On the functional role of visual and motor areas in the process of movement recognition

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I hereby declare that I have written this thesis autonomously and without any unauthorized help. The references and help used are cited in their entity. This thesis has not been submitted to any other faculty, and I guarantee that it will not be published before the completion of the promotion procedure.

Düsseldorf, on Dec. 2012

Anastasia Pavlidou

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Glossary

BM	Biological Motion
EEG	Electroencephalography
ERPs	Event Related Potentials
fMRI	Functional Magnetic Resonance Imaging
Hz	Hertz
IPL	Inferior Parietal Lobule
mA	Milliampere
MEG	Magnetoencephalography
MEPs	Motor Evoked Responses
MNS	Mirror Neuron System
ms	Milliseconds
MT+/V5	Motion Sensitive Area
PET	Positron Emission Topography
PLD	Point-light Display
PMC	Premotor Cortex
RTs	Reaction Times
SQUIDs	Superconducting Quantum Interference Devices
STG	Superior Temporal Gyrus
STS	Superior Temporal Sulcus
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation

Abstract

Movement recognition is thought to be a process in which visual information is integrated with one's own personal motor repertoire. This process is thought to take place within the mirror neuron system (MNS), which comprises of the superior temporal sulcus (STS), inferior parietal lobule (IPL), and premotor cortex (PMC). Here, three studies using the point-light display (PLD) method addressed open questions on the functional role of the MNS areas and their interactions during the recognition of biological movements (BM). In the first two studies we recorded magnetoencephalography (MEG) brain activity while participants differentiated between three variations of a single PLD movement varying in its degree of plausibility. The third study employed transcranial direct current stimulation (tDCS) on PMC to investigate PMC's role in the visual processing of different forms of BM (human vs. bird), as well as, its interpretation in the distinction between natural vs. unnatural PLD movements.

In the first study participants were asked to differentiate between plausible (natural human movements) and scrambled (random dot motion) movements. Significant differences were observed in gamma (55-95 Hz), beta (20-35 Hz) and alpha (9-13 Hz) power, between 500-1300 milliseconds (ms) in parieto-occipital, sensorimotor, and left temporal areas respectively for the plausible condition. Furthermore, positive trial-by-trial power coupling was observed only for the plausible movement between sensorimotor beta and parieto-occipital gamma, as well as, left-temporal alpha.

In the second study participants had to distinguish between two visually similar PLD movements, differing slightly in their degree of biomechanically plausibility. Significant differences were found in the beta power (~20 Hz), between 1650-2650 ms in left-temporal, parieto-occipital and sensorimotor areas successively.

Taken together these two MEG studies reveal that the dynamic modulations and temporal profiles between visual and motor areas are modulated by the degree of plausibility of the observed movement, and that beta band may provide a mechanism that combines visual and motor areas into a functional network during the process of movement recognition.

In the third study, real (anode and cathode) and sham tDCS was administered on PMC to examine the causal effects of PMC on the visual perception of human and non-human PLD movements. Participants performed 2 experiments: In Experiment 1, participants were asked to make a between category judgement; distinguishing between human, bird and random

movement. In Experiment 2, participants were asked to make a within category judgement; distinguishing between natural, unnatural and random movement. In Experiment 1, anodal tDCS on PMC facilitated the global processing of the bird movement, while cathodal tDCS on PMC severely decreased participants' accuracy in the recognition of human movements. In Experiment 2, anodal tDCS seem to increase PMCs visuomotor priming of natural movements, severely affecting the interpretation of the unnatural PLD movement.

The third study extends the importance of PMC in the visual processing of BM. Our results suggest that PMC is highly specialized in the visual percept of natural human BM, but extends to include other species, such as bird BM. Furthermore, it suggests that the PMC may act as an active interpreter rather than a submissive observer during higher form processes such as discriminating between correct and incorrect movements.

Taken together, the results of the three studies suggests that the process of BM recognition does not seem to purely depend on the overt visual information of the observed movement but rather uses premotor representations to further refine and in turn interpret the observed movement.

Zusammenfassung

Bewegungserkennung wird als ein Prozess verstanden, in dem visuelle Information in das eigene persönliche Motor-Repertoire integriert wird. Dieser Prozess findet im **Spiegelneuronen-System** (SNS) statt, das den **Sulcus temporalis superior**, den **inferioren parietalen Lobulus** und den **prämotorischen Kortex** (PMK) umfasst. In der vorliegenden Arbeit werden drei Studien vorgestellt, die mittels der **Lichtpunkt-Darstellungs-Methode** (LDM) verschiedene Fragestellungen zur funktionellen Rolle des SNS und dessen Interaktionen während der Wahrnehmung biologischer Bewegung untersuchen.

In den ersten zwei Studien wurde mittels Magnetenzephalographie (MEG) die Gehirnaktivität gemessen, während Probanden zwischen drei verschieden Lichtpunkt-Läufern unterscheiden mussten. Für die dritte Studie wurde **transkranielle Gleichstromstimulation** (tGSS) auf Teile des SNS angewendet, um den Einfluss von tGSS auf die visuelle Verarbeitung von verschiedenen Formen biologisch plausibler Bewegungsmuster (Mensch vs. Vogel) und der Unterscheidung zwischen natürlichen und unnatürlichen menschlichen Bewegungen zu untersuchen.

Im Rahmen der ersten Studie mussten die Probanden zwischen (biologisch) plausiblen menschlichen Bewegungen und Bewegungen einer zufälligen, strukturlosen LDM-Darstellung unterscheiden. Es wurden signifikante Unterschiede im Gamma- (55-95 Hz), Beta- (20-35 Hz) und Alpha-Frequenzband (9-13 Hz) gefunden. Diese Effekte wurden in parieto-okzipitalen, sensomotorischen und links temporalen Kortexbereichen lokalisiert. Weiterhin konnte eine **positive Kopplung des Leistungsspektrums über Versuchsdurchgänge** ausschließlich für die plausible Bedingung zwischen sensomotorischem Gamma-Band und parieto-okzipitalem Beta-Band aufgezeigt werden.

In der zweiten Untersuchung differenzierten Probanden zwischen zwei visuell ähnlichen LDM-Bewegungen, die sich geringfügig in ihrer biomechanischen Plausibilität unterschieden. Zwischen 1650 und 2650 ms zeigten sich signifikante Effekte im Beta-Band (~20 Hz) in links temporalen, parieto-okzipitalen und sensomotorischen Arealen.

Zusammengenommen zeigen diese MEG-Studien, dass die dynamischen Modulationen und zeitlichen Profile zwischen visuellen und motorischen Arealen durch den Grad der Plausibilität der beobachteten Bewegung moduliert werden. Zudem könnte das Beta-Band

einen Mechanismus darstellen, mit dem visuelle und motorische Areale während der Bewegungserkennung zu einem funktionierenden Netzwerk kombiniert werden.

Für die dritte Studie wurden reale (anodale und kathodale) und vorgetäuschte (*sham*) tGSS auf dem PMK appliziert, um die kausalen Effekte des PMK auf die visuelle Wahrnehmung von menschlichen und nichtmenschlichen LDM-Bewegungen zu untersuchen. Im ersten Experiment sollten die Probanden zwischen verschiedenen Stimuluskategorien unterscheiden: Mensch, Vogel und strukturlose LDM-Darstellung. Das zweite Experiment umfasste eine Unterscheidung innerhalb einer Kategorie: Probanden sollten zwischen natürlicher und unnatürlicher menschlicher Bewegung und strukturloser LDM-Darstellung unterscheiden. Im ersten Experiment konnte gezeigt werden, dass anodale tGSS des PMK die allgemeine Verarbeitung der Vogel-Bewegung erleichtert, während kathodale tGSS des PMK die Erkennungsleistung menschlicher Bewegungen stark herabsetze. Im zweiten Experiment führte anodale tGSS zu einer Verstärkung der visuomotorischer Bahnung von natürlichen Bewegungen im PMK, was einen starken Effekt auf die Erkennungsleistung der unnatürlichen LDM-Bewegungen hatte.

Die dritte Studie erweitert die Bedeutung des PMK für die visuelle Verarbeitung von biologischen Bewegungen. Die vorliegenden Ergebnisse deuten darauf hin, dass der PMK hoch spezialisiert für die visuelle Wahrnehmung von natürlichen menschlichen Bewegungen ist, jedoch auch andere Spezies umfasst, darunter die biologische Bewegung von Vögeln. Weiterhin zeigt sich, dass der PMK während komplexeren Stufen der Bewegungsunterscheidung – wie zwischen korrekten und inkorrekten Bewegungen - eher als eine aktive Schaltstelle zu verstehen ist, anstatt als nachgeschalteter, passiver Beobachter.

Zusammengefasst zeigen die Ergebnisse der vorliegenden drei Studien, dass der Prozess der biologischen Bewegungserkennung nicht ausschließlich auf der offenkundigen visuellen Information der beobachteten Handlung basiert. Vielmehr werden eher prämotorische Repräsentationen genutzt, um die beobachtete Bewegung konkreter einzugrenzen und anschließend zu interpretieren.

1. Introduction

In a constantly changing environment, recognizing and understanding actions or movements is essential and of high evolutionary significance such as social communication. The process of movement recognition includes the interpretation of the observed movements. This involves identifying the type of movement, who is performing the movement and where, and even a prediction of the intentions of the observed movement in its current state. The process of movement recognition is often used synonymously with the term "biological motion" (BM). The 'BM' phenomenon was first introduced by Johansson (Johansson, 1973), and since then many studies have attempted to understand the neural mechanisms involved in BM using a wide variety of neurophysiological and neuroimaging techniques. Owing to the numerous studies exploring BM a complex network, similar to the mirror neuron system (MNS) discovered in monkeys, has been observed in humans during the observation/execution of different movements. Furthermore, theoretical models of motor control suggest that visual and motor areas work together to process and predict future sequences of an observed movement.

The aim of this thesis was to extend works on movement recognition using BM to simulate representations of different variations of a single class of movement that varied in its degree of biomechanically plausibility as well as, different classes of movements. We examined neuronal oscillatory activity in visual and motor areas, in response to the visual representation of different movements and explored the functional role of these areas in the process of movement recognition.

1.1 Biological motion

Johansson (1973) was the first to explore the perception of BM in experimental psychology. According to Johansson, BM is characterized by highly complex spatiotemporal patterns that distinguish it from other types of motion (Johansson, 1973). Using point-lights attached to the main joints (e.g. knees, shoulders, etc.) of a human actor, Johansson recorded various types of movement (e.g. walking, running, and throwing) in a dark room. Using the recorded human movements he created the now well-known PLD stimulus.

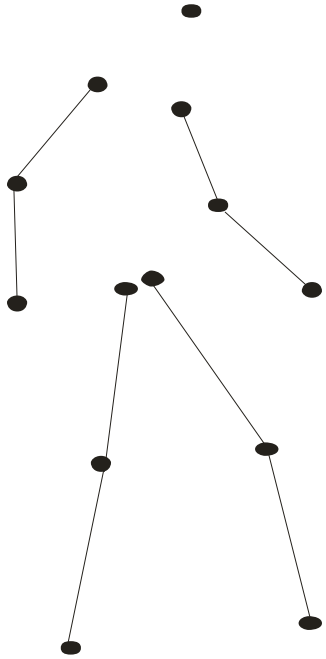

Figure 1: Static representation of a PLD stimulus. Point-light dots represent the main joints of the actor.

The PLD stimulus consists of only ~13 moving dots that make up a human figure performing different movements. The PLD stimulus has several features that make them useful stimuli. Despite their lack of visual cues such as colour or contours, the motion signals of the PLD alone are enough to convey the type of movement presented as well as who is performing the movement (Johansson, 1973; Mather et al., 1992; Neri et al., 1998). Johansson discovered that observing the motion of the point-lights alone, was quickly followed by the experience of observing a human walking or a human throwing. Johansson then speculated that the experience of a human moving reflected the observers' experience in watching other people move (Johansson, 1973). In contrast, static or inverted frames of the same movements prompt no such behaviour (Johansson, 1973; Sumi, 1984).

Since then, a large number of behavioural studies using Johansson's PLD stimulus have demonstrated that observers are able to recognize not only the type of movement presented but also the gender of the actor (Cutting and Kozlowski, 1977; Kozlowski and Cutting, 1977) as well as, their emotional expressions (Bassili, 1978; Dittrich et al., 1996). In addition, observers can easily discriminate a human walking towards the left or right even when the PLD stimulus was masked by additional point-light dots that shared the same motion signals as the human PLD (Bertenthal and Pinto, 1994).

These behavioural findings imply the existence of a specialized network involved in the perception of BM that might be separate from the perception of other types of motion. Strong evidence of such a network comes from neurophysiological and neuroimaging studies of human and non-human primates.

1.2 Biological motion in non-humans

One area known as the superior temporal sulcus (STS) has been the focus of extensive research ever since neurons in this area selective to the visual processing of BM were

identified in monkeys, using single-cell recordings (Perrett et al., 1985). The anatomical location of STS is known to be the meeting point of the ventral and dorsal visual pathways. This provides STS with the unique advantage of combining form and motion information received from ventral and dorsal streams respectively and integrating them with other sensory information (Jellema et al., 2000; Perrett et al., 1985). Motion information is presumed to reach the STS via the dorsal pathway ~20 ms earlier than form information via the ventral pathway, implying that synchrony between motion and form signals is not required to initialize a response in STS cells (Oram and Perrett, 1996). This was supported by the observation that a large population of STS cells strongly responded to the observation of PLD's of BM. In the absence of any form cues the motion signals of the point-light dots, relative to each other were enough to extract form-from-motion information (Oram and Perrett, 1994; Oram and Perrett, 1996). Cells responding to BM have been shown to be selective to the view-point of the moving body, and the direction of movement; most cells respond to movements of the body moving towards the direction it faces, but a small number of STS cells seem to respond to movements of the body moving opposite of the direction it faces (Oram and Perrett, 1996).

In addition to responding to full body movements, a population of STS cells have been shown to respond to hand object movements as well. These cells appear to be sensitive to the shape of the hand, manipulating the object to perform a movement, and remain unresponsive when the object manipulation is done so by tools (Oram and Perrett, 1996; Perrett et al., 1989). Furthermore, it was observed that hand movements alone miming a movement with no object visible, or object movements with no hands visible elicited little neuronal responses compared to the presence of both (Perrett et al., 1989). This suggests that this population of STS cells responds to the interaction between the hand and object movements the strongest.

The response of the STS cells to full body and hand movements suggests that STS's response to BM is explicitly guided by the visual illustration of the observed movement. In recent years however, information from motor areas have shown to modulate activity in STS in humans (Hietanen and Perrett, 1996; Nishitani and Hari, 2000). Motor areas have long been known to be the centre in storing, organizing, and controlling motor representations of body and limb movements (Rizzolatti and Luppino, 2001). Interestingly, a population of cells in motor areas of the macaque monkey have been discovered to respond to movements in a similar fashion to that observed in STS.

In the early 1990's, di Pellegrino and colleagues (Di et al., 1992) discovered neurons in area F5 of the monkey PMC known as mirror neurons. Mirror neurons are a particular class of visuomotor cells that fire when a monkey observes and executes a goal-directed movement (Di et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996). Mirror neurons have been subdivided into two different categories; strictly congruent and broadly congruent (Gallese et al., 1996). Strictly congruent mirror neurons are activated when the observed and executed movement is goal-directed (i.e. reaching for something). Broadly congruent mirror neurons are activated when the observation of a movement is not exactly the same as the one coded motorically (Rizzolatti and Craighero, 2004). Similar to STS cells, the display of an object alone or an experimenter mimicking a movement does not activate mirror neurons. The visual properties shared by both mirror and STS neurons have led researchers to believe that STS and PMC in monkeys, form a system that is highly effective in the processing of BM (Gallese et al., 1996; Rizzolatti et al., 1996; Rizzolatti and Craighero, 2004). The manner in which STS and PMC interact is suggested to be mediated by parietal areas (Gallese et al., 2002).

This role of inferior parietal lobule (IPL) as a mediator between STS and PMC was established when neurons in the IPL of the monkey have also been discovered to respond to the observation of movements done by others (Fogassi et al., 2005; Fogassi and Luppino, 2005; Gallese et al., 2002; Rizzolatti et al., 1997). These neurons are known as canonical neurons (Rizzolatti et al., 1997). Although, most neurons in IPL appear to respond to sensory stimuli, some appear to contain mirror neuron properties. The IPL receives input from both STS and PMC, and has been suggested to play an important role in the transferring of visual and motor information during the observation of a movement from STS to PMC (Gallese et al., 2002). This network in monkeys comprising of the STS, IPL, and PMC is known as the mirror neuron system (MNS).

1.3 Biological motion in humans

The breakthrough discovery of the MNS in monkeys has prompted scientists to ask if such a network existed within the human brain as well. While unable to record the responses of specific neurons themselves (due to ethical reasons), researchers started exploring the human brain by employing neurophysiological and neuroimaging techniques to further elucidate the areas involved and their roles in the visual processing of BM in humans. In the following

sections, evidence of the BM phenomenon in humans and its similarities to monkeys are discussed.

1.3.1 Lesion studies

In many cases, behavioural studies following brain lesions provide a correlation between an area within the brain and its function (or dysfunction). The logic being that if a patient cannot perform a particular task, the execution of that particular task depends on the lesioned area. Although lesion studies are based on clinical observation and may involve only one patient (case study) they are still considered a valid approach and have guided numerous studies in the production and creation of different applications using neuroimaging and electrophysiological tools.

The first important finding that BM was different from other motion processes was that of patient A.F. with bilateral lesions in the temporal-parieto-occipital junction (Vaina et al., 1990). After extensive psychophysical tests it was observed that patient A.F. was severely impaired in several motion tasks including the discrimination of speed and direction of a motion. Surprisingly, his perception of PLD performing different BM was faultless. This led to the interpretation that a separate visual pathway may exist, specializing in BM perception (Vaina et al., 1990). This interpretation was then supported by anatomical studies, discussing the presence of a separate pathway in the temporal lobe, independent from the motion sensitive area (MT+/V5), that may receive separate motion inputs (Colby et al., 1988). This interpretation was further supported by human studies using PLD's to point out that damage to area MT+/V5 led to an impairment of coherent motion but not BM, while damage to area STS led to an impairment in the perception of biological but not coherent motion (Schenk and Zihl, 1997a; Schenk and Zihl, 1997b). These findings imply that BM is processed differently from other motion processes receiving motion input separate from MT+/V5.

Furthermore, based on the investigation of 60 stroke patients with bilateral lesions to the brain it was concluded that normal activity in STS and PMC is necessary for the processing of BM (Saygin, 2007). In addition, lesions to the PMC seem to affect the processing of plausible but not implausible movements (Candidi et al., 2008). The perception of BM seems to be also affected in patients with bilateral lesions to IPL (Battelli et al., 2003). It was observed that patients with lesions to IPL could discriminate coherent motion but had a difficult time

distinguishing BM. The authors concluded a deficit in the global processing of the PLD human figure (Battelli et al., 2003).

With lesion studies however, due to the large size of the lesion, affected areas may overlap making it hard to narrow it down to a specific region in the brain. The dissociations discussed above however, further suggest the importance of STS, IPL, and PMC in the processing of BM, similar to that observed in monkeys. These dissociations suggest distinctive neural networks underlying this effect. Studies trying to uncover these networks are discussed below.

1.3.2 Neuroimaging studies

Studies using functional imaging methods have shown that the perception of BM is always accompanied with activity in STS. Howard and colleagues (Howard et al., 1996) were the first to observe such activity in STS following the visual representation of PLD of BM. Although the study aimed at examining the visual properties of MT+/V5, one of the stimuli used during the experiment was PLD of BM. In response to the BM stimuli activity was observed not only in area MT+/V5 but also in areas of the superior temporal gyrus (STG). This finding was very surprising as activity in STG was always thought to be evoked by speech perception. Since then, many studies using functional magnetic resonance imaging (fMRI) and positron emission topography (PET) as well as the PLD method have been carried out to further elucidate the areas involved in BM.

Depending on the type of stimulus used (full body or limb movements) activity in STS varies. However, PLD of BM versus a control condition, such as coherent motion, scrambled motion, object motion, inverted motion and static frames always evoked stronger activation of STS (Bonda et al., 1996; Grezes et al., 2001; Grossman et al., 2000; Grossman and Blake, 1999; Grossman and Blake, 2001; Grossman and Blake, 2002; Michels et al., 2005; Michels et al., 2009; Pelphrey et al., 2004; Pelphrey et al., 2005; Santi et al., 2003; Singh et al., 2002; Thompson et al., 2005).

The processing of BM, however, seems to be more pronounced in the right STS, irrespective of the visual field the PLD was presented (Grezes et al., 2001; Grossman and Blake, 1999; Grossman and Blake, 2002). Moreover, it has been shown that visual representation of BM using PLD elicits activity in PMC as well (Saygin et al., 2004). This observation is consistent with the discovery of mirror neurons in the monkey PMC, and their involvement in BM (Gallese et al., 1996; Rizzolatti et al., 2001; Rizzolatti and Craighero, 2004), which were

discussed at an earlier section (Section 1.2). Other areas responding to the visual representation of PLD of BM are the cerebellum, amygdala, IPL, inferior frontal gyrus, fusiform face area and kinetic occipital area (Bonda et al., 1996; Calvo-Merino et al., 2005a; Calvo-Merino et al., 2005b; Calvo-Merino et al., 2006; Grossman and Blake, 1999; Grossman and Blake, 2002).

Results from functional imaging studies are consistent with studies using neurophysiological methods such as electroencephalogram (EEG) and MEG. Such methods provide researchers' with the unique opportunity to measure changes in brain activity within a matter of ms, which is up to three magnitudes faster in comparison to hemodynamic techniques. These changes in neural oscillatory activity are characterized by their amplitude, phase and frequency, and reveal important timing information on the functional role of the areas engaged in response to a particular task.

Neural oscillations are periodic variations of neural activity generated by large groups of synchronized neurons and reveal oscillatory activity in specific frequency bands (Schnitzler and Gross, 2005a; Schnitzler and Gross, 2005b). The first discovered and best-known frequency band is alpha (8–12 Hz) which can be detected when an individual is at rest and increases when the eyes are closed. (Gastaut and Bert, 1954). Other frequency bands are delta (1–4 Hz), theta (4–8 Hz), beta (13–40 Hz) and gamma (40–100+ Hz) frequency band. The functional roles of these different frequencies of neural oscillations vary and are extensive, but have been linked to cognitive states, such as awareness and consciousness (Engel et al., 2001; Schnitzler and Gross, 2005b).

Studies examining the neural dynamics involved in BM perception often compare PLD's of BM and scrambled motion. In one such study, passive viewing of an upright point-light walker evoked an increase in gamma MEG activity (~30 Hz) over occipital (~100 ms), parietal (~130 ms) and temporal (~170 ms) areas in comparison to an inverted point-light walker and scrambled motion (Pavlova et al., 2004). The authors concluded that occipital areas showed sensitivity to the coherent structure of the PLD form, while temporal and parietal areas showed sensitivity to the BM (Pavlova et al., 2004). In addition, studies observed stronger event-related potentials (ERPs) for BM compared to scrambled motion in occipital and temporal areas (Hirai et al., 2005) between 240 and 330 ms respectively. The above temporal changes in neural activity suggest that temporal and parietal areas respond stronger to BM at different time points following visual representation of BM.

Changes in neural activity and more precisely changes in mu oscillations (8-13 Hz) have also been observed in motor areas during the observation of BM. Although gamma band activity is often associated with attention, the mu band is an oscillation generated over motor areas when individuals are at rest (Gastaut and Bert, 1954). If an individual however moves, observes or imagines a movement, the amplitude of the mu oscillation is reduced (Babiloni et al., 1999b; Babiloni et al., 1999a; Cochin et al., 1999; Hari et al., 1998; Pineda et al., 2000). Studies investigating the dynamic modulations of MNS in humans have linked mu suppression to MNS activity (Muthukumaraswamy et al., 2004; Muthukumaraswamy and Johnson, 2004; Pineda, 2005; Ulloa and Pineda, 2007).

A more direct indication of the existence of mirror properties in human motor areas comes from transcranial magnetic stimulation (TMS) studies. TMS over the brain area of interest can enhance or inhibit activity of that particular area offering a direct measure of causal observation. One study (Fadiga et al., 1995) using TMS recorded motor evoked responses (MEPs) to stimulation of left motor cortex, when participants observed goal-directed movements, meaningless arm movements, 3-D objects and a spot of light dimming. The results showed that both types of movements (goal-directed and meaningless) evoked stronger increases in MEPs when compared to the object or spot of light conditions. The increase in MEPs was selective to the muscles used in producing the observed movements. Likewise, Borroni and colleagues (Borroni et al., 2005) recorded MEPs of participants while they were observing a cyclic flexion-extension movement of the wrist performed by another individual. Results showed that movement observation modulated strong MEP responses following the same period as the observed movement. Surprisingly, modulations of MEPs came before the observed movement suggesting that motor areas expect movement execution rather than just simply reacting to it (Borroni et al., 2005). These TMS studies among others (Borroni et al., 2005; Fadiga et al., 1995; Gangitano et al., 2001; Gangitano et al., 2004) suggest that engagement of motor areas takes place also in the anticipation of a movement rather than simply reacting to the observed movement.

