonstrated increased cortical activity in areas including the IFG for the action and intention conditions. Moreover, the intention condition yielded significantly greater cortical activation than the action condition, suggesting that intention was selectively processed. Additionally, the intention to drink elicited significantly greater cortical activation compared to the intention to clean. In another study (described on page 17; Fadiga et al., 2006), it was demonstrated that observation of meaningful hand shadows resembling animals increases cortical activity in the IFG. These studies suggest that the IFG code the meaning behind observed motor actions, and not the motor action itself.

The involvement of activity in the IFG in relation to action understanding has also been suggested in several rTMS studies (e.g. Urgesi et al., 2007; Avenanti et al.,

2007). For example, Pobric and Hamilton (2006) demonstrated that action understanding depends on activity in the left IFG specifically. In this study, participants observed a video of a hand lifting a box and placing it on a shelf, or bouncing balls. rTMS or sham was applied to the IFG or to the occipital region prior to each presentation. Following each presentation, participants estimated the weight of the box and that of the bouncing balls. The results demonstrated that rTMS to the IFG but not in any other condition - impaired performance on judging the weight of the box, but not the bouncing balls. This finding was interpreted as evidence that the IFG is involved in understanding the meaning of actions observed. Furthermore, in a lesion study, Tranel and colleagues (2003) reported that the region with greatest overlap in lesion that is associated with impaired retrieval of conceptual knowledge for actions was the IFG. This finding suggests that the IFG is associated with interpreting meaning of actions observed. The notion that the IFG is involved in understanding actions and intentions is also supported by the observation that F5 mirror neurons in monkeys represent actions even when the final part of the action is hidden (Umilta et al., 2001). These studies suggest that the IFG is involved in understanding actions observed and inferring its intentions and meaning.

IFG and Imitation

The IFG has also been specifically related to the ability to imitate (e.g. Iacoboni & Wilson, 2006; Grèzes et al., 2003; Irwin et al., 2011). Cortical activity in the IFG is frequently reported during imitation (see meta-analysis by Caspers et al., 2010). However, the most convincing evidence in support of this notion comes from rTMS studies. For example, Heiser and colleagues (2003) disrupted the IFG and occipital region using rTMS, and reported that participants made more response-location errors

in a finger movement imitation task than in a control task during rTMS to the IFG, but not during occipital stimulation. Additionally, the authors did not report any effects of rTMS to the IFG on more subtle measures of perceptual-motor translation (i.e. response times, movement kinematics or accuracy of finger selection). This result demonstrates that the ability to imitate depends on activity in the IFG. In another study, Catmur and colleagues (2009) demonstrated that rTMS to the IFG but not posterior parietal cortex, selectively impaired imitation of index and little finger abduction. In this study, participants were required to move their index finger or the little finger of their right hand in response to a coloured circle (e.g. orange for index response and purple for little finger response). A task irrelevant action stimulus (image of little finger or index finger) was presented at the same time as the coloured circle. This image could be compatible (index stimulus and index response) or incompatible (little finger stimulus and index finger response). The results indicated that the tendency to perform an action faster when observing the same action than incompatible action was abolished after stimulation to the IFG. This finding corroborate that imitation depends on the IFG. Furthermore, lesions to areas involving the IFG has been associated with impaired performance on imitation tasks. For example, Goldenberg and Karnath (2006) demonstrated that lesions to the IFG were associated with impaired ability to imitate finger movements. In another study, Goldenberg and colleagues (2007) reported that some patients with lesions to areas including the IFG demonstrated impairment in imitating hand gestures, however, this was not indicated in all patients suggesting that the IFG is not the only region associated with imitation.

A relationship between hMNS-related activity and suppression in μ has been demonstrated by stimulating the IFG using rTMS (Keuken et al., 2011). This study

(previously described on page 58) involved observing two videos depicting non-biological movement and five videos depicting biological movements. In one video, the participants were required to imitate the movement. This movement was a right hand opening and closing. The results demonstrated that prior to rTMS, the imitation condition and biological movement elicited significantly greater suppression in μ compared to non-biological movement, but also that the imitation condition elicited significantly larger suppression in μ compared to biological movement. However, subsequent to rTMS to the IFG, the selective suppression in μ to imitation was abolished, as was the difference between biological movements and non-biological movement. This result suggests that disruption to the IFG modulates μ-reactivity, and that the μ-rhythm indicates activity in the IFG.

This section described evidence suggesting that the IFG is selectively involved in imitation and action understanding. However, similar evidence has also been reported linking the IPL with comparable functions as was described in the previous subsection. Only a few studies have investigated activity in the IFG and IPL with corresponding behaviour in the same experiment (e.g. Arnstein et al., 2011; Braadbart, Williams, & Waiter, 2013), and therefore, the role of each node in the hMNS is not adequately clear. However, because the hMNS is thought to function as an information sharing system that involves both the IPL and the IFG (e.g. Iacoboni & Wilson, 2001; Rizzolatti & Craighero, 2004; Kilner, Friston, & Frith, 2007), it is therefore not likely that one area is totally independent of the other. The current experiment investigated the contended relationship between the IFG and μ-reactivity, and the relationship between IFG activity and imitation-related processes. It was reasoned that the results of this study will enable a systematic investigation of the

IFG in relation to μ-reactivity and imitation, and consequently a comparison can be drawn with the IPL.

Summary and Predictions

The method was the same as in Experiment 4 (IPL), but the predictions were slightly different. Here it was predicted that tACS applied in IAF to the IFG would lead to less ERD during observation of moving hands. This was based on the observation that tACS to the IPL (Experiment 4) resulted in decreased β1-ERD, and with Keuken and colleagues' (2013) finding that rTMS to the IFG resulted in elimination of selective μ-suppression to biological movement and impaired performance on the associated behaviour. The next prediction was that tACS would modulate performance on the imitation task based on the findings of Experiment 4 (IPL), in which a positive correlation between β1-ERD and imitation performance was found post-tACS. It was suggested on page 141 that this correlation was driven by activity in the IFG and given that this correlation was positive: increased activity in the IFG should lead to increased performance. Furthermore, the effects of rTMS (Keuken et al., 2013) to the IFG has been shown to result in elimination of μ-reactivity, and consequently, it was predicted that imitation performance would decrease subsequent to tACS to the IFG. It was also expected to find a positive relationship between μ-reactivity and imitation performance in line with Bernier and colleagues' (2007; 2013) work suggesting that imitation ability correlates positively with μ-suppression during observation of a moving hand. Lastly, it was demonstrated in Experiment 4 that suppression was greatest for the longest sequences, and therefore, it was predicted that μ-reactivity would be dependent on sequence length.

Method

Participant selection

See general method section (see page 123).

Stimuli

See general method section (see page 124).

Procedure

See general method section (see page 124).

tACS procedure

The protocol for stimulation was the same as in Experiment 4 (page 127) except for the tACS electrode montage. One stimulation electrode was positioned over the left IFG (between F7 and C5) and the other over the contralateral frontal polar (FP2 on the 10/20 system) in line with several previous studies targeting this area (e.g. Wach et al., 2013; Moliadze, Antal, & Paulus, 2010; Nitsche & Paulus, 2000; Moliadze et al., 2012). A graphical representation of electrode montage is presented in Figure 21 below.

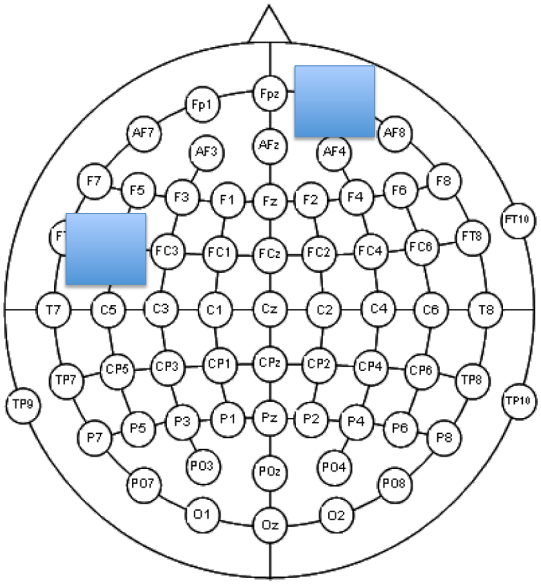

Figure 21: Graphical representation of electrode montage targeting the IFG

EEG data acquisition

Data were acquired as in Experiment 4 (see page 128).

EEG data preparation

Data were acquired as in Experiment 4 (see page 128).

Data Analysis

Data were treated and analysed like in Experiment 4 (see page 129). The behavioural data were normally distributed according to Kolmogorov-Smirnov statistics ($p > .05$), while the EEG data were not ($p < .05$).

Results

Imitation performance

Results of the repeated measures ANOVA indicated a significant main effect for the factor sequence length: $F(2, 56) = 64.91$, $p < .001$, $\eta_p^2 = .699$. Planned comparisons indicated that participants performed significantly better on the short length trials compared to both medium and long length trials (ps < .001). Additionally, the medium length trials elicited significantly more correct response compared to the long length trials ($p < .001$), suggesting that performance declined progressively with longer sequences. A significant interaction was also found between factors stimulation condition and time: $F(1, 28) = 8.97$, $p = .006$, $\eta_p^2 = .243$. Planned comparisons demonstrated that performance changed significantly pre- to post-stimulation for the tACS group ($p = .002$) but not for the sham group ($p = .295$). Furthermore, performance did not differ between groups pre-stimulation ($p = .600$), but performance differed post-stimulation as the tACS group performed significantly better compared to sham ($p = .017$), suggesting that tACS improved performance. This finding is presented in Table 7 and Figure 22.

Table 7: *t* (and *p*-values) for planned comparisons for: (a) main effect for the factor sequence length, (b-c) interaction between factors time and stimulation condition

(a)	6 vs. 7	7 vs. 8	8 vs. 6
	7.98 (< .001)	**14.17 (< .001)**	**22.15 (< .001)**

(b)	Sham	tACS
Pre vs. Post	3.56 (.204)	**8.02 (.007)**

(b)	Pre	Post
Sham vs. tACS	2.67 (.600)	**14.24 (.017)**

Note. Results in bold reached significance at .05 level or lower.

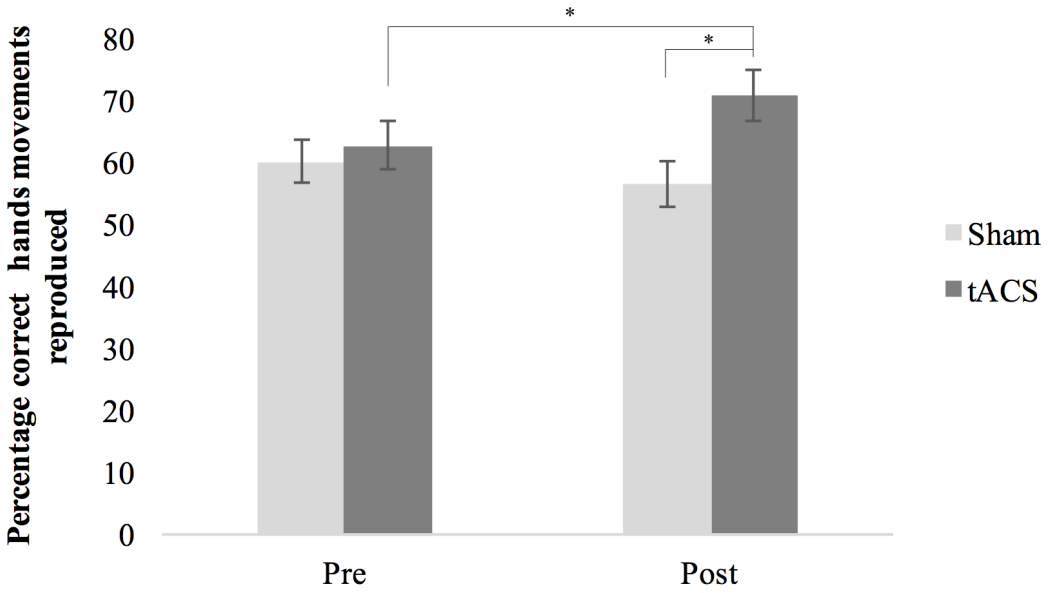

Figure 22: Results of the planned comparisons. Bars represent percentage correct movements reproduced for pre and post stimulation for sham and active tACS. Error bars indicate standard error. Note: * indicate significance level < .05.

Electrophysiological reactivity

Sensorimotor vs. occipital channels

Results of the repeated measures ANOVA indicated a significant interaction between the factors channels and sequence length in α1: F (4, 112) > 2.86, p < .027, η_p^2s > 0.093, but not α2 (ps > .570). Additionally, a significant main effect for the factor channels was found in all bandwidths: Fs (2, 58) > 5.03, ps < .010, η_p^2s > 0.148, indicating that ERD differed between regions. Therefore, investigations of μ-reactivity were investigated separately in FC, C, and O-channels.

Central channels

The results of the repeated measures ANOVA indicated no main effect or interaction for the factor stimulation condition (*p*s > .192), nor for the factor sequence length (*p*s > .136), suggesting that the C-channels did not differentiate between conditions and were not affected by tACS. However, the result of the ANCOVA indicated a significant effect of the covariate (pre-stimulation ERD values) in all bandwidths (α1, α2, β1): *F*s(1, 27) > 18.82, *p*s < .001, η_p^2s > 0.411, suggesting that the post-stimulation values were affected by pre-stimulation values. Furthermore, a significant difference in ERD post-stimulation between groups was almost reached when controlling for the influence of pre-stimulation ERD in β1: $F(1, 29) = 4.15$, $p = .052$, $\eta_p^2 = 0.133$. Although not significant at .05 level, the pairwise comparison indicated that the tACS group elicited significantly lower ERD compared to sham ($p = .052$), suggesting a tendency that tACS modulated μ-reactivity during observation of moving hands. This result is presented in Figure 23 below.

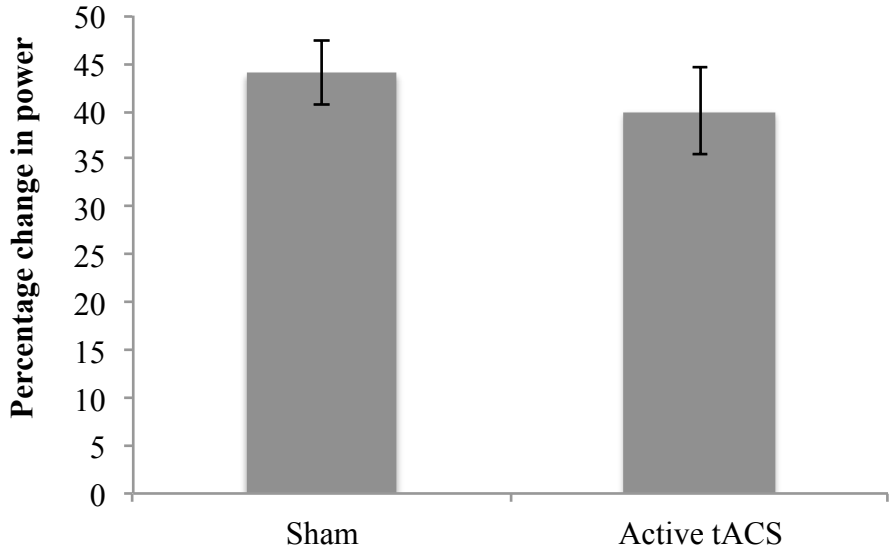

Figure 23: Results of ANCOVA. Bars represent percentage change in β1 in C-channels during observation of hands movements, post stimulation controlling for

pre-stimulation values for sham and active tACS. Error bars indicate standard error. Note: positive values represent ERD.

