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To mirror or not to mirror upon perceived eye contact?

A neurophysiological perspective on interpersonal motor resonance

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Frequently used abbreviations

ADM	abductor digiti minimi (hand muscle)
ANOVA	analysis of variance (statistical analysis)
AOI	area of interest (eye tracking parameter)
APB	abductor pollicis brevis (hand muscle)
ASD	autism spectrum disorders (neuropsychiatric condition)
EEG	electroencephalography (neural assessment technique)
EMG	electromyography (muscle tone assessment technique)
FC	fixation count (eye tracking parameter)
FDI	first dorsal interosseous (hand muscle)
FG	fusiform gyrus (brain region)
fMRI	functional magnetic resonance imaging (neural assessment technique)
IFG	inferior frontal gyrus (brain region)
IPL	inferior parietal lobule (brain region)
M1	primary motor cortex (brain region)
MEG	magnetoencephalography (neural assessment technique)
MEP	motor evoked potential (TMS parameter)
mPFC	medial prefrontal cortex (brain region)
OFC	orbitofrontal cortex (brain region)
PMC	premotor cortex (brain region)
RMSE	root mean square error (statistical construct)
rMT	resting motor threshold (TMS parameter)
STORM	social top-down response modulation (neural construct)
STS	superior temporal sulcus (brain region)
TFD	total fixation duration (eye tracking parameter)
TMS	transcranial magnetic stimulation (brain stimulation / assessment method)
TPJ	temporoparietal junction (brain region)

Samenvatting

Een van de grootste uitdagingen voor neurowetenschappers is om uit te zoeken hoe we precies de acties, gedragingen en emoties van anderen begrijpen tijdens interpersoonlijke interacties. Aan de grondslag van deze vaardigheden ligt een verzameling gespecialiseerde cellen in de hersenen, die we **spiegelneuronen** noemen, die actief zijn wanneer we zelf een handeling uitvoeren, maar ook wanneer we iemand anders een handeling zien uitvoeren. Door 'zien' automatisch te verbinden met 'doen', zorgen onze hersenen er voor dat we de acties en gedragingen van anderen bijna moeiteloos kunnen begrijpen, en hierop gepast kunnen reageren. Maar hoe beslist ons brein wie en wanneer we moeten spiegelen? In dit doctoraatsproject hebben we onderzocht hoe de blik van de interactiepartner dit neuraal proces beïnvloedt. Het correct interpreteren van de non-verbale signalen van anderen, zoals oogcontact, speelt immers een belangrijk rol in het alledaags sociale leven. Wanneer iemand naar je kijkt, geeft dit waarschijnlijk aan dat hij of zij met jou wil communiceren. Het is dus erg relevant voor jou om zijn of haar handelingen, emoties en gedragingen goed te spiegelen, zodat je hier gepast op kan reageren. Wanneer iemand echter zijn blik afwendt, betekent dit meestal dat zijn of haar handelingen niet aan jou gericht zijn. In dit geval is het dus minder relevant om zijn of haar acties te spiegelen.

In een **eerste fase** van dit project hebben we, met behulp van de hersenstimulatietechniek transcraniële magnetische stimulatie (TMS), een gevoelig paradigma ontwikkeld om het effect van oogcontact op de werking van het spiegelneuronensysteem in de hersenen na te gaan. Een eerste studie bevestigde onze hypothese en toonde aan dat oogcontact tussen de interactiepartner en proefpersoon inderdaad meer spiegeling uitlokt. Volgens ons werkmodel vormt deze flexibele activatie van het spiegelneuronensysteem tijdens het waarnemen van oogcontact een goede neurofysiologische index van sociale toenadering. De toepasbaarheid en validiteit van dit paradigma en bijbehorende hypotheses werden verder onderzocht door middel van twee bijkomende studies. In een tweede studie gingen we de impact van de ecologische validiteit van de getoonde stimuli na, en toonden we aan dat de observatie van 'live' personen resulteerde in robuustere bevindingen in vergelijking met het observeren van videostimuli. De derde studie onderzocht hoe deze neurofysiologische maat zich verhoudt tot andere markers waarvan bekend is dat ze worden beïnvloed door interpersoonlijke interactie (bijvoorbeeld huidgeleidbaarheid).

In de **tweede fase** toonden we aan dat dit vermogen van het spiegelneuronensysteem om zich aan te passen naargelang de non-verbale signalen van de interactiepartner gelinkt kan worden aan bepaalde sociale eigenschappen van de persoon, zoals de mate van sociale angst, sociale responsiviteit en (on)veilige hechting. Verder hebben we ook de functionaliteit van het spiegelneuronensysteem onderzocht bij mensen met een autisme spectrum stoornis (ASS), die worstelen met veel aspecten van dagdagelijkse sociale interactie en communicatie. Vanwege de veronderstelde rol van het spiegelneuronen-systeem in een breed scala van sociaal-cognitieve functies, werden de sociaal-interactieve moeilijkheden in ASS in eerste instantie toegeschreven aan een beschadigd neuraal spiegelmechanisme. Anderen hebben gesuggereerd dat enige onregelmatigheden in dit mechanisme een bijproduct zijn van defecten in andere hersengebieden die verantwoordelijk zijn voor de verwerking van sociale informatie. Desalniettemin tonen onze resultaten aan dat de omvang van eventuele dysfuncties in het spiegelneuronensysteem van personen met een ASS, zij het in het spiegelen van sociale of non-sociale stimuli, sterk afhangt van de ernst van de klinische ASS symptomen. Deze bevindingen vormen een belangrijke aanvulling op onze kennis van het spiegelneuronensysteem in ASS.