In response to PLD stimuli of BM, TMS administered over motor areas and more specifically PMC, led to a decrease in sensitivity and response bias (increase in false alarms) to the processing of coherent BM, and had no effect to non-BM and object movements (van Kemenade et al., 2012). Furthermore, TMS over STS compromised the processing of PLDs of BM and had no effect on scrambled motion (Grossman et al., 2005; van Kemenade et al.,

2012), without any significant changes in response bias as observed for PMC (van Kemenade et al., 2012). Compromise of BM was only observed for TMS over STS, and had no effect on TMS over MT+/V5, (Grossman et al., 2005). This further suggests that BM is processed differently in the brain compared to other forms of motion. Moreover, differences in behavioural performance of PMC and STS following TMS suggest that both areas have a unique role in the processing of BM.

All in all, the studies summarized above indicate that the perception of BM depends upon visual and motor processes. The union of these two processes suggests that the perception of BM in humans is different from other processes of motion. This implies that interactions between visual and motor areas may play an important role in higher cognitive functions such as movement recognition. This issue is addressed in greater detail below.

1.4 Biological motion and other processes

Humans are inherently highly social creatures, and naturally spend more time observing and interpreting other human movements than any other class of movements (e.g. animals or objects). Thereby understanding and recognizing movements are of great significance for social purposes. Movement recognition can be defined as the ability to internally simulate the observed movement and use it to determine future behaviours. This process in humans is thought to be done by mapping the visual representation of the observed movement, and comparing it to the motor representations stored in one's own personal motor repertoire (Rizzolatti et al., 2001; Rizzolatti and Craighero, 2004). The observation of a movement causes the motor system of the observer to resonate (Rizzolatti et al., 1999). The more familiar the movement the stronger the resonance.

For example, Orgs and colleagues (Orgs et al., 2008) observed stronger alpha/beta band suppression in motor areas when ballet dancers observed ballet dance movements vs. non-ballet dancers observing the same movements. This observation along with a similar finding using capoeira dancers and fMRI (Calvo-Merino et al., 2005a; Calvo-Merino et al., 2005b; Calvo-Merino et al., 2006) suggests that movements belonging to the motor repertoire of the observer is stored in their motor system (Rizzolatti and Craighero, 2004). If however, the observed movement does not belong to the observers' personal motor repertoire the

movement is predominantly recognized on a visual basis and does not excite the motor system (Rizzolatti and Craighero, 2004).

This assumption was examined by Buccino and colleagues (Buccino et al., 2004) using two different movement stimuli. The first movement stimuli was that of a human, monkey and dog biting. The second was communicative movements, such as barking and lip smacking. The results show that biting movements, irrespective of who is performing the movement activated IPL and PMC areas. Observation of barking movements however showed no such activity, implying that motor activity is predominantly dependent on whether or not the movement observed belongs to one's own motor repertoire.

Although humans show greater sensitivity to human BM they are still able to recognize movements they cannot perform. Behavioural studies comparing PLD movements of humans and animals illustrated that observers could easily identify each class of movement (Jokisch and Troje, 2003; Mather and West, 1993). Furthermore, a recent fMRI study reported PMC activity when participants observed moving pictures of real animals (Fadiga et al., 2006), close to that observed when participants viewed biomechanically plausible and implausible finger and body movements (Costantini et al., 2005; Craighero et al., 2008; Romani et al., 2005; Schurmann et al., 2011).

Taken together these findings suggest that the human analogue of the MNS discovered in monkeys is much more supple since it appears to be engaged not only in the observation and execution of goal-directed movements, but also to different classes of movements (e.g. animals), different representations of a single movement (e.g. implausible or scrambled movements), as well as, to meaningless arm movements. In the processing of these stimuli it is possible that visual areas categorize the observed movements and work with motor areas to further refine the observed movements via a template matching approach (Lange et al., 2006; Lange and Lappe, 2006).

2. Current work

The aim of the current work was to examine the neural mechanisms involved in movement recognition and the interactions between and within areas of the MNS in the frequency domain. Three studies addressed different questions using PLD stimuli and different methodological approaches. Data for the first two studies were collected in a conjunct MEG experiment. Each study however, focused on different experimental questions and used different subsets of the data. In the third study tDCS stimulation was used to modulate activity in an area of interest and examine its effects in movement recognition.

Study 1: Here we recorded brain activity using MEG while subjects viewed PLDs of plausible movements and its scrambled counterpart (random assortment of dots). A plausible PLD was an animation of a human figure, in its original form as recorded, performing different biological movements. We were interested in the modulations of neural oscillatory activity between these two distinctly different PLD movements, and more specifically, in the correlation of oscillatory activity between visual and motor areas.

Study 2: MEG brain activity was recorded while subjects viewed PLDs of biomechanically plausible and implausible versions of the same movement. In contrast to scrambled movements, the overall visual information and human structure of the implausible movements was only minimally changed. We were interested in how oscillatory activity is modulated between two seemingly similar PLD movements and compared it to the findings of our first study.

Study 3: Here we were interested in whether or not tDCS on PMC influenced the visual perception of PLD movements of different classes (human or bird) and different variations of a single movement (similar to study 1 and 2). Performance (reaction times (RTs) and accuracy) was measured before, during, immediately after, and 30 minutes after tDCS stimulation.

The overall goal of these studies was to extend the knowledge of the distinct roles of visual and motor areas, and the functional roles of the neuronal oscillations involved during the process of movement recognition.

3. Methodological approaches

For the purpose of this thesis two different methodologies were used to study the functional roles of visual and motor areas and investigate their interactions during the recognition of different PLD movements.

3.1 Magnetoencephalography (MEG)

MEG is a non-invasive method used to measure oscillatory brain activity from outside the brain. MEG records magnetic fields induced by electric currents of large groups of synchronized neurons using very sensitive magnetometers known as superconducting quantum interference devices (SQUIDs) (Hamalainen et al., 1993; Sato et al., 1991). The most recent MEG systems contain hundreds of SQUIDs covering the entire head. Magnetic fields are thought to be more sensitive to postsynaptic potentials from pyramidal cells, which are situated perpendicular to the surface of the cortex. Since the magnetic signals from the brain are in the order of only a few femtoteslas (~10^{-15}), shielding from external magnetic fields is essential (Cohen, 1972). MEG offers a very high temporal resolution (around 1 ms), and a good spatial resolution (around 2 to 3 mm) contributing to a direct measure of neuronal activity.

MEG can detect neural changes in a matter of ms. Other methods measuring brain activity such as fMRI and PET detect changes within seconds and minutes. MEG directly measures changes of magnetic fields, while PET records changes in metabolic activity and fMRI measures changes in blood flow, which are both indirect indicators of neural activity. To some extend, MEG is similar to EEG but with one important difference. The magnetic fields measured by MEG are less distorted by the skull and tissue surrounding the brain than the electric fields measured by EEG (Cohen and Cuffin, 1983). This offers a better localization of brain function for MEG.

The MEG lab at the Institute of Clinical Neuroscience and Medical Psychology at Heinrich-Heine-University is a 306-sensor Elektra Neuromag device. The 306 sensors are made up of 204 planar gradiometers and 102 magnetometers (SQUIDs) in a helmet configuration covering the whole head. This system allows for the delivery of visual and auditory stimuli, and recordings can be acquired in either a sitting or supine position.

3.2 Transcranial direct current stimulation (tDCS)

tDCS differs from other brain stimulation methods such as TMS, in that it does not induce action potentials, but rather it modulates the area of interest using a low constant electrical current delivered directly to the brain area of interest via small electrodes soaked in saline solution (Nitsche and Paulus, 2000). Based on the type of stimulation used the current flow delivered can either increase or decrease the neuronal excitability of the stimulated area (Nitsche et al., 2008). A positive stimulation causes the resting membrane to depolarize increasing neuronal excitability and spontaneous cell firing. In contrast, a negative stimulation causes the resting membrane to hyperpolarize decreasing neuronal excitability and spontaneous cell firing. These changes in current flow easily allow for a bi-directional investigation of causal influence.

An important aspect of tDCS is that it has the ability to achieve cortical changes in the stimulated area even after stimulation has ended (Nitsche et al., 2008). The duration of these cortical changes is dependent on two factors: the length and intensity of the stimulation. The longer and more intense the current is applied on the area of interest the longer these cortical changes last (Nitsche et al., 2008). Numerous studies have been done to determine the maximum time and current intensity of tDCS stimulation in order to reduce side effects and eliminate risks felt by individuals receiving tDCS stimulation. Currently, the acceptable time is ~20 mins of stimulation using a maximum current of 2mA.

The tDCS device used in the third study was a neuroConn DC-Stimulator, which is a micro-processor-controlled constant current source. It allows for continuous monitoring of electrode impedance. If insufficient contact with the skin is detected, stimulation is automatically terminated.

4. Study 1: Interactions between visual and motor areas during the recognition of plausible actions as revealed by MEG.

Study 1 used the PLD stimulus, with which the spatial position of the point-light dots was manipulated while keeping the motion signals of the dots the same, thereby changing the coherent structure of the human PLD figure. By employing the PLD stimulus and using MEG changes in neuronal oscillatory activity were measured across the brain. The locations of the strongest oscillatory activity in visual and motor areas were examined, and cross-frequency coupling between them was determined.

4.1 Introduction

Our ability to recognize movements is important in interacting with the people around us. Movement recognition can occur at different levels and over distinct time scales. On a lower level and a shorter time visual information is processed (Blake and Shiffrar, 2007; Michels et al., 2009). This relies on the ability to differentiate form and motion (Lange and Lappe, 2006; Oram and Perrett, 1996). In recent years, following the discovery of mirror neurons in the monkey PMC, and the engagement of motor areas during the observation of movements in humans, movement recognition is thought to rely on both visual and motor processes (Schippers and Keysers, 2011; Urgesi et al., 2010).

The proposed mechanism on how movement recognition is mediated in the brain is to compare visual information to motor representations stored in one's own personal motor repertoire of possible movements (Rizzolatti and Craighero, 2004). Most studies using the PLD method to examine the process of BM have been performed using functional magnetic resonance imaging (Grossman et al., 2000; Michels et al., 2005; Michels et al., 2009; Pelphrey et al., 2005; Saygin et al., 2004). Very little is known, however, about the functional role of neuronal oscillatory activity in visual and motor areas, and how they interact during the process of movement recognition.

4.2 Methods

Three conditions with different variations of a movement were created using the PLD method; plausible, scrambled and implausible. The plausible condition was of a biomechanically probable BM in its original form as recorded. The scrambled condition was a random assortment of dots, in which the human figure was completely destroyed while the net movement of the dots remained unchanged. The implausible condition involved in randomizing the starting position of four point-light dots, altering the human figure only minimally, while the net movement of the dots remained the same. For each stimulus, each cycle of movement was repeated five times. Each trial started with a fixation cross, before a point light movement appeared for a period of 3600-5000 ms. After a random period, in which a black screen was visible, instructions were visually presented. 12 participants were asked to rate the movement observed using a 1-4 rating scale as either plausible (1), implausible (2, 3) or scrambled (4) (please see Appendix 1; Figure 1). While participants performed the task, brain activity was recorded continuously with a 306-channel whole head MEG system (Neuromag Elekta Oy, Helsinki, Finland). The data collected were then offline analyzed with respect to time course, strength, sources, and cross-frequency correlation of neuronal oscillatory activity, and compared between conditions. Based on our main research question and for the sake of comparability to other studies done to date on BM, the first study focused on the main contrast of plausible vs. scrambled movements.

4.3 Results

4.3.1 Behavioural

All participants could easily distinguish between plausible and scrambled movements with an average rating of 1.5 and 3.8 respectively. Statistical testing revealed highly significant differences between both movements ($p<0.001$).

4.3.2 Stimulation effects

Effects of visual stimulation were first determined by pooling all trials together irrespective of the condition (plausible, scrambled, and implausible). Sensors revealing the strongest fluctuations in oscillatory activity were then selected for further analysis. Strong sustained changes in alpha (7-13 Hz), beta (13-35 Hz) and gamma (55-100 Hz) power were bilaterally observed in sensors over parieto-occipital, sensorimotor and temporal areas (please see Appendix 1; Figure 2). Strongest cortical sources of these effects were identified in parieto-occipital, sensorimotor and bilateral temporal areas. (please see Appendix 1; Figure 3). To further examine the different roles of our four regions of interest in movement recognition we assessed differences in neuronal oscillatory activity between plausible and scrambled movements.

4.3.3 Condition contrast

Differences between plausible vs. scrambled movements were assessed in the above four regions of interest (parieto-occipital, sensorimotor and bilateral temporal). A significant increase for plausible in comparison to scrambled movements was observed in gamma (55-90 Hz), beta (20-35 Hz) and high alpha (9-13 Hz) power between 500-1300 ms in parieto-occipital, sensorimotor and left temporal areas respectively. Furthermore, a significant decrease was observed in gamma (50-90 Hz) and alpha/low beta (10-22 Hz) power between 1300-2000 ms in right temporal and parieto-occipital areas respectively (please see Appendix 1; Figure 4A). Cortical sources of these effects were more pronounced in posterior visual areas including STS and frontal motor areas including PMC (please see Appendix 1; Figure 4B).

4.3.4 Cross-frequency correlations

Interactions between visual and sensorimotor areas during the recognition of movements were assessed by calculating the trial-by-trial cross-frequency correlation between the significant time-frequency clusters mentioned in our condition contrast section above (see also Appendix 1; Figure 4A). A positive correlation was observed between sensorimotor beta (averaged between 20-35 Hz and 700-1200 ms) and parieto-occipital gamma power (averaged

between 55-90 Hz and 500-800 ms) power (r = 0.09; $p<0.05$) as well as between sensorimotor beta and left temporal alpha (9-13 Hz and 900-1300 ms) power (r = 0.20); $p<0.05$) for the plausible movements but not the scrambled ones.

4.4 Discussion

Visual presentation of PLDs (across all conditions) elicited sustained effects in alpha (7-13 Hz), beta (15-25 Hz) and gamma (50-100 Hz) power within visual and motor areas of the mirror neuron system (MNS). Suppression of alpha and beta power in parieto-occipital areas is in line with previous reports on visual stimulation (de Lange et al., 2008; Hoogenboom et al., 2006). This suppression of alpha/beta power was also observed in sensorimotor areas, in line with earlier studies of movement observation, execution and imagery (Hari and Salmelin, 1997; Koelewijn et al., 2008; Oberman et al., 2008; Schnitzler et al., 1997). An increase of high alpha was also observed in sensorimotor areas. While a decrease of alpha/beta-band power has been linked to the engagement of sensorimotor areas, an increase has been suggested to reflect inhibition of the sensorimotor system (Hummel et al., 2002; Jensen et al., 2002; Nachev et al., 2008; Neuper and Pfurtscheller, 2001). This increase of high alpha power in our study might thus reflect participants' active inhibition of eye and/or finger movement. Finally, an increase of high gamma power was observed in a wide array of posterior and frontal areas, in line with previous reports involving visuomotor tasks (Pavlova et al., 2004; Pfurtscheller and Neuper, 1992).

When comparing plausible to scrambled movements, we observed an early increase of gamma (55-90 Hz) band power in right visual and parietal cortices between 500-800 ms. This observation is in line with previous findings of gamma band activity in response to PLD walkers over temporal-parieto-occipital areas (Pavlova et al., 2004; Pavlova et al., 2006; Singh et al., 2002), and hemodynamic responses (Grossman and Blake, 2002; Michels et al., 2005). Activity in gamma frequency over right visual and parietal areas is often thought to reflect the global processing of the PLD (Battelli et al., 2003; Pavlova et al., 2004). This early gamma cluster suggests that both plausible and scrambled movements are first distinguished on an early visual basis (Pavlova et al., 2004)

This gamma increase was followed by an increase in sensorimotor beta (20-35 Hz) between 700-1200 ms. Previous hemodynamic studies demonstrated that sensorimotor areas and more

specifically the PMC, responded to both human and scrambled movements but was more pronounced for human BM (Buccino et al., 2004; Saygin et al., 2004). This positive sensorimotor beta cluster reflects a stronger suppression of power for the scrambled movements. In contrast, a previous study observed stronger beta suppression when subjects viewed movements more familiar to them, compared to other BM (Orgs et al., 2008). On the other hand, stronger beta suppression of sensorimotor areas has been reported for the observation of incorrect vs. correct movements (Koelewijn et al., 2008). The stronger beta-band suppression observed in our study might thus reflect the recognition of the scrambled movements as incorrect.

The increase in sensorimotor beta was followed by an increase in left STS alpha (9-13 Hz) between 900 and 1300 ms. STS is known to be involved in the processing of biological movements (Allison et al., 2000). An increase in alpha power might thus reflect active inhibition of STS. Although, previous studies have reported activity in right STS, the observed left STS activity might reflect the differentiation of the local details between the plausible and scrambled PLD, which is thought to be processed in the left hemisphere of the brain (Bonda et al., 1996; Lamb and Robertson, 1988).

Interestingly, a significant positive trial-by-trial correlation was observed between sensorimotor beta, and parieto-occipital gamma, as well as, left temporal. This positive correlation between sensorimotor beta and other frequencies was only observed for the plausible movements, suggesting a functional interaction from visual to motor and back to STS. Beta oscillation might thus provide a mechanism that combines visual and motor into a functional network (Brovelli et al., 2004; Schnitzler and Gross, 2005a).

4.5 Conclusion

In summary, our results reveal a wide array of areas involved in the recognition of plausible movements, including the STS and PMC areas of the MNS, operating at different frequency bands, extending previous neuroimaging studies. Interactions between visual and motor areas were revealed by positive power correlations during the recognition of plausible movements at distinct spatial-temporal scales predominantly coupled to sensorimotor beta. This is in support to current models of motor control, which propose the presence of inverse and forward models involving visual and motor interactions.

5. Study 2: Distinct spatio-temporal profiles of beta-oscillations within visual and sensorimotor areas during action recognition as revealed by MEG.

In the first study, we observed spatio-temporal profiles of gamma, beta and alpha power in parieto-occipital, sensorimotor and left-temporal areas respectively, between 500-1300 ms when participants had to distinguish between plausible and scrambled movements. In addition, interactions between visual and motor areas during the recognition of plausible BM were predominantly coupled in the sensorimotor beta frequency. In this study, we investigated the dynamic modulations between two seemingly similar PLD movements varying in the degree of plausibility, to assess the role of MNS in the refinement of similar movements. Temporal modulations in beta power where of particular interest.

5.1 Introduction

Movement recognition is thought to be a process in which visual information is intergrated with one's own personal motor repertoire of possible movements (Rizzolati and Craighero, 2004). Previous electrophysiological and neuroimaging studies have identified three areas to play an important role in the process of BM known as the MNS; STS, IPL, and PMC (Oram and Perret, 1996; Rizzolatti et al., 1997; Grossman et al., 2000; Saygin et al., 2004).

MEG and EEG studies have observed neuronal changes in areas of the MNS in alpha (9-13 Hz) and beta (13-30 Hz) frequency. Changes in alpha/beta frequency have been attributed to increase of visual attention in visual areas (Wrobel, 2000). In motor areas, decrease of alpha power has been observed during the preparation and excecution of a movement, while attenuation of beta power has been interpreted as MNS activity (Kilner et al., 2009).

Although beta activity is abundant in sensorimotor areas it is still unknown how beta activity is modulated when the degree of plausibility of BM is varied. Here we used two different versions of the same PLD movement (plausible and implausible) and examine the neural modulations, in visual and motor areas of the MNS. In contrasts to a scrambled movement in which the configural human figure is completely destroyed, overall visual information and human configural structure of the implausible PLD movement is only minimally changed.

5.2 Methods

The data for this study were collected in combination with the first study. Therefore, the stimuli and experimental procedures as well as the methods of data acquisition and data analysis were the same as in the first study. The data analysis in this study however, focuses on the plausible and implausible condition, in contrasts, to plausible and scrambled in the first study. Twelve participants were asked to rate the movement observed using a 1-4 rating scale as plausible (1), implausible (2, 3) or scrambled (4) while brain activity was continuously recorded with a 306-channel whole head MEG system (Neuromag Elekta Oy, Helsinki, Finland). The data collected were then offline analyzed with respect to temporal profiles of power changes, strength, and sources between plausible and implausible conditions.

5.3 Results

5.3.1 Behavioural results

All participants could easily distinguish between plausible and implausible movements with an average rating of 1.5 for plausible and 2.3 implausible. Statistical testing revealed highly significant differences between both movements ($p<0.001$).

5.3.2 Stimulation effects

The strongest effects in neuronal oscillatory activity were examined by pooling all three conditions together (plausible, implausible and scrambled). Clear perturbations of oscillatory activity were observed in alpha (7-13 Hz), beta (13-35 Hz) and gamma (50-100 Hz) power in parieto-occipital, sensorimotor and bilateral temporal areas (please see Appendix 2; Table 1).

5.3.3 Condition contrast

Differences between plausible and implausible movements were assessed in sensors over parieto-occipital, sensorimotor, and bilateral temporal. A significant increase in alpha/low beta (9-21 Hz) power was observed between 1650-2050 ms in left temporal areas, followed

by a significant increase in alpha (5-11 Hz) and low beta (13-21 Hz) power, between 1950-2350 ms in parieto-occipital areas as well as a significant increase in low beta (15-21 Hz) power at 2400-2650 ms in sensorimotor areas (please see Appendix 2; Figure 2A). No significant effects were observed in right temporal areas. Cortical sources of these significant effects were observed in visual and motor areas including STS and PMC (please see Appendix 2; Figure 2B).

5.3.4 Temporal changes in alpha/beta power

Temporal changes in alpha/beta suppression were assessed separately for plausible and implausible movements in parieto-occipital, sensorimotor and left temporal areas. Although both conditions showed and initial decrease in alpha/beta power, it was stronger for implausible movements (please see Appendix 2; Figure 3).

5.4 Discussion

Visual stimulation of PLD movements elicited changes in alpha (7-13 Hz), beta (13-30 Hz) and gamma (50-100 Hz) power in parieto-occipital, sensorimotor and bilateral temporal, in agreement with previous fMRI (e.g. Grossman et al., 2000; Singh et al., 2002; Saygin et al., 2004; Michels et al., 2009) and electrophysiological (e.g. Pavlova et al., 2004; Pavlova et al., 2006; Singh et al., 2002) studies on BM.

Differences between plausible and implausible movements showed significant modulations in alpha/beta power in successive order between 1650-2650 ms in left temporal, parieto-occipital and sensorimotor areas respectively. These late spatio-temporal profiles suggest that both plausible and implausible movements activate visual and motor areas but do so at different times, suggesting that beta power may provide a functional network of communication between these areas.

Beta suppression in visual areas has been linked to an increase in visual attention (Kaminski et al., 2012; Wrobel et al., 2007). In contrast to previous neuroimaging studies, which report right temporal activity (Grossman et al., 2000; Pavlova et al., 2004) the observed left temporal activity in our study might reflect participants' increase in visual attentiveness to the local details of the PLD when trying to differentiate between two very similar stimuli. This process is thought to take place in the left hemisphere (Bonda et al., 1996; Robertson et al., 1988).

In contrasts, to our first study, and other electrophysiological studies (e.g. Pavlova et al., 2004) that compared plausible and scrambled movements, we did not observe an increase in gamma power in this study. This suggests that in the presence of two very similar stimuli there is no visual distinction between the two or differences in gamma power are too small for MEG to detect.

The late temporal profiles observed in sensorimotor areas and stronger suppression of alpha/beta power for implausible movements is in line with studies that have used correct vs. incorrect movements (Koelewijn et al., 2008), as well as in relation to motor imagery (de Lange et al., 2008). The duration of beta band suppression is consistent with the complexity of the task (de Lange et al., 2008). The late alpha/beta spatio-temporal profiles in our study might thus reflect the recognition of the implausible movement over a longer period of time.

This is in contrasts to the findings of our first study in which differences between plausible and scrambled movements in sensorimotor areas were observed between 700-1200 ms. Therefore, the process to distinguish between two very similar PLD movements requires more time to interpret and thus more time to activate the sensorimotor areas.

5.5 Conclusion

In summary, we observed late spatio-temporal profiles in beta power which distinguish between the recognition of plausible and implausible movements. This suggests that the beta band may provide a functional network of communication between visual and motor areas of the MNS. The sequential order of beta power from temporal, parietal, to motor areas suggests a directed flow of information, in line with inverse models of motor control. The later activation of motor areas in comparison to visual areas suggests their involvement in higher form processes when interpreting the biomechanically plausibility of the observed movement. This suggests that the MNS acts more like an active interpreter than a submissive observer during the recognition of a movement.