The result of the one-samples t-tests indicated that all sequence lengths (6, 7, 8) differed significantly from zero in all bandwidths, both before and after tACS and sham: *t*s(29) > 10.38, *p*s < .001.

Fronto-central channels

The results of the repeated measures ANOVA indicated a significant main effect for the factor sequence length in β1: $F(2, 54) = 7.24$, $p = .002$, $\eta_p^2 = 0.211$. Planned comparisons indicated that ERD was significantly larger during the long length trials compared to short length (*p*s < .001), but no difference was detected between the short length trials and the medium length (*p*s > .134), or between medium length and the long length (*p*s > .592). No other main effects or interactions were detected (*p*s > .138), suggesting that the FC-channels were not affected by tACS to the IFG.

The result of the ANCOVA indicated a significant effect of the covariate (pre-stimulation ERD values) in all bandwidths: *F*s(1, 27) = 18.62, *p*s < .001, η_p^2s > 0.408, but no significant difference was detected for the factor stimulation condition (*p*s > .192), suggesting that the lack of effect in FC-channels were not simply due to pre-stimulation ERD confounding the results. Consequently, it appears that tACS did not modulate μ-reactivity in the FC-channels.

The result of the one-samples t-tests indicated that all sequence lengths (6, 7, 8) differed significantly from zero in all bandwidths, both before and after tACS and sham: *t*s(29) > 12.65, *p*s < .001.

Occipital channels

The results of the repeated measures ANOVA demonstrated no main effects and no interactions (*p*s > .264) in any bandwidth, suggesting that ERD in the occipital region did not differentiate between any of the conditions, and it is therefore more likely that the ERD observed reflects sensorimotor activity and not visual reactivity.

μ-reactivity and imitation performance

Pearson's correlation was used to assess whether performance on the imitation task was related to μ-ERD during observation of hands movements. Because the factor stimulation condition did not elicit a significant effect in any specific bandwidth or channel, correlation analysis was performed on both C and FC, in all bandwidths, for both tACS and sham. However, to reduce the number of comparisons, the factor sequence length was collapsed. The results are presented in Table 8 below and suggest that performance on the imitation task positively correlated with μ-ERD.

Table 8: Pearson's *r* (and *p*-values) for μ-ERD correlated with the percentage correct hands movements reproduced.

		Sham			
		C		FC	
		Left	Right	Left	Right
α1	pre	.114, .685	.220, .431	.206, .480	.138, .623
	post	**.533, .041**	.288, .298	.432, .123	.363, .183
α2	pre	.098, .729	-.035, .901	.120, .684	-.151, .590
	post	.288, .299	-.028, .921	.381, .179	.075, .792
β1	pre	-.174, .534	.188, .503	-.076, .796	.054, .849
	post	.273, .325	.015, .958	-.229, .432	-.059, .836

Note: ERD was correlated pre-ERD with pre-behaviour, and post-ERD with post-behaviour. Results in bold reached significance at .05 level. Note that multiple comparisons were not controlled for.

		tACS			
		C		FC	
		Left	Right	Left	Right
α1	pre	-.271, .329	-.293, .290	-.454, .089	-.254, .361
	post	-.159, .572	-.079, .779	-.231, .408	-.207, .458
α2	pre	-.203, .468	-.295, .286	**-.526, .044**	-.294, .288
	post	-.323, .241	-.110, .697	-.359, .189	-.370, .175
β1	pre	-.301, .275	.219, .434	-.405, .134	.034, .903
	post	-.181, .520	-.059, .835	-.272, .326	.112, .692

Note: ERD was correlated pre-ERD with pre-behaviour, and post-ERD with post-behaviour. Results in bold reached significance at .05 level. Note that multiple comparisons were not controlled for.

Interim Discussion

The current study investigated the relationship between the μ-rhythm and activity in the IFG, and the effect on imitation when this relationship is modulated with tACS. It was reasoned that the results of this experiment would enable a comparison between the frontal (IFG) and posterior (IPL) nodes of the hMNS in terms of cortical activity and in the ability to imitate. The IFG was stimulated using the same method and procedure as was used in Experiment 4 (IPL) in order to make comparisons more meaningful. The results of the current experiment confirmed that activity in the IFG is central in processes relating to the ability to imitate, as performance increased

significantly subsequent to tACS, but not to sham stimulation. Furthermore, a tendency was detected suggesting that tACS lead to a decrease in β1-ERD during observation of hands movements. Although not significant at the traditional alpha level, this trend is similar to the finding in the IPL in that the effect was found in C-channels in β1-bandwidth. These results may enlighten the current understanding of the involvement of the IPL and IFG in observing and imitating hand movements.

The results of the current experiment failed to demonstrate a significant effect of tACS to the IFG. However, on closer inspection, a difference between groups post-stimulation was observed that almost reached significance. Although this finding should be treated with caution, a tendency to decrease ERD subsequent to tACS was apparent and therefore does not totally exclude the IFG as an influence of μ-reactivity. This tendency is in line with the study prediction and with Keuken and colleagues' (2013) study demonstrating that rTMS to the IFG leads to elimination of selective μ-suppression to biological movement and corresponding behaviour. Therefore, the finding that tACS elicited less ERD compared to sham – although not significant at the traditional level – is more likely to reflect involvement of IFG in observation of hands movements rather than that of a mere epiphenomenon. Given that the suppression in μ during action observation is considered an index of hMNS-related activity (e.g. Muthukumaraswamy & Johnson, 2004; Pineda, 2005) and the observation that the mu-rhythm is modulated by activity in the IFG (e.g. Arnstein et al., 2011; Braadbart, Williams, & Waiter, 2013), the current finding can be interpreted as evidence that hMNS-related processes were indicated by β1-ERD during observation of hands movements. This interpretation is supported by the large number of studies demonstrating that the IFG is activated during observation of hands movements (see page 14; e.g. Caspers et al., 2010), in addition to several recent

studies reporting that activity in the IFG is directly related with suppression in μ during observation and imitation of hands movements (Babiloni et al., 2016; Braadbart, Williams, & Waiter, 2013; Arnstein et al., 2011). Given this literature, the results can be interpreted as evidence that IFG was involved in observation of hands movements albeit to a lesser degree than the IPL. Despite that the sensorimotor reactivity patterns were weaker in this experiment compared to the previous (IPL), there were effects found for sequence length, and for stimulation condition that were not present in the occipital region. The absence of effect in occipital electrodes strengthens the claim that sensorimotor μ reflected recruitment of motor systems and possibly hMNS-related activity, and not mere excitation of the visual cortex.

Alternatively, given that the tACS effect observed for the IFG is similar to that observed for the IPL, it is possible that the suppression observed is partly due to activity in the IPL. This argument is based on several assumptions: Firstly, the μ-rhythm is thought to involve activity of both the IPL and IFG via cortico-cortical connections (Muthukumaraswamy and Johnson, 2004; Pineda, 2005); secondly, the hMNS is thought to enable forwards and backwards communication between its nodes (e.g. Iacoboni & Wilson, 2001; Rizzolatti & Craighero, 2004; Kilner, Friston, & Frith, 2007). Consequently, modulating activity in one is likely to influence the other. Therefore, it is possible that tACS to the IFG also modulated activity in the IPL (via cortico-cortical connections), consequently affecting summation of μ-reactivity recorded over the sensorimotor area. Another consideration is that the effect of the stimulation may have included the IFG given that electrical currents passing through the scalp and skull are dispersed (Rossi et al., 2009). This possibility is however unlikely given the relative distance from the IPL to the IFG. Lastly, the IFG could be related to language processing (e.g. Hecaen & Consoli, 1973), but this possibility

alone seems unlikely to explain β1-ERD observed in the current experiment.

The assumption that the IFG is central in processes related to imitation performance was suggested by the correlation between μ-ERD and imitation performance post-tACS in the previous experiment. This finding led to the prediction in the current experiment that tACS would affect imitation performance. More specifically, it was predicted that tACS would lead to a decrease in imitation performance. This prediction was based on the finding that rTMS to the IFG leads to elimination of μ-reactivity and impaired performance on corresponding behaviour (Keuken et al., 2013). The results of the current experiment suggest that performance was significantly enhanced subsequent to tACS, and therefore, although the direction in which tACS modulated performance was not supported, the finding confirms that the IFG is directly involved in processes related to preparing to imitate. This finding is consistent with a large number of studies demonstrating that cortical activity in the IFG is involved in processes relating to imitation as demonstrated using fMRI (see review by Caspers et al., 2010), brain stimulation (e.g. Heiser et al., 2003; Catmur et al., 2009), and lesion studies (e.g. Golenberg & Karnath, 2006; Goldenberg et al., 2007). Additionally, this finding supports the proposition (see page 141) that activity in the IFG was driving the correlation between μ-reactivity and imitation performance subsequent to tACS to the IPL.

The behavioural results in relation with the EEG results, indicate that tACS to the IFG induced processes that resulted in enhanced imitation performance, however, μ-reactivity to observation of moving hands were less affected. The reverse pattern was indicated when the IPL was stimulated. This pattern may suggest that observation and imitation are governed by different but interacting nodes, that is, μ-suppression

during observation is related to activity in the IPL, while imitation performance is related to activity in the IFG. This interpretation is in line with the notion that the hMNS is an information sharing system that enables forward and backward communication between nodes (Kilner, Friston, & Frith, 2007), and with Arnstein and colleagues (2011) finding that μ-suppression during hand movement observation correlates with activity in the IPL but not with the IFG.

It was predicted that μ-reactivity would correlate positively with imitation performance in line with Bernier and colleagues' (2007; 2013) work suggesting that imitation performance correlates positively with μ-suppression during observation of a moving hand. The results of the current experiment detected two significant correlations suggesting that μ-reactivity was related to performance on the imitation task. Although these correlations were somewhat sporadic (one was in α1 post-sham and the other in α2 pre-tACS), they were both indicated in the left hemisphere. Cortical activity in the IFG, but specifically in the left hemisphere has repeatedly been associated with imitation performance (e.g. Pobric & Hamilton, 2006; see review by Caspers et al., 2010), and supports the current finding that μ-reactivity correlated with imitation performance. Incidentally, the correlation found in the IPL was also in the left hemisphere, which was interpreted as evidence that the IFG was involved. The proposition made in this thesis that the IPL is more strongly recruited for observation of hands movements, and the IFG for imitation performance, is supported by these correlations.

In line with the study prediction, and with results reported in Experiment 4 (IPL), the length of the sequences modulated μ-suppression. The longest sequence (8) induced the largest suppression in μ, and significantly greater suppression compared to the

shortest sequences (6). No difference was observed between the short and medium or the long and medium. This finding is comparative to the IPL finding that the greatest suppression was detected for the longest sequences, but in the IPL, a significant difference was detected between the long and the medium trials. This could suggest that IFG activity was less selective to subtle differences in sequence lengths compared to the IPL. This could suggest that the IPL is more strongly recruited for observation of hands movements, and the IFG is only indirectly involved.

In summary, the current study demonstrated that tACS significantly improved performance on the imitation task, confirming that the IFG is central in the ability to imitate. This was interpreted as evidence that the IFG is more strongly related to preparing to imitate than the IPL. Furthermore, a tendency was detected towards tACS modulating β1-ERD during observation of hands movements similar to the findings of Experiment 4 (IPL). This finding was interpreted as evidence that the IFG plays a smaller role in observation of hands movements compared to the IPL as the effect was less prominent in the IFG. Moreover, significant correlations between μ-suppression and imitation performance were found in the left hemisphere, suggesting that activity in the IFG is related to imitation performance. Additionally, activity in one node appears to affect activity in the other, suggesting that the hMNS is not a strictly linear system, and that considering one node without the other is not meaningful.

Experiment 6: Stimulating the Primary Motor Cortex (M1)

Introduction

In the previous two experiments, the relationship between μ-rhythms and the IPL (Experiment 4) and the IFG (Experiment 5) were investigated. The results of Experiment 4 indicated that activity in the IPL modulated β1-ERD, but related indirectly to the ability to imitate. Experiment 5 indicated the reverse pattern, i.e. that activity in the IFG related to the ability to imitate, but modulated β1-ERD indirectly. The current experiment may enlighten the role of the IFG and IPL in observing and imitating hands movements, as it investigates the effect of tACS to the primary motor cortex (M1), which is considered the source for generating μ-rhythms (Salmelin et al., 1995; Hari, 2006; Avanzini et al., 2012). It is known that the M1 is directly involved in the control and generation of voluntary movement (See review by Hatsopoulos & Suminsky, 2011), however, it has remained elusive whether the M1 is involved in observation of movement and therefore the M1 has not been considered as a hMNS related area (Lepage, Lortie, & Champoux, 2008). Recently, several studies suggest that activity in the M1 is mirror like and should be considered as a hMNS core area. This literature will be reviewed next.

It was reported in several early studies using PET (Rizzolatti et al., 1996; Decety et al., 1997) and single-cell studies in monkeys (Gallese et al., 1996; Fogassi et al., 2001) that mirror like activity was absent in the M1 during mirror tasks. It was reasoned that the absence of M1 activity was evidence that mirror-like activity elsewhere was not the product of mere motor facilitation or covert movement (Gallese et al., 1996; Iacoboni et al., 1999; Fogassi et al., 2001). There were however

many human studies at the time indirectly demonstrating mirror like activity in M1 using brain stimulation (e.g. Fadiga et al., 1995; Baldissera et al., 2001) and EEG/MEG (e.g. Hari et al., 1998; Cochin et al., 1998; Nishitani & Hari, 2000). The M1 activity in these studies were however not related to hMNS, instead it was assumed that the activity detected was relating to the mirror input to the M1, and not mirroring activities in the M1 per se.

It was later reported that neurons in the M1 in monkeys do respond to action observation (Tkach et al., 2007; Wahnoun et al., 2006), but this activity was interpreted as *mental rehearsal* of a learned motor action and not processes relating to mirror properties. Mental rehearsal is considered to be a replay of an internal movement plan, in which neurones re-enact the movement activity as if the learned action itself were being performed, but in a weaker way (Cisek & Kalaska, 2004). Similarly to mirror neurons, mental rehearsal neurons exhibit activity during action execution and observation, but they become active earlier. It was therefore assumed that they reflected a prospective mental rehearsal of an upcoming learned action rather than mirroring (Cisek & Kalaska, 2004). However, in a more recent single-cell study in monkeys, subpopulations of M1 neurons were identified that related to both mirroring properties and mental rehearsal. Some of these cells' activation was defined as mirroring rather than mental rehearsal (Dushanova & Donoghue, 2010). These studies are compelling evidence suggesting that M1 contains mirror neurons in monkeys.