Tot slot hebben we in de **derde fase** van dit doctoraat onderzocht of dit neuraal spiegelmechanisme in functie van oogcontact gemoduleerd kan worden door middel van intranasale toediening van een enkele dosis oxytocine. Oxytocine is een neuropeptide dat wordt aangemaakt in de hersenen, en dat een sleutelrol speelt in de regulering van complexe sociale gedragingen, aangezien het specifiek inwerkt op de neurale circuits die hieraan ten grondslag liggen. Vanwege zijn ‘pro-sociale’ reputatie wordt het therapeutisch potentieel van oxytocine dan ook steeds meer onderzocht, vooral in patiëntpopulaties met bijzondere moeilijkheden in het sociale domein zoals personen met een ASS. Met deze laatste studie hebben we aangetoond dat, vooral bij personen die minder sociale vaardigheden rapporten en ook een verlaagde neurale spiegelrespons tijdens sociale interacties vertonen, een enkele dosis oxytocine in staat is om deze respons te verhogen. Bij personen die reeds voldoende spiegel-activiteit vertonen, verhoogt oxytocine deze respons niet verder.

Samengevat, dit doctoraatsproject draagt bij tot een beter begrip van de flexibele werking van het spiegelneuronensysteem tijdens het waarnemen van een combinatie van dynamische non-verbale sociale signalen, zoals dit in het dagelijkse leven gebeurt. Bovendien benadrukt dit proefschrift de rol van relevante persoonskenmerken zoals sociale eigenschappen in deze processen, zowel voor typische als voor ASS participanten, en bieden we veelbelovend initieel bewijs voor een modulerende rol van oxytocine over het neurale spiegelmechanisme.

Summary

One of the great challenges in cognitive neuroscience is to explore how exactly we comprehend other people's actions, behaviors and emotions during social interactions. The presumed neural basis of these abilities is the so-called '**mirror neuron system'**, which is active not only when we perform an action ourselves, but also when we see someone else performing that action. By automatically bridging the gap between 'seeing' and 'doing', this mechanism supports a variety of socio-cognitive functions that are important for everyday life, such as action comprehension and intention understanding. But how does our brain decide who and when to mirror? In this PhD project, we investigated how the interaction partner's gaze direction modulated the propensity to mirror others' actions. Indeed, correctly interpreting the non-verbal signals from others, such as eye contact, plays an important role in everyday social life. Whenever someone is looking at you, it probably indicates that he or she wants to communicate with you. Therefore, it is relevant to adequately mirror his/her actions, emotions and behaviors, so that you can respond appropriately. However, when someone displays averted gaze while performing an action, this means that his or her actions are not addressed to you. It is therefore less relevant to process and mirror these actions.

In a **first phase** of this project we developed a sensitive and relatively easily applicable paradigm to measure the effect of eye contact on mirror system activation. Using the non-invasive brain stimulation technique transcranial magnetic stimulation (TMS), our first 'proof of principle' study demonstrated that mirror system activation was significantly enhanced when eye contact between the interaction partner and participant was established. We propose that this modulation of the mirror system during the observation of eye contact cues provides a sensitive neurophysiological index of the individual's propensity to interact with others. The applicability and validity of this paradigm and associated hypotheses were further investigated by means of two additional studies. The second study demonstrated the impact of the ecological validity of the presented stimuli, and indicated that observation of a live stimulus person resulted in more robust findings compared to video stimuli. The third study investigated how this neurophysiological measure relates to other markers known to be impacted by interpersonal interaction (i.e. electrodermal response, gaze behavior of the participant and mu rhythm suppression).

In the **second phase** of this project, we demonstrated that one’s ability to show adaptive mirror system adjustment in accordance to observed eye contact cues is associated with certain social characteristics of the individual, such as social anxiety, social responsiveness and attachment style. Furthermore, we also investigated the extent of mirror system (dys)functioning in individuals with an autism spectrum disorder (ASD), who struggle with many aspects of social interaction and communication. Due to the role of the mirror system in a broad range of socio-cognitive functions, the various socio-interactive deficits that characterize ASD were initially attributed to a dysfunctional mirror system (a notion known as the "Broken Mirror Theory"). Others have suggested that any irregularities in the mirror system are a byproduct of deficits in other brain regions responsible for social information processing. Nevertheless, our results demonstrate that the extent of any mirror system (dys)functioning in ASD, be it in a social or non-social context, heavily depends on ASD symptom severity, and thus provide an important contribution to the knowledge about the mirror system in ASD.

Finally, in the **third phase** of this PhD project, we investigated whether the propensity to mirror others’ actions upon perceived eye contact could be modulated by a single dose of intranasally administered oxytocin. Oxytocin is a hypothalamic neuropeptide that has a key role in the regulation of complex social behaviors, as its action mechanism specifically targets the neural circuits in the brain that underlie these behaviors. Due to its ‘prosocial’ behavioral and neural effects, its potential as an effective treatment for improving socio-cognitive functioning, also in populations with particular difficulties in the social domain (such as individuals with ASD), is increasingly investigated. With this study, we provided first neurophysiological evidence that a single dose of oxytocin is able to selectively augment the mirror response in socially relevant situations (i.e. only when direct gaze from the interaction partner is perceived). Importantly, significant moderations of the treatment effect were noted, indicating that participants with less self-reported social skills displayed a stronger oxytocin-related effect (enhancement of motor resonance facilitation by direct gaze), compared to participants with high self-reported social skills.