6. Study 3: Anodal stimulation of premotor cortex facilitates the recognition of different forms of movements.

In our previous studies using MEG we observed different spatio-temporal profiles in sensorimotor areas including PMC during the observation of varying degrees of plausibility of a single movement. When participants were asked to differentiate between two distinctly different forms of PLD movements such as plausible and scrambled movements, significant differences were observed between 700-1200 ms. In contrast, when distinguishing between two seemingly similar forms of PLD movements such as plausible and implausible movements significant differences were observed between 2400-2650 ms. The differences in PMC activity observed between the two contrasts suggest that PMC activity is dependent on the degree of plausibility of the observed movement. In this study, PMC activity was modulated using tDCS to examine the causal effect of PMC during the recognition of different movements. Performance in terms of RTs and accuracy was measured to investigate influence of PMC in the visual percept during the recognition of different PLD movements.

6.1 Introduction

Since the discovery of mirror neurons in the monkeys' PMC during the observation/execution of goal-directed movements, area PMC in humans has received much attention. Several neuroimaging studies have reported PMC activity during the observation and execution of BM as well as during motor imagery (Calvo-Merino et al., 2005b; Saygin et al., 2004; Schnitzler et al., 1997; Singh et al., 2002; Ulloa and Pineda, 2007). PMC is considered an important part of the MNS. The more the observed movement matches the personal motor repertoire of the observer the more the MNS, and in particular the PMC, resonates (e.g. Orgs et al., 2008). If the observed movement however, does not match the observers' personal motor repertoire the movement is thought to be recognized only on a visual basis (Rizzolatti and Craighero, 2004).

Although the monkey PMC is only activated when monkeys observe or execute a goal-directed movement, the human PMC appears to be more flexible. Images of real moving animals (Fadiga et al., 2006), and observed movements of implausible full body and finger movements (Candidi et al., 2008; Urgesi et al., 2007b; Urgesi et al., 2007a) have also been reported to activate the PMC. This suggests that PMC might be involved in higher form

processes such as interpreting the observed movement, rather than simply reacting to it. Sensitivity to PLDs of BM has been shown to decrease when administering TMS on PMC (van Kemenade et al., 2012). Further research however is needed to determine the human PMC's role in the processing of BM of different species other than human, and its effects in the process of correct vs. incorrect movements. Here, we employed the PLD stimulus and created movements of different classes of species (human walking and bird flying) as well as different variations of a single movement varying in their degree of plausibility (similar to study 1 and 2). tDCS was administered on PMC to examine the role of PMC during the visual perception of the above movements.

6.2 Methods

Real and sham tDCS was administered over the left PMC, while participants were asked to distinguish between different PLD movements. Each type of tDCS stimulation (anode, cathode, sham) had four testing sessions: before (pre), during (tDCS), one immediately after (post), and 30 minutes post (30-post) tDCS stimulation. This allowed us to examine the effects on PMC before and after modulation and compare the results. 10 participants performed 2 experiments. In Experiment 1, five subjects were asked to make a between category judgment; distinguishing between human, bird, and random PLD movements (please see Appendix 3; Figure 1A). In experiment 2, five subjects were asked to make a within category judgment; distinguishing between natural, unnatural, and random PLD movements (please see Appendix 3; Figure 1B). Participants were asked to respond as quickly and as accurately as possible, following visual representation of each PLD movement. Performance (RTs and accuracy) was recorded and false reports for each PLD movement were accounted for across all testing sessions. Results were averaged across participants for each experiment and the effects of tDCS on PMC were compared between the two.

6.3 Results

6.3.1 Experiment 1 (between class discrimination)

The human PLD was recognized the fastest across all testing session regardless of the stimulation used (~563 ms). Anodal stimulation significantly decreased RTs in the processing

of bird and random PLD movements. In addition, there was a significant decrease of bird false reports as random suggesting recognition of bird PLD movements significantly improved following tDCS. Cathodal stimulation significantly decreased participants' accuracy in the recognition of human PLD movements (please see Appendix 3; Figure 2). This effectively increased participants' tendency to recognize human PLD movements as random PLD. Sham stimulation however, showed no such effects on PMC during the recognition of human, bird and random PLD movements (please see Appendix 3; Table 1).

6.3.2 Experiment 2 (within class discrimination)

In contrast to experiment 1, RTs in experiment 2 (between ~1050 and 1100 ms) showed that discriminating between natural and unnatural movements required more time across all stimulation sessions. Anodal stimulation resulted in a significant decrease in RTs during the processing of natural movements (please see Appendix 3; Figure 3). Frequency count of false reports showed a significant increase in participants' tendency to report unnatural PLD movements as natural effectively decreasing participants' accuracy in recognizing unnatural movements (please see Appendix 3; Table 2). Cathodal and sham stimulation showed no significant effects on PMC during the recognition of natural, unnatural and random movements.

6.4 Discussion

This study aimed to reconcile the findings of previous studies involving PMC and movement recognition. We administered tDCS on PMC to explore its role in the visual perception of both human and non-human movements. We used the PLD method and created different categories of movement (Experiment 1) and different variations of a single movement (experiment 2). By using a single stimulus class and a single paradigm we could effectively evaluate the role of PMC in movement recognition within and across movement categories. Experiment 1 consisted of three different sessions of tDCS to the PMC (anodal, cathodal and sham) and four different time points (pre, tDCS, post and 30 minutes post). We measured speed and accuracy of the recognition of human, bird and random PLD movements to test PMCs role in the visual perception of BM of other species. In experiment 2 however, which

also consisted of three tDCS sessions and four time point measurements, we investigated the effects of tDCS on PMC's interpretation during the visual perception of natural and unnatural movements.

Consistent with previous TMS studies, decreasing neuronal excitability of PMC by cathodal stimulation significantly reduced sensitivity to human BM (Candidi et al., 2008; Urgesi et al., 2007b; Urgesi et al., 2007a; van Kemenade et al., 2012). This observation is also in line with lesion studies on PMC, which report that normal activity of PMC in essential in the processing of human BM (Candidi et al., 2008; Urgesi et al., 2007b). PMC in humans is theorized to be an area in which visual information are integrated with the internal motor representations of movement (Rizzolatti and Craighero, 2004). The more familiar the observed movement the faster it is processed in the PMC. The human PLD movement was recognized the fastest in comparison to bird and random PLD movements. High performance across the human, bird and random movement however suggest that some of the same global processes are attributed to the recognition of all three PLD movements. The global processing of BM is thought to be derived from changes of structural information of the movement over time (Lange et al., 2006). This might suggests that PLD in PMC are globally processed via a template matching approach that is highly effective in the recognition of human BM extending to other form of PLD movements such as a bird flying (Lange et al., 2006; Lange and Lappe, 2006). Anodal stimulation over PMC seems to facilitate this process, since we observed a decrease in participants' tendency to report bird PLD movements as random.

In experiment 2 participants were asked to differentiate between two human PLD movements differing in their degree of plausibility. Following anodal stimulation participants had an increase tendency to report unnatural movements as natural suggesting that increasing excitability of PMC cells prompts stronger visuomotor priming to natural human movements. Visuomotor priming is thought to be an automated process within PMC during the observation of a movement. Familiar movements have been observed to prompt stronger visuomotor priming (Gowen et al., 2010). The observation of faster RT and increase in accuracy during the recognition of natural PLD movements, in this study suggests that anodal stimulation facilitated the global processing of the most familiar PLD movement. This was at the expense of the recognition of unnatural but not random movements, implying that visuomotor priming is strongly influenced by the structural properties of the observed PLD movement.

The differences in RTs during the processing of human PLD movements between our 2 experiments are in line with our two MEG studies. When differentiating between two distinctly different forms of PLD movement (e.g. plausible vs. scrambled; human vs. bird), PMC activity is faster than when differentiating between two very similar movements (e.g. natural vs. unnatural). This further suggests PMC's active role in interpreting the observed movement and not just visually reacting to it.

6.5 Conclusion

In summary, we presented PLD movements and applied tDCS on PMC to investigate its role on the visual processing of different classes of movement, and different variations of a single movement. Both polarities of tDCS stimulation (anode and cathode), and a control (sham) were used to compare their effects. Anodal tDCS facilitated the distinction of less familiar BM movements, suggesting a key role for PMC in the visual percept of the global processing of the PLD using the template matching approach. Cathodal stimulation significantly reduced PMC's sensitivity in the visual discrimination of a human PLD. In addition, anodal tDCS over PMC increased visuomotor priming to natural movements severely affecting the visual interpretation of an unnatural PLD movement. These findings underline the importance of PMC in the visual processing of human BM.

7. General Discussion

The current thesis aimed to further elucidate the roles of the visual and motor areas of the MNS during the recognition of BM. We employed the PLD method together with MEG and tDCS to investigate the neural mechanisms of the MNS and examine the functional roles and interactions between visual and motor areas during the visual presentation of different BM. Three studies investigated the roles of visual and motor areas; Study 1 and 2 recorded MEG brain activity and uncovered the neural mechanisms within and between MNS areas when participants were asked to differentiate between two contrasts, with varying degrees of plausibility in their movement. Study 3 employed tDCS to modulate activity in PMC, to determine PMC's role in the visual processing of different classes of movement, as well as, different variations of a single movement.

Study 1 compared plausible (normal human movements) and scrambled (random assortment of dots) PLD movements. Significant differences were observed in gamma (55-90 Hz), beta (20-35 Hz) and alpha (9-13 Hz) in parieto-occipital, sensorimotor and left temporal areas respectively between 500-1300 ms for the plausible condition. The changes in gamma power suggest that differences between both PLD movements are first distinguished on a visual basis. Further analysis of the plausible PLD movement is processed in motor and temporal areas. Furthermore, we observe a positive trial-by-trial correlation between sensorimotor beta, and parieto-occipital gamma, as well as, left temporal alpha for the plausible but not the scrambled PLD movement. This suggests that beta-band may provide a functional network of communication between visual and motor areas during the recognition of plausible BM. This is in line with the theory that BM recognition in the brain is a process in which visual information is integrated with ones own motor repertoire of possible movements.

Study 2 compared two visually similar PLD movements that varied in their degree of plausibility. In this study, we compared plausible and implausible PLD movements and compared the effects of visual and motor areas to that observed in the first study. Significant changes were observed in the beta-band in successive order between 1650-2650 ms in left temporal, parieto-occipital and sensorimotor areas respectively for the plausible condition. In contrast to study 1, no changes in gamma power were observed due to the fact that low-level information between the two PLD movements is very similar. The late temporal profiles observed for the sensorimotor areas in comparison to visual areas suggest that the

interpretation between plausible and implausible PLD movements happens over a longer period of time, taking longer to activate sensorimotor areas. Notably, all effects were observed in beta band further suggesting that beta provides a functional network integrating visual and motor information during the recognition of BM.

The first two studies suggests that activity in sensorimotor areas is modulated depending on the degree of plausibility of a BM. Recruitment of sensorimotor areas is much faster for the plausible vs. scrambled contrast than the plausible vs. implausible movements. This suggests that motor activity is sensitive to the visual illustration of the BM. In the third study we employed tDCS on PMC and examined causal effects to the visual perception of human and non-human movements. Anodal tDCS on PMC appeared to facilitate the global processing of non-human BM such as a bird flying, while cathodal tDCS on PMC severely affected participants' accuracy in the recognition of human PLD movements. Visual presentation of natural and unnatural PLD movements was processed slower than human and bird PLD movements. Anodal tDCS increased PMC's automated response to natural PLD movements, severely affecting the interpretation of the unnatural PLD movement. This further suggests the importance of PMC in the processing of human BM, suggesting its role as an active interpreter rather than a submissive observer during the discrimination between correct and incorrect movements.

Taken together, the results of the three studies included in this thesis contribute a better understanding of the functional roles of visual and motor areas during the perception of BM. Theories on movement recognition have proposed a strong interaction between visual and internal motor representations of an observed movement (Rizzolatti and Craighero, 2004; Shiffrar et al., 1997). This view was supported by the neuronal modulations observed between visual and motor areas when observing plausible movements compared to movement patterns that varied in their degree of plausibility. Therefore, recognition of a BM does not seem to depend purely on visual processing but seems to be facilitated by the activation of premotor representations, using a template matching approach to further interpret the observed movement.

8. Outlook

The current work extended previous research on the functional roles of visual and motor areas during the perception of BM. However, the results of these studies leave open questions, and imply other mechanisms that may also be involved during the recognition of BM.

In this work, we established the importance of visual and motor areas during the perception of BM. This observation of visual and motor activity in our study suggests a similar network involvement to that observed during motor imagery. Motor imagery reflects the internal simulation of a movement. In an earlier study by de Lange and colleagues (de Lange et al., 2008) activity in visual and motor areas was observed during motor imagery of hand movements. The prolonged motor activity in our second study and the increase of RTs observed in our third study, when participant's had to distinguish between two very similar movements might suggest an increase in motor imagery. An interesting task for future studies would be to produce a carefully designed paradigm in which both movement recognition as well as motor imagery processes can be extracted independently. This will serve to further elucidate the role of visual and motor areas of the MNS during the recognition of a movement as well as the prediction of the forthcoming movement sequences. MEG can be employed to determine and compare the neuronal modulations between the two processes and investigate the temporal profiles of beta power.

Furthermore, the distinct spatio-temporal profiles observed in our MEG studies suggests a directed flow of information, which appears to be modulated by the degree of plausibility of the observed movement, in line with internal models of motor control (Kawato and Wolpert, 1998; Wolpert et al., 2003). Internal models, which include forward and inverse representations, have been suggested to play a key role in movement recognition. During movement recognition both forward and inverse models will work together to predict the observed movement (Gazzola and Keysers, 2009). The observed movement is first internally simulated in the PMC via the inverse model. The forward model is then used to predict the future sequences of the observed movement by comparing them to the observer's personal repertoire of possible movements (Rizzolatti and Craighero, 2004; Schippers and Keysers,

2011; Wolpert et al., 2003). The efference copy of the predicted movement is then transported back to temporal areas.

We observed temporal changes, which suggest a net flow of information from visual, to motor PMC, projecting back to STS in temporal areas when comparing plausible to scrambled PLD movements. In contrast, when comparing plausible to implausible PLD movements the observed temporal changes were from temporal via parieto-occipital to premotor areas in line with inverse models. These spatio-temporal profiles are in line with previous research that suggests that the MNS is a dynamic system employing both forward and inverse models in the recognition and prediction of the observed movement (Kilner et al., 2007a; Kilner et al., 2007b; Schippers and Keysers, 2011). However, very little information is given on the neural mechanisms involved during movement planning and control of the internally simulated movements. Unfortunately, the nature of our paradigm does not allow for a differentiation between internal simulation of a movement and prediction of future motor sequences of the observed movement. Future research can focus on gathering more information on the complex nature of these mechanisms. It would be interesting to know to what extent recognition of BM for different intent recruits the same neural networks or relies on different ones.

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Appendix

This work is based on:

Publication 1 (Appendix 1)

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Study 2 (Appendix 2)

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Personal Contribution: 80%

Study 3 (Appendix 3)

Pavlidou A, Edwards M, Lange J, Schnitzler A & Bell, J (submitted) Anodal stimulation of premotor cortex facilitates the recognition of different forms of movements (submitted).

Personal Contribution: 80%

Interactions Between Visual and Motor Areas During the Recognition of Plausible Actions as Revealed by Magnetoencephalography

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Abstract: Several studies have shown activation of the mirror neuron system (MNS), comprising the temporal, posterior parietal, and sensorimotor areas when observing plausible actions, but far less is known on how these cortical areas interact during the recognition of a plausible action. Here, we recorded neural activity with magnetoencephalography while subjects viewed point-light displays of biologically plausible and scrambled versions of actions. We were interested in modulations of oscillatory activity and, specifically, in coupling of oscillatory activity between visual and motor areas. Both plausible and scrambled actions elicited modulations of θ (5–7 Hz), α (7–13 Hz), β (13–35 Hz), and γ (55–100 Hz) power within visual and motor areas. When comparing between the two actions, we observed sequential and spatially distinct increases of γ (∼65 Hz), β (∼25 Hz), and α (∼11 Hz) power between 0.5 and 1.3 s in parieto-occipital, sensorimotor, and left temporal areas. In addition, significant clusters of γ (∼65 Hz) and α/β (∼15 Hz) power decrease were observed in right temporal and parieto-occipital areas between 1.3 and 2.0 s. We found β-power in sensorimotor areas to be positively correlated on a trial-by-trial basis with parieto-occipital γ and left temporal α-power for the plausible but not for the scrambled condition. These results provide new insights in the neuronal oscillatory activity of the areas involved in the recognition of plausible action movements and their interaction. The power correlations between specific areas underscore the importance of interactions between visual and motor areas of the MNS during the recognition of a plausible action. *Hum Brain Mapp 00:000–000, 2012.*

Key words: MEG; mirror neurons; oscillatory activity; power correlations; point-light displays

INTRODUCTION

Action recognition plays an important role for effective communication and interaction with other people [Blakemore and Frith, 2005; Kokal et al., 2009; Schippers and Keysers, 2011]. Action recognition occurs at different levels and over distinctive time scales. On a lower level and a shorter time period, sensory information will be processed [Blake and Shiffrar, 2007; Grossman et al., 2000; Michels et al., 2009; Pavlova and Sokolov, 2003]. This incorporates the ability to integrate form and motion but it can also rely on the ability to distinguish form from motion [Lange et al., 2006; Michels et al., 2005; Oram and Perrett, 1994]. Several recent studies have argued that action recognition also relies on higher, nonsensory areas of the mirror

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neuron system (MNS) [Schippers and Keysers, 2011; Urgesi et al., 2010]. Mirror neurons were first discovered in area F5 of the macaque monkey premotor cortex (PMC) [Di Pellegrino et al., 1992]. They are a particular class of neurons that fire when a monkey performs a goal-oriented action but also when it passively observes that same action [Gallese et al., 1996; Rizzolatti et al., 1996]. Areas frequently considered as being part of the MNS in humans are the PMC, supplementary motor area, somatosensory areas, the inferior parietal lobe, inferior frontal gyrus, and indirectly the superior temporal sulcus (STS), a visual area known to respond to biological actions without being a standard part of the MNS [Bonda et al., 1996; Buccino, 2004; Dinstein et al., 2007; Filimon et al., 2007; Gazzola et al., 2007; Pelphrey et al., 2005; Rizzolatti and Craighero, 2004; Schippers and Keysers, 2011].

The proposed mechanism of how mirror neurons mediate recognition of actions is to compare visual information of an action to one's own motor repertoire [Rizzolatti and Craighero, 2004]. In other words, when one observes an action performed by another person, neurons that represent that action in the observer's repertoire of possible actions are triggered in the PMC [Buccino et al., 2004a; Rizzolatti et al., 2001]. Actions belonging to the movement repertoire of the observer are mapped in their PMC. Actions that do not belong to this repertoire are recognized predominantly on a visual basis. In line with this model, studies have shown that the observers' ability to perform an observed action modulates activation in mirror neuron areas (e.g., Calvo-Merino et al., 2005; Orgs et al., 2008).

An effective and frequently used method for studying action recognition is the point-light display (PLD) method [Johansson, 1973]. Although PLD represents a human body and its action with only a handful of dots, observers can easily recognize the actions of these PLD (e.g., Grossman et al., 2000; Johansson, 1973). As PLDs are easy to present and manipulate, they are a useful tool in neuroimaging to study the cortical areas involved in action recognition. By changing the spatial configuration of the dots, while keeping the motion trajectories intact, the configural and holistic impression of the action can be destroyed while keeping low-level information such as motion signals, stimulus size, and number of point-light dots constant. Such "scrambled" PLDs are often used as control stimuli to unravel action recognition from basic low-level visual perception [Grossman et al., 2000; Michels et al., 2005; Pavlova et al., 2004]. Neuroimaging studies in human and nonhuman primates have identified the visual areas to be primarily involved in the process of PLD actions compared to scrambled PLD [Grossman et al., 2000; Michels et al., 2005; Oram and Perrett, 1994; Pavlova et al., 2004]. More recently, studies have also identified the PMC to be involved in the recognition of PLD actions compared to scrambled PLD [Candidi et al., 2008; Kemenade et al., 2012; Saygin et al., 2004]. These findings have led to the interpretation that visual as well as motor areas contribute to the recognition of actions. Most of these studies have been performed using functional magnetic resonance imaging (fMRI). Little is known, however, about the role of neuromagnetic oscillatory activity and how these cortical areas dynamically interact during the process of action recognition.

To investigate the dynamic modulations and interactions between visual and motor areas during the process of action recognition, we used the PLD method similar to the above-mentioned fMRI studies and magnetoencephalography (MEG). We created different PLD action representations and scrambled versions of these PLD actions. MEG's high temporal and good spatial resolution enabled us to examine the dynamics in the frequency domain within- and between-sensory and motor areas during the process of action recognition.

METHODS

Subjects

Twelve right-handed subjects with normal or corrected to normal vision (six males, mean age $\pm$ SD $= 27.6 \pm 2.87$) and with no known neurological disorders participated in this study. All subjects gave informed consent in accordance to the declaration of Helsinki and the local Ethics Committee.

Stimuli

Point-light biological motion animations were generated by recording the movements of human actors with sensors attached to their main joints (head, shoulders, elbows, wrists, hips, knees, and feet) using a motion tracking system (MotionStar; Ascension Technology, Burlington, VT; [Lange and Lappe, 2007]). The main joints were represented by 14 small white dots (5×5 pixels) against a black background.

Stimuli were offline manipulated using MATLAB (MathWorks, Natick, MA). First, actions were cut into segments representing one cycle of the action, lasting between 0.6 and 1.0 s. Next, cycles of each action were repeated five times. To compute a seemingly continuous movement of each action, transitions between cycles were smoothed [Lange et al., 2006]. We manipulated the different stimuli to create three different stimulus conditions with different degrees of action representation, whereas leaving low-level visual information as constant as possible (Fig. 1).

Originally 20 animations depicting a human action were recorded. In a pretest, we presented plausible, implausible, and scrambled versions of the animations and asked subjects to rate the stimuli as plausible, implausible, or scrambled. Eight animations, which were clearly distinguished based on the three-scale rating, were selected and used in the MEG experiment. The selected animations depicted eight actions: walking (viewed from the front, walking toward the screen), walking (viewed from the

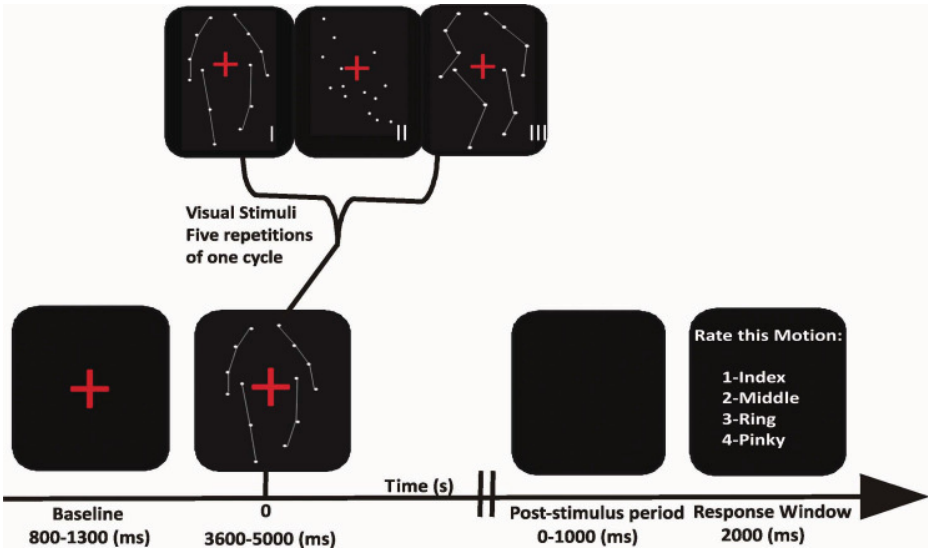

Figure 1.

Experimental setup. Examples of stimuli used (I) Plausible, (II) Scrambled, (III) Implausible. Connecting lines were not present in the actual experiment. For details, see **Experimental Procedures** section. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

side walking either toward the left or toward the right), running, throwing, boxing, skipping (on one leg), skipping (side to side), and a high kick into the air.

Plausible condition (I): Each animation in its original form as recorded. In the pretest, subjects reported to perceive the stimuli as normal, biomechanically probable biological motion.

Scrambled condition (II): Scrambled versions of each animation were created by randomizing the spatial positions of all dots within the field of the original figure [Grossman et al., 2000; Pavlova and Sokolov, 2003; Saygin et al., 2004]. Again, the net movement of the dots is unchanged, whereas the spatial configuration of a human figure is completely destroyed. In the pretest, subjects rated these stimuli as meaningless movements of dots.