A growing number of human studies have also indicated that the M1 is involved in action observation similar to mirror neurons in monkeys (e.g. Babiloni et al., 2016; Montagna, et al., 2005; Boroni et al., 2005; Press, et al., 2011; Szameitat et al., 2012).

For example, it is well-known that β-oscillations are generated in areas including the M1 and that α-oscillations are generated more posteriorly in the somatosensory cortex (Cheyne et al., 2003; Hari et al., 1998; Rossi et al., 2002; Salmelin & Hari, 1994), and that suppression in sensorimotor rhythms are associated with increased cortical activity (Goldman et al., 2002; Steriade & Llinas, 1988). As μ-rhythms include both α and β-oscillations, it is logical that suppression in μ during action observation reflects engagement of the M1 and the somatosensory cortex. Despite the number of studies reporting suppression in μ during action observation (e.g. Muthukumaraswamy & Johnson, 2004; Cochin et al., 1999; Oberman et al., 2005; Perry & Bentin, 2009; Puzzo et al., 2010), these results have been attributed to activity in hMNS areas (IPL and IFG) via downstream cortico-cortical connections (e.g. Muthukumaraswamy & Johnson, 2004; Pineda, 2005) rather than mirror activity within the M1 itself. There is however support for both interpretations, that μ-suppression reflects M1 mirror activity and mirror activity from core areas of the hMNS. For example, Press, Cook, Blakemore and Kilner (2011) demonstrated using MEG that β-power during action observation (intransitive moving arm and hand) has its source in the sensorimotor cortex and not core areas of the hMNS. Furthermore, more direct evidence that μ-rhythms relate to M1 activity was recently reported by Babiloni and colleagues (2016). In this study, electrocorticography (EcoG) activity was investigated in epilepsy patients undergoing pre-surgical invasive investigation by subdural electrodes. EcoG is considered a more direct approach to measure neuronal activity than is EEG because it is intracranial and therefore not affected by the poor electrical conduciveness of the skull (Rossi et al., 2009). Babiloni and colleagues (2016) demonstrated that α and β-suppression during action observation and imitation occurs in areas including the M1. In contrast, Braadbart, Williams and Waiter (2013) reported that suppression in μ during observation and imitation of

object directed hand movements, correlates with fMRI BOLD signal in areas including the IFG and IPL, but not the M1.

Although the studies mentioned above support the notion that μ-suppression during action observation reflects mirror like activity in M1, they cannot reject the notion that the suppression recorded reflects mirror like input from other hMNS-related areas (Muthukumaraswamy & Johnson, 2004; Pineda, 2005). There is however more compelling evidence that challenges the notion that the sensorimotor cortex is activated postsynaptically during action observation given the anatomical connection between premotor cortex and sensorimotor cortex (Matelli et al., 1986; Dum & Strick, 2005). This line of evidence comes from brain stimulation studies. The earliest evidence for M1 involvement in action understanding is the observation that MEPs (see page 10 for explanation of approach) can be recorded from the muscle corresponding to the muscle used to execute the observed action (Fadiga et al., 1995). Similar results have been reported by many others since (e.g. Hardwick, McAllister, Holmes, & Edwards, 2012; Borroni, et al., 2005; Baldissera, 2005; Montagna et al., 2005; Maeda et al., 2002) and confirm M1 involvement in action observation. These studies collectively suggest that the sensorimotor cortex may be an intrinsic part of the hMNS rather than merely receiving input from hMNS-related areas.

Summary and Predictions

The current experiment was designed to investigate whether activity in the M1 is on par with that of the IPL and IFG in relation with hMNS-related activity and with the ability to imitate. For this purpose, the M1 was stimulated using the same method as was used in experiments 4 and 5 to make comparisons meaningful. Given that μ-

suppression during action observation is recorded over M1 (C-channels), signal from this area is inevitably involved in action observation. However, whether this signal reflects mirror-like activity in the M1, or mirror-like input to the M1 from hMNS core areas remains elusive. It was reasoned that if the M1 is involved in the same processes that are related to the IFG and the IPL (i.e. mirroring and not simply mental rehearsal) during observation of hands movements, then stimulating the M1 should affect μ-reactivity to the same degree. However, if the M1 is unrelated to mirroring and is more related to simple motor facilitation or mental rehearsal, then stimulating the M1 should affect μ-reactivity to a lesser degree. Based on the substantial evidence suggesting that the M1 is involved in the hMNS (e.g. Babiloni et al., 2016), it was predicted that tACS to M1 would modulate μ-reactivity. Furthermore, based on the results of experiments 4 and 5, it was predicted that the effect of tACS would be a decrease in suppression. Next, based on findings of experiment 5 (IFG) it was predicted that tACS to the M1 would lead to enhanced performance on the imitation task. It was predicted in line with experiments 4 and 5 that μ-reactivity correlates positively with imitation performance, but additionally that this correlation will be found in the left hemisphere. This prediction was based on the fact that the correlations observed in experiments 4 and 5 were found in the left hemisphere only and that previous studies have specifically linked the left hemisphere to imitation performance (e.g. Decety et al., 2002; Goldenberg & Karnath, 2006; Pobric & Hamilton, 2006). Lastly, it was predicted that suppression in μ would depend on sequence length. This prediction was supported in experiments 4 and 5.

Method

Participant selection

See general method section (page 123).

Stimuli

See general method section (page 124).

Procedure

See general method section (page 124).

tACS procedure

The protocol for stimulation was the same as in Experiment 4 (page 127) except for tACS electrode montage. One stimulation electrode was positioned over the left premotor cortex (C3) and the other over the contralateral frontal polar (FP2 on the 10/20 system) in line with several previous studies targeting this area (e.g. Wach et al., 2013; Moliadze, Antal, & Paulus, 2010; Nitsche & Paulus, 2000; Moliadze et al., 2012). A graphical representation of electrode montage is presented in Figure 24 below.

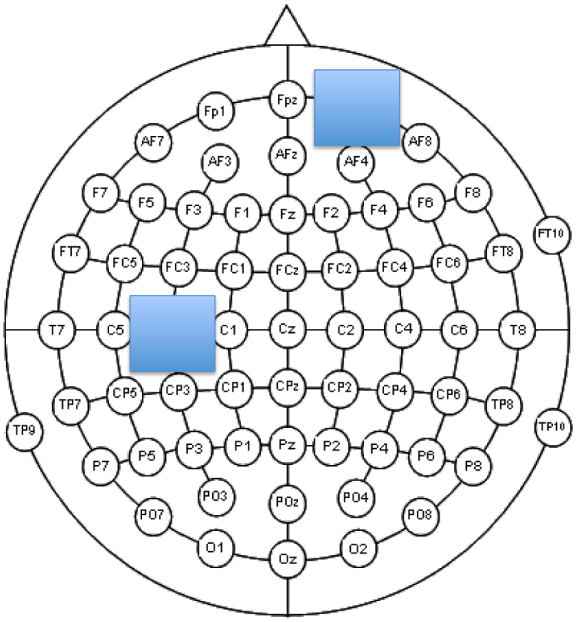

Figure 24: Graphical representation of electrode montage targeting the M1.

EEG data acquisition

Data were acquired as in Experiment 4 (see page 128).

EEG data preparation

Data were acquired as in Experiment 4 (see page 128).

Data Analysis

Data were treated and analysed as in Experiment 4 (see page 129). The behavioural data were normally distributed according to Kolmogorov-Smirnov statistics ($p > .05$), while the EEG data were not ($p < .05$).

Results

Imitation performance

Results of the repeated measures ANOVA indicated a significant main effect for the factor sequence length: $F(2, 56) = 43.22$, $p < .001$, $\eta_p^2 = .607$. Planned comparisons indicated that performance declined progressively with more movements, that is, participants performed significantly better on the short sequences compared to both medium and long (ps < .034), and significantly better on the medium sequences compared to the long ($p < .001$). A significant interaction was also observed between factors sequence length and time: $F(2, 56) = 24.10$, $p < .001$, $\eta_p^2 = .463$. This finding is similar to that in Experiment 4 and was further analysed using the same method. Bonferroni corrected pairwise comparisons revealed that there were no differences in performance pre to post-stimulation on the short sequences ($p = .964$), however performance increased significantly post stimulation on the medium sequences ($p <$ .001), and significantly decreased on the long sequences ($p < .001$). The results of these comparisons are presented in Table 9 and Figure 25, and suggest that learning-related changes in performance are dependent on the number of movements in a sequence. No main effect or interaction was observed for the factor stimulation condition (ps > .163), suggesting that tACS did not modulate performance on the imitation task.

Table 9: t (and p-values) for planned comparisons for: (a) main effect for the factor sequence length, (b) interaction between factors time and sequence length

(a)	6 vs. 7	7 vs. 8	8 vs. 6
	5.98 (.034)	**14.07 (< .001)**	**20.05 (< .001)**

(b)	6	7	8
Pre vs. Post	**5.98 (.034)**	**14.07 (< .001)**	**20.05 (< .001)**

Note. Results in bold reached significance at .05 level or lower.

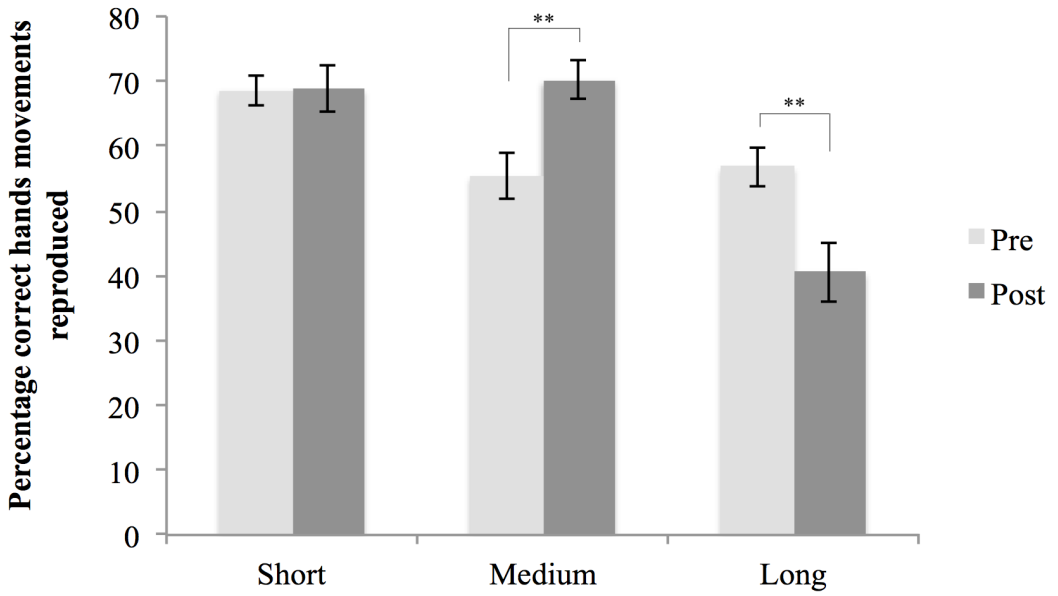

Figure 25: Results of Bonferroni corrected pairwise comparisons. Bars represent percentage correct movements reproduced for short (6), medium (7), and long (8) sequences by time. Error bars indicate standard error. Note: ** indicates significance level < .001.

Electrophysiological reactivity

Sensorimotor vs. occipital channels

Results of the repeated measures ANOVA indicated no significant interaction between the factors channels and length (*p*s > .397), however, a significant main effect for the factor channels was found in all channels: *F*s (2, 58) > 7.51, *p*s < .001, η_p^2s > 0.206, indicating that ERD differed between regions. Therefore, investigations of μ-reactivity were investigated separately in FC, C, and O-channels.

Central channels

Results of the repeated measures ANOVA demonstrated that an interaction between

the factors time and stimulation condition was close to significance in β1: $F(1, 28) = 3.79$, $p = .062$, $\eta_p^2 = .119$. Although not significant, this interaction was investigated further due to the similarity with findings in the IPL. The results mirror that in the IPL as β1-ERD differed significantly from pre to post-stimulation for the sham condition ($p = .038$) but not for the active tACS condition ($p = .571$), suggesting that tACS moderated μ-reactivity during observation of moving hands. The result of planned comparisons are presented in Figure 26 below.

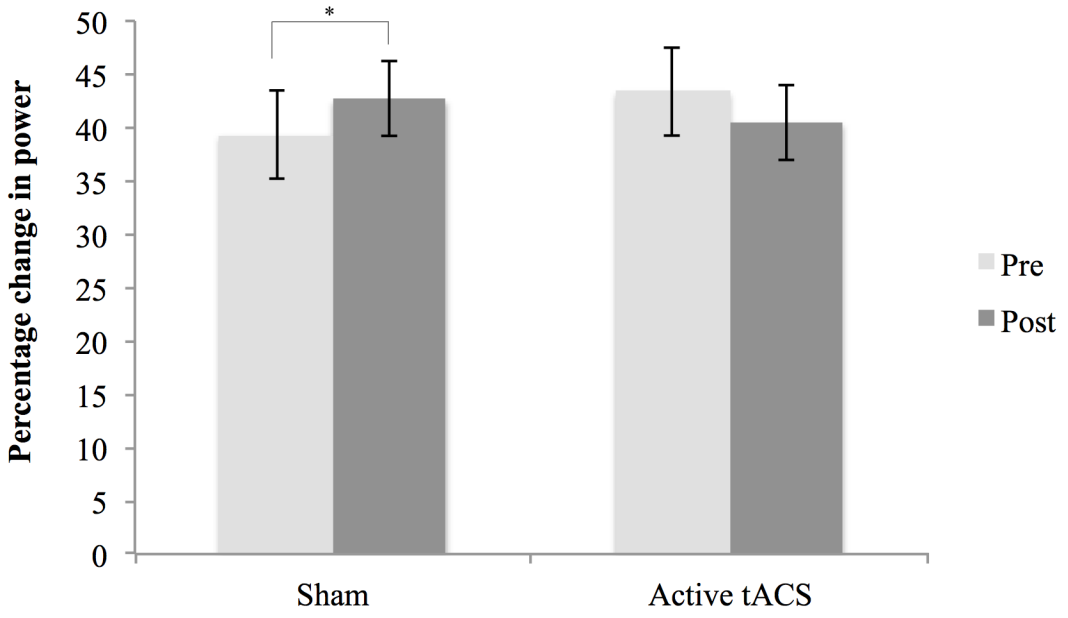

Figure 26: Results of planned comparisons. Bars represent percentage change in lower beta in C-channels during observation of hands movements, pre and post stimulation for sham and active tACS. Error bars indicate standard error. Note: positive values represent ERD.

The result of the ANCOVA indicated a significant effect for the covariate (pre-stimulation ERD values): $F(1, 29) = 49.43$, $p < .001$, $\eta_p^2 = 0.647$, and close to significant effect of the stimulation condition: $F(1, 29) = 4.09$, $p = .053$, $\eta_p^2 = 0.132$. Although not significant at the 0.05 level, this result suggests that there was an effect of stimulation condition on post-stimulation ERD after controlling for pre-stimulation

ERD. Planned comparisons maintained that active tACS elicited less ERD compared to sham (p = .053) although not significant at the traditional level. See Figure 27 below.