In summary, this doctoral project contributes to a better understanding of the modulation of the mirror system while observing a combination of multimodal, dynamic and contextually-embedded non-verbal social signals, such as this happens in everyday life. In addition, this dissertation emphasizes the role of relevant social characteristics in these processes, both in the neurotypical and autistic population, and provides first indications of a possible action of intranasal oxytocin in modulating the neural mirror mechanism.

General Introduction

Preamble

Social interaction and communication are defining features of human social life. Yet, social information processing is extremely complex, as it involves the integration of dynamic, multimodal (i.e. verbal and non-verbal information) and contextually embedded cues. Accordingly, one of the great challenges in cognitive neuroscience is to explore how exactly we comprehend other people's behaviors during interpersonal interactions. Yet, the problem of how we understand others' behaviors is not new in the field of philosophy. Traditionally, it was accepted that the capacity to understand other minds depends on the cognitive ability of inferential reasoning. In this view, known as **Theory Theory**, information about others is compared to an internal body of causal-explanatory knowledge (or 'theory'), which allows the observer to infer the mental states that generate others' behaviors (i.e. "she is laughing, thus she must be happy").

In contrast, **Simulation Theory** suggests that the understanding of others is achieved by using one's own mind as a model to compare the behavior of others with (i.e. "putting yourself in the other person's shoes"). The discovery of a so-called "**mirror mechanism**" in the monkey brain, and later also in humans, has provided a neurophysiological substrate for the latter. The essence of this mechanism is the following: each time an individual observes another individual performing an action, a special set of neurons that encodes this action is activated in the observer's own motor system. By bridging the gap between 'seeing' and 'doing', this mirror mechanism allows an individual to rather intuitively and effortlessly understand the actions, motor goals and intentions of others (Gallese & Goldman, 1998).

The aim of this general introduction is to provide a broad overview of the field of mirror neuron research and mirror-based action understanding. In the next sections, we will **first** briefly discuss the discovery and basic properties of the mirror neuron system in humans. In the **second** section, the putative functional relevance of the mirror system in humans will be outlined. Here, we will also touch upon the relevance of this neural system for neuropsychiatric disorders such as autism spectrum disorders. The **third** section will elaborate on the role the mirror system plays when perceiving multimodal and socially embedded action cues. **Finally**, in the last sections, we will describe the methodology and general aim of the present PhD project, and provide a comprehensive overview of the studies presented in the following chapters of this doctoral thesis.

1 The mirror system: a primer

1.1 Discovery of mirror neurons in monkeys

While doing research on the neural representation of motor movements in the ventral premotor cortex (area F5) of the macaque monkey (*macaca nemestrina*), an Italian team of neuroscientists accidentally identified a special type of neuron that fired not only when the monkey performed a movement (e.g. grasping a peanut), but – surprisingly so – also when the monkey observed another individual (monkey or human) performing that movement (Rizzolatti et al., 1988; **figure 1A**). Neurons with similar properties were later also found in other parts of the monkey brain (area PF/7b; Rizzolatti, Fogassi & Gallese, 1997; **figure 1B**). The team of neuroscientists aptly named these cells 'mirror neurons' (di Pellegrino, Fadiga, Fogassi, Gallese & Rizzolatti, 1992), and their discovery has profoundly influenced the field of cognitive neuroscience, psychology and other associated disciplines. The interested reader is referred to Fabbri-Destro and Rizzolatti (2008) for a detailed description of the basic functional properties of monkey mirror neurons. The focus of the current dissertation is the human mirror system and its implicated role in human social cognition.