Implausible condition (III): Implausible versions of each animation were created by randomizing the starting positions of two dots from the upper body and two from the lower body, whereas leaving their motion paths unchanged. This manipulation leaves the overall movement of all dots unchanged and alters the configural structure only minimally. In the pretest, subjects reported to perceive the stimuli as "somehow human" but the actions as biomechanically implausible.

Experimental Procedure

Subjects were seated comfortably with their head placed inside the MEG helmet. Visual stimuli were projected on the backside of a translucent screen positioned 100 cm in front of the subjects using a projector (PT-DW700E; Panasonic) with a refresh rate of 60 Hz placed outside the shielded room. Each trial started with the presentation of a central red cross (0.4 × 0.4 cm; visual angle. 0.23°). After a randomized period of 800–1,300 ms, in which only the red fixation cross was visible, the point-light animation (8.4 × 3.4 cm; visual angle, 4.81° × 1.95°) appeared for a period of 3,600–5,000 ms (five cycles). The red fixation cross was centrally present throughout the duration of the stimuli to minimize eye movements. After another random period of 0–1,000 ms, in which only a black screen was visible, response instructions were visually presented on the screen. Subjects were asked to rate the animation using a 1–4 rating scale as either plausible (1), implausible (2–3), or scrambled (4) by button presses. Once a response was given, a new trial started. The assignment of the four-fingers to the four configurations of the rating scale was randomized for each trial and response hands were balanced across subjects (Fig. 1). If no response was given within 2,000 ms, or if a response was given too quickly (before the response instructions appeared), the trial was discarded from analysis and repeated at the end of the block. No feedback was given. The *estimated* duration of a trial was 4,400–7,300 ms, followed by the individual response period (maximum of 2,000 ms). Stimuli were presented in pseudo-random order within a block. One block consisted of 31 trials, so that each block had an *estimated* duration of 136.4–226.3 s, respectively, without individual response times (max. 2,000 ms) taken into account (no. of trials × duration). If response times are taken into account,

each block had an estimate duration of ~5 min. Overall, five blocks were presented, with self-timed breaks of ~2 min in between blocks. On the whole, the experiment lasted ~25–30 min. Subjects performed a training session of ~5 min before the start of the MEG experiment. Stimulus presentation was controlled using Presentation Software (Neurobehavioral Systems, Albany, NY).

Data Acquisition and Analysis

While subjects performed the task, neuromagnetic activity was recorded continuously at a sampling rate of 1,000 Hz with a 306-channel whole head MEG system (Neuromag Elekta Oy, Helsinki, Finland). This system includes 204 planar gradiometers and 102 magnetometers arranged in a helmet configuration. In the present study, data analysis was carried out only with the planar gradiometers. In addition, vertical and horizontal electrooculograms were recorded simultaneously for offline artifact rejection. Subjects' head position within the MEG helmet was registered by four coils placed at subjects' forehead and behind the left and right ear. A 3T MRI scanner (Siemens, Erlangen, Germany) was used to obtain individual full brain high-resolution standard T1-weigthed structural magnetic resonance images (MRIs). MRIs were offline aligned with the MEG coordinate system using the coils and anatomical landmarks (nasion, left, and right preauricular points).

Data were analyzed offline with the open source toolbox FieldTrip for Matlab (http://www.ru.nl/donders/fieldtrip) [Oostenveld et al., 2011]. Continuously recorded data were cut into epochs as defined by the trials. All epochs were first semi-automatically and then visually inspected for artifacts. Artifacts caused by eye movements or muscle activity were removed. Power line noise was removed by applying a Fourier transformation of 10-s long signal periods and subtracting the 50, 100, and 150 Hz components.

Time–Frequency Analysis

Time–frequency representations were computed separately for two frequency windows: For frequencies ranging from 4 to 40 Hz (in steps of 2 Hz), we applied a Fourier transformation on 500-ms windows moved in steps of 50 ms. Data segments were tapered with a single Hanning taper, resulting in a spectral smoothing of ±2.0 Hz. For the frequencies from 40 to 100 Hz (in steps of 5 Hz), a Fourier transformation was applied on 400-ms windows moved in steps of 50 ms, using the multitaper approach [Walden et al., 1995]. Data segments were tapered with seven tapers, resulting in a spectral smoothing of ±10.0 Hz around each center frequency.

As we were interested in the development of power over time and power correlations across frequencies (for details, see correlation analysis), we used a Fourier transformation on constant time window and tapering for all frequencies within a frequency band. This approach ensures that the same data set and same tapers are used within a frequency band. Any changes observed are thus attributed to the frequency components, rather than changes in time windows and/or tapers. We used different time windows and tapering for low and high frequencies because low-frequency bands are relatively narrow and closely spaced. We therefore aimed at a high spectral resolution in the low frequency range of roughly ±2 Hz (i.e., 1/500 ms). In the higher frequency range, frequency bands are broader and spaced more far apart so that we applied a spectral smoothing of ±10.0 Hz. This approach provided an acceptable trade-off between capture of physiological frequency bands and comparability within- and between-frequencies. Previous studies have identified parieto-occipital, left and right temporal, and sensorimotor areas as crucial areas in the recognition of PLD actions [Grossman et al., 2000; Michels et al., 2009; Saygin et al., 2004; Schippers and Keysers, 2011]. To identify these regions of interest in sensor space in our study, we applied a combined data driven and a priori approach. First, we pooled all trials together irrespective of stimulus conditions (plausible, implausible, and scrambled) and determined which sensors showed clear perturbations of oscillatory activity in response to PLD relative to baseline (−400 to −250 ms). Six sensors in the right hemisphere showing a sustained decrease in α (7–13 Hz) and β (13-23 Hz) power as well as a selective sustained increase in γ (55–95 Hz) power where selected over parieto-occipital areas (Fig. 2A). In addition, 10 sensors, five in the left hemisphere and symmetrically five in the right hemisphere, showing a sustained decrease in low α (7–11 Hz) and a selective increase in high α (11–13 Hz) power as well as a sustained decrease in β (15–23 Hz) power where selected over the sensorimotor cortex (Fig. 2B). Finally, owing to the vast reports on the importance of STS and temporal areas in action recognition (e.g., Dinstein et al., 2007; Grossman and Blake, 2001, 2002; Grossman et al., 2000; Pavlova et al., 2004; Pelphrey et al., 2004), eight sensors in the left and symmetrically eight sensors in the right hemisphere were selected over the temporal cortices. Although temporal cortices showed similar effects in the lower range frequencies (4–40 Hz) as observed in parieto-occipital areas, the difference in the γ-band effects between the two suggests that both process action representations differently. To asses the different roles of the parieto-occipital, temporal, and sensorimotor areas in action recognition, we next investigated the contrasts between the conditions.

Condition Contrasts

We assessed differences in spectral power between stimulus conditions in the four above-mentioned regions of interest (parieto-occipital, left and right temporal, sensorimotor). To this end, we averaged spectral power over the sensors of interest for each stimulus condition

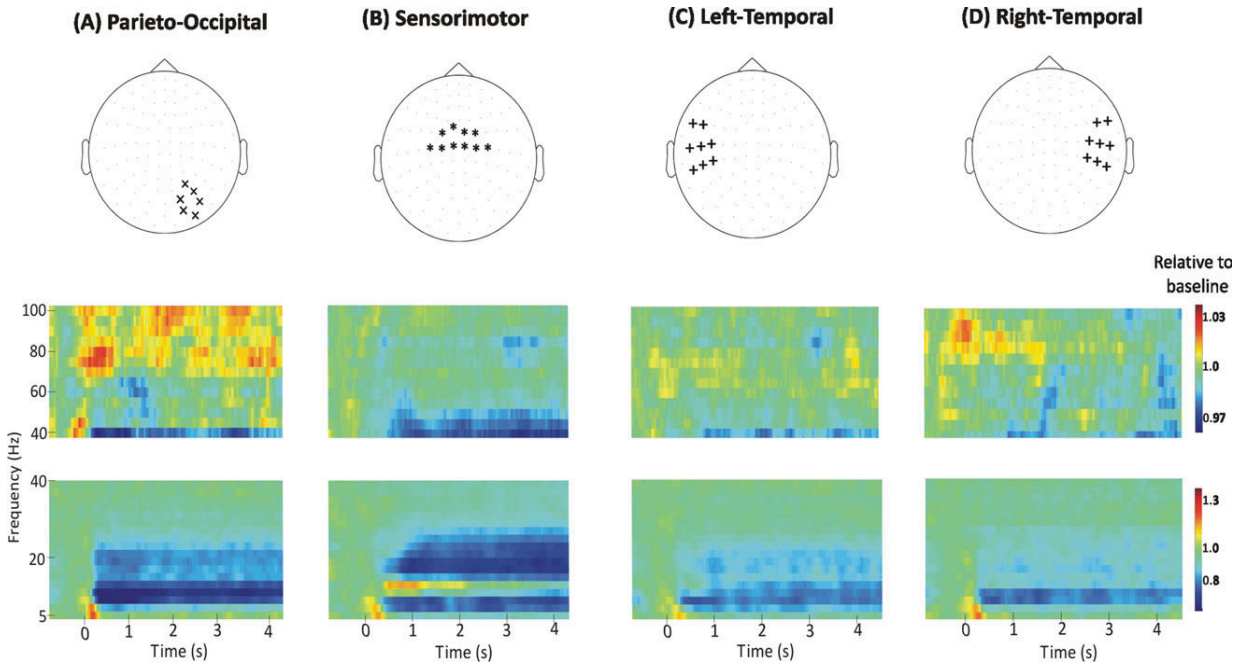

Figure 2.

Stimulation effects of PLDs. Top row shows our sensors of interest for (**A**) parieto-occipital (x), (**B**) sensorimotor (*), (**C**) left-temporal (+), and (**D**) right-temporal (+) areas, respectively. Color maps illustrate changes in power relative to baseline (−400 to −250 ms), which were calculated separately for low (4–40 Hz) and high frequencies (40–100 Hz) by pooling all trials together irrespective of conditions.

separately. Next, we compared stimulus conditions for each subject by subtracting power of both conditions and dividing the difference by the variance (equivalent to an independent-sample *t*-test). This step serves as a normalization of interindividual differences [Hoogenboom et al., 2010; Lange et al., 2011]. This comparison was carried out for each time–frequency sample independently, resulting in a time–frequency map of pseudo-*t*-values for each subject. To minimize influences of motor activity owing to response preparation, statistical analyses were restricted to the first 3 s. Next, we analyzed the consistency of pseudo-*t*-values over subjects by means of a nonparametric randomization test. This statistical test effectively corrects for multiple comparisons [Maris and Oostenveld, 2007]. To this end, time–frequency pseudo-*t*-values exceeding a threshold ($P < 0.05$) were identified and neighboring significant time–frequency pseudo-*t*-values were combined to a cluster. For each cluster, the sum of the *t*-values was used in the second-level cluster-level test statistics. We used the Monte Carlo approach to estimate the permutation *P*-value of the cluster by comparing the cluster-level test statistic with a randomization null distribution. The null distribution was computed by randomly assigning data to different conditions, under the null hypothesis of no difference between conditions and thus exchangeability of the data. The random reassignment of the data to conditions was performed 1,000 times. For each of these 1,000 repetitions, a group *t*-value was calculated. Finally, a *P*-value was estimated for each cluster as the proportion of the elements in the randomization null distribution exceeding the observed maximum cluster-level test statistic (for details, see Lange et al., 2011). This group level statistics results in time–frequency clusters which reveal differences between conditions that were significant at the random effects level after correcting for multiple comparisons along both the time and the frequency dimension [Maris and Oostenveld, 2007].

In the present study, we were interested in how processing of plausible actions differs from processing of nonactions. As discussed in the **Introduction** section, most fMRI studies, to date, on PLD action recognition have dealt with a similar question by comparing actions to scrambled versions of these actions. Based on our main research question and for the sake of comparability, we will focus in our present study on the main contrast of plausible (actions) versus scrambled (nonactions) conditions. The comparison between plausible and implausible actions engages different research questions and thus presumably different cortical networks and mechanisms which lie beyond the scope of the present study.

Source Analysis

To determine the neuronal sources, we applied dynamic imaging of coherent sources (DICS), an adaptive spatial

filtering beamforming technique [Gross et al., 2001]. To this end, a regular three-dimensional 1-cm grid in the Montreal Neurological Institute (MNI) template brain was created and the structural MRI of each subject was linearly warped onto this template brain. The inverse of this warp was applied to the template grid, resulting in individual grids. This approach allowed us to average source parameters over subjects by simply averaging over grid points. For each grid point then, a forward model based on a realistic single shell volume conductor based on the individual MRI was used to calculate the lead-field matrix [Nolte, 2003]. We next applied a Fourier transformation on time–windows of interest and computed the cross-spectral density (CSD) matrix between all MEG sensor pairs for the frequency bands of interest, which were determined by the significant time clusters on sensor level. Spatial filters were constructed for each individual grid point using the CSD and lead field matrix. These filters pass activity from the location of interest, whereas suppressing activity from all other locations. Spatial filters $w(r,f)$ were computed from the following formula:

$$w(\mathrm{r},\mathrm{f}) = (L'(r)C(f) + \lambda\, x\, I)^{-1}L'(r)^{-1}L'(r)C(f) + \lambda\, x\, I)^{-1},$$

where $L'(r)$ is the inverse of lead-field matrix (forward model) at location of interest r, $C(f)$ is the CSD matrix between all MEG signals at frequency f, λ is the regularization parameter, and I is the identity matrix [de Lange et al., 2008; Gross et al., 2001].

First, we pooled all conditions (pre- and post-stimulus period for stimulation effects; plausible and scrambled conditions for condition contrast) and computed common filters. Next, CSD matrices of single trials were projected through those filters, providing single trial estimates of source power [Hoogenboom et al., 2010; Lange et al., 2011]. In line with the analysis on sensor level, we computed a relative change to baseline for stimulation contrasts and a between-condition t-value for condition contrasts for each subject. Statistical testing on group level for time–frequency representations of stimulation effects ($P < 0.05$, cluster corrected) and condition contrasts ($P < 0.05$, uncorrected) was carried out in the same way as on sensor level (see above). Results were displayed on the MNI template brain and neuronal sources were identified using the AFNI atlas (http://afni.nimh.nih.gov/afni), integrated into FieldTrip.

Cross-frequency Correlations

To investigate the interaction between visual and motor areas during the recognition of plausible actions, we calculated the crossfrequency coupling over the specific time course of our significant clusters. Cross-frequency coupling refers to the coupling of the neuronal signal between distinct frequency bands in the same or different cortical regions [Jensen and Colgin, 2007]. Here, we investigated the power correlation between the significant time–frequency clusters of the above-mentioned time–frequency analysis. For each trial, we averaged spectral power across the time and frequency bins defined by the significant clusters on group level (Fig. 4A). Next, we computed correlations between sensorimotor β-power on the one hand and parieto-occipital γ and temporal α-power on the other hand. Power correlations were determined per subject on a trial-by-trial basis by computing Pearson correlation coefficient. Individual correlation coefficients were converted to z-values using the Pearson's r-to-z transform to attain a normally distributed variable [Choi, 1977]. The distribution of correlation coefficients across subjects was statistically tested against the null hypothesis of no correlation, that is, $r = 0$ by using a two-sided t-test. To test for a temporal specificity of the correlations, frequency bands of interest were shifted in steps of ±100 ms and correlations were recomputed as described above.

RESULTS

Behavioral Data

The subjects rating of the PLD motion as plausible or scrambled indicated that they could easily distinguish both stimuli with an average rating of 1.5 (±0.19) for all plausible, and 3.8 (±0.14) for all scrambled. Statistical testing revealed highly significant differences between both conditions ($P < 0.001$).

Stimulation Effects

We first determined the effects of stimulation by pooling all trials irrespective of condition (plausible, implausible, and scrambled) and computing time–frequency representations of neural oscillatory activity in response to the PLD relative to baseline (−400 to −250 ms). We focused on four main areas, which showed clear perturbations of spectral activity in response to visual stimulation:

Parieto-occipital areas: PLD elicited an increase of power in the θ-band (5–7 Hz) immediately after stimulus onset (0–0.3 s). In addition, we observed a sustained decrease in the α (7–13 Hz) and β (13–21 Hz) band power after stimulus onset (0.2–4.5 s), as well as a sustained increase in γ-power (70–95 Hz) between 0.1 and 4.5 s poststimulus onset. All stimulation effects showed a clear bilateral distribution in parieto-occipital areas, with the γ-band effect more strongly pronounced to the right hemisphere (Fig. 2A).

Sensorimotor areas: PLD elicited a weak increase in θ-band (5–7 Hz) power after stimulus onset (0.0–0.3 s), which, however, is most likely owing to spatial smearing from the parieto-occipital areas. In addition, we observed a distinct and sustained increase in high α (11–13 Hz) power between 0.4 and 4.0 s and a sustained decrease in low α (7–11 Hz) and β (15–23 Hz) power between 0.5 and 4.5 s poststimulus onset in bilateral sensorimotor areas (Fig. 2B).

Temporal areas: PLD elicited a bilateral increase in θ-band (5–7 Hz) power after stimulus onset (0.0–0.3 s). In addition, we observed a sustained bilateral decrease in low α

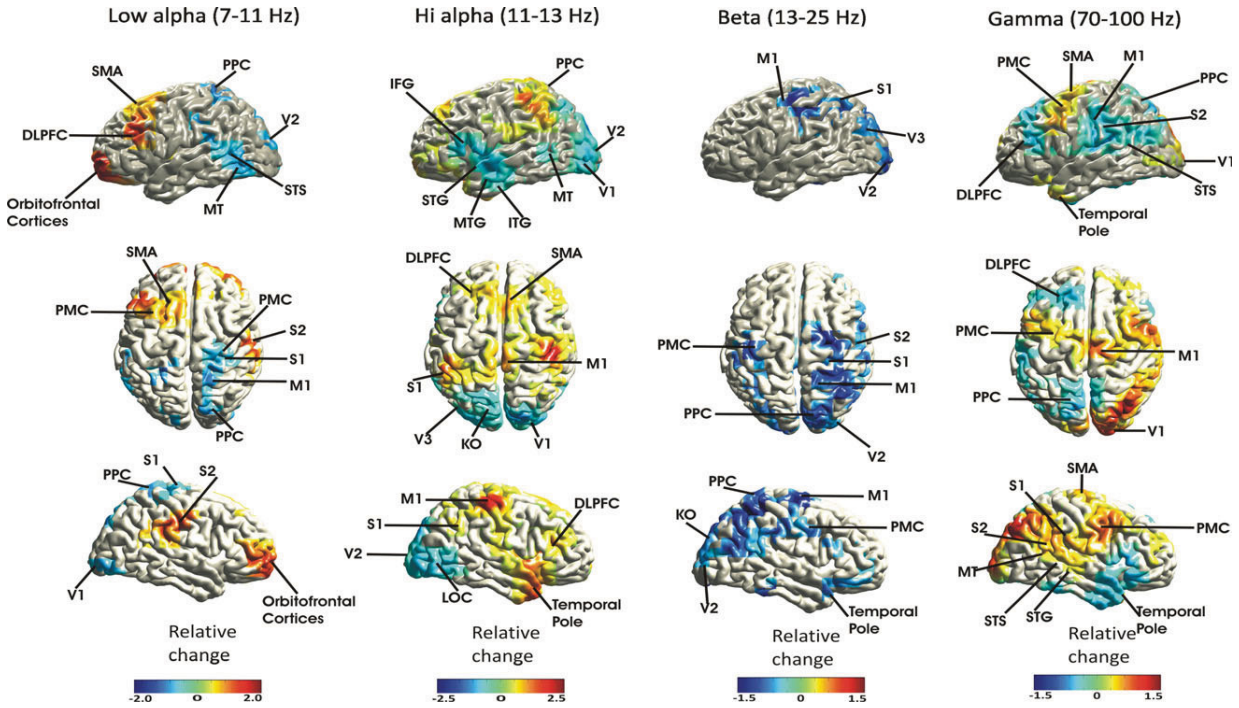

Figure 3.

Stimulation effects on source level. Cortical sources of relative change for low α (7–11 Hz), high α (11–13 Hz), β (13–25 Hz), and γ (70–100 Hz) power, respectively. Color maps illustrate changes in power relative to baseline. Only significant sources ($P < 0.05$; cluster corrected) are shown.

(7–11 Hz) and β-power (13–23 Hz) between 0.5 and 4.5 s, as well as a bilateral increase in high α (11–13 Hz) between 0.3 and 0.6 s (Fig. 2C,D). These effects are highly similar to the effects found in sensors over parieto-occipital and sensorimotor areas (see above) but with lower amplitude. In contrast to the results from parieto-occipital sensory, we observed a robust early increase (90–100 Hz) between 0 and 1.5 s and a sustained decrease in oscillatory γ-power (50–80 Hz) between 0 and 4.5 s poststimulus onset in right temporal cortex (Fig. 2D).

Next, we identified the cortical sources of the sustained effects, found in the time–frequency representations on sensor level. To this end, we performed source localization using a beamformer on four distinct frequency bands, based on the results on sensor level (i.e., for low α [7–11 Hz], high α [11–13 Hz], β [13–25 Hz], and γ [50–100 Hz] band). Strongest cortical sources were identified in visual as well as sensorimotor areas (for details, see Fig. 3).

Condition Contrast

We assessed differences between plausible versus scrambled stimuli in the four regions of interest (parieto-occipital, left, and right temporal, and sensorimotor areas). We found a significant increase in γ (55–90 Hz) power at 500–800 ms poststimulus onset in parieto-occipital areas ($P < 0.05$), followed by a significant increase in β (20–35 Hz) power at 700–1,200 ms poststimulus onset in sensorimotor areas as well as a significant increase in high α (9–13 Hz) power at 900–1,300 ms in left temporal areas (Fig. 4A). In addition, we found a significant decrease in γ (50–80 Hz) and α/low β (10–22 Hz) power, between 1,300 and 2,000 ms in right temporal and parieto-occipital (Fig. 4A) areas ($P < 0.05$), respectively.

Next, we identified the cortical sources of these significant clusters. For the increase in γ-power (55–90 Hz) between 500 and 800 ms, the sources were identified in the primary visual cortex (V1). Additional sources were identified in the right medial and inferior temporal gyrus, as well as right dorsolateral prefrontal cortex (DLPFC) (Fig. 4B).

The sources of the significant effects between 20–35 Hz and 700–1,200 ms were located in the bilateral sensorimotor areas of the brain and more specifically the PMC and right primary motor cortex (M1) (Fig. 4D).

The sources for the significant effects between 9–13 Hz and 900–1,300 ms were located in the left temporal areas of the brain and more specifically the STS. Additional sources were identified in the left somatosensory areas (Fig. 4F).

The sources for the significant effects between 50–80 Hz and 1,300–1,600 ms were located in the right temporal areas of the brain and more specifically the right medial

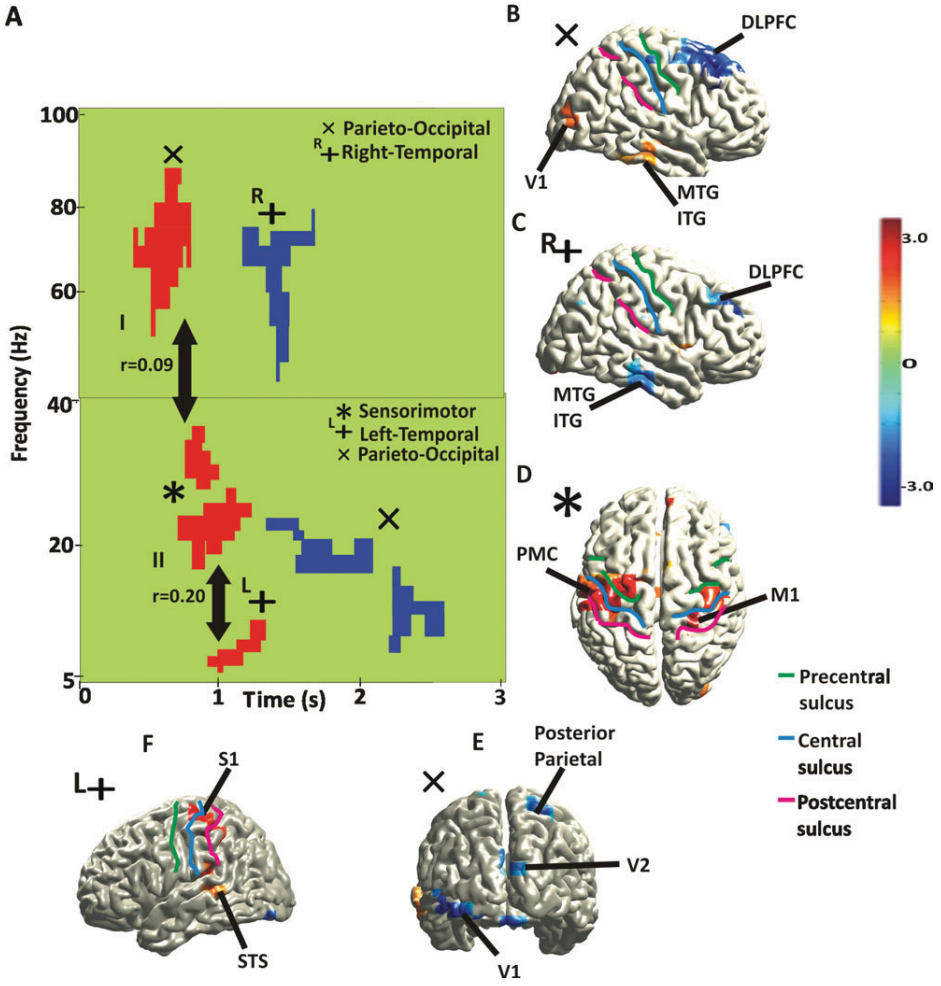

Figure 4.