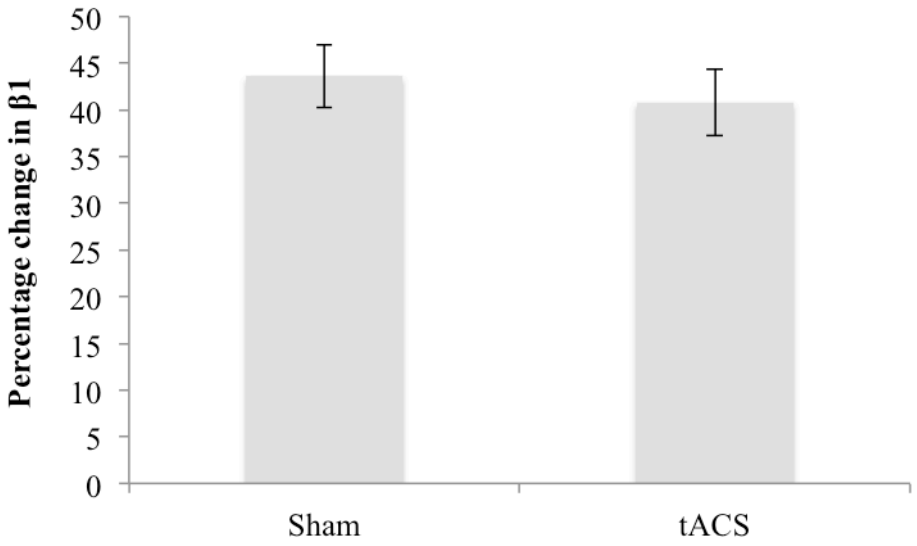

Figure 27: Results of ANCOVA. Bars represent percentage change in β1 in C-channels during observation of hands movements, post stimulation controlling for pre-stimulation values for sham and active tACS. Error bars indicate standard error. Note: positive values represent ERD.

The result of the one-samples t-tests indicated that the ERD observed in both pre and post stimulation differed significantly from zero for both the sham and the active tACS: ts(14) > 18.93, ps < .001.

Fronto-central channels

Results of the repeated measures ANOVA indicated a significant main effect for the factor sequence length in α2: $F(2, 54) = 3.79$, $p = .029$, $\eta_p^2 = .123$, but not in α1 or β1 (ps > .085). Planned comparisons indicated that ERD was significantly larger for long sequences compared to short (p = .05), but no difference was detected between short sequences and medium (p = .131), or between medium sequences and long (p = .989),

suggesting that ERD was affected by the length of the sequence. No other main effects or interactions were detected in the FC-channels (*p*s > .093).

Occipital channels

Results of the repeated measures ANOVA indicated no main effects (*p*s > .240) and no interactions (*p*s > .373) in any bandwidth, suggesting that the occipital region did not distinguish between sequences of hand movements, and was not affected by tACS. It is therefore more certain that the suppression pattern observed in sensorimotor region reflected recruitment of motor systems rather than visual reactivity.

μ-reactivity and imitation performance

Pearson's correlation was used to assess whether performance on the imitation task was related to μ-ERD during observation of hands movements, as it was for IPL. Because the effect of time by stimulation condition was found in the β1 only, only β1 was investigated here. The results are presented in Table 10 below and suggest that subsequent to tACS, performance positively correlated with μ-ERD.

Table 10: Pearson's *r* (and *p*-values) for μ-ERD correlated with percentage correct hands movements reproduced.

	Sham		Active tACS	
	Left	Right	Left	Right
Pre	-.174, .534	.188, .503	.218, .434	.107, .703
Post	.273, .325	.015, .958	**.553, .033**	.370, .174

Note: ERD was correlated pre-ERD with pre-behaviour, and post-ERD with post-behaviour. Results in bold reached significance at .05 level. Note that multiple comparisons were not controlled for.

Interim Discussion

The current experiment investigated whether the M1 is involved in action observation prior to imitation on a par with the IPL and the IFG. To do this, the M1 was stimulated using tACS employing the same method and procedure as was used in experiments 4 and 5. Based on the findings in the IPL and IFG, it was assumed that tACS to the M1 would decrease μ-ERD and increase performance on the imitation task. The results indicated a tendency for β1-ERD to decrease subsequent to tACS to the M1, and, although performance on the imitation task was not affected by tACS, a significant and positive correlation between β1-ERD and imitation performance was indicated subsequent to tACS in the left hemisphere. These results are strikingly similar to those found in the IPL, although less robust. Moreover, although the effect of tACS on imitation performance was not significant, it was visible that performance was improved subsequent to tACS. This finding is similar to that in the IFG, but also less robust. The implications of these findings will be discussed next.

The finding that β1-ERD is modulated by tACS is consistent with the study prediction and with the notion that activity of the M1 is involved in observation of movement (e.g. Lepage, Lortie, & Champoux, 2008). As such this result can be viewed as supporting the view that activity in the M1 contains mirror-like properties (e.g. Babiloni et al., 2016; Montagna, et al., 2005; Boroni et al., 2005; Press, et al., 2011; Szameitat et al., 2012). There is compelling evidence supporting this notion, such as brain stimulation studies (e.g. Fadiga et al., 1995; Hardwick et al., 2012; Baldissera, 2005), EEG/MEG (e.g. Hari et al., 1998; Cochin et al., 1998; Nishitani & Hari, 2000), and single-cell studies in monkeys (e.g. Tkach et al., 2007; Wahnoun et al., 2006; Dushanova & Donoghue, 2010). However, the effect of tACS on β1-ERD could also reflect other motor system processes relating to movement preparation

(e.g. Hatsopoulos & Suminsky, 2011) or mental rehearsal (Cisek & Kalaska, 2004) in response to observation of moving hands. This possibility cannot readily be refuted because mirror like processes are not easily differentiated from other motor related processes using correlative tools like EEG (see page 19). This is a common issue for all studies investigating hMNS. Although it has been proposed that mental rehearsal is quicker than mirroring (see page 166), speed of neuronal response between two conceptually different neuronal assemblies cannot easily be investigated using EEG, because the signal recorded is the summation of activity in all nearby cells (see page 19). Therefore, there is no way to differentiate whether the β1-ERD recorded in the current study reflects one or the other.

The difficulty in differentiating which conceptual neuronal population was affected by tACS may be enlightened by the similarity between the current findings and that of the IPL and IFG. It is likely that the tACS induced decrease in β1-ERD in the IPL and IFG reflects mirror-like processes rather than motor preparation and mental rehearsal because activity in the IPL and IFG is not associated with either. Given the similarity in the tACS induced effects on β1-ERD, it is likely that the M1 also reflected mirror like activity. However, the effect of tACS on β1-ERD observed in the M1 was less robust than that recorded in the IPL, but comparative to the IFG. Although this finding may be interpreted as evidence that activity in the M1 and IFG are less involved in observation of movements, there is little evidence that would lead to the interpretation that activity in these regions is not involved at all. Conceding the possibility that it is activity in the IPL that is reflected by the modulation observed to β1-ERD subsequent to tACS, it could then be construed as supporting the notion that the M1 receives mirror-like input, rather than containing mirror-like properties (e.g. Lepage, Lortie, & Champoux, 2008). This possibility is conceptually possible given

the assumption that μ reflects cortical activity in hMNS areas via downstream cortico-cortical connections (e.g. Muthukumaraswamy & Johnson, 2004; Pineda, 2005). Additionally, there is some evidence that would support this interpretation for example, absence of mirror like activity has been reported in human PET studies (Rizzolatti et al., 1996; Decety et al., 1997), fMRI BOLD activity correlates with activity in the IPL and IFG during observation and imitation of hands movements, but not M1 (Braadbart, Williams & Waiter, 2013) – although note that such correlation has been reported by others (Arnstein et al., 2011; Babiloni et al., 2016) – and single-cell studies in monkeys (Gallese et al., 1996; Fogassi et al., 2001) suggesting that mirroring does not occur in M1.

It appears from the data and from evidence mentioned above that the effect of tACS on β1-ERD reflects mirror like input to the M1 rather than mirror like activity in the M1. However, if this logic is applied to the M1, it should also be applied to the IFG given the similarity of the effect. However, the IFG has a substantial literature indicating that the IFG is directly involved in action observation (see page 14). Therefore, the M1 is not likely to be the same as the IFG. The effect of tACS on M1, IPL and IFG was however not only similar in terms of β1-ERD, but also in terms of imitation performance: Both the IPL and M1 data demonstrated a lack of effect on imitation performance, yet revealed a significant and positive correlation between β1-ERD and imitation performance subsequent to tACS. This finding may support the possibility that β1-ERD observed in the M1 was driven by activity in the IPL. Moreover, on closer inspection, the effect of tACS on imitation performance in the M1 was an increase (although not significant) and that is more like the finding of the IFG than the IPL, which suggested tACS impaired performance (although not significantly). This finding could be interpreted as evidence that M1 was influenced

by activity in the IFG relating to imitation performance, given that the IFG influences μ-rhythms. It is then a logical assumption that activity in the M1 reflects input from the IPL (in relation to action observation) and input from the IFG (in relation to the ability to imitate). However, the data presented in this thesis cannot fully establish whether μ-reactivity observed in the current study reflected mirror like activity in the M1 itself, or mirror input from the IPL and IFG. This issue will be explored further in the chapter discussion and in the general discussion chapter.

It was predicted that tACS would improve imitation performance based on the finding that the tACS to the IFG improved performance, and because tACS to the IPL revealed a significant positive correlation between β1-reactivity and imitation performance. The results of the current experiment were similar to the previous two experiments in several ways. Although the results did not demonstrate a significant effect of tACS on imitation performance, the effect of tACS on imitation performance was visibly improved subsequent to tACS similarly to the IFG results. Furthermore, a correlation was observed between β1-ERD and imitation performance subsequent to tACS, similar to the IPL results. Additionally, as in experiments 4 and 5, imitation performance correlated positively with β1-ERD in the left hemisphere. This finding supports Bernier and colleagues' (2007; 2013) work suggesting that imitation performance correlates positively with μ-suppression during hands movements observation, and studies demonstrating that the left hemisphere is particularly involved in imitation, for both the IPL (e.g. Decety et al., 2002; Goldenberg, 1995; Goldenberg & Karnath, 2006) and the IFG (e.g. Pobric & Hamilton, 2006; Caspers et al., 2010).

Lastly, the length of the sequence presented modulated both μ-ERD during

observation of hands movements and performance on the imitation task, similar to experiments 4 and 5. This finding is therefore consistent across all experiments, and supports the literature indicating that sensorimotor suppression (Klimesch, Schimke, & Pfurtscheller, 1993; Klimesch, 1998; Brinkman et al., 2014; Pfurthscheller & Lopes da Silva, 1999) is enhanced by task demands and cognitive load. The finding that performance progressively declines with more movements is consistent with well-known performance related decline with increased cognitive load (e.g. Leppink et al., 2014; Kalyuga, Chandler, & Sweller 2000).

In summary, the current experiment demonstrated β1-ERD during observation of moving hands. Significantly less β1-ERD was observed subsequent to tACS compared to sham, suggesting that tACS decreased β1-ERD. No direct effect was observed subsequent to tACS to the M1, but a significant and positive correlation was observed between β1-ERD and imitation performance subsequent to tACS, suggesting that M1 activity is at least partially or indirectly involved in this relationship. The findings of the M1 was on par with the IPL but also reminiscent to the IFG, suggesting that the M1 reflects input from both the IPL and IFG. In order to further investigate the effects of tACS on these areas, the results of experiments 4, 5 and 6 will be compared in an omnibus analysis. The aim of this analysis is to compare the effects of each node in comparison to each other. It is possible that this analysis can enlighten the role of M1 in relation to observation of hand movements and the ability to imitate them.

Omnibus Analysis

Data Analysis

The data were treated and analysed similarly to Experiment 4 (see page 129) with some differences as pointed out below. As the behavioural data were all normally distributed, no transformation was performed on this data. However, the majority of the EEG data were not normally distributed, and therefore the EEG data used for the omnibus analysis were log transformed. A repeated measures ANOVA was conducted for the behavioural data with three factors: "time" with two levels (pre-stimulation, post-stimulation), "sequence length" with three levels (6, 7, 8), and one between-subjects factor "stimulation condition" with four levels (sham, tACS IPL, tACS IFG, tACS M1) in order to compare the effects of tACS to each stimulation condition on imitation performance. It was expected to observe a significant interaction between the factors stimulation condition and time based on findings in experiments 4, 5 and 6. Planned comparisons (Bonferroni corrected pairwise comparisons) in this case were pre vs. post for each stimulation condition. Subsequently, in order to compare post-stimulation differences between groups whilst controlling for pre-stimulation differences, ANCOVA was conducted with performance pre-stimulation as the covariate, performance post-stimulation as the dependent variable, and stimulation condition as the fixed variable. Planned comparisons (Bonferroni corrected pairwise comparisons) were conducted on the following pairs to further investigate between-group differences: (a) sham vs. IPL; (b) sham vs. IFG; (c) sham vs. M1; (d) IPL vs. IFG; (e) IPL vs. M1; (f) IFG vs. M1.

A repeated measures ANOVA was conducted for the EEG data in order to investigate

the effect of tACS on β1-ERD to observation of hands movements. Because the main findings in the previous experiments were indicated in C-channels in β1, the ANOVA was conducted for β1 in C-channels only. Factors for this ANOVA included: "time" with two levels (pre-stimulation, post-stimulation), "sequence length" with three levels (6, 7, 8), "hemisphere" with two levels (left, right), and one between-subjects factor "stimulation condition" with four levels (sham, tACS IPL, tACS IFG, tACS M1). It was expected to find an interaction between factors time and stimulation condition based on the previous results, and similar planned comparisons were conducted as for the behavioural data. Subsequently, ANCOVA was conducted with a similar set up as for the behavioural data, and with similar planned comparisons.

Results

Imitation performance

Results of the repeated measures ANOVA indicated a significant main effect for the factor sequence length: $F(2, 112) = 101.979$, $p < .001$, $\eta_p^2 = .646$. This main effect was not further investigated because it is considered outside the interest of the current analysis. A significant interaction was detected between the factors time and stimulation condition $F(3, 56) = 3.67$, $p = .018$, $\eta_p^2 = .164$. Results of the planned comparisons indicated that a significant change in performance pre to post stimulation was only recorded for the group receiving tACS to the IFG ($p = .004$), and not for all other groups ($ps > .186$). This result is presented in Figure 28 below. No other main effects or interactions were detected ($ps > .343$).

The results of the ANCOVA revealed a significant effect for both the covariate (pre-

stimulation ERD values): $F(1, 60) = 75.46$, $p < .001$, $\eta_p^2 = 0.578$, and for the stimulation condition: $F(3, 60) = 4.13$, $p = .036$, $\eta_p^2 = 0.184$, suggesting that there was a significant effect of stimulation condition on post-stimulation performance after controlling for pre-stimulation performance. Results of the planned comparisons indicated that post-stimulation performance in the sham group was significantly worse than in the IFG group ($p = .011$), and a tendency for participants in the IFG group to perform better than in the IPL group ($p = .058$). No other comparison reached significance ($ps > .753$).