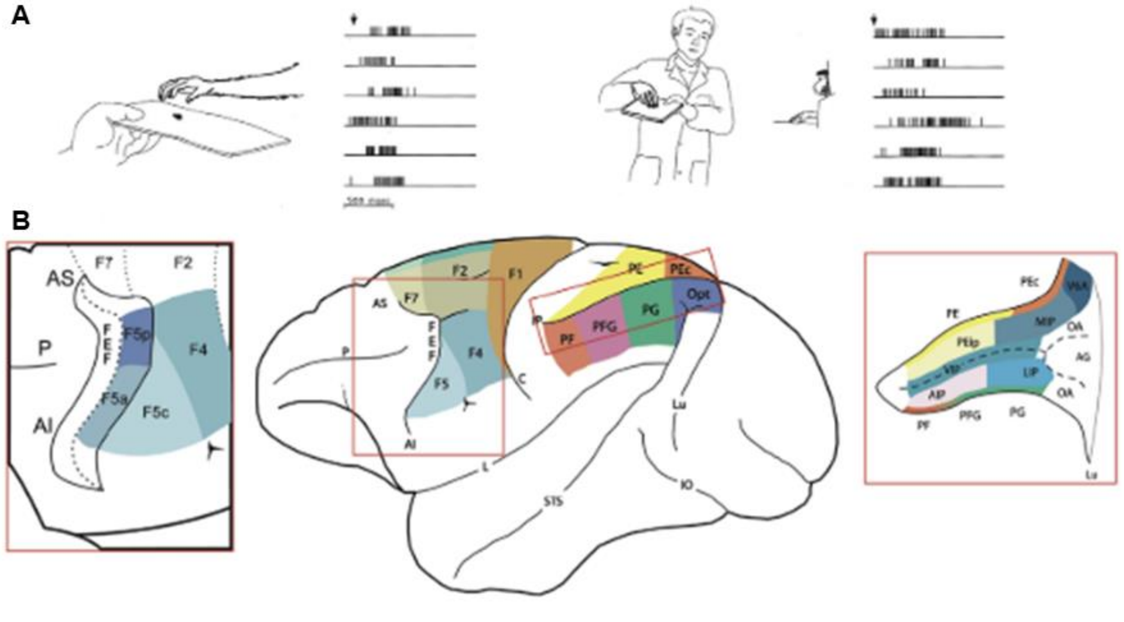

Figure 1. The monkey mirror neuron system. (A) Example of the behavior of a mirror neuron. The neuron discharges when the monkey grasps an object and when it observes the experimenter grasping it. (**B)** Lateral view of the monkey brain showing the cytoarchitectonic parcellation of the motor areas of the frontal lobe (F1–F7) and posterior parietal areas (PE, PEc, PF, PFG, PG, PF op, PGop and Opt). Figure and figure legend adapted from Rizzolatti & Fabbri-Destro, 2008, *Current Opinion in Neurobiology*.

1.2 Description of a homologue mirror system in humans

Prompted by the discovery of monkey mirror neurons, the search for a comparable mirror neuron system in the human brain emerged. Single-neuron recordings, which require the opening of the skull to insert microelectrodes in the brain, are however very invasive and rarely feasible in human participants. Nevertheless, a rich amount of data proves, indirectly, the existence of a human mirror system. This evidence comes from brain imaging studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI; for reviews, see Caspers, Zilles, Laird & Eickhoff, 2010; Molenberghs, Cunnington & Mattingley, 2012), transcranial magnetic stimulation (TMS; reviewed by Fadiga, Craighero & Olivier, 2005), and magneto-/electro-encephalography (M/EEG; summarized in Bowman et al., 2017; Hobson & Bishop, 2017). Each technique has a different mechanism of action, as well as its own advantages and disadvantages. In the following paragraphs, a short description of these methods is provided.

1.2.1 Localization of the human mirror system

Over the last decade, a large number of brain imaging studies (i.e. fMRI and PET) have tried to pinpoint the neural substrate of action processing in the human brain. Studies comparing neural activity during perceived and executed actions have consistently revealed overlapping activation in the rostral part of the inferior parietal lobule (IPL), the inferior frontal gyrus (IFG; also known as *pars opercularis*) and the adjacent dorsal and ventral premotor cortex (PMC), appointing them as the **core nodes** of the “classic” fronto-parietal mirror system (fore reviews, see Caspers, Zilles, Laird & Eickhoff, 2010; Molenberghs, Cunnington & Mattingley, 2012). The localization of the human mirror system is shown in **figure 2**, and corresponds closely to the monkey brain areas in which mirror neurons were originally described. Furthermore, brain imaging studies also revealed that the observation of others’ actions induces a gross-scale somatotopic activation in both the premotor and the parietal areas of the mirror system, similar to that of the classical motor cortex homunculus (Buccino et al., 2001).

Another important brain region during action processing is the **superior temporal sulcus** (STS). Although the STS is not involved in the execution of motor functions, it consistently responds to the perception of biological motion (Grèzes et al., 2001; Grossman & Blake, 2002). As such, the STS region forms an integral part of the “extended” mirror system network by providing visual input about the dynamics of others’ actions to the upstream mirror regions (Rizzolatti & Craighero, 2004).