Condition contrasts for plausible versus scrambled PLD: (**A**) Representations of significant clusters ($P < 0.05$) found on sensor level for (x) parieto-occipital, (*) sensorimotor cortex, and (+) left-temporal, and (+) right-temporal. Red denotes higher power for plausible, whereas blue denotes higher power for scrambled. Source reconstruction of the significant clusters found on sensor level for (**B**) parieto-occipital γ increase, (**C**) right temporal γ decrease, (**D**) sensorimotor β-increase, (**E**) parieto-occipital β-decrease and, (**F**) left temporal α increase. Color map represents *t*-values for source reconstruction. Red denotes higher power for plausible, whereas blue denotes higher power for scrambled. Arrows and *r*-values represent significant ($P < 0.05$) positive trial-by-trial correlations for the plausible condition between sensorimotor β and (I) parieto-occipital γ-power as well as (II) left temporal α-power.

and inferior temporal cortex. Additional sources were identified in the right DLPFC (Fig. 4C).

Finally, the sources of the significant cluster between 10–22 Hz and 1,600–2,000 ms were localized in bilateral parieto-occipital areas and more specifically visual areas V1 and V2 as well as right parietal posterior (Fig. 4E).

Cross-frequency Correlations

To assess the interactions between visual and sensorimotor areas during the recognition of actions, we calculated the trial-by-trial cross-frequency correlation between the significant time–frequency clusters (Fig. 4A). A significant positive correlation was observed between sensorimotor β (averaged between 20–35 Hz and 700–1,200 ms) and parieto-occipital γ (averaged between 55–75 Hz and 500–800 ms) power ($r = 0.09$; $P < 0.05$) as well as between sensorimotor β and left temporal α (9–13 Hz and 900–1,300 ms) power ($r = 0.20$; $P < 0.05$) for the plausible action condition, but not for the scrambled one. No significant correlation was observed when the time windows of the significant clusters were moved in steps of $\pm$100 ms. In

addition, a significant positive trial-by-trial correlation was observed between parieto-occipital β (10–22 Hz and 1,600–2,000 ms) and right temporal γ (50–80 Hz and 1,300–1,600 ms) power ($r = 0.16$; $P < 0.05$) for the scrambled condition. A significant positive trial-by-trial correlation was still visible when the time windows of the significant clusters were simultaneously moved in steps of −100 ms ($r = 0.19$; $P < 0.05$), but not for other time shifts. Finally, a significant negative trial-by-trial correlation was observed between sensorimotor β (20–35 Hz and 700–1,200 ms) and parieto-occipital β (10–22 Hz and 1,600–2,000 ms) power ($r = 0.08$; $P < 0.05$) for the scrambled condition that was not present when the time windows were moved in steps of ±100 ms.

DISCUSSION

The present study aimed at determining the dynamic modulations of neuronal oscillatory activity in the cortical networks involved in the recognition of plausible actions. PLDs elicited sustained effects in θ (5–7 Hz), α (7–13 Hz), β (15–25 Hz), and γ (50–100 Hz) power within cortical areas of the MNS. We were particularly interested how these dynamic modulations as well as the interactions between areas of MNS changed when we compared plausible and scrambled actions. We will first discuss the observed stimulation-induced effects with respect to earlier hemodynamic and electrophysiological reports. The main focus of this article is the comparison of our two conditions and their interactions between cortical areas of the MNS, which will then be applied to current theories of the action recognition process.

Presentation of PLD (pooled over all conditions) induced a sustained decrease of spectral power in the α- and β-band in parieto-occipital regions. The decrease started at ∼200 ms poststimulus onset and was sustained throughout the trial. The decrease as well as its timing is in line with the previous reports on visual stimulation (e.g., de Lange et al., 2008; Hoogenboom et al., 2006; Koelewijn et al., 2008; Singh et al., 2002). The decrease of α/β-power was also found in sensorimotor areas, starting at around ∼500 ms poststimulus and lasting until the end of the trial, in agreement with the earlier reports of α/β suppression during action preparation, action execution, and motor imagery tasks (de Lange et al., 2008; Hari and Salmelin, 1997; Koelewijn et al., 2008; Oberman et al., 2005; Orgs et al., 2008; Schnitzler et al., 1997; Ulloa and Pineda, 2007). Moreover, somatosensory areas have been suggested to play a role in the internal simulation of the sensory consequences of observed actions or embodiment [Caetano et al., 2007; de Lussanet et al., 2008]. In contrast to the suppression of low α band-power, sensorimotor areas revealed an increase of high α (11–13 Hz) band power between 400 and 4,000 ms poststimulus. While a decrease of α/β-band power has been linked to engagement of sensorimotor areas, an increase has been suggested to reflect inhibition or disengagement of the sensorimotor system [Hummel et al., 2002; Jensen et al., 2002; Nachev et al., 2008; Neuper and Pfurtscheller, 2001]. The early observed increase in high α-band power might thus reflect subjects' active inhibition of finger and/or eye movements during stimulus presentation or suppression of task-irrelevant areas during initial stimulus presentation. Finally, we observed a sustained increase of high γ-band power in a wide range of areas including frontal and posterior regions of the brain (for details, see Fig. 3). This increase of γ-power is visible between 100 and 4,500 ms, that is it starts slightly earlier than the other sustained effects, similar to the previous reports involving visuomotor tasks [Aoki et al., 1999; de Lange et al., 2008; Pavlova et al., 2004, 2006; Pfurtscheller and Neuper, 1992].

When comparing plausible to scrambled condition, we observed an early increase of γ (55–75 Hz) band power between 500 and 800 ms in right V1 and temporal cortex. Other electrophysiological studies report an increase in γ-power as early as 80–170 ms when subjects passively viewed point-light walkers [Pavlova et al., 2004, 2006]. One reason for the differences in timing might be owing to the different definition of γ-band activity: Although we observed γ-band effects between 55 and 75 Hz, Pavlova et al. found effects in the lower γ-band between 25 and 40 Hz. In addition, differences might be owing to the different experimental designs between Pavlova et al. (passive viewing of normal, scrambled, inverted PLD) and our study (active evaluation of normal, implausible, and scrambled PLD). This increase of γ-band, however, is in line with increased hemodynamic responses in parieto-occipital and temporal areas for plausible versus scrambled PLD (e.g. Grossman and Blake, 2002; Grossman et al., 2000; Michels et al., 2005, 2009; Pelphrey et al., 2004, 2005). Neuronal activity, especially γ-band activity, in right temporal areas reflects the processing of the global form of the PLD, which is only recognizable in the plausible condition [Michels et al., 2005, 2009; Pavlova et al., 2004]. As the γ-band effect was the earliest significant cluster, the result suggests that discrimination between plausible and scrambled PLD starts at early, low-, and high-level visual stages of the action recognition process (e.g. Pavlova et al., 2004).

The increase of γ-band power was followed by an increase of power in the β (20–35 Hz) band between 700 and 1,200 ms in bilateral sensorimotor areas (PMC and M1). Similar to the timing of the sensorimotor β-effect, previous electrophysiological studies reported sensorimotor α/β decreases to differentiate during action observation or motor imagery in the time period of ∼450–1,500 ms poststimulus onset [de Lange et al., 2008; Orgs et al., 2008; Schnitzler et al., 1997]. Previous fMRI studies demonstrated that sensorimotor areas and more specifically the PMC, responded to both human (plausible) and nonhuman (scrambled) actions, but much stronger for human actions belonging to the observer's own motor repertoire [Buccino et al., 2004b; Saygin et al., 2004]. In addition, Calvo-Merino et al. (2005) observed a stronger hemodynamic response in

STG, premotor, and parietal areas when capoeira and classical ballet dancers observed movements from their own repertoire. The observed positive β-cluster in sensorimotor areas reflects a stronger suppression of power for scrambled than plausible actions. In contrast to this observation, one previous study revealed a stronger suppression of sensorimotor β-power when subjects viewed actions within their own repertoire compared to other plausible, but clearly distinguishable movements [Orgs et al., 2008]. Interestingly however, stronger suppression of sensorimotor β-band power has been reported for the observation of incorrect versus correct button presses [Koelewijn et al., 2008]. Although subjects in the study by Koelewijn et al. had to distinguish between correct and incorrect button presses, subjects in our study had to distinguish between normal and scrambled actions. Despite these notable differences in the experimental setup, we observed a similar pattern of β-decrease as reported by Koelewijn et al. We therefore speculate that stronger β-band suppression in our study might thus be related to the recognition of the scrambled action movements as incorrect. Future studies, however, are needed to support this speculation.

The sensorimotor β-increase was followed by an α (9–13 Hz) band increase between 900 and 1,300 ms in left S1 and STS. An increase in α power might reflect suppression of task-irrelevant areas during initial stimulus presentation, as well as active inhibition or disengagement of the cortical areas involved (e.g. Jensen and Mazaheri, 2010; Jensen et al., 2002). The observed α-power increase over left STS and somatosensory areas, two areas known to be involved in the processing [Allison et al., 2000] and internal simulation [de Lussanet et al., 2008] of biological actions, might thus reflect active inhibition of these areas. Previous electrophysiological studies reported α activity of temporal areas peaking at around ∼750 ms during a visual attention task [Pantazis et al., 2009]. The observed left hemisphere activity might reflect visual attention of the local details of the PLD when differentiating between plausible and scrambled conditions [Bonda et al., 1996; Fink et al., 1997; Lamb and Robertson, 1988].

Interestingly, we observed a significant positive trial-by-trial correlation between sensorimotor β-power and parieto-occipital γ-power as well as left temporal α-power. This correlation was observed only for plausible PLD but not for scrambled PLD, and the correlation was observed only at specific time points, namely at time points where we found the significant power increase for plausible PLD. This finding illustrates a crossfrequency coupling between visual and motor areas during recognition of plausible actions operating at large spatio-temporal scales. The temporal profiles of the power changes suggest a functional interaction proceeding from visual areas to sensorimotor areas and back projecting to STS.

At a later time point, we observed an additional negative cluster in the β-band in parieto-occipital areas, reflecting a stronger β-band power for the scrambled than the plausible condition. This finding is in line with fMRI studies which suggest that parieto-occipital areas are more sensitive to image scrambling (for review, see Grill-Spector and Malach, 2004). Trial-by-trial correlations between this late parieto-occipital β-band power and early sensorimotor power revealed a negative correlation for the scrambled PLD, but no significant correlation for the plausible PLD. This finding reveals crossfrequency coupling between sensorimotor and visual areas over several hundred milliseconds. We suggest that this effect reflects feedback projections from sensorimotor areas to visual areas, possibly updating visual processing [Schippers and Keysers, 2011]. Interestingly, all correlations have been observed between sensorimotor β-power and other frequencies in other areas. Oscillations in the β-band have been widely observed in sensorimotor areas in relation to motor behavior [Haegens et al., 2011; Salenius and Hari, 2003] and have been proposed as a mechanism for synchronization over long transmission delays and long ranges [Bibbig et al., 2002; Gross et al., 2004; Kopell et al., 2000; Schnitzler and Gross, 2005]. We suggest that β-oscillations supply a mechanism that combines visual and motor areas into a functional network [Brovelli et al., 2004].

The power correlations, although low in absolute value, are statistically significant and consistent across all subjects. Studies investigating working memory with intracranial EEG (iEEG) have reported correlation with absolute values >0.3 (e.g. Axmacher et al., 2010). This difference might be owing to a higher signal-to-noise ratio for iEEG when compared to MEG. The absolute values of the correlation values (0.07–0.20) of our study, however, are in line with the previous MEG studies, reporting power correlations in the range of 0.01–0.07 (e.g., Hipp et al., 2012; Hoogenboom et al., 2010).

Interestingly, we also observed a much stronger γ-power for scrambled PLD in right DLPFC. DLPFC activity has been linked to the process of evaluating other people's behavior (e.g. Saygin, 2007; Saygin et al., 2004). It has been, therefore, suggested that DLPFC is an important contributor to cognitive control in a social domain, as its role is to maintain intentions of our actions in working memory, and subsequently using feedback to evaluate whether our actions match those intentions [Weissman et al., 2008]. The stronger suppression of γ-power for plausible than scrambled actions might thus reflect DLPFC efforts in trying to evaluate the intentions of the scrambled actions that do not match the intentions stored in working memory.

In summary, our results reveal a widespread cortical network involved in the recognition of plausible actions, including areas of the MNS operating at different frequency bands, extending previous fMRI and MEG studies. We demonstrate interactions between these areas by revealing power correlations between visual and motor areas during the recognition of plausible and scrambled actions at specific spatial-temporal scales. We propose that these results reveal a functional coupling of visual and motor areas, predominantly coupled to the sensorimotor β-frequency, in support to current models of motor control that propose the presence of internal models (inverse and forward) involving visual and motor interactions.

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Title Page

Title:

Distinct spatio-temporal profiles of beta-oscillations within visual and sensorimotor areas during action recognition as revealed by MEG.

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Running title: Beta-oscillations reflect plausibility of actions.

Abstract

The neural correlates of action recognition have been widely studied in visual and sensorimotor areas of the human mirror neuron system (MNS). However, the role of neuronal oscillations involved in MNS during the process of action recognition remain unclear. Here, we were interested in how the plausibility of an action modulates neuronal oscillations in visual and sensorimotor areas. Subjects viewed point-light displays (PLD) of biomechanically plausible and implausible versions of the same actions. Using magnetoencephalography (MEG), we examined dynamic changes of oscillatory activity during these action recognition processes. While both actions elicited oscillatory activity in visual and sensorimotor areas in several frequency bands, a significant difference was confined to the beta-band (~20 Hz): An increase of power for plausible actions was observed in left temporal, parieto-occipital and sensorimotor areas of the brain, in the beta-band in successive order between 1650-2650 ms. These distinct spatio-temporal beta-band profiles suggest that the action recognition process is modulated by the degree of biomechanical plausibility of the action, and that spectral power in the beta-band may provide a functional interaction between visual and sensorimotor areas of the MNS in humans.

Keywords point light displays, oscillatory activity, actions, mirror neuron system

Introduction

Understanding others' actions is essential to communicate effectively with the people around us. Understanding an action is essentially preceded by the ability to recognize an action. Action recognition is a process in which visual information is integrated with motor representations (Craighero et al., 2007; Jeannerod, 2001; Rizzolatti and Craighero, 2004). The underlying cortical sources of this process have been studied by single-cell recordings in macaque monkeys (di Pellegrino et al., 1992; Fadiga et al., 1995; Oram and Perrett, 1994) and extensively in humans using hemodynamic and electrophysiological techniques (Buccino et al., 2004b; Grossman et al., 2000; Pavlova et al., 2004; Saygin et al., 2004; Michels et al., 2005; 2009; Schippers and Keysers, 2011). In summary, these studies show a widespread cortical network involved in the observation/recognition of actions known as the mirror neuron system (MNS), mainly comprising the superior temporal sulcus (STS), inferior parietal lobule (IPL) and premotor cortex (PMC). Mirror neurons are a particular class of visuomotor neurons first discovered in area F5 of the macaque monkey, which fire when a monkey observes and executes an action (di Pellegrino et al., 1992; Gallese et al., 1996).

Electrophysiological studies have demonstrated changes of neuronal oscillatory activity in areas of the MNS, especially sensorimotor areas, during periods of action observation/recognition in the alpha (9-13 Hz) and the beta range (13-30 Hz) (Babiloni et al., 2002; Cochin et al., 1998; de Lange et al., 2008; Hari et al., 1998). Such changes of beta activity in sensorimotor areas have been associated with two processes: Asynchrony or decrease of beta power occurs during the preparation and execution of movements, while synchrony or increase of beta power reflects active inhibition (Pfurtscheller and Lopes da Silva, 1999; Pfurtscheller et al., 2005; Salmelin et al., 1995). Furthermore, attenuation of oscillatory beta activity in the primary motor cortex (M1) has been interpreted as evidence of a MNS in humans (Kilner et al., 2009; Rizzolatti and Craighero, 2004). Although M1 is not considered to be part of the MNS, cortical activity in M1 has been argued to be influenced by strong activity in PMC, which is part of the MNS, due to the strong cortical connections that exist between the two areas (Kilner et al., 2009).

While beta activity in sensorimotor areas is abundant, little is known, however, how oscillatory activity in sensorimotor areas is modulated by the plausibility of an observed action and how sensorimotor areas interact with other cortical areas during action recognition.

In a previous study, we examined how oscillatory activity in visual and sensorimotor areas is modulated by the presence or absence of a human action (Pavlidou et al., under second revision, Human Brain Mapping). Actions were represented by point-light displays (PLD), a method in which the human body is portrayed by just a handful of moving dots (Johansson, 1973; Grossman et al., 2000; Saygin et al., 2004, Michels et al., 2005). Subjects were asked to differentiate between plausible (e.g. walking) and scrambled (random assortment of dots) versions of different action representations. We observed changes in gamma (~70 Hz), beta (~25 Hz) and alpha (~10 Hz) oscillatory activity between 0.5 and 1.3 s in widespread network of cortical areas, including the sensorimotor areas and parts of the MNS. Further research however, is needed to determine whether the MNS is involved in higher form processing such as distinguishing between natural and unnatural forms of action movements when the degree of plausibility of an action is manipulated.

In the current study, we compare plausible human PLD actions to implausible human PLD actions. An implausible PLD action leaves the overall movement of the dots unchanged, i.e. in contrast to scrambled PLD, overall visual information and human configural structure is only marginally altered. Subjects were asked to differentiate between the two PLD actions. We were interested whether MNS will be engaged differently when we manipulate the biomechanical plausibility of a human PLD action. More specifically, we were interested whether beta-band activity was sensitive to the degree of plausibility of the observed action within and between the MNS network.

Methods

Data were collected in a conjunct experiment with data from an earlier study (Pavlidou et al., under second revision, Human Brain Mapping). Accordingly, the details of the stimuli and experimental procedures as well as the methods of data acquisition and data analysis have been described in great detail elsewhere (Pavlidou et al., under second revision, Human Brain Mapping). Here, we provide a concise overview of the experimental procedure and analysis. Data analysis in the present study, however, focuses on different experimental questions and uses different subsets of the data.

Subjects, experimental procedure and stimuli

12 right handed subjects with normal or corrected to normal vision (6 males, mean age (± SD) 27.6 ± 2.87 years) participated in the study. Each trial started with a presentation of a red fixation cross (visual angle 0.23°). After a jittered period (800-1300 ms), a point-light display (PLD) (visual angle 4.81° x 1.95°) appeared for a period of 3600-5000 ms (5 cycles of one action). After another randomized period (0-1000 ms), where only a black screen was visible, instructions were visually presented for a duration of 2000 ms. Subjects were asked to rate the PLD as either 1-plausible, 2,3-implausible and 4-scrambled (Figure 1). Stimuli were presented with a projector (PT-DW700E; Panasonic) with a refresh rate of 60 Hz. Stimulus presentation was controlled using Presentation Software (Neurobehavioral Systems, Albany, USA).

Data acquisition and analysis

Neuronal activity was recorded with a 306-channel whole head MEG system (Neuromag Elekta Oy, Helsinki, Finland). Vertical and horizontal electrooculogram (EOG) were recorded for offline artifact rejection. The subjects' head position relative to the sensor array was determined before the MEG recording. For source reconstruction, we obtained structural magnetic resonance images (MRI) from each subject using a 3T MRI scanner (Siemens, Erlangen, Germany) and then co-registered the MRIs with the MEG data. The MEG data were analyzed offline using the Fieldtrip toolbox (http://www.ru.nl/donders/fieldtrip; Oostenveld et al., 2011). Epochs with artifacts were discarded and power line noise was removed as previously described (Pavlidou et al., under second revision, Human Brain Mapping).

Time frequency analysis

Time frequency representations (TFRs) of power were calculated using windows of 500 ms moved in steps of 50 ms for frequencies between 4 and 40 Hz. Time windows were tapered with a Hanning window with a spectral application of ±2.0 Hz. For frequencies between 40 and 100 Hz, we used windows of 400 ms moved in steps of 50 ms. Time windows were multiplied with 7 tapers, resulting in a spectral smoothing of ±10 Hz. Regions of interest were first determined on sensor level by pooling all conditions together. Sensors revealing the strongest perturbations in oscillatory activity were then selected for further analysis. Strong changes in alpha (7-13 Hz), beta (13-35 Hz) and gamma (55-100 Hz) power were bilaterally observed in sensors over parieto-occipital, temporal, and sensorimotor areas (for details of sensor selection see table 1 and Pavlidou et al., under second revision, Human Brain Mapping). To further examine the different roles of the four regions of interest in action recognition, we assessed differences in oscillatory activity between plausible and implausible actions as described below.

Condition Contrast

Differences in spectral power between plausible and implausible PLD were assessed for parieto-occipital, temporal and sensorimotor areas. Per subject, we performed an independent samples t-test between power values of the plausible and implausible conditions averaged across sensors for each of the four regions of interest. This resulted in a time-frequency t-map for each subject. The consistency of t-values across subjects was analysed in a second step using a nonparametric randomization test. This statistical test effectively corrects for multiple comparisons (Maris and Oostenveld 2007), and thresholds the individual time–frequency maps of t-values at a value of 1.96 (alpha = 0.05). Neighbouring t-values exceeding the threshold were combined to time–frequency clusters. For each time-frequency cluster the sum of the t-values were used in the second-level cluster-level test statistics (Maris and Oostenveld, 2007). The *p*-value of the cluster in the second-level test statistics was then estimated using the Monte Carlo approach by comparing cluster test statistic with a randomization null distribution. The null distribution was computed by randomly permuting the data 1000 times and calculating the maximum cluster test statistic (see Lange et al., 2011 for details). Statistical analysis was done for the first 3 seconds to minimize influence of motor preparation.

Temporal evolution of alpha/beta power

Based on the significant clusters on sensor level, we assessed changes in alpha/beta power for plausible and implausible PLD for parieto-occipital (5-21 Hz), left temporal (9-21 Hz) and sensorimotor areas (15-21 Hz). Per subject, we averaged across sensors for each of the three areas (parieto-occipital, left temporal and sensorimotor) and their respective significant frequency clusters, across all trials for each of our two conditions. This resulted in a temporal evolution of alpha/beta power change for each subject. Finally, we averaged the results across subjects.

Source Analysis

Based on the significant clusters on sensor level, we determined neuronal sources by applying Dynamic Imaging of Coherent Sources (DICS), an adaptive spatial filtering technique (Gross et al., 2001). This takes into account the forward model at the location of interest (the leadfield matrix) and the crossspectral density (CSD) between all MEG sensor pairs for the frequency of interest determined by the significant time-clusters on sensor level. The leadfield matrix was calculated based on a realistically shaped single-shell volume conduction model (Nolte, 2003), derived from each individual subject's structural MRI. The headmodel was reduced to a regular three-dimensional grid (1 cm resolution) and spatial filters $w(r,f)$ were constructed for each grid point using the following formula:

$$w(r,f)= (L'(r)C(f) + \lambda \times I)^{-1} L'(r)^{-1}L'(r)C(f) + \lambda \times I)^{-1},$$

where $L'(r)$ is the inverse of the lead-field matrix (forward model) at location of interest r, $C(f)$ is the CSD matrix between all MEG sensor pairs at frequency f, λ is the regularization parameter, and I is the identity matrix (de Lange et al., 2008; Gross et al., 2001). Plausible and implausible conditions were pooled to compute common filters. Next, CSD matrices of single trials were projected through those filters, providing single trial estimates of source power (p) using the following formula (Bauer et al., 2006)

$$p(r, f)= w(r, f)C(f)' w^*(r, f).$$

In line with the analysis on sensor level, a between-condition t-value for condition contrasts was computed for each subject. Statistical testing on group level for condition contrasts was carried out in the same way as on sensor level (see above). Results on group level (p<.05 uncorrected) were displayed on the MNI template brain and neuronal sources were identified using the AFNI atlas (http://afni.nimh.nih.gov/afni), integrated into Fieldtrip.