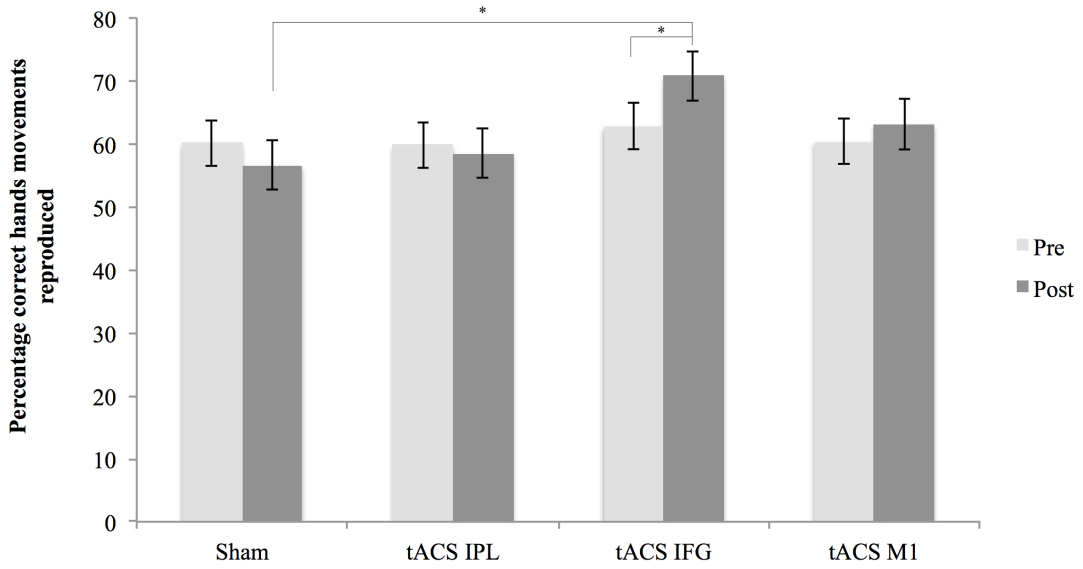

Figure 28: Results of the omnibus analysis of imitation performance. Bars represent percentage correct hands movements reproduced by each stimulation condition by time. Error bars indicate standard error. Note: * indicate significance level < .05.

Electrophysiological reactivity

Results of the repeated measures ANOVA indicated a main effect for the factor sequence length: $F(2, 112) = 3.32$, $p = .040$, $\eta_p^2 = .052$. This effect was not further investigated as it is considered outside the interest of the current analysis. A significant interaction was detected between factors stimulation condition and time:

$F(3, 56) = 2.79$, $p = .049$, $\eta_p^2 = .130$. Planned comparisons indicated that ERD changed pre to post only for the sham group ($p = .021$), but not for any other group ($ps > .129$). This result is presented below in Figure 29.

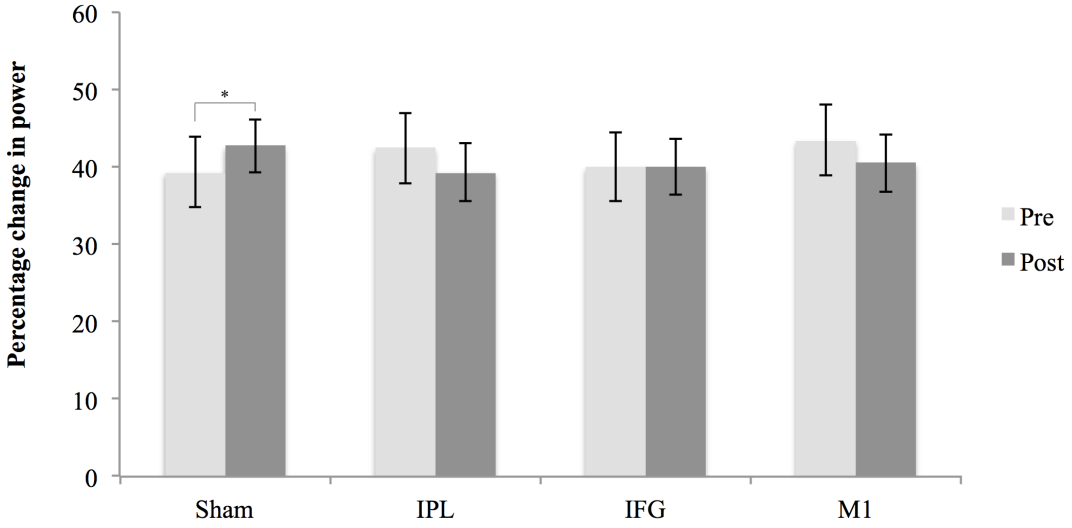

Figure 29: Results of the omnibus analysis of the EEG data. Bars represent percentage change in lower beta in C-channels during observation of hands movements, pre and post stimulation for all stimulation conditions. Error bars indicate standard error. Note: positive values represent ERD.

The results of the ANCOVA revealed a significant effect for both the covariate (pre-stimulation ERD values): $F(1, 60) = 131.76$, $p < .001$, $\eta_p^2 = 0.706$, and for the stimulation condition: $F(3, 60) = 2.77$, $p = .050$, $\eta_p^2 = 0.131$, suggesting that there was a significant effect of stimulation condition on post-stimulation performance after controlling for pre-stimulation performance. However, results of the planned comparisons indicated no significant differences in any comparison ($ps >. 073$).

μ-reactivity and imitation performance

In order to compare the correlations observed between ERD and imitation performance between stimulation conditions, Fisher's z test was conducted. Because

the results of the single analysis indicated mostly significant (IFG was non-significant) correlations in the left hemisphere in β1 in C-channels, these were the parameters for the current analysis. The correlation coefficient observed in the single analysis are reproduced in table 11 below:

Table 11: Correlation coefficients and *p*-values in brackets, between ERD and imitation performance for each stimulation condition.

Stimulation site	*r*-value (*p*-value)
IPL	.553 (.032)
IFG	-.272 (.326)
M1	.553 (.033)

The correlation coefficients for the IFG and M1 are virtually the same suggesting that they are reflecting the same relationship (i.e. the relationship between observation and imitation in the M1 is the same as in the IPL). Due to the similarity of the effect, it was unsurprising that there were no significant difference was detected between the correlation coefficient for the IPL and M1: $z = 0$, $p = .1$. Furthermore, no difference was detected between correlation coefficients for the IFG with either IPL or M1: zs = 0.84, ps = .400, suggesting that the relationships between ERD and imitation performance was not significantly different from each other.

Interim Discussion

The omnibus analysis was conducted in an attempt to compare the effects of tACS on performance and β1-ERD in all the stimulation conditions. In terms of imitation performance, a change in performance pre- to post-stimulation was visible in all groups, although only the IFG group demonstrated a significant change (improved

performance). These findings are consistent with the findings of experiments 4, 5 and 6. When controlling for pre-stimulation differences in performance and comparing the post-stimulation performance between groups, only the IFG group demonstrated significantly higher performance compared to the sham group and compared to pre-stimulation performance. These findings are also consistent with previous experiments, and further support the proposition that the IFG is more related to imitation performance than any of the other nodes.

Moving on to the EEG data, the sham group was the only group that demonstrated a significant change in ERD pre- to post-stimulation. This finding is consistent with previous experiments. However, when comparing post-stimulation ERD between groups controlling for the pre-stimulation ERD, no differences were detected between any groups. Given Experiment 4 (IPL) in which the ANCOVA revealed significantly less ERD post-stimulation in the tACS group compared to sham, and tendency for the same effect on experiments 5 and 6, the failure to detect similar effects in the comparisons here was unexpected. This finding suggests that there were no differences post-stimulation between groups. However, the lack of effect observed in the planned comparisons here could be explained by the stringency of the Bonferroni corrections. In the current analysis, there were substantially more comparisons conducted than in the previous experiments, and as consequence, the effects observed in previous experiments were not robust enough to survive the Bonferroni correction.

Chapter Discussion

In this chapter, an expanded behavioural version of Experiment 1 (observing intransitive hand movement) was developed and tested with respect to three different

areas of the brain associated with the hMNS. The purpose of this was to investigate the contended relationship between μ-suppression during action observation and activity in areas associated with the hMNS (IPL, IFG and M1), with relation to corresponding behaviour (imitation). This relationship was tested in order to investigate whether suppression in μ during action observation is a valid indicator of hMNS-related activity. The EEG literature contains a wealth of studies demonstrating indirectly that μ-suppression during action observation reflects activation of hMNS-related activity, particularly when considering that the BOLD signal correlates with μ-suppression (e.g. Arnstein et al., 2011; Braadbaart, Williams, & Waiter, 2013). However, these studies are correlational in nature and lack causal and more direct evidence, and therefore, the argument that μ-suppression is a valid indicator for hMNS-related activity remains controversial. Causal (and more direct) evidence can be established using brain modulation techniques such as tACS, in which intrinsic neuronal networks can be modulated within a specific frequency. The current chapter presented three experiments in which the method and the control group was the same, but active tACS was applied to three different sites (IPL, IFG, M1). Effects of tACS was assessed by consequent changes in μ and on imitation performance, and compared with sham stimulation for each stimulation site separately. Finally, all were compared in an omnibus analysis.

The results of Experiment 4 (IPL) demonstrated β1-ERD during observation of moving hands, and significantly less ERD subsequent to tACS compared to sham stimulation. No direct effect of tACS was observed on imitation performance, but a significant and positive correlation between β1-ERD and imitation performance was detected following tACS. The results of Experiment 5 (IFG) demonstrated β1-ERD during observation of moving hands, and a tendency was observed suggesting that

tACS decreased β1-ERD. Furthermore, imitation performance was significantly improved subsequent to tACS. Additionally, positive correlations were detected between β1-ERD and imitation performance. The results of Experiment 6 (M1) demonstrated β1-ERD during observation of moving hands, and significantly less ERD was observed subsequent to tACS compared to sham stimulation. Although no significant effect was observed on performance subsequent to tACS, performance visibly improved. Additionally, a significant and positive correlation was detected between β1-ERD and imitation performance post-tACS. These results show that tACS to the M1 and IPL affected β1-ERD more than imitation performance, whilst the IFG demonstrated that tACS affected behaviour more than β1-ERD. When comparing the effects of tACS on all brain sites in an omnibus analysis, it was confirmed that imitation performance was more strongly related to activity in the IFG as no change in performance was detected in any other stimulation group. When comparing the EEG data, only the sham group maintained a significant difference pre to post-stimulation as was found in previous experiments. However, when comparing post-stimulation ERD controlling for pre-stimulation ERD, the effect observed in Experiment 4 (IPL) and the trends observed in experiments 5 (IFG) and 6 (M1) that the tACS groups elicited significantly less ERD compared to sham was absent. It was visible that the effect of tACS applied to the IFG and M1 was substantially weaker compared to the IPL. Lastly, the correlation between β1-ERD and imitation performance detected post-tACS for IPL and M1 was compared with Fishers' z test. The result of this test revealed no difference in effect, suggesting that the relationship between β1-ERD and imitation performance was equally strong in the M1 and IPL. These findings suggest that the IPL and M1 may be responsible for the same processes in regards to observation of hands movements, and that the M1 and IFG are related to similar processes in relation to the ability to imitate. Some implications of

these data are discussed next.

One interpretation of the results is that the IFG and IPL play different roles in the process of matching observations with execution of the same action. Activity of the M1 appears to reflect input from both the IPL and the IFG (although in different aspects), however, it cannot be established whether the μ-ERD pattern observed reflects hMNS-related activity in M1 itself, or whether μ reflected mirror input from the IPL. There is evidence that could lead to either interpretation, including studies reporting that mirror neurons exist in M1 in monkeys (e.g. Tkach et al., 2007; Wahnoun et al., 2006; Dushanova & Donoghue, 2010) and evidence reporting that mirror neurons do not exist in monkeys' M1 (Gallese et al., 1996; Fogassi et al., 2001). Likewise, there are studies in humans suggesting that M1 is involved in hMNS (e.g. Fadiga et al., 1995; Hardwick et al., 2012; Borroni, et al., 2005; Baldissera, 2005; Babiloni et al., 2016; Press et al., 2011), and studies suggesting that the neuronal response in M1 reflects input from hMNS areas (e.g. Braadbart, Williams, & Waiter, 2013) and that mirror-like activity is absent in human M1 (Rizzolatti et al., 1996; Decety et al., 1997). Additionally, it has been proposed that M1 activity reflects mental rehearsal rather than mirroring (Cisek & Kalaska, 2004), and because there is no way to disentangle this possibility, it cannot be rejected as an explanation of M1 results. Note that this issue is further addressed in the future directions section. Activity of the M1 will be further addressed in the general discussion chapter under future directions.

The data in this thesis can be interpreted as evidence that the IPL is more strongly recruited for observation of hand movements, while the IFG is more strongly recruited for processes relating to imitation. This interpretation is in line with several

conceptions: firstly, the IFG and IPL are thought to be responsible for different actions in the process of matching observed actions with existing motor representations (e.g. Rizzolatti & Craighero, 2004); the hMNS circuit has been proposed to contain reciprocal connections enabling both forward and backward communication within the system, and as such, the nodes can be sharing and adjusting information at different levels of processing (Kilner, Friston, & Frith, 2007); it has been demonstrated that parietal and premotor cortices are intra and interhemispherical functionally connected during motor imagery (Szameitat, McNamara, Shen, & Sterr, 2012) and therefore it is possible that the IPL has a more direct route to motor areas where the μ-rhythm was recorded, and lastly; the observation that cortical activity in the IPL but not the IFG correlates with μ-suppression during action observation and execution (Arnstein et al., 2011).

It is proposed that perhaps the activation pattern observed in the current experiments should be considered as an indivisible whole rather than being constructed by its parts. Collectively, tACS induced a decrease in β1-ERD in all of the nodes investigated during observation of moving hands. In addition, β1-ERD was related to performance on the imitation task, suggesting that β1-ERD reflected hMNS-related activity. Although it cannot be said that individual cells (or even assemblies of cells) are doing both - as is the definition of mirror neurons in monkeys (see page 1; e.g. Di Pellegrino et al., 1992) – the activity pattern and the behavioural data presented in this thesis can be interpreted as evidence that the hMNS have separate but interconnected nodes that functionally match activation patterns of mirror neurons in primates. Alternatively, the data can be interpreted as not relating to mirror like activity at all, as none of the nodes demonstrated clearly that observation was related to execution. Instead, the results observed could reflect other motor related processes

such as motor preparation or mental rehearsal (see page 166; Cisek & Kalaska, 2004). However, if this possibility were true, one would expect activity in the M1 to be affected substantially more by tACS than the IPL and IFG as these are not strictly motor areas and this was not the case in the data presented. The results of the current experiments are therefore unlikely to reflect mere motor preparation or mental rehearsal as our results demonstrated that the greatest effect of tACS was in the IPL. Additionally, a trend was observed (in the analysis of individual sites) for tACS to improve performance in the M1, while in the IPL the reverse was observed. The interpretations of the data presented are subject to several methodological limitations as will be discussed in the last chapter.