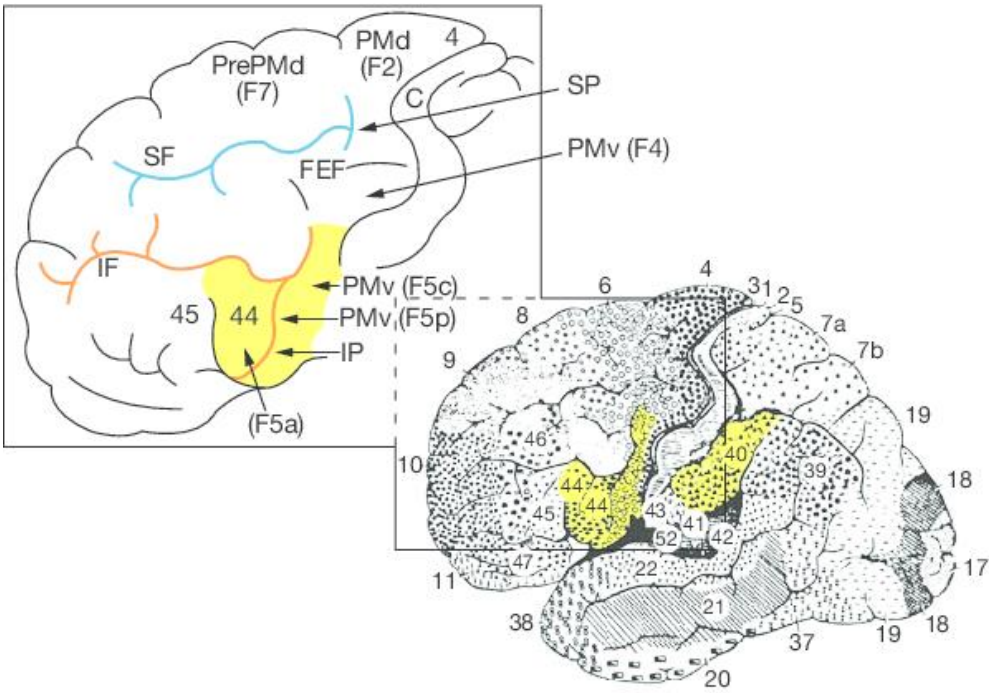

Figure 2. The fronto-parietal mirror system in humans. The areas in yellow correspond to areas that respond to the observation and execution of hand motor acts. The possible homology between monkey and human premotor cortex is indicated by arrows. Abbreviations: C, central sulcus; FEF, frontal eye field; IF, inferior frontal sulcus; IP, inferior precentral sulcus; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; PrePMd, pre-dorsal premotor cortex; SF, superior frontal sulcus; SP, upper part of the superior precentral sulcus. Numbers indicate Brodmann cytoarchitectonic subdivision. Figure and figure legend adapted from Rizzolatti & Fabbri-Destro, 2008, *Current Opinion in Neurobiology*.

1.2.2 Neurophysiological evidence

The first evidence of a homologue mirror system in the human brain was obtained by a study using **TMS** (Fadiga, Fogassi, Pavesi & Rizzolatti, 1995). TMS is a non-invasive technique to magnetically stimulate cortical areas of the brain. When TMS is applied to the primary motor cortex (M1), which employs a somatotopic organization, small muscle twitches or motor evoked potentials (MEPs) can be recorded from the contralateral extremity muscles. Importantly, it was demonstrated by Fadiga et al. (1995) that the amplitude of these MEPs is enhanced during action observation compared to rest. Moreover, this increase was selective for those muscles that are intrinsically recruited when producing the observed movements; i.e. MEPs from the hand muscles were only facilitated during observation of hand actions whereas MEPs from the arm muscles were enhanced only during the perception of arm gestures. This facilitation of MEPs during movement observation is thought to result from a facilitation of M1 activity by excitatory cortico-cortical connections between M1 and mirror regions in the brain (Fadiga, Craighero & Olivier, 2005).

The initial results from Fadiga et al. (1995) have since been successfully replicated and extended by other studies using the TMS technique (reviewed by Fadiga, Craighero & Olivier, 2005), thereby successfully demonstrating the existence of a mirror mechanism in the human brain. Moreover, TMS has provided us with some unique insights in the properties of the human mirror system at work. First, as demonstrated by Fadiga et al. (1995), and later also confirmed by numerous other studies (e.g. Alaerts, Swinnen & Wenderoth, 2009; Maeda, Kleiner-Fisman & Pascual-Leone, 2002; Strafella & Paus, 2000), the human mirror system encodes others' actions in a strictly **muscle-specific way**: MEPs are facilitated during the observation of a given action, but only in the same muscles that are also recruited during the execution of that movement. Second, TMS research has also indicated that the **lateralization** pattern of M1 facilitation is similar to the known lateralization pattern for motor control (Aziz-Zadeh, Maeda, Zaidel, Mazziotta & Iacoboni, 2002). In other words, each hemisphere is activated most by observation of the contralateral body part, i.e. the one it controls. This implies that when TMS is applied to the left M1, MEPs are larger while observing right hand actions. Third, due to its relatively high temporal resolution TMS has also been used to address the temporal dynamics of action observation and simulation. For instance, by applying TMS and recording MEPs at different time intervals during the observation of a grasping movement, Gangitano, Mottaghy, and Pascual-Leone (2001) showed that the time course of M1 facilitation during action observation follows the grasping movement phases of the observed action: MEPs were maximal during the finger aperture phase, and became gradually smaller during the closure phase. This clearly demonstrates the strict **temporal coupling** between changes in M1 excitability and the dynamics of action execution. Lastly, also different **kinematic features** of the observed motor acts, such as movement height, velocity and grip force (e.g. Alaerts, de Beukelaar, Swinnen & Wenderoth, 2012), are encoded by the observer's motor system.

Since the current PhD project is centered around the TMS technique to study the human mirror system at work during action perception, the application of this technique is discussed in more detail in the last section. In addition to TMS, also **EEG** and **MEG** can be adopted to study the human mirror mechanism by inspecting modulations in the sensorimotor mu frequency (i.e. neural oscillations in the 8-13 Hz frequency band over the sensorimotor cortex) upon action execution and observation. In general, the sensorimotor mu rhythm is significantly suppressed during both action observation and execution, which is indicative of neural activity in the underlying cortical areas (Bowman et al., 2017; Hobson & Bishop, 2017). Since the present thesis adopted the EEG method in **chapter 3**, the reader is referred to this chapter for a more detailed explanation on the mu rhythm.