Results

Behavioural data

Subjects could easily distinguish between plausible and implausible PLD with an average rating of 1.5 and 2.3 respectively. Statistical testing revealed highly significant differences between the two conditions ($p<0.001$).

Stimulation effects

Visual stimulation (pooling all conditions) of PLD showed clear perturbations of spectral activity in the low alpha (7-11 Hz), high alpha (11-13 Hz), beta (13-35 Hz) and gamma (50-100 Hz) frequency bands in four areas (parieto-occipital, bilateral temporal and sensorimotor) (see Table 1 and Pavlidou et al., under second revision, Human Brain Mapping).

Condition contrast

We assessed differences between plausible and implausible PLD actions in the four regions of interest on sensor level (sensors over parieto-occipital, left and right temporal, and sensorimotor areas; see Table 1 for details on sensor selection).

We found a significant increase in alpha/low beta (9-21 Hz) power at 1650-2050 ms post-stimulus onset in sensors over left temporal areas ($p<0.05$). In addition, we found a significant increase in alpha (5-11 Hz) and low beta (13-21 Hz) power, between 1950-2350 ms in sensors over parieto-occipital areas ($p<0.05$), and a significant increase in low beta (15-21 Hz) power at 2400-2650 ms in sensors over sensorimotor areas ($p<0.05$) (Figure 2A). No significant clusters were found in sensors over right temporal cortex.

Next, we identified the cortical sources of these significant clusters. For the increase in high alpha/low beta power (9-21) between 1650-2050 ms, the sources were identified in the left temporal pole, STS, and motion sensitive area (MT). Additional sources were identified in the left IFG, SMA and lateral occipital sulcus (Figure 2B, left panel).

The sources of the significant effects between 5-21 Hz and 1950-2350 ms were located in bilateral parieto-occipital regions of the brain more specifically the V1, V2, V3, and KO, and in superior and medial temporal gyrus (Figure 2B, middle panel).

Finally, the sources of the significant cluster between 15-21 Hz and 2400-2650 ms were localized in bilateral M1, PMC, SMA and somatosensory areas. Sources were also identified in bilateral prefrontal cortex, IFG and in the left superior frontal gyrus. (Figure 2B, right panel).

The temporal evolution of alpha /beta power change was assessed separately for plausible and implausible conditions in left temporal (Figure 3A), parieto-occipital (Figure 3B), and sensorimotor areas (Figure 3C). For both conditions, we observed an initial strong decrease of power in all areas directly after stimulation onset. Stronger alpha/beta suppression was observed for implausible vs. plausible across all areas. Significant time point differences (where the difference between plausible and implausible conditions was greater; $p<0.05$) were observed for left temporal (Figure 3A), parieto-occipital (Figure 3B) and sensorimotor areas (Figure 3C).

Discussion

We investigated the modulations of neuronal oscillatory activity elicited by two seemingly similar point-light display (PLD) actions (plausible vs. implausible). Plausible and implausible PLD actions are highly similar in low-level visual information and both actions are clearly recognized as a human figure. The subtle modification, however, had an effective influence on the configural recognition so that subjects perceived the actions as biomechanically plausible or implausible action.

We found that PLD (pooled over all conditions) elicited power changes in alpha (7-13 Hz), beta (15-25 Hz) and gamma (50-100 Hz) bands in several cortical areas including areas of the MNS. Activation of MNS during the visual processing of PLD action representations is consistent with earlier studies of action observation (Buccino et al., 2004a; Calvo-Merino et al., 2005; Dinstein et al., 2007; Grossman et al., 2000; Saygin et al., 2004).

Our main finding is that subtle changes in the configuration of the human PLD elicited modulations of beta-band power in a distinct spatio-temporal profiles within the above mentioned network of action recognition. Normal, plausible actions showed a significant increase of beta-band power relative to implausible actions in left temporal sensors between 1650-2050 ms, followed by an increase in parieto-occipital sensors between 1950-2350 ms, and finally in sensorimotor sensors between 2400-2650 ms post-stimulus onset. We identified the left superior temporal sulcus (STS), motion sensitive area (V5/MT+), temporal pole as the cortical sources of the effects found in left temporal sensors. As cortical sources of the effects in parieto-occipital sensors, we identified bilateral kinetic occipital (KO), primary visual area (V1), lateral occipital complex (LOC), Finally, the effects in sensorimotor sensors were localized to premotor cortex (PMC), primary motor area (M1), supplementary motor area (SMA), somatosensory areas, and left inferior frontal gyrus (IFG).

These positive beta clusters reflect a stronger suppression of power for implausible than plausible actions (Figure 3). Stronger suppression of beta-band-power in sensorimotor areas has been found for incorrect relative to correct movements (Koelewijn et al., 2008). Implausible actions might therefore be processed similar to incorrect movements. Another potential explanation for the stronger suppression of power for the implausible PLD might be increased internal motor imagery when differentiating between two very similar stimuli. Motor imagery has been found to suppress beta-band-power in sensorimotor areas (de Lange et al., 2008; Schnitzler et al., 1997). The complexity of the imagery task correlates with

the duration of the beta-suppression (de Lange et al., 2008). Recognition of an implausible action might therefore require more mental imagery, reflected in prolonged beta-suppression in sensorimotor areas. A previous study found suppression of alpha/beta power to correlate with the observation of actions belonging to the observer's motor repertoire (e.g. ballet dancing observed by professional ballet dancers) but not if ballet dancing was observed by non-professional dancers (Orgs et al., 2008). In addition to this study, our results demonstrate that beta-band power is not only involved in the recognition of familiar actions (plausible) but also in the recognition of unfamiliar actions (implausible).

Suppression of beta-band power in visual areas has been linked to the increase of visual attention (Kaminski et al., 2011; Wrobel, 2000; Wrobel et al., 2007). Previous hemodynamic (Allison et al., 2000; Grossman and Blake, 2002; Michels et al., 2005; 2009; Pelphrey et al., 2005) and electrophysiological (Pavlova et al., 2008; Singh et al., 2002) studies reported right-temporal activity in response to PLD. Activity in the right hemisphere reflects the processing of the global form of the PLD (Lamb and Robertson, 1988). In their global form both plausible and implausible PLD appear very similar. The differences of the two PLD exist in the spatial position of only a few of the overall number of dots that make up the human form (4 of 13 dots). This subtle manipulation of the human PLD form requires the process of the PLD local details, which is generally thought to be involved in the left hemisphere (Bonda et al., 1996; Lamb and Robertson, 1988). The observed left temporal activity in our study when differentiating between plausible and implausible stimuli might thus reflect visual attention to the local details of the PLD, when differentiating between two seemingly similar PLD forms.

Earlier studies found activity in visual cortices as early as ~200 ms after stimulus onset when observing a PLD walker relative to scrambled displays (Pavlova et al., 2004). Similarly, in a recent study, we observed first differences in gamma-band (55-95 Hz) power in parieto-occipital areas between 500-800 ms post stimulus onset. This early gamma difference suggests that plausible and scrambled stimuli are first distinguished on an early visual basis (Pavlidou et al., under second review, Human Brain Mapping). Due to the highly similar low-level visual information, both plausible and implausible PLD appear to be very similar, and thus there is no visual distinction between the two PLD actions in the present study. This similarity is reflected in the absence of any differences in gamma-band activity, as observed

e.g. for plausible vs. scrambled PLD (Pavlova et al, 2004; Pavlidou et al., under second review, Human Brain Mapping).

Notably, the observed distinct spatio-temporal profiles were all found in the beta-band. These effects reveal that both plausible and biomechanically implausible human actions both activate the visual and motor areas of MNS but at different spatio-temporal scales. These findings provide supporting evidence that during action recognition the beta band provides a functional network for long-range communication between visual and motor areas of the MNS. In the present study, the significant differences in sensorimotor areas in the beta-band were found between 2400-2650 ms in line with studies that have used correct vs. incorrect actions (Koelewijn et al., 2008). This suggests that MNS is involved in higher form processes that include an evaluative component for the observed action operating at a slower rate. The involvement of the sensorimotor areas therefore, implies that the MNS does not depend on the overt visual information of an observed action. Rather, the MNS may interact with the visual system by integrating prior motor representation stored in the observer's motor repertoire (Borroni et al., 2005; Craighero et al., 2007; Fadiga et al., 2006). Previously, we found for plausible vs. scrambled actions differences in sensorimotor beta-band power between 700-1200 ms. The process to distinguish two seemingly similar actions, however, requires more time to activate sensorimotor areas and thus more time is required to interpret the observed action than a simple plausible vs. scrambled action discrimination.

Another possible explanation to the present results is a reflection of motor imagery. Motor imagery is a process in which an internal formation of a movement plan takes place. de Lange and colleagues (de Lange et al., 2008) observed activity in visual and sensorimotor areas during motor imagery of hand movements. This observation of visual and sensorimotor activity suggests a similar network involvement to that observed during action recognition. Future research can produce a carefully designed paradigm in which both action recognition and motor imagery processes can be extracted independently, to further understand the MNS role in the recognition as well as the prediction of actions. MEG can be used to determine the time course and dynamic modulations involved in the frequency domain (e.g. beta power) of both processes.

In summary, we found distinct spatio-temporal profiles in the beta-band when subjects had to distinguish plausible and implausible actions. The beta-clusters revealed a sequential order suggesting a directed flow of information. Notably, all significant effects were found in

the beta-band, suggesting that the beta-band might provide a functional network of long-range communication between visual and motor areas of the MNS in the differentiation of plausible and implausible action movements. The later activation of the sensorimotor areas in comparison to visual areas suggests their involvement in higher form processes when interpreting the plausibility of the observed actions, which further suggests that the MNS acts more like an active interpreter than a submissive observer when recognizing an action.

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Captions

Table 1: Sensor selection based on stimulation effects observed during PLD visual stimulation pooled over all conditions. Sustained effects (0-4.5 s) observed in our four regions of interest (parieto-occipital, sensorimotor, and bilateral temporal) in alpha (9-13 Hz), beta (13-25 Hz) and gamma (50-100 Hz). * Gamma increase observed between 0-1.5 s in right temporal.

Figure 1: Experimental setup. Examples of stimuli used (I) Plausible, (II) Implausible, (III) Scrambled. Connecting lines were not present in the actual experiment. Participants first fixated on a red cross. PLD stimuli appeared at time point 0. After a black screen, response instructions visually appeared. See Experimental Procedures for details.

Figure 2: Condition contrasts: Plausible vs. Implausible actions. (A) Representations of significant clusters ($p<0.05$) found on sensor level for (chi) parieto-occipital, (cross) left-temporal, and (star) sensorimotor areas. Red denotes positive clusters, i.e. higher power for plausible stimuli. (B) Source reconstruction of the significant clusters found on sensor level. The precentral (green), central (blue), and postcentral (pink) sulci are displayed for reference. Colour maps illustrate t-values for the source reconstruction.

Figure 3: Temporal evolution of alpha/beta power change for plausible (dotted line) and implausible (black) for left temporal (A), parieto-occipital (B) and sensorimotor (C) sensors. Gray pattern area denotes the significant time points, i.e. highest differences between plausible and implausible. Power is represented on a log scale.

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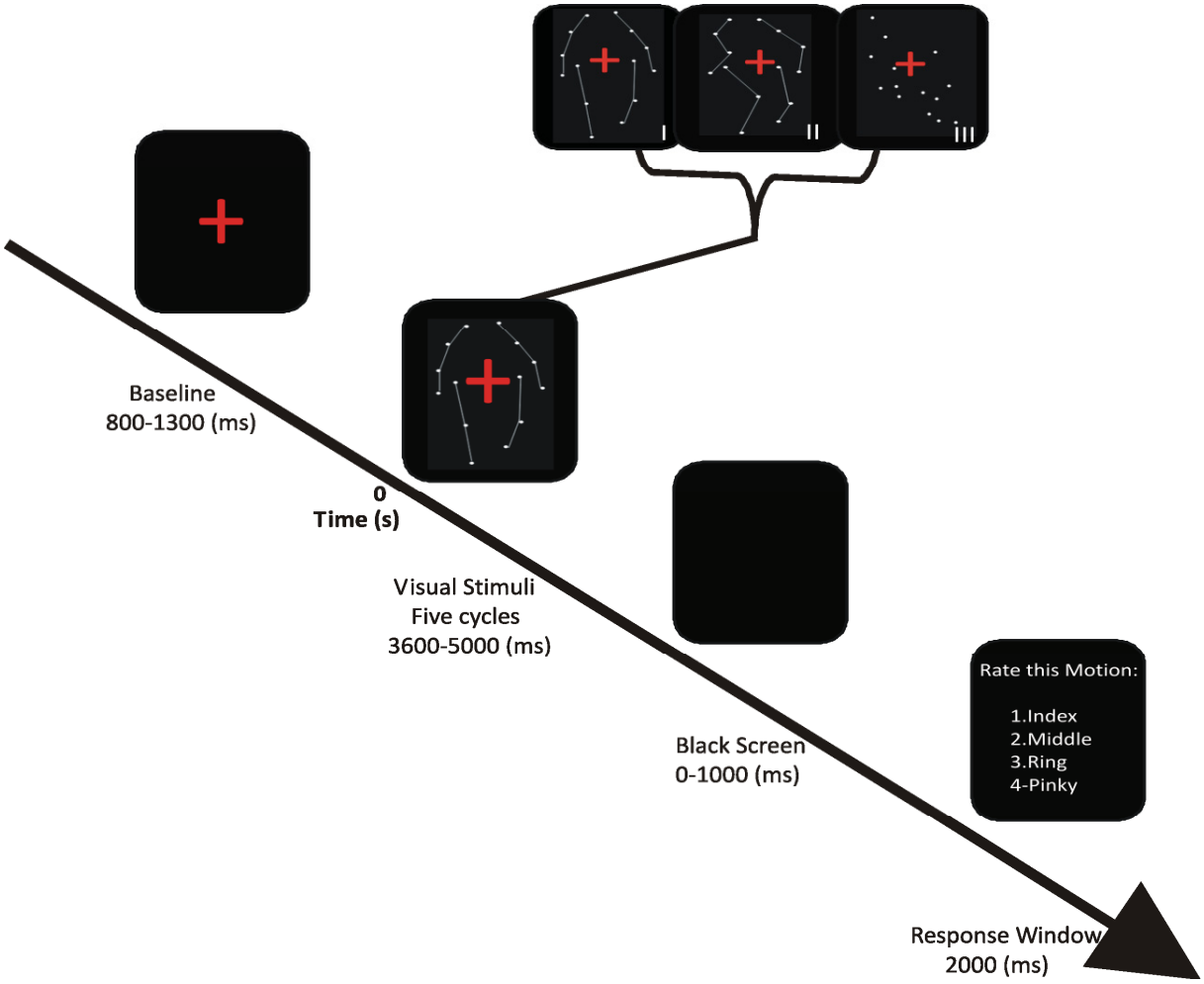
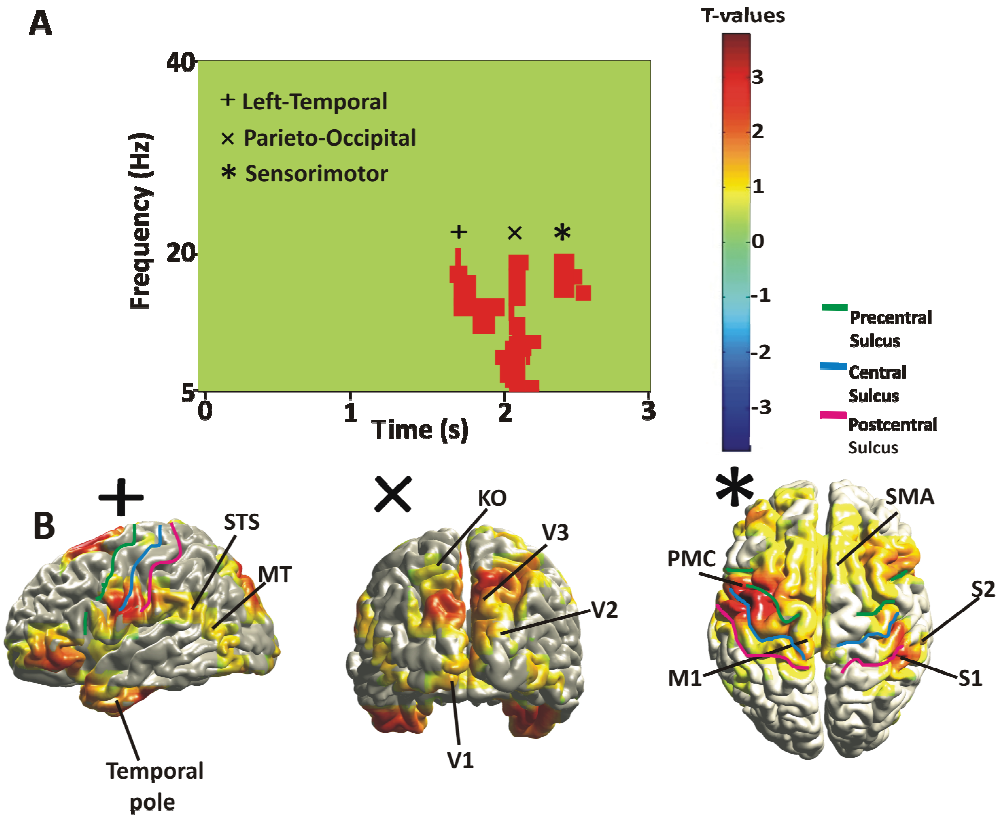
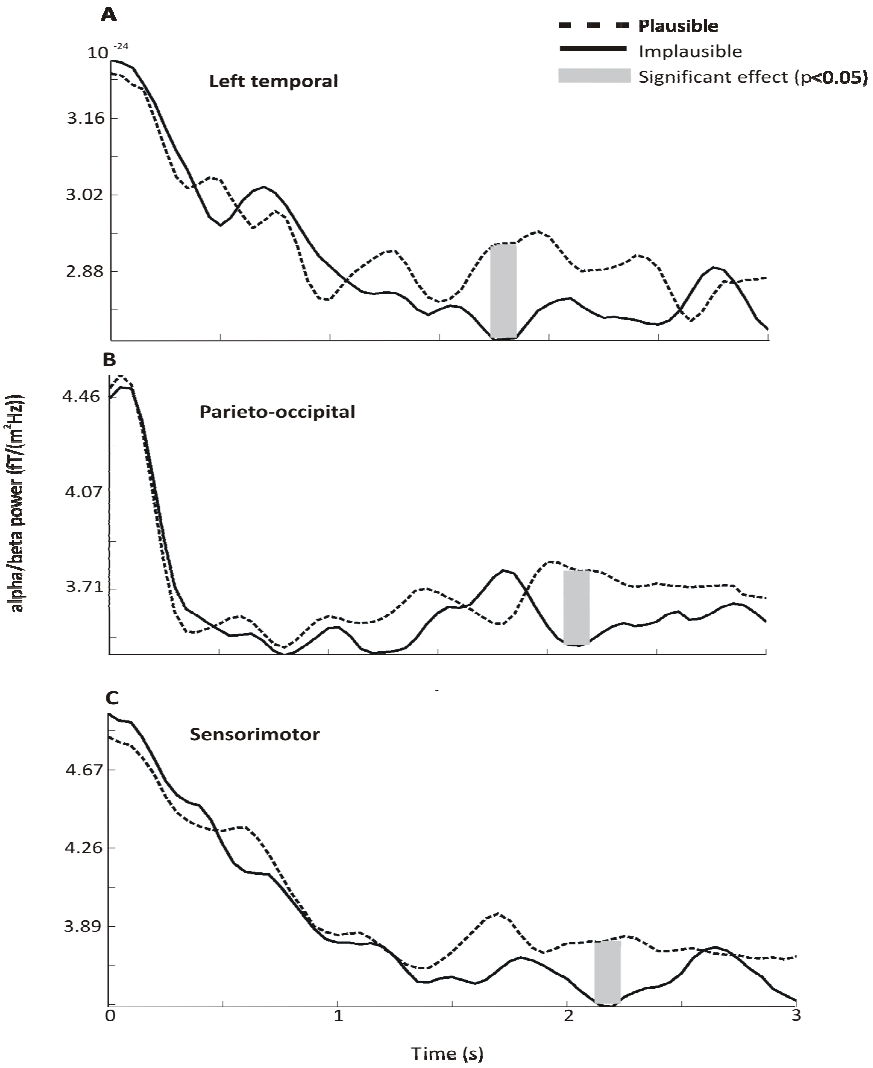
Stimulation effects pooled over all conditions

Regions of Interest	Frequency range
Parieto-occipital	↑ gamma (55-95 Hz) ↓ beta (13-23 Hz) ↓ alpha (7-13 Hz)
Sensorimotor	↓ beta (15-23 Hz) ↑ high alpha (11-13 Hz) ↓ low alpha (7-11 Hz)
Left Temporal	↓ beta (13-23 Hz) ↑ high alpha (11-13 Hz) ↓ low alpha (7-11 Hz)
Right Temporal	↑ gamma (90-100 Hz)* ↓ gamma (50-80 Hz) ↓ beta (13-23 Hz) ↑ high alpha (11-13 Hz) ↓ low alpha (7-11 Hz)


I
II
III
Baseline
800-1300 (ms)
0
Time (s)
Visual Stimuli
Five cycles
3600-5000 (ms)
Black Screen
0-1000 (ms)
Rate this Motion:
1.Index
2.Middle
3.Ring
4-Pinky
Response Window
2000 (ms)

Plausible vs. Implausible




A
Plausible
Implausible
Significant effect (p<0.05)
10 -24
Left temporal
3.16
3.02
2.88
B
Parieto-occipital
4.46
4.07
3.71
C
Sensorimotor
4.67
4.26
3.89
alpha/beta power (fT/(m²Hz))
0
1
2
3
Time (s)

Title

Anodal stimulation of premotor cortex facilitates the recognition of different forms of movements.

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Running title: Anodal stimulation aids movement recognition.

Abstract

Recently, the role of the premotor cortex (PMC) has been extended beyond the processing of human biological motion. This includes the differentiation between correct and incorrect biological movements, and also, the processing of biological movements of different species. Here, we were interested in whether transcranial direct current stimulation (tDCS) of PMC influences the visual perception of point light displays (PLD) movements differing in their form and degree of plausibility. Real and sham tDCS over left PMC was administered to 10 subjects while performing a PLD recognition task. Performance (Reaction times (RT) and accuracy) was measured before, during, immediately after, and 30 minutes post tDCS stimulation. Subjects performed 2 experiments. In Experiment 1, five subjects were asked to make a category judgment; distinguishing between human, bird, and random movements. Human PLDs were recognized fastest across all testing sessions. Interestingly, anodal tDCS significantly facilitated RTs for bird and random PLD. In contrast, cathodal tDCS significantly impaired accuracy of human PLD movements. In Experiment 2, five subjects were asked to make a within category discrimination; to distinguish between 3 variations of a single movement; a biomechanically natural, unnatural, and random movement. Anodal tDCS led to a significant increase in false reports of unnatural movements as natural as well as effectively increasing accuracy and speed in recognising natural movements. These results extend previous reports on the role of PMC in movement recognition. Experiment 1 findings imply that PMC is involved in the visual perception of the global form of human and non human biological movements. In addition Experiment 2 shows that anodal tDCS over PMC severely affects the discrimination between natural and unnatural movements, increasing participants' tendency to report unnatural as natural movements.

Keywords: point-light displays, global form, tDCS, visuomotor priming, template matching approach

Introduction

Our ability to recognize different forms of movement is important and of high evolutionary significance for purposes such as communication and social interaction. Movement recognition is a process involving a widespread network of cortical areas. In human studies, the superior temporal sulcus (STS), inferior parietal lobule (IPL), and premotor cortex (PMC) have all been identified as areas involved in the visual processing of actions or movements (Allison, Puce & McCarthy, 2000; Grossman & Blake, 2002; Pavlidou, Schnitzler & Lange, 2012; Saygin, Wilson, Hagler, Bates & Sereno, 2004). In monkey studies, all three areas have been shown to respond to the observation/execution of a goal-directed movement (Fogassi & Luppino, 2005; Gallese, Fadiga, Fogassi & Rizzolatti, 1996; Oram & Perrett, 1996). Of the three areas, IPL and PMC have been reported to contain mirror neurons that fire when a monkey observes a particular movement, and when it executes that same movement (Di, Fadiga, Fogassi, Gallese & Rizzolatti, 1992; Fogassi, Ferrari, Gesierich, Rozzi, Chersi & Rizzolatti, 2005). These mirror neuron areas make up a highly specialized network known as the mirror neuron system (MNS) (Rizzolatti & Craighero, 2004).