Some alternative interpretations are necessary to point out in regards to the interpretation made: i) EEG is an indirect tool for investigating neuronal activity and the signal recorded is a summation of activity in nearby neuronal assemblies (Dickter & Kieffaber, 2014). Therefore, the signal cannot differentiate which conceptual neuronal population is responsible for the signal recorded. Furthermore, given that the paradigm involved observing a motor movement with the intention to imitate it, the activity recorded is inevitably involving motor preparation and possibly mental rehearsal. However, that is not to say that the activity did not reflect hMNS activity at least partly. One problem in discounting the possibility that the signal reflected motor preparation exclusively is that; ii) the effects of tACS were observed in β1 even though tACS was applied in IAF. Although both α and β1 have been related to the hMNS (e.g. Puzzo et al., 2010; Cooper et al., 2013; Babiloni et al., 2002), the extent to which this is true is considered controversial given that EEG is an indirect way to investigate neuronal activity. Additionally, it is the narrow bandwidth (8 – 13 Hz) that has been more commonly associated with the hMNS (e.g. Oberman et al., 2005; Perry

& Bentin, 2009; Muthukumaraswamy & Johnson, 2004), while β1 has typically been related to exclusive motor processes such as preparing motor responses (Zhang et al., 2008). Therefore, hMNS sceptics may interpret this finding as evidence that the signal recorded reflected motor preparation. Nevertheless, the fact that β1 reflects motor preparation is not to say that it doesn't reflect hMNS activity. It is known that α and β1 rhythms are correlated and often work in complementary ways (e.g. Crone et al., 1998), and it is therefore interpreted in this thesis that because the task used in experiments 4 – 6 was exclusively motor, it tapped into basic mirroring with little confounding influence of other systems not exclusively motor; therefore, involvement of α was not required. Instead, in experiments 4 – 6, β1 was triggered by the paradigm and further influenced by stimulation in the α-band; lastly, iii) the fact that μ was recorded over M1 could be a problem because the signal recorded could reflect activity from the M1 more strongly than from any other region (based on relative distance). Although this is a possibility, it is not considered likely given that the results suggested that μ was less affected by tACS to the M1 than to the IPL.

In summary, the results of the current three experiments suggest that activity in the IPL, IFG - and possibly the M1 - are interacting during observation and imitation of hands movements as activity in one node was not clearly dissociated from activity in another. This finding is interpreted as evidence that the activity pattern observed reflects hMNS-related activity. Furthermore, activity in the IFG and IPL appears to be responsible for different aspects in the process of matching observed actions with existing motor representations. The question whether the M1 should be included in an extended hMNS cannot adequately be answered based on these findings. In the last chapter, the implications for these findings will be discussed further.

CHAPTER 4: General Discussion

Study Rational and Aims

Direct evidence for the existence of mirror neurons in humans have been documented in the medial frontal cortex and temporal cortices (Mukamel et al., 2010). Activity of mirror neurons on a systems level (hMNS) rather than behaviour of individual cells has long been indicated using TMS (see page 9), neuroimaging (see page 13), and EEG/MEG (see page 19). The focus of this thesis however, was the use of EEG to indicate hMNS-related activity. The EEG index of hMNS-related activity is suppression in the μ-rhythm during action observation (e.g. Muthukumaraswamy & Johnson, 2004; Pineda, 2005). The rationale for this contention is as following: μ is suppressed during both movement and action observation (e.g. Oberman et al., 2005; Perry & Bentin, 2009; Puzzo et al., 2010); cortical activity is observed in hMNS core areas during action observation (e.g. Caspers et al., 2010; Grosbras, Beaten & Eikhoff, 2011; Molenbergs, Cunnington, & Mattingley, 2012); suppression in μ indicates cortical excitation (e.g. Goldman et al., 2002; Steriade & Llinas, 1988); suppression in μ coincides with cortical activation in hMNS core areas (e.g. Arnstein et al., 2011; Babiloni et al., 2016; Braadbaart, Williams, & Waiter, 2013). However, EEG is an indirect measure of neuronal activity and the signal recorded is a summation of neuronal activation in nearby cell assemblies (Kirschstein & Köhling, 2009). Therefore, EEG cannot distinguish between neuronal populations relating to mirror neuron activity or other motor-related activities such as motor preparation or mental rehearsal (Cisek & Kalaska, 2004; Rizzolatti et al., 2014). Consequently, the extent to which the EEG signal recorded in hMNS studies indicates activation of mirror neuron activity is controversial.

The evidence relating μ-suppression to hMNS-related activity includes an abundance of neuroimaging studies (fMRI and PET) demonstrating increased cortical activation in core areas of the hMNS during action observation (Caspers et al., 2010; Grosbras, Beaten & Eikhoff, 2011; Molenbergs, Cunnington, & Mattingley, 2012), and the observation that suppression in μ during action observation coincides with cortical activity in core areas of the hMNS (Arnstein et al., 2011; Braadbaart, Williams, & Waiter, 2013). However, neuroimaging is also an indirect measure of neuronal activation and correlational to behaviour, therefore, more direct and causal evidence may strengthen the claim that μ-suppression is an indication of hMNS-related activity. The aim of this thesis was therefore to validate the use of EEG to indicate hMNS-related activity. This was achieved by stimulating core areas of the hMNS, and relating consequential changes in μ to both observation of movement and execution of movement. A summary of the experiments conducted for the purpose of this thesis is reviewed next.

Summary of Research

Establishing Protocol

Three separate experiments were conducted with the main purpose of identifying a hMNS-related task that efficiently induces μ-suppression (ERD), and also to investigate μ-ERD in relation to behavioural performance on a corresponding task. Relating μ-reactivity with both observation and performance on corresponding behaviour is important in order ascertain that the activity recorded includes mirror neuron activity. Additionally, empathy was investigated in all of these protocols given the contended relationship between empathy and hMNS-related activity. Three experimental protocols were tested to this end, and after data analysis, the most

efficient protocol was selected and used for the next sets of experiments. Each of these experimental protocols are described next.

Experiment 1

Experiment 1 investigated μ-ERD during observation of a hand opening and closing. The movement did not include an object and did not convey a goal, and as such, this protocol tested rudimentary motor mirroring with less ambiguity relating to other processes not exclusively motor in nature. The benefit of this protocol is that its efficiency inducing μ-suppression is well documented (e.g. Oberman et al., 2005; Puzzo et al., 2011; Bernier et al., 2007), but also, the most basic principles of mirror neurons (in monkeys and humans) were addressing simple motor processes (see Chapter 1). Arguably, the literature on basic motor mirroring contains the most convincing evidence because all other functions associated with hMNS have been generalized from the basic principles. As predicted, the results of Experiment 1 indicated selective ERD in μ (α2 and β1) during observation of a moving hand, suggesting that hMNS-related activity was present. Empathy did not affect the ERD observed, and it was speculated whether this finding was a result of the protocol lacking social relevance.

Despite the success in inducing μ-ERD, this protocol did not involve a behavioural measure. Because mirror neurons by definition responds to both observation and execution (Rizzolatti et al., 1996), experiments 2 and 3 included a behavioural measure. Socially relevant stimuli were also used in order to more fully explore the putative role of empathy in modulating μ-reactivity. These are reviewed in turn next.

Experiment 2

Experiment 2 investigated μ-ERD in relation to a social-cognitive task that involved mental state recognition (Reading the Mind in the Eyes Test; RMET), this test however did not include observation of movement per se, but movement indicated by facial muscles in formation of various mental states. It has been reported that observation of movement induces more μ-suppression than static images (see page 38). This protocol was chosen given Pineda and Hecht's (2009) study demonstrating μ-suppression in response to the RMET. In the Experiment 2, μ-ERD during correct and incorrect trials of the RMET were assessed and related to speed and accuracy of the response on the word-matching task. It was reasoned here that if the hMNS is involved in the processing of the RMET, then greater ERD in μ would be indicated for RMET trials that participants got correct. Given that μ-suppression has been reported during the RMET, and related to performance on the RMET (Pineda & Hecht, 2009), the results were not quite as predicted. Firstly, ERD was indicated in β1 while ERS was indicated in α. The ERD in β1 may suggest recruitment of hMNS-related activity, however, ERD in β1 were not selective for correct trials, suggesting that β1 was not sensitive to the understanding of mental states.

Secondly, performance was not correlated with μ-reactivity in any bandwidth, suggesting that observation and recognition of mental states were distinct processes. This finding is not consistent with Pineda & Hecht's (2009) finding. Furthermore, relating ERD in β1 to the hMNS is then difficult given the hMNS theory claiming that motor actions are understood by simulating the movement in the observer's own motor repertoire (Gallese & Goldman, 1998). Based on this theory, the interpretation of mental states should be triggered as a consequence of simulating the muscles used to produce the mental state depicted. However, there are also indications that

different nodes of the hMNS are responsible for different aspects of the observation/matching process (e.g. Hamilton & Grafton, 2006; Decety et al., 2002; Iacoboni et al., 2005; Fadiga et al., 2006), and therefore it is plausible that observation and execution of corresponding behaviour (in this instance inferring mental states) are distinct processes but also related to the same system.

Furthermore, the results of Experiment 2 were difficult to interpret for the following reason: ERS was observed in sensorimotor α rather than ERD, yet α-ERD was observed in the occipital area. ERS in α has been related to cognitive inhibition processes (e.g. Klimesch et al., 2007; Cooper et al., 2002), and therefore, this result can be interpreted as sensorimotor α indicating inhibitory processes rather than hMNS-related ones as. In this instance, inhibitory processes may have been complementing suppression in β1 as it is known that observation of facial expressions tends to result in automatic imitation of the expression observed (e.g. Dimberg, Thunberg, & Elmehead, 2000; Neumann, et al., 2014; Heyes, 2011). The lack of predictability and consistence with previous research led to this experimental protocol being excluded from the selection process of the most efficient protocol. Lastly, as in Experiment 1, individual level of empathy did not modulate μ-reactivity in this experimental protocol and therefore it appears that actions observed in a socially relevant context are not more optimal in demonstrating a relationship between hMNS-related activity and affective empathy, than actions that are not.

Experiment 3

Experiment 3 investigated μ-ERD in relation to a social-perceptive task that involved recognising social interactions that were either positive or negative from point-light biological motion videos. This protocol was based on two approaches inducing

hMNS-related activity: social interactions (e.g. Iacoboni et al., 2004; Oberman, Pineda, & Ramachandran, 2007) and point-light biological motion displays (PLBM; e.g. Saygin et al., 2004; Ulloa & Pineda, 2007). It was reasoned that the hMNS is involved in inferring meaning from motion cues and in social interactions, and so μ-reactivity should be involved in inferring meaning of social interactions depicted by motion cues (PLBM). Additionally, it has previously been demonstrated that hMNS-related activity is more sensitive to positive expressions compared to negative (e.g. Niedenthal et al., 2010; O'Doherty et al., 2003), and therefore it was predicted that positive PLBM social interactions would trigger greater ERD in μ.

As predicted, greater μ (α2) ERD was observed during observation of PLBM social interactions compared to scrambled versions, suggesting that hMNS-related activity may have been involved. Additionally, the ERD observed was selective for positive social interactions, corroborating previous research. However, μ-reactivity and performance on the word-matching task were not related, suggesting that observation and the ability to interpret PLBM social interactions were distinct processes. This finding is in line with Experiment 2 in that there was no relation observed between μ-reactivity and performance on the corresponding task. Therefore, relating the observed α2-ERD to hMNS-related activity is then difficult given the same reasons provided in the discussion above relating to Experiment 2. However, these findings are not interpreted here as evidence that hMNS-related activity was absent. Instead, it is suggested that the tasks used in these experiments, i.e. mental state inference (Experiment 2) and meaning of social interactions (Experiment 3), may have been too far removed from the basic principle of mirror neurons (i.e. single cells respond to both observation and execution of the same movement). What was actually tested in experiments 2 and 3 was not the neuronal response to both observation and execution,

it was neuronal response during observation with performance on corresponding behaviour.

Furthermore, although it has previously been indicated that social-cognition is more related to hMNS-related activity than is social-perception (Pineda & Hecht, 2009), the data presented in experiments 2 and 3 is not consistent with this claim, as the results of Experiment 3 were more predictable and clear compared to Experiment 2. However, note that the protocol used in Experiment 2 did not include movement, and this may have been the reason that the protocol performed less well. Lastly, empathy did not modulate μ-reactivity or performance in either experiments 2 nor 3. It was speculated in Experiment 1 that a lack of social relevance was the reason μ-reactivity was not modulated by levels of affective empathy. Given that neither experiments 2 nor 3 indicated that empathy modulated μ-reactivity, it is unlikely that social relevance is responsible for the lack of such modulation. Instead, it is assumed that affective empathy is not related to μ-reactivity in either of these conditions. It may well be that another type of empathy would be more suitable, such as cognitive type empathy, but a discussion of this is outside the scope of the thesis.

In summary, all of these three experimental protocols could be interpreted as inducing μ-ERD albeit to varying extents. Whether or not this μ-ERD reflects hMNS-related activity is debateable and was not the purpose of these initial experiments. Whether μ-ERD reflects hMNS-related activity was the purpose of the next sets of experiments. The selected experimental protocol to use as a basis for the next set of experiments was Experiment 1 given that the outcome of this experiment was the clearest and most predictable.

Investigating Causal Evidence

The experimental protocol used for these experiments were developed based on Experiment 1 in which a single hand opened and closed. It was decided to extend this into an imitation task for following reasons: although indirect, a relationship between imitation and hMNS-related activity is well-documented (e.g. Iacoboni et al., 1999; Iacoboni, 2005; Liepepelt, Prinz, & Brass, 2010); imitation of movement is closer related to the basic principles of mirror neurons (that activity relates to both observation and execution of the same movement); and lastly, incorporating an imitation task following observation of a movement is relatively easy and provides an objective measure of behaviour.

The modified protocol included two hands (left and right) in order to increase task demands. The two hands opened and closed (one at the time) in sequences ranging from 6 to 8 movements based on a pilot study that suggested that 5 movements in a sequence was too easy, and 8 movements in a sequence was too hard. The results of the pilot study therefore fit nicely with George Miller's (1956) classic theory of working memory (see page 115). The imitation aspect was included directly after presentation. This protocol was designed to investigate the contended relationship between μ-ERD and hMNS-related activity. For this purpose, tACS was applied to each of the core areas of the hMNS (IPL, IFG, M1) and the consequential changes in μ-reactivity and in the ability to imitate were assessed and compared to sham stimulation. It was reasoned that if μ-ERD indicates hMNS-related activity, then both μ-reactivity and performance on the imitation task should be affected by the stimulation. Stimulation to the hMNS core areas were investigated separately in three different experiments, using a similar method, and the same control group. Each experiment is described next.