Finally, following years of intense research after the discovery of mirror neurons in monkeys, Mukamel et al. (2010) provided direct electrophysiological evidence that humans have mirror neurons. In 21 epileptic patients waiting for brain surgery, they recorded **single-neuron activity** from a total of 1177 neurons in medial frontal (including supplementary motor area and anterior cingulate cortex) and temporal areas (including amygdala, hippocampus, para-hippocampal gyrus and entorhinal cortex) while executing and observing two types of hand actions (precision grips and whole hand grips). Mukamel et al. (2010) found that a significant proportion of these neurons behaved exactly like the mirror neurons found in monkeys; discharging during both the observation and the execution of hand actions.

Together, these results indicate that upon action observation, whether this is watching someone grasping a cup of tea, biting an apple or kicking a football, an internal motor replica of that action is automatically generated, activating the same regions of our brain that would fire if we were doing the same action.

1.3 Properties of the human mirror system

Many types of **biological movement** activate the mirror system in humans. This includes purposeful or goal-related (i.e. transitive) actions such as grasping and reaching, but also non-object- or non-goal-related (i.e. intransitive) actions that are non-specific (e.g. finger tapping), symbolic (e.g. performing the OK sign) or mimicked (e.g. pretending to knock on a door; Lui et al., 2008). Also the perception of **impoverished motion stimuli**, such as shadow animations (Alaerts, Van Aggelpoel, Swinnen & Wenderoth, 2009), robotic arms (Gazzola, Rizzolatti, Wicker & Keysers, 2007) or point-light displays (Grèzes et al., 2001; Ulloa & Pineda, 2007) have shown to be sufficient to recruit mirror areas in the brain. Furthermore, as indicated by the original mirror neuron experiment by Rizzolatti et al. (1988) showing active mirror neurons in the monkey not only when it observed another monkey, but also when it observed a human experimenter grasping a peanut, mirror system activation can happen **regardless of species**.

However, when robotic actions or point-light displays do not closely match biological movement, the mirror system will not respond (Gazzola et al., 2007), indicating that only motor acts that belong to the **motor repertoire** of the observer are able to trigger resonant activity in the motor system. This property was also confirmed in an fMRI experiment using video clips of two types of mouth actions (biting to eat and oral communicative actions) performed by humans, monkeys and dogs (Buccino et al., 2004). Human mirror regions only responded to actions that were part of the motor repertoire of the human observer (i.e. biting), also when executed by a nonhuman performer. For the communicative actions, human (speech reading) and to some extent monkey (lip smacking) gestures evoked activity in human mirror areas, but not barking, presumably due to the absence of a motor representation for barking in the human motor repertoire.

These studies prove that the human mirror mechanism is extremely sensitive to the movement kinematics in itself, irrespective of the goal, actor or visual properties of the observed movement. The only prerequisite for the human mirror system to become active appears to be that: (i) the observed motion pattern is interpreted as being biologically possible and (ii) belongs to the motor repertoire of the observer. The observation that the human mirror system is not only triggered in response to visuomotor cues, but also to action-related sounds (Aziz-Zadeh, Iacoboni, Zaidel, Wilson & Mazziotta, 2004; Gazzola, Aziz-Zadeh & Keysers, 2006), music (Bangert et al., 2006), reading of action verbs (Pulvermüller, Härle & Hummel, 2001) and motor imagery (Grèzes & Decety, 2000) further confirms its **high degree of generalization**.

Lastly, activity within the mirror system is also shaped by the observer’s **personal experience**. For example, mirror regions are more activated when expert ballet and capoeira dancers observe videos of dance actions belonging to their own versus the other dance style (Calvo-Merino, Glaser, Grèzes, Passingham & Haggard, 2005). Similarly, participants who are initially naive to certain dance steps were shown to display an increase in mirror activation over time if they underwent a period of **motor training** in which they became skillful in performing those steps (Cross, Hamilton & Grafton, 2006).

2 Functional role of the mirror system

Though it is hard to dispute the existence of a human mirror system, its function has yet to be clearly delineated. The essence of the “classic” fronto-parietal mirror system is that actions performed by others, irrespective of their sensory format, are directly mapped onto the observers’ own motor representations. This is confirmed by numerous human (and primate) studies showing that although the mirror system does encode the basic motor features of an observed action, its core ability is to understand the goals and ‘intentions’ behind the occurring actions of others (Rizzolatti & Sinigaglia, 2010). However, several fMRI experiments (reviewed by Caspers et al., 2010; Molenberghs et al., 2012) and also the single-neuron recordings by Mukamel et al. (2010) indicate that mirror neurons may not be restricted to the regions traditionally identified as part of the classic fronto-parietal mirror network (i.e. PMC, IFG, IPL), but extend to other areas involved in somatosensory, auditory and emotional processing as well. By extension, the mirror system has been proposed to contribute to various other socio-cognitive phenomena, such as imitation (Iacoboni et al., 1999), language (Rizzolatti & Arbib, 1998), empathy (Preston & de Waal, 2002) and Theory of Mind (Gallese & Goldman, 1998), but these claims are more speculative.

2.1 Delineating mirror system function

In the next paragraphs, we will briefly discuss the role of mirroring vs. ‘emotional mirroring’ (i.e. empathy) and ‘cognitive mirroring’ (i.e. Theory of Mind) during social information processing. Although these abilities are often used as synonyms, the capacities and skills they represent rely on different neuronal circuitry. Empathy refers to our ability to share the feelings, emotions and sensations of others and relies on sensorimotor cortices as well as limbic and paralimbic structures (Baird, Scheffer & Wilson, 2011). In contrast, Theory of Mind refers to our ability to understand mental states such as others’ intentions, goals and beliefs, and relies on neural structures in the temporal lobe and prefrontal cortex (Kliemann & Adolphs, 2018).