Observed activity in the MNS has often been regarded within the framework of motor resonance (Rizzolatti & Craighero, 2004). In other words, observation of a movement matching the internal motor representation causes the PMC to resonate. More recently, it has been argued that the more the observed movement matches the internal motor representations the stronger the resonance. For instance, Calvo-Merino and colleagues (Calvo-Merino, Glaser, Grezes, Passingham & Haggard, 2005) observed stronger BOLD activation in PMC when capoeira dancers observed capoeira dance movements vs. non-dancers observing the same movements. Similarly, observation of human biting movements but not dog barking movements activate the PMC, indicating that activity of PMC depends on movements that belong to the observer's motor repertoire (Buccino et al. 2001). If the movement observed does not belong to the observer's motor repertoire, the movement is recognized on a visual basis and does not trigger activity in the PMC (Schippers & Keysers, 2011).

On the other hand, humans are able to perceive and understand movements they cannot perform. A recent fMRI study (Fadiga et al. 2006) showed PMC activity when participants observed moving images of real animals close to the PMC activity observed by Buccino and colleagues (Buccino et al. 2004) when participants viewed mouth movements of dogs and

monkeys. Similarly, an analogous PMC activation has been reported when participants viewed biomechanically implausible finger (Craighero, Bonetti, Massarenti, Canto, Fabbri & Fadiga, 2008; Romani, Cesari, Urgesi, Facchini & Aglioti, 2005) and implausible body movements (Costantini et al. 2005; Pavlidou, Schnitzler & Lange, 2012). This suggests that PMC activity might not depend on the overt visual representation of a movement, but rather to the general meaning of the movement. Implausible movements prompt higher activation of temporal and parieto-occipital areas than plausible movements (Costantini et al. 2005). This finding accords with that observed for image scrambling in visual regions (for review see Grill-Spector & Malach, 2004). Activation of PMC might thus be attributed to varying signals received from parieto-occipital and temporal areas in response to implausible movements. With neuroimaging studies however, it is difficult to establish a causal link between neural activation of PMC and behaviour.

One method to modulate brain activity and measure its causal influence on behaviour is transcranial magnetic stimulation (TMS). TMS over PMC has been reported to lead to an increase in false alarms when distinguishing between biological and non-biological motion (van Kemenade, Muggleton, Walsh & Saygin, 2012). TMS over PMC also led to an impairment in the visual discrimination of plausible body actions but not implausible body actions (Candidi, Urgesi, Ionta & Aglioti, 2008; Urgesi, Calvo-Merino, Haggard & Aglioti, 2007; Urgesi, Candidi, Ionta & Aglioti, 2007)

These TMS studies validate the importance of PMC in the processing of human biological actions as previously suggested (Buccino et al. 2001; Rizzolatti, Fadiga, Gallese & Fogassi, 1996; Saygin, Wilson, Hagler, Bates & Sereno, 2004). PMC's role in the processing of biological motion of other species (i.e. bird flying) and its role in the visual discrimination of natural vs. unnatural movements is still elusive.

Transcranial direct current stimulation (tDCS) over PMC can help elucidate this role because changes in current flow (anodal/cathodal) easily allow for a bi-directional investigation of influence. tDCS is a form of neuromodulation that delivers a low intensity current to the brain area of interest via small electrodes (Nitsche et al. 2008). In contrast to TMS, it does not induce neuronal action potentials, but rather it modifies neuronal excitability and spontaneous cell firing by de-or-hyperpolarization of the resting membrane potential, depending on the direction of the electric field (Nitsche et al. 2008). A positive current stimulation (anodal) causes the resting membrane potential to depolarize, resulting in an

increase of neuronal excitability and more spontaneous cell firing. A negative current stimulation (cathodal) will cause the resting membrane potential to hyperpolarize, decreasing neuronal excitability and spontaneous cell firing (Nitsche et al. 2008; Nitsche & Paulus, 2000). Anodal stimulation has been reported to facilitate learning, whilst cathodal stimulation has been reported to decrease performance (Antal, Nitsche & Paulus, 2003; Nitsche, Liebetanz, Antal, Lang, Tergau & Paulus, 2003). Sham stimulation is used as a control for comparison to anodal and/or cathodal stimulation. tDCS also has the ability to achieve cortical changes in the stimulated area even after stimulation has ended (Nitsche et al. 2008).

Here, we studied the causal role of PMC in the recognition of human and non-human movement using tDCS. We used point-light displays (PLD), a presentation method in which a type of movement is reduced to just a handful of moving dots (Johansson, 1973). We used PLDs to create different categories of movement (Experiment 1) and different types of a single movement (experiment 2). Experiment 1 consisted of three different types of tDCS delivered to the PMC (anodal, cathodal, and sham) and four different time points (pre, tDCS, post, and 30 minutes post). We measured speed and accuracy of recognition of PLDs to test whether PMC's role in the visual perception of biological motion extends to other species besides humans. In experiment 2, we investigated if tDCS over PMC is specific to a natural biological movement or whether it might generalize to unnatural or random movement. By using a single stimulus class and a single paradigm we can effectively evaluate the role of PMC in movement recognition within and across movement categories.

Methods

Subjects

12 healthy, right handed volunteers (6 males; mean age ±SD 22.3 ± 5.57 years) with normal or corrected to normal vision and with no history of neurological or psychiatric disorders or head trauma participated in the experiment. Eleven adults completed all three tDCS sessions (anodal, cathodal and sham) and received payment after each session. Each tDCS session was spaced at a three day interval to ensure no remaining effects from the previous sessions (Nitsche et al. 2008). Six participants completed Experiment 1 but one participant was excluded from analysis for pressing the wrong buttons in one session. Four additional participants, plus one participant from Experiment 1 completed Experiment 2. The sixth participant in Experiment 2 did not return to complete the last session for unspecified reasons. Written informed consent was attained from all participants, and the study was approved by the Australian National University Human Research Ethics committee and thus complies with the tenants of the declaration of Helsinki.

Stimuli and Procedure

Point-light displays (PLD) of human movement were generated by attaching sensors to the main joints of a human actor and recording their movements (MotionStar; Ascension Technology, Burlington, VT; (Lange & Lappe, 2006). The main joints (head, shoulders, elbows, hands, hips, knees, and feet) were represented by 13 small white dots (5 x 5 pixels). The point-light bird animations were attained from the point-light archives of Temple University (http://astro.temple.edu/~tshipley/mocap.html, Philadelphia, USA). Stimuli were offline manipulated using Matlab (Mathworks, Natick, MA, USA). All PLDs subtended approximately 6.2° × 4.4° of visual angle when viewed from a distance of 1.1 metres.

Individual images of our PLDs were loaded through Cambridge Research systems Visage graphics system and displayed on a ViewSonic Professional Series PF817 monitor, with a screen resolution of 1024 x 768, a refresh rate of 100 Hz and a mean luminance of 50.4 cd/m^2. The luminance display of the monitor was Gamma corrected using a CRS optical device. Luminance of the dots on the stimuli was 100.8 cd/m^2 against a grey background screen (50.4 cd/m^2). Thus, the Michelson contrast of each dot element was 33.3%. Each image frame of the motion sequence was shown for 10 frames (100 ms) and the sequence was terminated once a participant made a response. A 1 s inter-stimulus interval then

preceded the next trial. In both experiments there were five stimuli for each condition moving along, either towards the left or right of the screen before looping back to the starting position. The starting frame was randomised over a couple of frames, so as to avoid any systematic form cue on the opening frame. Each PLD was presented 24 times in a pseudorandom order within a block. Each block was repeated five times in random order. Each testing session had a total of 360 trials (120 trials of each class of PLD) and lasted for ~15 mins. For each tDCS stimulation condition (anode, cathode, sham) there were four testing sessions: before (pre), during (tDCS), one immediately after (post), and 30 minutes post (30-post) tDCS stimulation. All in all each testing session lasted for ~90 minutes. Participants were instructed to respond as quickly and as accurately as possible using a 3-alternative finger choice by pressing the corresponding button when identifying the form of the PLD movement (Experiment 1) or the type of a single PLD movement (Experiment 2). Responses were recorded using a Cedrus RB530 5 button response box.

Experiment 1 (Between category judgments)

Three different PLD forms were used as stimuli with each representing a unique biological movement. The first was of a human walking either towards the left or right of the screen. The second was of a bird flying either towards the left or right of the screen. The third was the scrambled counterparts of human and bird PLD. In the scrambled version the spatial position of the dots was randomized, altering the configuration of a human or bird PLD to that of a random assortment of moving dots whilst preserving the local motion content. The human, bird, and scrambled counterpart PLD were matched in terms of the number of dots and movement trajectory. Participants were asked to recognize a PLD movement as either human, bird, or random dots (Figure 1A). Each condition had five different individual PLD versions presented in random order in a testing session.

Experiment 2 (Within category judgments)

Three different representations of a human PLD movement were employed (similar to Pavlidou, Schnitzler & Lange, 2012). The first was a representation of a natural human movement. The second was a representation of an unnatural human movement. An unnatural movement consisted of the spatial manipulation of only a few dots (~4 dots from each stimulus) of the natural PLD movement to give the notion of a biomechanically

implausible movement, without destroying the overall human figure (Figure 1B). The third was a representation of a random movement. A random movement was the scrambled counterpart of the natural human movement (Figure 1B). Participants were asked to discriminate between the three movements and respond to them as either: natural, unnatural or random. Each condition was presented in the same way as in Experiment 1.

Transcranial direct current stimulation (tDCS)

Current stimulation was delivered by a battery driven constant current stimulator (NeuroConn GmbH, Ilmenau, Germany) via conductive rubber electrodes, placed in saline soaked pads (35 cm^2). The middle of the active electrode pad was placed over left PMC between C3 and F3 according to the 10–20 international system for EEG electrode placement (Lagerlund et al. 1993). The other electrode pad was placed on the back of the neck to avoid stimulation effects on visual processing, and decision making processes associated with certain areas of the brain. An EEG reference cap (Bio-Medical Instruments Inc: Michigan) was used to ensure that the location of the stimulation was identical for each participant and stable across testing sessions. Caps were referenced to the nasion and inion to aid this aim.

Real tDCS stimulation (anode and cathode) was performed for 15 min. tDCS was applied with a current strength of 2 mA. The current was linearly ramped up or down over the first and last 30 s, respectively. During sham stimulation current ramped up for 30 s, before ramping down and remaining thus. All participants reported that they felt a mild tingling sensation under the electrode pads with both polarities and for sham. tDCS stimulation order was counterbalanced across all our participants for both experiments and participants were blinded to the tDCS conditions.

Data Analysis

The same data analysis was carried out for both experiments. Matlab (Mathworks, Natick, MA, USA) was used to calculate accuracy as a percentage, record reaction times (RTs) in milliseconds, tally false reports in frequency counts and, to filter the RT data. Analysis of RTs was only administered for correct trials. Prior to analysis, RT data less than 300 ms were removed as they were deemed too fast to be indicative of effective processing of the stimulus. In addition, for the data set in each condition, RTs greater than the mean + 2.5 SDs

were removed from further analysis. Since our data were not normally distributed the median RT was calculated from the filtered data set as it was in principle a better measure of central tendency in our data. The mean of the median across all participants was then computed across all testing sessions for each type of stimulation. We used individual medians for the analysis and plotted the mean of all individual medians.

Statistical analysis and presentation of the data was performed using GraphPad Prism V5 (GraphPad Software, California, USA). Repeated measure ANOVA's were used to test for differences in RT and accuracy as a function of stimulation condition, or test session. Post-hoc paired t-tests were used to test for differences in specific comparisons (e.g. is there a significant difference in RT or accuracy between tDCS and post testing sessions?). We used the bonferroni correction method to effectively correct for multiple comparisons. A chi square test was used to analyse the false report data because these were not normally distributed but rather are frequency counts.

Results

Experiment 1

Participants were asked to differentiate between three different categories of PLD movements while real and sham tDCS was administered over left PMC. Participants were asked to respond as quickly and as accurately as possible when recognizing a human, bird or random PLD movement. Results are presented separately below, for each stimulation type.

Anodal stimulation

RT Analysis as a function of testing sessions

We found a significant main effect of RT for birds *(F(3,12)=4.398; p=0.036)*. Post-hoc analysis revealed a significant difference between pre and 30-post *(t(4)=2.730; p=0.026)* and a trend for tDCS vs. post *(t(4)=1.62; p=0.089),* suggesting a positive effect of stimulation on bird RTs (Figure 2A).

A similar pattern was observed for the random PLD. We found a significant main effect for RTs *(F(3,12)= 8.031; p=0.003).* Post-hoc analysis revealed significant differences between tDCS and post *(t(4)= 2.730; p=0.026)* and tDCS and 30-post *(t(4)= 3.162; p=0.017)* testing sessions. No significant main effects in RT were observed for the human condition *(F(3,12)= 1.278; p=0.326).* Thus anodal stimulation led to an improvement in RTs for unfamiliar non human movements (bird and random) but not for human PLDs, where observers' performance appeared to asymptote within the pre testing session.

RT Analysis as a function of conditions

The fastest RT times were found for the human PLD (mean RT = 568 ms ± 59.1) across testing sessions, followed by random (mean RT = 633 ms ± 100.5) and bird PLD (mean RT = 698 ms ± 111.5) (Figure 2A). We found a significant main effect of RTs between the three PLD movements *(F(2,8)=10.55; p=0.0057).* Planned paired-samples t-tests revealed a significant difference between human PLD and bird PLD in the pre *(t(4)=5.31; p=0.0001)*, tDCS *(t(4)=4.543; p=0.001)* and 30-post *(t(4)=3.427; p=0.05)* testing sessions. RT for the random condition were significantly different from the human condition only for the pre condition *(t(4)=3.108; p=0.05).* No significant effects (all with a $p>0.05$) were observed between bird and random conditions.

Accuracy

Participants could easily categorize the three conditions with mean accuracy scores of 95.6 % ± 1.86 for humans, 82.5 % ± 2.45 for birds, and 97.2 % ± 0.67 for random movements (Figure 2B) across testing sessions. No significant main effects in accuracy were observed for human, bird and random PLD movements as a function of testing sessions *(F(3,12)=2.025; p=0.164), (F(3,12)=0.799; p=0.517), (F(3,12)=0.775; p=0.530)* respectively*),* or as a function of conditions *(F(2,8)=3.469; p=0.0823).*

False Reports

Anodal stimulation led to a significant decrease in false reports of birds as noise. Frequency count significantly decreased from 111 false reports in the pre testing session to 76 false reports in the post test session out of possible 600 errors *(X^2(2,N=285)= 6.58; p=0.037)* (Table 1). Human and noise false reports were low (≤ 22 false reports in each session; Table 1). No other significant effect (p>0.05) were observed.

Cathodal stimulation

RT analysis as a function of test session

RT data during cathodal stimulation decreased across test session for all three conditions (human, bird, and random movement) (Figure 2C). No significant main effects on RT were observed as a function of test sessions (human *F(3,12)=0.812; p=0.511*, bird *F(3,12)=1.030; p=0.414* and random *F(3,12)=1.732;p=0.213)*.

RT analysis as a function of condition

We found a main effect of RTs between conditions during cathodal stimulation between PLD conditions *(F(2,8)=8.566; p=0.0103)* (Figure 2C), similar to anodal stimulation. Planned paired-samples t-tests revealed significant differences in RT between human PLD and bird PLD in pre *(t(4)=4.263; p=0.01)*, tDCS *(t(4)=3.210; p=0.05)* and post *(t(4)=3.212; p=0.05)* testing sessions. Furthermore, human and random PLD were significantly different from each other in the pre testing session *(t(4)=3.308; p=0.05)*. No other significant differences (p>0.05) were observed for human and random PLD. In addition, no significant differences were observed between bird and random PLD (all with a p>0.05).

Accuracy

Participants could easily recognize the three conditions with a mean accuracy score of 96.1 % ± 1.59 for humans, 90.1 % ± 1.63 for birds, and 96.6 % ± 0.52 for random movements (Figure 2D) across testing sessions. No significant main effects were observed between testing sessions for human *(F(3,12)=3.152; p=0.064)*, bird *(F(3,12)=1.122; p=0.379)*, and random *(F(3,12=0.491; p=0.695)* PLD movements or between conditions *(F(2,8)=2.143; p=0.179)*. The main effect of human PLD showed a strong trend towards significance *(p=0.064)* and in line with this, post hoc analysis between tDCS and 30-post revealed a significant decrease in human accuracy *(t(4)=2.409; p<0.0368)*.

False reports

The frequency count of human PLD as noise significantly increased from 7 false reports to 20 in frequency count (X^2 *(2,N=35)=8.97; p=0.011*; Table 1). This significant increase suggests that cathodal stimulation of PMC led to a response uncertainty when categorizing human movement. No other significant effects (p>0.05) were observed for noise or bird false reports.

Sham stimulation

RT analysis as a function of test session

RT analysis revealed a significant main effect for the bird PLD *(F3,12)=6.027; p=0.0096)* (Figure 2E; dark grey line). Post-hoc analysis revealed that RTs significantly decreased from pre to post *(t(4) =2.865; p=0.0457)*. This effect however was primarily due to longer RTs for one subject in the pre testing session and was not consistent across participants. No significant main effects were observed for human *(F(3,12)=2.763; p=0.088)* or random *(F(3,12)=2.262; p=0.133)* PLD movements (Figure 2E).

RT analysis as a function of condition

We found a significant main effect of RTs between conditions during sham stimulation between PLD conditions *(F(2,8)=5.318; p=0.0340)* (Figure 2E). Planned paired-samples t-tests revealed significant differences in RT only between the human and noise PLD in the pre *(t(4)=3989; p=0.01)* testing session. No other significant differences (p>0.05) were observed between the two conditions. In addition, no significant differences were observed between human and bird or between bird and noise PLD movements (all with a p>0.05).

Accuracy

Participants could easily differentiate between the three conditions with a mean accuracy score of 94.6 % ± 1.71 for humans, 90.8 % ± 1.70 for birds, and 96.0 % ± 1.39 for random movements (Figure 2F) across testing sessions. No significant main effects were observed between testing sessions for human *(F(3,12)=2.127; p=0.150)*, bird *(F(3,12)=0.884; p=0.467)*, and random *(F(3,12=1.477; p=0.270)* PLD movements or between conditions *(F(2,8)=1.210; p=0.347)*.

False reports

No significant effects (p>0.05) in the frequency count of false reports were observed for the sham stimulation session across all three conditions (Table 1).

Summary

In summary, results from Experiment 1 show that human PLD movements were the fastest to be recognized across all stimulation types. Following anodal tDCS over PMC, a significant decline of RTs was observed for both bird and random PLD movements. Moreover, participants' tendency to falsely report bird PLD as random PLD significantly decreased following anodal tDCS, suggesting a significant improvement in the recognition of bird PLD movements. In contrast, cathodal tDCS over PMC significantly decreased participants' accuracy in the recognition of human PLD movements, effectively increasing participants' tendency to recognize human PLD movements as random PLD.

Experiment 2

Participants were asked to differentiate between three different representations of a single human PLD movement while real and sham tDCS was administered over left PMC. Participants were asked to respond as quickly and as accurately as possible when recognizing a natural, unnatural or random human PLD movement. We were specifically interested in the effects of tDCS over PMC during the visual presentation of natural and unnatural movements. Results are presented in the same way as Experiment 1.

Anodal Stimulation

RT analysis as a function of test session

We found no significant main effects of RTs across all testing sessions for natural *(F(3,12)=2.547; p=0.105)*, unnatural *(F(3,12)=0.785; p=0.524)* and random *(F(3,12)=1.454; p=0.276)* PLD movements. Interestingly, when plotting the results a strong decrease in RT was observed for the natural PLD (Figure 3A; black line). Post-hoc analysis revealed a significant decrease in RTs for the natural PLD between pre and post *(t(4)=3.136; p=0.0175)* as well as between pre and 30-post *(t(4)=2.785; p=0.0248)* conditions (Figure 3A; black line). This suggests that anodal stimulation of PMC has a positive, facilitating effect on RTs to natural movement. On the other hand, RT for the unnatural PLD significantly decreased between pre and post *(t(4)=3.826; p=0.0093)* but not between pre and 30-post *(t(4)=2.169; p=0.0960)* as observed for the human PLD (Figure 3A; dark grey line).

RT analysis as a function of condition

We observed a significant main effect of RTs (F(2,8)=13.84; p=0.002). Planned paired-samples t-tests revealed significant differences in RTs between random (mean RT= 767.4 ms ± 111.1) and unnatural (1093 ms ± 181.7) PLD movements across all testing sessions (pre *(t(4)=4.990= p=0.001)*, tDCS *(t(4)=4.262); p=0.01)*, post *(t(4)=3.593; p=0.05)* and 30-post *(t(4)=4.112; p=0.01)*. In addition, random PLD was significantly different from natural human PLD in the pre and tDCS testing session *(t(4)=4.757; p=0.001)* and *t(4)=3.826; p=0.01)*. Furthermore, post-hoc analysis revealed that natural (1014 ms ± 283.8) and unnatural PLD movements were significantly different from each other in the post anodal stimulation session *(t(4)= 2.543; p=0.031).* The difference was not significant for the 30-post session *(t(4)= 1.793; p=0.073)*, suggesting that prior to anodal stimulation both natural and unnatural movements were processed in a similar manner, that altered following anodal stimulation.

Accuracy

Participants could easily distinguish between the three PLD movements with a mean accuracy of 97.2 % ± 0.97 for the random PLD, 95.0 % ± 1.81 for natural PLD and 88.7 % ± 3.22 for the unnatural PLD (Figure 3B). No significant main effects in accuracy were observed for the natural *(F(3,12)=2.273; p=0.132)* and random *(F(3,12)=2.144; p=0.147)* PLD movements. Interestingly we observed a significant main effect in accuracy for the unnatural

movements *(F(3,12)=8.947; p=0.002)*. Post hoc analysis revealed a significant decrease of accuracy for the unnatural PLD *(t(4)=6.086; p=0.0018*); Figure 3B, dark grey line) between pre and post anodal stimulation suggesting a negative effect on the recognition of an unnatural PLD movement. In contrast, post hoc analysis revealed a significant improvement in human PLD accuracy between pre and post anodal stimulation *(t(4)=3.295; p=0.0150)* suggesting a positive effect in the recognition of a natural PLD movement following anodal stimulation (Figure 3B; black line). No significant effects were observed between pre and post testing sessions *(t(4)=1.951; p=0.122)* for the random PLD. Furthermore, no significant main effects were observed in accuracy as a function of condition *(F(2,8)=1.987; p=0.1993)*.

False reports

Anodal stimulation yielded a significant increase in false reports of unnatural PLD as natural. Frequency count significantly increased from 31 false reports in the pre testing session to 60 in the 30-post session *(X^2 (2,N=139)=6.11; p=0.047;* Table 2). No significant effects (p>0.05) were observed for the natural or random PLD false reports.

Cathodal Stimulation

RT analysis as a function of test session

We found no significant main effects of RTs across all testing sessions for natural *(F(3,12)=1.748; p=0.210)*, unnatural *(F(3,12)=2.633; p=0.097*) and random *(F(3,12)=1.968; p=0.172)* PLD movements (Figure 3C). In contrast to anodal stimulation however, post hoc analysis revealed no significant differences between pre and post testing sessions for natural *(t(4)=1.099; p=0.333)* and unnatural *(t(4)=1.583; p=0.1887)* PLD movements. In addition, we observed no significant differences between pre and post testing sessions for the random PLD *(t(4)=1.013; p=0.3684).*

RT analysis as a function of condition

We observed significant main effects in RTs *(F(2,8)=8.959; p=0.009)*. Planned paired-samples t-tests revealed significant differences in RTs between random and unnatural PLD movements across all testing sessions (pre *(t(4)=4.467= p=0.001)*, tDCS *(t(4)=3.703); p00.01)*, post *(t(4)=3.561; p=0.05)* and 30-post *(t(4)=3.458; p=0.05)*. Furthermore, random PLD was significantly different from natural PLD only in the pre testing session *(t(4)3.607; p=0.01)*. In contrast to anodal stimulation however no significant differences were observed between natural and unnatural PLD (all with p>0.05).

Accuracy

Participants could easily recognize random (mean 97.1% ± 1.19), human (mean 95.3% ± 1.53) and unnatural (mean 86.2% ± 2.68) movements (Figure 3D). No significant main effects in accuracy were observed between testing sessions for natural *(F(3,12)=0.943; p=0.451)*, unnatural *(F(3,12)=0.370; p=0.775)*, and random *(F(3,12=1.447; p= 0.278)* PLD movements or between conditions *(F(2,8)=1.995; p=0.198)*.

False reports

Cathodal stimulation had no significant effects on frequency count of false reports in our three PLD conditions (p>0.05).

Sham Stimulation

RT analysis as a function of test session

We found no significant main effects in RTs across all testing sessions for natural *(F(3,12)=1.337; p=0.308)*, unnatural *(F(3,12)=0.316; p=0.813)* and random *(F(3,12)=0.297; p=0.826)* PLD movements (Figure 3E). Post hoc analysis revealed no significant differences before and after stimulation for natural *(t(4)=0.317; p=0.766)* and unnatural *(t(4)=0.533; p=0.621)* PLD movements, in contrast to anodal stimulation. In addition, no significant differences were observed for random PLD *(t(4)=0.738; p=0.504)*.