Experiment 4

Experiment 4 investigated the effects of tACS to the IPL and consequential changes in μ-reactivity and on the ability to imitate. The sham group demonstrated a significant increase in β1-ERD subsequent to stimulation, but no change was detected in the tACS group. Despite this, significantly less β1-ERD was observed subsequent to tACS compared to sham, suggesting that tACS interrupted processes involved in facilitating β1-ERD. This finding is consistent with the finding that rTMS to the IPL leads to a decrease in β1-ERD (Puzzo et al., 2013), and therefore the effect was considered more robust. Furthermore, no effect of tACS was observed on imitation performance directly, but a significant and positive correlation between β1-ERD and imitation performance was observed post-tACS in the left hemisphere. Therefore, activity in the IPL was related to the ability to imitate at least indirectly. This finding supports the finding that imitation performance relates to suppression in μ (Bernier et al., 2007; 2013), and that the μ-ERD observed is hMNS-related. However, given that μ-rhythms are modulated by activity in both the IPL and the IFG, and the correlation was only apparent after the IPL was disrupted; it was proposed that activity in the IFG might be driving this correlation. This issue was further investigated in the next experiment, investigating the IFG.

Experiment 5

Experiment 5 applied the same method and control group as Experiment 4, but stimulated the IFG rather than the IPL. The results here indicated that β1-ERD decreased subsequent to tACS in a similar vein to the IPL, although the effect was weaker. Given that μ reflects activity in both IPL and IFG, and that the effect in IFG was weaker than in the IPL; the finding was interpreted as evidence that the IPL was more strongly related to activity during the observation than the IFG. Furthermore,

the ability to imitate was significantly improved subsequent to tACS, confirming the proposition that the IFG is more strongly related to the ability to imitate. Consistent with the work of Bernier and colleagues (2007; 2013), performance on the imitation task correlated with β1-ERD in the left hemisphere in this experiment. This finding corroborates the notion that imitative abilities are controlled by the left IFG (see meta-analysis by Caspers et al., 2010; Pobric & Hamilton, 2006), and that the μ-reactivity observed reflects hMNS-related activity. However, note that the tACS was delivered to the left hemisphere, and therefore tACS could be confounding this interpretation.

Experiment 6

Experiment 6 applied the same method as experiments 4 and 5, but stimulated M1. The rationale for stimulating M1 - even though M1 is not traditionally considered a hMNS core area - was that μ-rhythms are generated in the M1, and as such reflect motor-related processes. Additionally, it has been suggested that mirror neurons exist in the M1 (e.g. Montagna et al., 2005; Press et al., 2011; Szameitat et al., 2012). This is partly based on the discovery of mirror neurons in M1 in monkeys (e.g. Tkach et al., 2007; Dushanova et al., 2010). However, it has been argued that in the human M1, the reactivity to action observation may reflect mirror input from hMNS core areas, and not mirror activity per se (e.g. Lepage, Lortie, & Champoux, 2008; Muthukumaraswamy & Johnson, 2004; Pineda, 2005). This experiment was therefore conducted to enlighten the role of M1 in action observation, and whether its activity can be related to the hMNS or whether it receives hMNS input. It was expected to observe an effect of tACS to the M1 given that the M1 generates μ-rhythms, and because it is known that the M1 is directly involved in the control and generation of voluntary movement (Hatsopoulos & Suminsky, 2011). Given that the presentation

involves hands movements, it is inevitable that activity in the M1 is involved. What could help disentangle motor-related properties with mirror properties is the relation between μ-ERD and imitation performance, and also the pattern of μ-ERD in relation with the IPL and the IFG. It was reasoned that if the M1 includes hMNS-related activity, then the amount of change after tACS and the relation between μ-reactivity and imitation performance should be larger than what was observed in the IPL and the IFG. However, if the M1 receives input from the IPL and IFG then the pattern of μ-reactivity after tACS and the relation with imitation performance should reflect the patterns seen in the IPL and the IFG. That is, imitation performance should resemble the finding observed in the IFG, and μ-ERD should resemble the finding observed in the IPL. The results indicated that while the EEG data were similar to the IPL, the behavioural data were more similar to the IFG. It is therefore tempting to conclude that the M1 receives mirror-like input from the IPL and the IFG rather than reflecting mirror like activity itself. However, it is also possible that the M1 was involved in both aspects. The type of data presented in this thesis cannot disentangle with much certainty which possibility is more likely, and therefore it is concluded that this issue must be further investigated in future studies.

In all of these experiments, a link with imitation performance and μ-ERD was observed on the left hemisphere, albeit to differing extents. Note that this effect could have been due to electrode position as the stimulation electrodes were always positioned over the left hemisphere, and the other electrode over the contralateral frontal polar region.

Interpretations

There are a number of interpretations offered in this thesis, but in relation to the overarching aim, the main interpretation of the data were that EEG is a valid tool for indicating hMNS-related activity. This interpretation is based on experiments 1 – 3 in which suppression in μ was observed during action observation, but more importantly, experiments 4 – 6 in which demonstrated that suppression in μ relates to both observation and execution of the same movement (imitation). This was demonstrated by applying tACS to core areas of the hMNS in which resulted in establishing a direct relationship between core areas of the hMNS and μ, and between μ and imitation performance. There is however a caveat to this interpretation, and that is as follows: although μ-ERD related to both observation and execution of the same movement, it was indicated that activity in each of the nodes were more related to one aspect than the other. That is, the IPL appeared to be more strongly related to observation of hands movements while the IFG appeared to be more strongly related to the ability to imitate the hands movements. Because the current findings did not clearly demonstrate that activity in one area were related to both observation and execution, one cannot conclude that the data are supportive of mirror neurons per se, as the definition of mirroring requires both (Rizzolatti et a., 1996). However, despite that μ is modulated by activity in both the IPL and the IFG, μ-reactivity is interpreted in this thesis on a systems level, rather than on its individual components. As such, μ-ERD related to both observation of movement and execution of the same movement, and that is consistent with the defining features of hMNS.

Furthermore, the reactivity patterns of these nodes could not be completely dissociated, suggesting that the activity in one node was influencing activity in the other. Therefore, it is argued here that it is useful for the hMNS to be considered in its

entirety and not by its components. That is because if activity patterns within one node are considered to the exclusion of the other, the essential property of the system is lost; the observation/execution matching (mirroring).

The finding that individual nodes of the hMNS are responsible for different aspects of observation/matching is consistent with previous literature (e.g. Hamilton & Grafton, 2006; Decety et al., 2002; Iacoboni et al., 2005; Fadiga et al., 2006), and with the view that the hMNS is different to that in monkeys (e.g. Hickok, 2008; Oztop, Kawato, & Arbib, 2013; Gazzola et al., 2007). Granted that the IPL was more strongly related to observation while the IFG was more strongly related to imitation, it can also be inferred that the hMNS is not a rigid system in which information is received by the STS and sent to the IPL, which subsequently transmits information to the IFG, and eventually to the M1 (see page 3). Rather, based on the data presented in experiments 4 – 6, the hMNS appears to be a system that allows both forwards and backwards communication as was suggested by Kilner, Friston, and Frith (2007). This can be inferred based on the finding that the correlation observed in the IPL relating μ-reactivity to imitation is a backward communication rather than forward, because the correlation is assumed to be driven by activity in the IFG.

The data gathered from M1 suggested that M1 may receive mirror input rather than containing mirror activity per se, because the activity pattern resembled the IPL in regards to β1-ERD during observation, and in regards to imitation performance the results resembled the IFG. However, this conclusion cannot be drawn with much certainty given that the results were at trend levels. In addition, tACS induced effects on μ (particularly β1) in response to motor movements is inevitable given that M1 is responsible for control and generation of voluntary movement (Hatsopoulos &

Suminsky, 2011), and because μ-rhythms are generated in M1 (e.g. Cheyne et al., 2003; Hari et al., 1998; Rossi et al., 2002). It is known that β-suppression is involved in preparing motor responses and inhibition (Zhang et al., 2008), and therefore it was not surprising that the effect of tACS was β1 specific. The results for the M1 part of the study are rendered inconclusive and more research is needed to disentangle whether the M1 is involved in the hMNS or whether it receives hMNS-related input.

Moving away from the M1 data specifically, another interpretation offered is in relation to the theory suggesting that the understanding of observed movements is the product of simulating the movement in the observer's own motor repertoire (Gallese & Goldman, 1998). Results of experiments 2 and 3 are inconsistent with this theory because neither of these experiments demonstrated a relationship between μ-reactivity during observation with performance on the tasks. However, results of experiments 4 – 6 were consistent with the simulation theory. The discrepancy between these results are interpreted as a consequence of the different tasks used: in experiments 2 and 3 the task did not involve execution of the action observed and may therefore have been too far removed from the basic principles of mirroring. In contrast, the task used in experiments 4 – 6 was directly related to observation of the movement, and therefore these experiments may have tapped more directly into basic mirror properties. The implication of this is that the application of basic mirroring properties to higher cognitive functions such as action understanding per se is unsupported by the data presented in this thesis, and consequently urges caution in relating hMNS-related activity beyond basic mirror properties. Others have reported similar interpretations of the application of basic properties to higher order functions (e.g. Hickok, 2008; 2013; Hickok & Hauser, 2010).

The finding that experiments 2 and 3 failed to demonstrate a relationship between μ-reactivity and performance on corresponding tasks is consistent with several other studies demonstrating that action understanding deviates from action observation. For example, mirror-like responses can be learned without affecting understanding of the action (e.g. Catmur, Welsh, & Heyes, 2007; Venezia, Matchin, & Hickok, 2012), and likewise that understanding of an action is not dependent on the ability to perform the action in question (e.g. Buccino et al., 2004; Hare & Woods, 2013). Therefore, if it is possible to alter a motor response without affecting its perception, then the motor responses cannot be at the basis of its perception.

The relationship observed between μ-ERD during hands movements observation with performance on the imitation task can however be considered in line with Hamilton's (2013) view that the hMNS may function to prepare a social response. Although observation of two hands opening and closing does not require a social response; imitating another individual performing this movement arguably is of social relevance (in the context of the experiment as a whole). Participants in experiments 4 - 6 observed hands movements with the intention to imitate the sequence of movements, and therefore it can be said that they were preparing a social response as they were preparing to do what was asked of them.

The last interpretation offered is in regards to the roles α and β play in relation to action observation. It is known that generators of α and β differ (Hari, 2006; Avanzini et al., 2012) and that they are responsible for different tasks: β suppression has been related to response preparation and inhibition (Zhang, Chen, Bressler, & Ding, 2008) and maintenance of the current sensorimotor or cognitive state (Engel & Fries, 2010), while α-suppression has been associated with processes not exclusively motor in

nature such as goal-directed movements and transitive movements (Muthukumaraswamy & Johnson, 2004; Oberman et al., 2005; Decety et al., 1997; Grèzes, Costes, & Decety, 1998). It was highlighted on page 51 that the lower end of the μ-spectrum may be more related to goal-directed and transitive hand movements, while the higher end of the spectrum may be more related to intransitive hand movements. The data presented in this thesis corroborates this view as experiment 1 demonstrated ERD in α2 and β1 during observation of a single hand opening and closing and experiments 4 – 6 demonstrated ERD in β1 during observation of two hands opening and closing. The other experiments did not involve observation of hands movements and were therefore not included in this discussion. Despite that μ consists of both α and β1, the majority of the literature using EEG as a tool to investigate hMNS-related activity investigates a narrow bandwidth (i.e. 8 – 13 Hz). Based on the data presented in this thesis, it is advocated for the use of the full μ spectrum (i.e. both components of μ) when investigating EEG as a tool to indicate hMNS-related activity.

Contributions to Literature

The specific contribution of this thesis to the hMNS literature (particularly as indicated by EEG) is that it validates the use of EEG in indicating hMNS-related activity. It also demonstrates that both α and β1 should be investigated to this end rather than the commonly used narrow bandwidth (8 – 13 Hz). Furthermore, this thesis enhances current understanding of the hMNS, its mechanism, and highlights the specific role of the IPL and the IFG within this system. Although it is rendered inconclusive whether M1 receives mirror-like input or contains mirror activity, the thesis provided evidence that may guide future research. The data presented in this thesis extends the majority of previous research in this area, as it investigated activity

in all of the core areas of the hMNS in relation to action observation and execution/imitation, rather than one individual node exclusively. The benefit of investigating all of the core areas is that it enabled an investigation of the hMNS in its entirety and on a systems level. The outcome of doing so demonstrated that the hMNS is a system that collectively contains mirror properties. The hMNS should therefore be investigated as a whole rather than by its parts.

Limitations

The limitations in regards to experiments 1 – 3 were discussed in their respective chapter discussions, and will not be reiterated here because the main interpretation of the thesis was mostly based on the results of experiments 4 – 6. The first limitation in regards to these experimental protocols were that EEG was recorded during observation only, and execution was indirectly measured by performance on an imitation task. It is possible that this method was not tapping directly into mirroring properties as the defining feature of mirroring is that activity overlaps for observation and execution. In defence of the protocol used, it is tricky to record EEG during execution due to movement related artefacts, and because the activity observed is inevitably going to show motor related activity and therefore the signal recorded is likely to overshadow any hMNS-related activity. Using a measure of performance rather than reactivity during actual performance may be a limitation, yet the results demonstrate that reactivity during observation relates to performance. And therefore, the interpretation that this activity pattern reflects hMNS is still substantial.

Another possible limitation is that the imitation task used required more memory processes than mirroring processes. The task inevitably required working memory, as the task involved participants remembering a list of items (in this case movements) in

a specific order, and then reproducing the movements in the correct order. Such serial recall is a classic example of working memory, which refers to the temporary storage and manipulation of information necessary for a current cognitive task (Baddeley, 1992). It is known that both α and theta (θ) is involved in working memory processes (Klimesch, 1999). During such memory tasks, event-related suppression is observed in α while an increase is observed in θ. Note that for the interest of this thesis, the focus here is on α. During actual task demands, α-suppression correlates positively with task demands, that is, the greater the cognitive load, the larger the suppression (Stipacek, Grabner, Neuper, Fink, & Neubauer, 2003; Klimesch, 1999; Boiten, Sergeant, & Geuze, 1992). Given that the results of experiments 4-6 all demonstrated that larger suppression coincided with greater number of items to remember and reproduce, it is possible that a considerable amount of the suppression observed reflected task demand rather than mirroring. It may have been more fruitful to investigate movement kinematics as opposed to movement sequences. This way, task demand may have confounded the results less.

Lastly, the use of offline tACS could be construed as a limitation given that online effects are better understood (see page 104). Offline effects were described on page 105 and are possibly related to long-term potentiation such as STDP rather than local processing. It is conceivable that the consequence of stimulating neuronal assemblies using the current methodology lead to the activity patterns between nodes appearing more similar than what they could have appeared as, if applying tACS online. However, given that after-effects remain poorly understood, this methodology may not even be a limitation as it is not yet clear exactly what tACS after-effects reflects. It is also possible that tACS induced after-effects are more appropriate than online tACS for the purpose intended. The only thing that is clear in this regard is that more

research is needed to determine the consequences of tACS after-effects on neuronal networks.