2.1.1 Mirroring vs. empathy

Empathizing denotes the ability to share the feelings and emotions of others in absence of any direct emotional simulation for oneself. The emotional account of the mirror system, proposed by Preston and de Waal (2002), posits that a second, emotional mirror mechanism is located in areas that mediate emotion-related behaviors, such as the anterior cingulate cortex (ACC) and anterior insula (AI). Empathy-related mirroring has mainly been investigated in the context of vicarious responding to other people’s pain or emotions, and is endorsed by brain imaging studies showing a similar neural substrate and similar autonomic and somatic responses for both feeling emotions and recognizing them in others (e.g. Wicker et al., 2003). Since this topic is not the focus of the current thesis, the interested reader is referred to Baird, Scheffer, and Wilson (2011) for a critical overview.

2.1.2 Mirroring vs. mentalizing (Theory of Mind)

Gallese and Goldman (1998) suggested that the mirror system might underlie our ability to understand other people's intentions by providing us with an automatic simulation of their action goals and intentions. Note however the difficulty in distinguishing between these concepts without a strong framework (indeed, the terms ‘actions’, ‘goals’ and ‘intentions’ are often used interchangeably in the field). In this respect, Grafton and Hamilton (2007) ordered actions hierarchically according to their functional level of abstractness, discriminating between: (i) *motions,* involving a particular pattern of muscle activity (e.g. opening of the hand), (ii) *actions,* involving the conjunction of different motions (e.g. grasping), (iii) immediate action *aims* (e.g. grabbing a cup of coffee), and (iv) task *goals* (e.g. grabbing a cup of coffee to drink). In a social context, goals with more long-term perspectives are often termed *intentions* (e.g. grabbing a cup of coffee to bring it to a colleague).

Reviews summarizing fMRI research suggest that the mirror system is mainly recruited for automatic lower-level goal interpretation, whereas the mentalizing system, consisting of the precuneus (PC), temporoparietal junction (TPJ) and the medial prefrontal cortex (mPFC), is recruited for more cognitive reflections about higher-level intentions (Van Overwalle & Baetens, 2009; Zaki & Ochsner, 2009). Also in the light of newer research data (see General Discussion, p. 200), this notion of a mirror-mentalizing gradient seems feasible. Furthermore, based on the meta-analytic finding that these two systems are never concurrently active, these reviews suggest that mirroring and mentalizing may be two distinct systems, each specialized in processing one type of social information, neither aiding nor subserving the other (Van Overwalle & Baetens, 2009). These reviews also rightly point out that this conclusion is however based on the research tradition to design isolating experimental tasks (i.e. providing action-related input with little to no social context for probing the mirror system vs. more abstract descriptions to test the mentalizing system); and/or hampered by the methodological limitation that not all tasks adopted to identify mentalizing abilities can be easily used to identify mirroring properties and vice versa. Behaviors involving higher-level goals (e.g. ‘cleaning the kitchen’ or ‘having a tea party') can easily be described verbally, but are more difficult to present in their totality in terms of actions within an experimental setting, meaning that often only some of their more immediate subcomponents can be presented (e.g. grabbing a cup to drink or to put in a dishwasher; as illustated by for example Iacoboni et al., 2005).

In sum, although previous research tasks have proven to be valuable in specifying the essence of each system, the adopted tasks and paradigms do not fully approximate the kind of real-word social interactions social neuroscientists are most interested in. As such, how these two systems interact while processing multimodal, dynamic and contextually-embedded social information is still an underexplored avenue. In **section 3**, this issue will be further discussed.

2.2 Broken mirrors in autism spectrum disorders?

Individuals with an autism spectrum disorder (ASD) struggle with many aspects of social interaction and communication, both in formal testing and in their everyday lives. Although the exact etiology of this disability remains unknown, many different cognitive and brain-based theories have been proposed to account for these difficulties. Due to the role of the mirror system in basic social information processing, as well as its speculated function in a broad range of socio-cognitive functions, it seems intuitively appealing to attribute the various socio-interactive deficits that characterize ASD to a dysfunctional mirror system. This is commonly known as the **Broken Mirror Theory of ASD** (Oberman & Ramachandran, 2007). There are several variants of this theory that make slightly different claims (see Hamilton, 2013), but the dominant view in the literature (also adopted in the current thesis) comprises a simulation version of the Broken Mirror Theory, which builds on the idea that the mirror system provides a basis for simulating others' actions, emotions and mental states (Iacoboni & Dapretto, 2006). Possibly due to its intuitive explanation, the Broken Mirror Theory of ASD has received considerable attention, including in the popular media (e.g. Ramachandran & Oberman, 2006).