RT analysis as a function of condition

We observed significant main effects in RTs *(F(2,8)=11.59; p=0.004), similar to results from anodal and cathodal stimulation*. Planned paired-samples t-tests revealed significant differences in RTs between random and unnatural PLD movements across all testing sessions (pre *(t(4)=4.325= p=0.01)*, tDCS (*t(4)=3.580); p=0.05)*, *post (t(4)=3.499; p=0.05)* and 30-post *(t(4)=4.290; p=0.01)*. Furthermore, random PLD was significantly different from natural PLD only in the 30-post testing session *(t(4)=4.064; p=0.01)*. Similar to cathodal stimulation however, no significant differences were observed between natural and unnatural PLD (p>0.05).

Accuracy

Participants could easily distinguish between random, natural and unnatural PLD (mean 97.4 % ± 0.85; 96.5% ± 0.88; 91.1% ± 2.99 respectively; Figure 3F) during sham stimulation. No significant main effects were observed as a function of testing sessions for natural

(F(3,12)=0.390; p=0.762), unnatural *(F(3,12)=1.807; p=0.199)* and random *(F(3,12)=0.980; p=0.434)* PLD movements or as a function of conditions *(F(2,8)=3.083; p=0.101).*

False reports

No significant effects (p>0.05) on the frequency count of false reports were observed during sham stimulation session in any of our three PLD conditions.

Summary

In summary, results from Experiment 2 showed that anodal tDCS significantly decreased RTs and significantly improved accuracy for natural movements. Furthermore, participants' tendency to report unnatural PLD movements as natural significantly increased following anodal tDCS, effectively decreasing participants' accuracy in recognizing unnatural movements. No significant effects were observed for cathode or sham stimulation.

Discussion

We used transcranial direct current stimulation (tDCS) to study the functional role of premotor cortex (PMC) in the recognition of different classes of movement. We used the point light display (PLD) method, where movements are reduced to just a handful of moving dots to represent these different classes of movement. The participants' task was to recognise as quickly and as accurately as possible the PLD representations of different movements.

In Experiment 1 participants had to make a between category assessment; distinguishing between a human, bird and random PLD, while undergoing tDCS on PMC. Decreasing neuronal excitability of PMC by using cathodal tDCS significantly decreased participants' accuracy in recognizing a human PLD movement with no significant effect on RTs (Figure 2C, D; black line). This effectively increased participants' tendency to report human movements as random (Table 1). This is in line with recent studies that reported decreased sensitivity and response bias (increased false alarms) to biological PLD movements following TMS on PMC (Urgesi, Calvo-Merino, Haggard & Aglioti, 2007; Urgesi, Candidi, Ionta & Aglioti, 2007; van Kemenade, Muggleton, Walsh & Saygin, 2012). A crucial role of normal functioning PMC for biological movement perception has also been reported in lesion studies (Candidi, Urgesi, Ionta & Aglioti, 2008; Saygin, 2007).

PMC is theorized to be an area in which visual information is compared and matched to internal premotor representations of movement (Rizzolatti & Craighero, 2004). RTs from experiment 1 indicate that human movements are recognized faster than bird or random movement, irrespective of stimulation type (~563 ms). Evidently, human observers are quicker at recognizing a human form performing movements than movements performed by a bird form or a random assortment of dots. This suggests that visual perception of movements depends on the degree of form similarity of the performed movement (Funk, Shiffrar & Brugger, 2005; Lee & Wong, 2004). However, the high accuracy scores across all participants in the recognition of human, bird, and random movement suggests that at least some of the same visual global form processes attributed to the recognition of all three.

Increased PMC excitability following anodal stimulation improved RTs for all three PLD movement categories, but this was only significant for bird and random movements (Figure 2A). While human movements were processed the fastest across all conditions, anodal stimulation had no significant effect on RTs and accuracy. We suggest that due to their high

familiarity to human observers, processing of human movements have already reached maximum levels in the pre condition, leaving hardly any room for further improvements by anodal tDCS. On the other hand, bird and random PLD movements are less familiar than human movements and their processing is less optimized in the pre condition, leaving room for improvement by anodal tDCS. Lange and Lappe (Lange & Lappe, 2006) suggested the presence of templates in the human brain specific to the processing of human biological motion. Observed movements are matched to these existing templates to interpret that movement. Such templates are suggested to be generated by a learning process (Lange & Lappe, 2006). In principle, such templates might exist also for other classes of biological stimuli. Our results are in line with such a template matching approach in PMC that seems to be highly specialized to a particular class of biological movements (e.g. humans) but which also extends to other classes of biological movements (e.g. bird) (Lange, Georg & Lappe, 2006). The significant decrease in our participants' tendency to report bird PLD as random PLD suggests that following anodal stimulation the templates generated to match and interpret the observed biological movement of birds were activated faster and with greater precision. This subsequently decreased RTs of random movements with no significant effect on how it was interpreted (accuracy and response bias). As random movements offer no identity as to who or what (no global form) is performing the movement, templates do not exist, therefore no interpretation of the movement is necessary and it is simply recognized as meaningless.

In contrast to Experiment 1, participants in Experiment 2 were asked to make a within class discrimination. We presented three different variations of a single class of movement, and again administered tDCS over PMC to examine its effects during the visual processing of a single movement differing in its degree of plausibility. We were specifically interested in the effects of tDCS on PMC in the visual processing of natural and unnatural PLD movements as both share very similar global forms when compared to the random PLD movement (Figure 1B). While cathodal and sham stimulation revealed no significant effects on RTs or accuracy (Figure 3C-F), anodal stimulation led to both faster RTs and more accurate responses to natural movements. In contrast, RTs for unnatural movements improved following anodal stimulation, but accuracy significantly decreased (Figure 3A-B). In addition, frequency counts of false reports revealed that following anodal stimulation participants had an increased tendency to recognize both natural and unnatural movements as natural (Table 2).

PMC has been previously suggested to play an important role in the interpretation of an incorrect movement (Craighero, Bonetti, Massarenti, Canto, Fabbri & Fadiga, 2008; Koelewijn, van Schie, Bekkering, Oostenveld & Jensen, 2008; Urgesi, Candidi, Ionta & Aglioti, 2007). However, the significant increase of false reports of unnatural as natural movements following anodal tDCS, suggests a shift in response bias within PMC during this process. A possible explanation for this shift is that anodal tDCS produced an increase in visuomotor priming within PMC, and this priming facilitated recognition of the most familiar action; natural movement.

Visuomotor priming is explained by positing that the observation of a movement automatically activates PMC cells, that in turn influence the observer's motor system (Gowen, Bradshaw, Galpin, Lawrence & Poliakoff, 2010). In the presence of biological vs. non biological movements, biological movements produces stronger priming effects (Brass, Bekkering & Prinz, 2001; Kilner, Paulignan & Blakemore, 2003). Furthermore, visuomotor priming has been shown to depend to some degree upon personal experience (Gowen, Bradshaw, Galpin, Lawrence & Poliakoff, 2010). This is in line, with the assumption that although activity in PMC extends to include pictures or videos of mouth, hand, full body, and different species movements, it seems to optimally respond to whole-body human movements (Craighero, Fadiga, Umilta & Rizzolatti, 1996; Gowen, Bradshaw, Galpin, Lawrence & Poliakoff, 2010; Gowen & Poliakoff, 2012; Hesse, Sparing & Fink, 2009). Our participants' tendency to report unnatural movements as natural suggests that increased excitability of PMC cells prompts stronger visuomotor priming to natural human movements, as indicated by the faster RT and increase accuracy in the recognition of natural PLD movements. This was at the expense of the recognition of unnatural but not random movements. Given that both natural and unnatural movements share very similar global forms an increase in visuomotor priming caused a shift in response bias within PMC affecting the interpretation of the unnatural movement as natural. This further suggests that visuomotor priming is strongly influenced by the visual properties of the observed stimulus similar to the template matching approach (Lange, Georg & Lappe, 2006; Lange & Lappe, 2006).

Interestingly, we observed a substantial difference in the reaction times (RTs) to natural human movement between our two experiments. When participants were asked to distinguish between three different categories of PLD movement (human, bird, random;

experiment 1), RTs were below 700 ms, with the human PLD movement having the fastest RTs (~563 ms). When participants were asked to discriminate between two similar representations of a single movement (natural and unnatural human movements; experiment 2) however, RTs increased to ~1014 and ~1091 ms for natural and unnatural movements respectively. This suggests that in the presence of two very similar global forms (natural vs. unnatural movement in contrast to human vs. bird movement) activity in PMC includes an evaluative component over a longer period of time (Craighero, Bonetti, Massarenti, Canto, Fabbri & Fadiga, 2008; Fadiga et al. 2006). A potential explanation for the increase in human PLD RTs in Experiment 2 is increased internal imagery when trying to distinguish between two very similar PLDs rather then a simple human vs. bird discrimination. If we consider that the templates are more likely to exist for learned or often seen (natural) movements (Lange, Georg & Lappe, 2006), the observation of both natural and unnatural movements will generate a matching process to the same templates at around the same time. Since no templates exist for unnatural movements observation of an unnatural movement might require the rejection of a template, requiring more time to interpret and in turn more time to recognize.

In summary, we presented PLD movements and modulated PMC to investigate its causal role on the visual processing of different movements, differing in form and degree of plausibility. To our knowledge the current study is the first to explore the functional role of PMC in discriminations between and within categories of movement, using a single paradigm (PLDs and tDCS). We used both polarities of stimulation (anode and cathode), and a control (sham) over PMC and compared their effects on movement recognition. Increasing neuronal excitability (anodal) facilitated the distinction of unfamiliar non human biological movements. This suggests a key role for PMC in the visual percept of different biological movements using the template matching approach, which involves the global processing of the PLD form. In contrast, decreasing neuronal excitability (cathodal) significantly affected PMC's role in the visual discrimination of a human PLD, underlining the importance of PMC in the visual processing of human movement. In addition, increasing excitability of PMC cells increased visuomotor priming and a shift in response bias to natural movement severely affecting the visual discrimination of an unnatural PLD movement.

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Captions

Table 1: Total number of false report for each condition (human, bird and random) for anode, cathode and sham across participants for Experiment 1. * Significant difference ($p<0.05$) between testing sessions.

Table 2: Total number of false report for each condition (natural, unnatural and random) for anode, cathode and sham across participants for Experiment 2. * Significant difference ($p<0.05$) between testing sessions.

Figure 1: Examples of our PLD stimuli for both experiments. (A) Representation of a human, bird and random PLD movement. (B) Representation of a natural, unnatural and random movement. Participants had to respond as quickly and as accurately as possible following visual representation of our stimuli. Connecting lines were not present in the actual experiment.

Figure 2: Experiment 1 results for mean RT and accuracy *(± SEM*) across all stimulation and testing sessions. (A, B) Anode RT and accuracy (C, D) Cathode RT and accuracy (E, F) Sham RT and accuracy for human (black line), bird (dark grey line) and random (light grey) PLD movement. A star denotes a significant effect between testing session for human (black), bird (dark grey) and random (light grey) PLD movements; * $p<0.05$ and ** $p<0.01$.

Figure 3: Experiment 2 results for mean RT and accuracy *(± SEM*) across all stimulation and testing sessions. (A, B) Anode RT and accuracy (C, D) Cathode RT and accuracy (E, F) Sham RT and accuracy for natural (black line), unnatural (dark grey line) and random (light grey) PLD movement. A star denotes a significant effect between testing session for human (black), bird (dark grey) and random (light grey) PLD movements; * $p<0.05$ and ** $p<0.01$.

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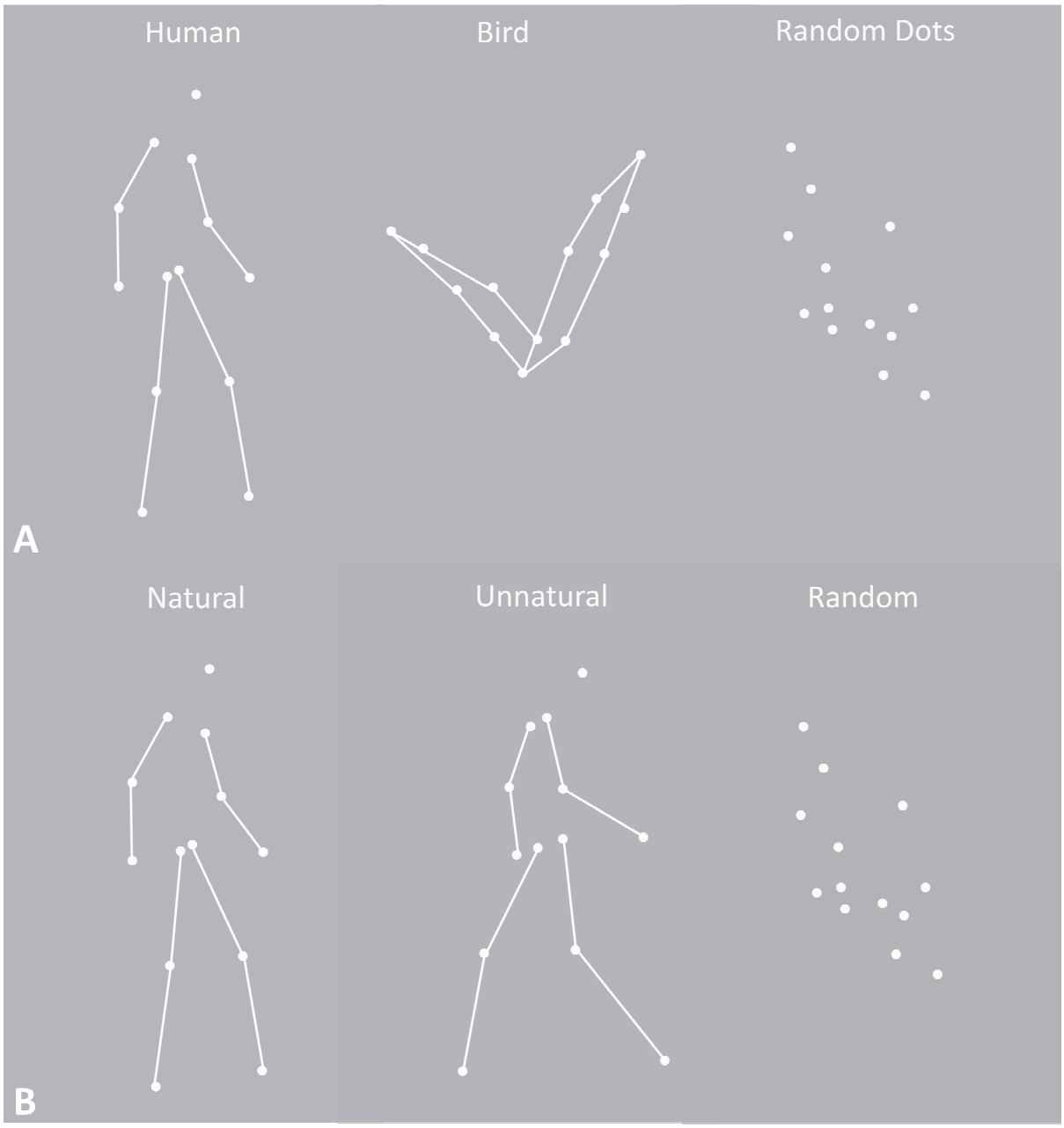
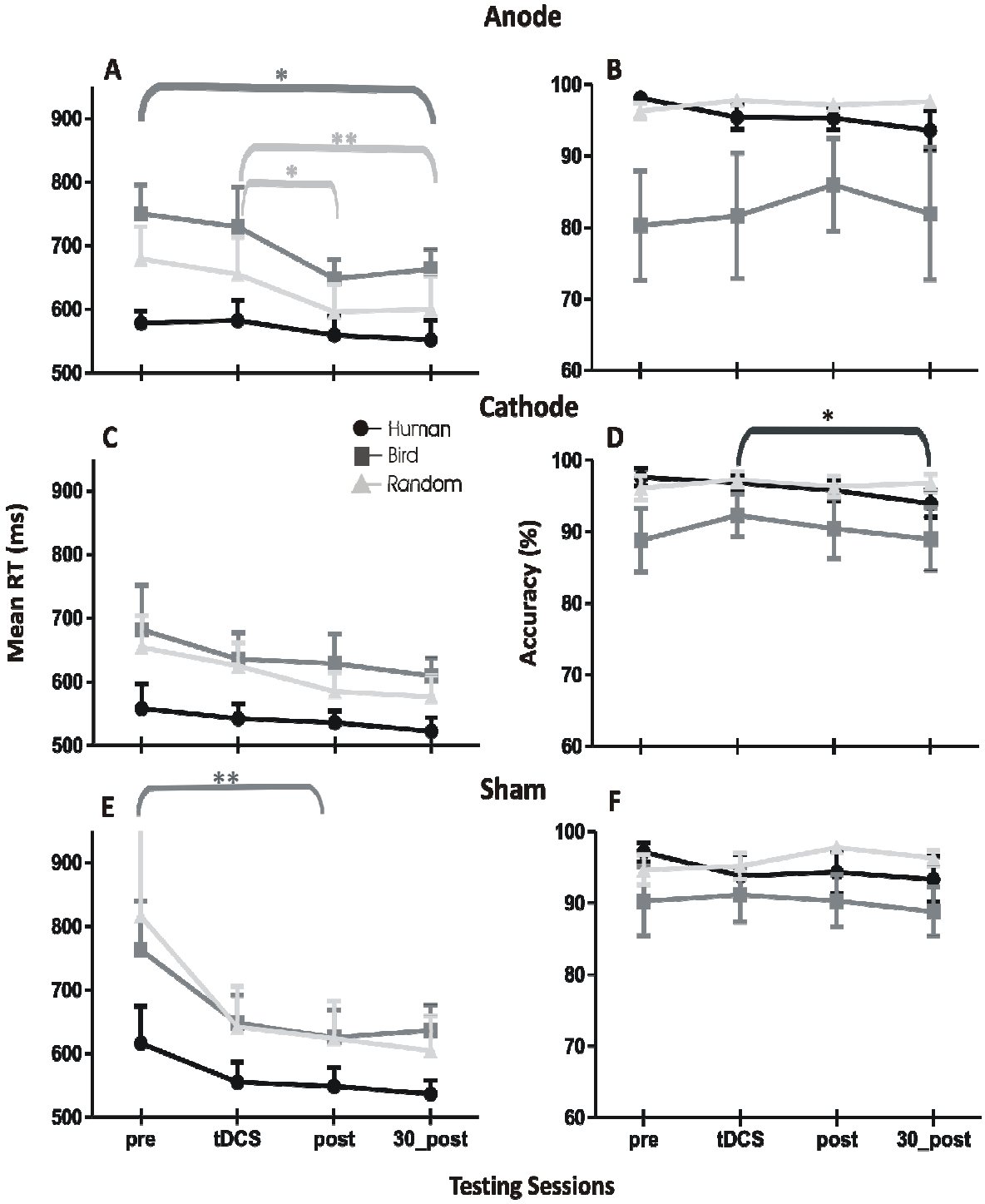
False reports for all three stimulation sessions (out of a total of 600)

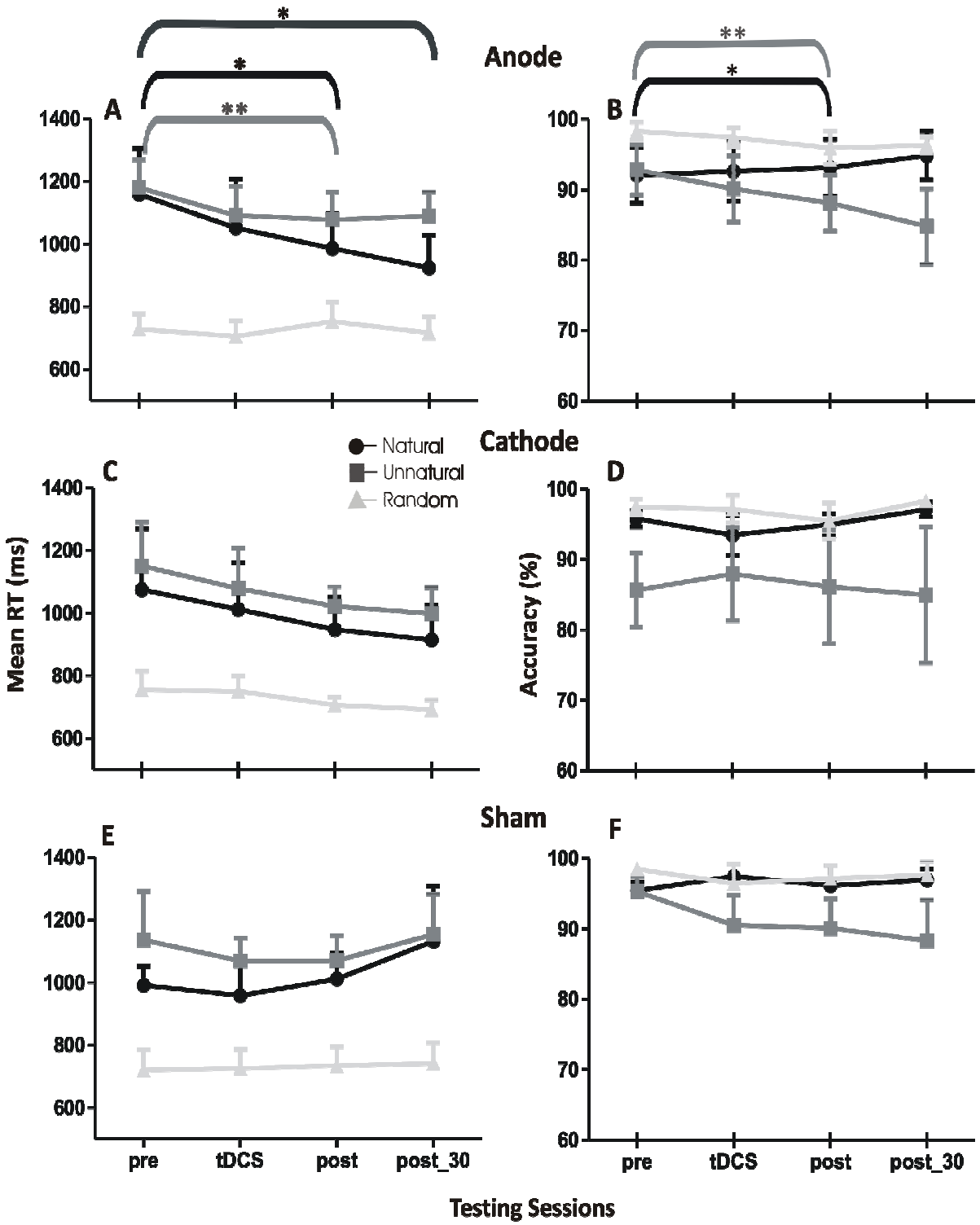
	Human False Reports		**Bird False Reports**		**Random False Reports**	
Anode	Bird	Random	Human	Random	Human	Bird
Pre	7	4	6	111*	14	8
tDCS	15	12	12	98	5	8
Post	10	19	8	76*	9	8
30-post	12	22	11	97	6	8
Cathode						
Pre	7	7	12	55	8	16
tDCS	12	7*	6	40	7	9
Post	17	8	11	46	6	16
30-post	16	20*	9	57	9	10
Sham						
Pre	9	9	8	50	10	22
tDCS	14	23	10	43	15	14
Post	15	19	13	45	4	9
30-post	16	20	16	51	11	11

False reports for all three stimulation sessions (out of a total of 600)

	Natural False Reports		**Unnatural False Reports**		**Random False Reports**	
Anode	Unnatural	Random	Natural	Random	Natural	Unnatural
Pre	43	4	31*	12	1	9
tDCS	40	4	40	19	0	13
Post	32	8	49	21	3	22
30-post	27	3	60*	29	8	18
Cathode						
Pre	22	3	72	14	1	14
tDCS	34	4	51	21	5	12
Post	21	9	63	14	6	21
30-post	13	4	68	23	3	7
Sham						
Pre	27	0	19	9	1	8
tDCS	13	2	22	12	1	20
Post	16	7	30	15	2	16
30-post	15	3	31	23	0	14


Human
Bird
Random Dots
A
Natural
Unnatural
Random
B


Anode
A
B
Cathode
C
D
Sham
E
F
Human
Bird
Random
Mean RT (ms)
Accuracy (%)
pre
tDCS
post
30_post
Testing Sessions


Anode
Cathode
Sham
A
B
C
D
E
F
Natural
Unnatural
Random
Mean RT (ms)
Accuracy (%)
1400
1200
1000
800
600
100
90
80
70
60
pre
tDCS
post
post_30
Testing Sessions