Future Directions

Given the difficulty differentiating neuronal activation relating to hMNS and motor properties, and likewise differentiating mental rehearsal from mirroring, future research should aim to investigate more directly the relation between EEG signal and cortical activations of different conceptual neuronal activity. Studies of this nature would need to involve intracortical investigations, and studies of this type are rare given the invasiveness. Studies like these are only justified in cases which require investigations of faulty neuronal activation in relation to neuronal conditions such as epilepsy (e.g. Babiloni et al., 2016; Mukamel et al., 2010). There are however other more indirect approaches that may further investigate the interpretation offered in this thesis, such as neuronal habituation in which it is assumed that sensory neurons habituate (adapt) and become less active when the stimuli they code are presented repeatedly (Grill-Spector & Malach, 2001). It may be possible to investigate such neuronal activation suppression in relation with μ-suppression. The outcome of such demonstration could potentially discount the possibility that μ-suppression during observation of movements relates to motor preparation exclusively, and consequently strengthen then interpretation that the μ-ERD observed in the current experiments reflects hMNS activity.

Another future direction relates to the question whether M1 receives mirror input or contains mirror activity. Again, single cell studies would be the optimal way of investigating this further, but because such experiments are implausible; other less invasive methods can be suggested in its place. The literature is currently lacking

studies investigating all of the core areas of the hMNS in relation to each other, with regards to action observation and execution. EEG in combination with fMRI could potentially clarify the role of M1 during observation and execution further than was possible in the current study. With this approach, it could be possible to relate μ-suppression with cortical activity in each area at the same time. There are currently only two such studies (Arnstein et al., 2011; Braadbart, Williams, & Waiter, 2013) and one other which investigated electrocorticography (Babiloni et al., 2016). The results of these studies are however not consistent, and therefore more research is required to understand what role M1 plays in relation to hMNS.

In order to further investigate the hMNS in its entirety, another brain stimulation approach could be used. The effect of stimulating the core areas on the relationship between observation and execution could also be investigated by online tACS effects. That is, applying tACS directly preceding the behavioural task. Such an approach could potentially ascertain the finding that the core areas of the hMNS are responsible for different aspects of the hMNS yet involved in the same system, rather than being a manifestation of long-term potentiation that remains poorly understood.

Lastly, the extent to which observation and execution of hands movements in the context of this thesis can be related to Hamilton's (2013) view that the hMNS functions to prepare a social response should be investigated in studies that applies motor movements that are in a socially relevant context as opposed to basic motor movements as was investigated here. Such designs could focus on hands movements that indicates social gestures. Social gestures are hand movements that do not require involvement of an object, yet depict a social intention. The outcome of this approach

may enable a comparison between simple motor preparation and social response preparation.

Final Summary and Conclusion

This thesis in summary included six experiments designed to validate the use of EEG to indicate hMNS-related activity. The first three experiments investigated three different experimental protocols with the aim of establishing one protocol that induces μ-suppression efficiently to be used in the next three experiments. The experimental protocol that most efficiently induced μ-suppression was the observation of an intransitive hand movement (Experiment 1). It was proposed that this protocol involves basic motor mirroring and was therefore more suitable than the other protocols. As this protocol did not include an objective measure of behavioural performance, for the purpose of the next three experiments, the hand movement observation protocol was altered to include an imitation task. Experiments 4 - 6 were designed to test the relationship between core areas of the hMNS and μ-reactivity, and the relation between μ-reactivity during observation of hands movements and the ability to imitate them. This was achieved by stimulating each node with tACS and assessing consequent changes in μ-reactivity during observation of hands movements and on imitation performance. In conclusion, the data presented in this thesis is interpreted as evidence that EEG is a valid tool for indicating hMNS-related activity. This interpretation is under the condition that μ reflects mirroring on a systemic level including influences from both the IPL and the IFG. Furthermore, it is urged that careful considerations should be taken when applying basic mirroring properties to higher order functions as action understanding. Lastly, the full spectrum of μ should be considered when indicating hMNS-related activity using EEG.

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Appendices

Appendix 1: Interpersonal Reactivity Index (IRI: Davis, 1983)

Below is a list of statements. Please read each statement very carefully and rate how well they describe you.

	The following statements describe me…				
	NOT WELL	LESS WELL	NOT SURE	WELL	VERY WELL
1. I daydream and fantasize, with some regularity, about things that might happen to me.					
2. I often have tender, concerned feelings for people less fortunate than me.					
3. I sometimes find it difficult to see things from the "other guy's" point of view					
4. Sometimes I don't feel sorry for other people when they are having problems.					
5. I really get involved with the feelings of the characters in a novel.					
6. In emergency situations, I feel apprehensive and ill-at-ease.					
7. I am usually objective when I watch a movie or play, and I don't often get completely caught up in it.					
8. I try to look at everybody's side of a disagreement before I make a decision.					
9. When I see someone being taken advantage of, I feel kind of protective toward them.					
10. I sometimes feel helpless when I am in the middle of a very emotional situation.					
11. I sometimes try to understand my friends better by imagining how things look from their perspective.					
12. Becoming extremely involved in a good book or movie is somewhat rare for me.					
13. When I see someone get hurt, I tend to remain calm.					
14. Other people's misfortunes do not usually disturb me a great deal.					
15. If I'm sure I'm right about something, I don't waste much time listening to other people's arguments.					
16. After seeing a play or movie, I have felt as though I were one of the characters.					
17. Being in a tense emotional situation scares me.					
18. When I see someone being treated unfairly, I sometimes don't feel very much pity for them.					
19. I am usually pretty effective in dealing with emergencies.					

20. I am often quite touched by things that I see happen.					
21. believe that there are two sides to every question and try to look at them both.					
22. I would describe myself as a pretty soft-hearted person.					
23. When I watch a good movie, I can very easily put myself in the place of a leading character.					
24. I tend to lose control during emergencies.					
25. When I'm upset at someone, I usually try to "put myself in his shoes" for a while.					
26. When I am reading an interesting story or novel, I imagine how I would feel if the events in the story were happening to me.					
27. When I see someone who badly needs help in an emergency, I go to pieces.					
28. Before criticizing somebody, I try to imagine how I would feel if I were in their place.					

Appendix 2: Reading the Mind in the Eyes Test (RMET: Baron-Cohen et al., 2001)

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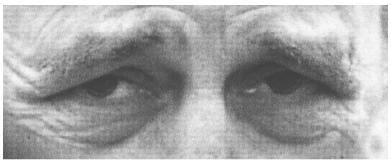
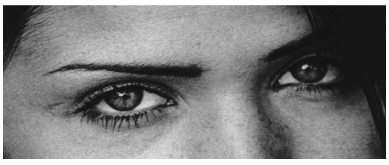
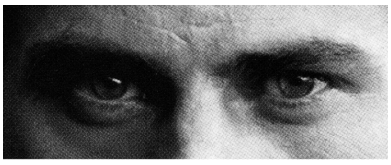
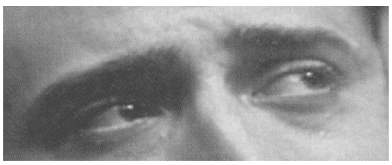
2 

3 
4 

5 
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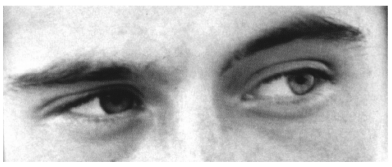
7 
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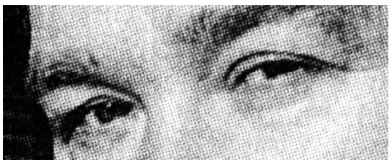
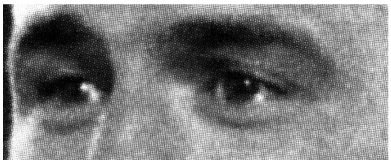
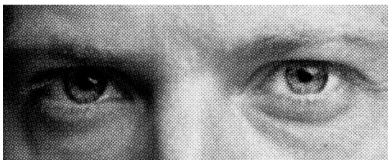
12 

13 
14 

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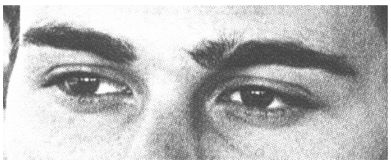
16 

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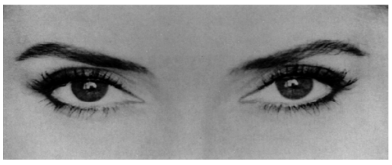
18 

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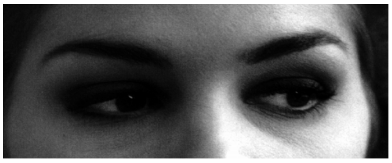
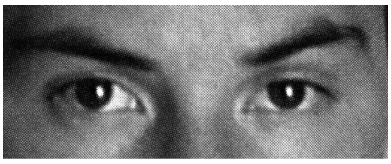
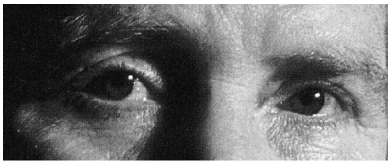
22 

23 

24 

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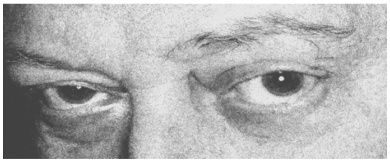
26 

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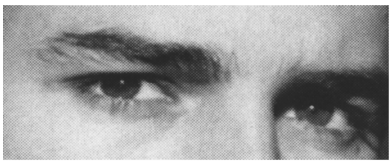
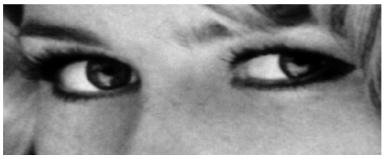
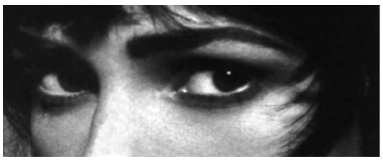
33 

34 

35 

36 

		Answers - Adults			
P	jealous	**panicked**	arrogant	hateful	**M**
1	**playful**	comforting	irritated	bored	**M**
2	terrified	**upset**	arrogant	annoyed	**M**
3	joking	flustered	**desire**	convinced	**F**
4	joking	**insisting**	amused	relaxed	**M**
5	irritated	sarcastic	**worried**	friendly	**M**
6	aghast	**fantasizing**	impatient	alarmed	**F**
7	apologetic	friendly	**uneasy**	dispirited	**M**
8	**despondent**	relieved	shy	excited	**M**
9	annoyed	hostile	horrified	**preoccupied**	**F**
10	**cautious**	insisting	bored	aghast	**M**
11	terrified	amused	**regretful**	flirtatious	**M**
12	indifferent	embarrassed	**sceptical**	dispirited	**M**
13	decisive	**anticipating**	threatening	shy	**M**
14	irritated	disappointed	depressed	**accusing**	**M**
15	**contemplative**	flustered	encouraging	amused	**F**
16	irritated	**thoughtful**	encouraging	sympathetic	**M**
17	**doubtful**	affectionate	playful	aghast	**F**
18	**decisive**	amused	aghast	bored	**F**
19	arrogant	grateful	sarcastic	**tentative**	**F**
20	dominant	**friendly**	guilty	horrified	**M**
21	embarrassed	**fantasizing**	confused	panicked	**F**
22	**preoccupied**	grateful	insisting	imploring	**F**
23	contented	apologetic	**defiant**	curious	**M**
24	**pensive**	irritated	excited	hostile	**M**
25	panicked	incredulous	despondent	**interested**	**F**
26	alarmed	shy	**hostile**	anxious	**M**
27	joking	**cautious**	arrogant	reassuring	**F**
28	**interested**	joking	affectionate	contented	**F**
29	impatient	aghast	irritated	**reflective**	**F**
30	grateful	**flirtatious**	hostile	disappointed	**F**
31	ashamed	**confident**	joking	dispirited	**F**
32	**serious**	ashamed	bewildered	alarmed	**M**
33	embarrassed	guilty	fantasizing	**concerned**	**M**
34	aghast	baffled	**distrustful**	terrified	**F**
35	puzzled	**nervous**	insisting	contemplative	**F**
36	ashamed	nervous	**suspicious**	indecisive	**M**

Appendix 3: Transcranial Magnetic Stimulation Adult Safety Screen (TASS; Keel, Smith, & Wassermann, 2001).

TRANSCRANIAL MAGNETIC STIMULATION ADULT SAFETY SCREEN

Please read all questions carefully and answer all questions honestly.
All responses will be kept **strictly confidential**.

	Yes	No
1. Have you ever had an adverse reaction to TMS?		
2. Have you ever had a seizure?		
3. Have you ever had an EEG?		
4. Have you ever had a stroke?		
5. Have you ever had a head injury (include neurosurgery)?		
6. Do you have any metal in your head (outside of the mouth) such as shrapnel, surgical clips or fragments from welding or metal work?		
7. Do you have any implanted devices such as a pacemaker, medical pump, or intracrdiac lines?		
8. Do you suffer from frequent or severe headaches?		
9. Have you ever had any other brain-related condition?		
10. Have you ever had any illness that caused a brain injury?		
11. Are you taking medications?		
12. If you are a woman of childbearing age, are you using any method of birth control?		
13. Does anyone in your family have epilepsy?		
14. Do you require further explanation of TMS and its associated risks?		

Appendix 4: Number of hands movements, and order of left vs. right movement in a sequence used in experiments 4 – 6.

6 sequences	Pre-stimulation	LRRLLR
		LLLRLL
		RLRRLR
		LRLRLL
	Post-stimulation	LRLLLL
		LLLRLR
		RRLLRR
		RLRLRR
7 Sequences	Pre-stimulation	LRLRRRR
		RRLLLRL
		LLRRRLR
		RRLRLRR
	Post-stimulation	RLRLLLR
		LRLRRRL
		RRRLLRR
		LLRLRLL
8 sequences	Pre-stimulation	RLLLRLRL
		LRLRRRRL
		LLLRLRLL
		LRLRRRLL
	Post-stimulation	RLRRLLLL
		RRLRLLRL
		LRRRLRLR
		LLRLRLLL

Appendix 5: Publications and presentations

Berntsen, M., Cooper, N., & Romei, V. (In Review). tACS to the IPL decreases mu suppression to egocentric, but not allocentric hands movements. *Neuroscience*

Berntsen, M., Cooper, N., & Romei, V. (submitted). Mu suppression relates to observation but not interpretation of social interactions. *Brain and Behaviour*

Berntsen, M., Cooper, N., & Romei, V. (2015). Stimulating the parietal node of the human mirror neuron system (hMNS). Poster presented at the BACN Annual Conference Essex, September 2015.

Berntsen, M., Cooper, N., & Romei, V. (2014). The effect of modulating the inferior parietal node of the human mirror neuron system on imitation performance. Poster presented at the BACN Annual Conference Nottingham, September 2014.

Berntsen, M., Cooper, N., & Romei, V. (2013). Point-light motion cues of mental states on the human mu rhythm. Poster presented at the BACN Annual Conference Nottingham, April 2013.