The suggestion that the mirror system may be impaired in ASD has its origin in studies investigating ASD-related imitation deficits (Williams, Whiten, Suddendorf & Perrett, 2001). Yet, as imitation requires more than only the automatic simulation of others' actions but also involves several other cognitive processes (see also further), the strongest evidence for a dysfunctional mirror system in individuals with ASD comes from studies reporting functional (fMRI: Dapretto et al., 2006; TMS: Oberman et al., 2005; TMS: Théoret et al., 2005) and anatomical (Hadjikhani, Joseph, Snyder & Tager-Flusberg, 2006) abnormalities in these brain areas. However, systematic reviews of brain-based studies on the integrity and function of the mirror system in ASD suggest that evidence in favor of an overall mirror system dysfunction is mixed (e.g. Hamilton, 2013). One possible explanation raised by Hamilton (2013) is that the broader social context in which mirroring occurs has a substantial impact on the obstained results. Indeed, studies using emotional stimuli suggest differences between neurotypical and ASD participants (e.g. Dapretto et al., 2006), whereas studies using goal-directed or other non-emotional stimuli did not find clear group differences (e.g. Williams et al., 2006).

An explanation for this pattern of mixed results – also provided by Hamilton (2013) – is that information processing in the visuomotor stream is abnormally controlled by the various social signals encountered during social interactions in ASD. In the next section, we will further consider this model, known as the **social top-down response modulation** (STORM) model (Wang & Hamilton, 2012), both in typical individuals and in individuals with ASD.

3 Social modulation of the mirror system

Although the classic mirror neuron literature clearly describes who, what and why we mirror, it does not specify *when* it is advantageous to simulate others' actions. Indeed, in daily life we are often in situations in which it is not relevant to fully activate the mirror system upon movement observation (for example, when watching television). An MEG study by Kilner, Marchant and Frith (2006) was the first to show that the mirror response is modulated by the social salience or relevance of the observed movements; i.e. oscillatory activity in the 7–12 Hz frequency range was more suppressed when the actor faced towards the observer, compared to when the actor had their back turned. They suggested that this modulation reflects a 'filter' mechanism that – depending on the social saliency of the information – selects which information to pass into the mirror system (Kilner et al., 2006). Further evidence for such a mechanism has been provided by previous TMS studies indicating that mirror system engagement upon action observation is – although presumed automatic – not impervious to other processes, but can be flexibly modulated by, amongst others: emotional body language of the actor (Borgomaneri, Gazzola & Avenanti, 2015), social reciprocity (Sartori, Cavallo, Bucchioni & Castiello, 2012), and the level of social interaction between actor and observer (Hogeveen & Obhi, 2012). The current dissertation will specifically look into the role of dyadic eye contact between actor and observer in shaping motor simulation in the observer.

3.1 The importance of eye gaze

"*The significance of eyes in human relationships fascinated writers and philosophers as well as scientists for centuries"* (Kleinke, 1986, p. 78).

Perceived eye contact is perhaps one of the most engaging and powerful components of interpersonal communication and interaction (Kleinke, 1986). Some researchers have even referred to gaze processing as "the core of social cognition" (Itier & Batty, 2009). As such, the function, evolution and neurobiology of social gaze processing has been studied extensively, and is still a topic of active investigation.

Perceiving others' gaze induces many widespread affective, attentional and neural processes in the observer, which have been excellently summarized elsewhere. Briefly, (i) other people's gaze cues are an important source for attentional orienting and trigger reflexive shifts of attention (i.e. we have the tendency to look where other people are looking; also denoted as 'joint attention'; Frischen, Bayliss & Tipper, 2007); (ii) the eyes are the most important area to various aspects of face processing and social information extraction (e.g. emotion recognition, identity processing; Itier & Batty, 2009); and (iii) perceived direct gaze elicits a strong affective response in the observer (Hietanen, 2018). Moreover, brain imaging studies (reviewed by Itier & Batty, 2009 and Senju & Johnson, 2009) indicated that dyadic eye contact activates various regions belonging to the social brain, i.e. a network of structures that is specialized to process social information such as facial expressions, emotions, but also biological motion, action and goal direction. These regions include the fusiform gyrus, anterior and posterior part of the STS, the medial prefrontal and orbitofrontal cortex, and amygdala.

One important function for social interaction and communication is that gaze cues convey the interaction partner's direction of attention and communicative intentions (Grossmann, 2017). Seeing someone's gaze directed at me signals that he or she is attending to me and shows communicative intent. Seeing someone's averted gaze on the other hand indicates that his/her attention is directed elsewhere at the moment. As such, perceived eye gaze is an important modulator of the social salience of the observed social scene for the observer. Combining both eye contact and visuomotor cues may therefore be a meaningful signal system to investigate how the perceived *relevance* of the observed actions influences motor system engagement in the observer.

3.2 Taking a look at actions: control of mimicry by eye contact

3.2.1 Behavioral findings

The first study to demonstrate the effect of perceived eye contact on the mirroring of others' actions did this in terms of *automatic imitation*, a behavioral marker strongly associated with the mirror system. It denotes the automatic (i.e. not depending on the actor's conscious intentions) facilitation of action execution upon observation of otherwise task-irrelevant similar actions, and automatic interference upon observation of dissimilar actions. In this study Wang, Newport and Hamilton (2011) employed a stimulus-response compatibility (SRC) paradigm, in which participants were shown a series of video clips of an actress performing a hand opening or hand closing movement. Critically, while performing the movement the actress gazed either directly towards or away from the participant. Participants were instructed to perform as quickly as possible a pre-specified motor response (e.g. perform 'hand opening') as soon as the actress' hand in the video clips began to move, irrespective of the presented stimulus. Thus, the participant's motor response could be congruent or incongruent with the observed movement.

In line with the mimicry literature, a clear congruency effect was found, with faster reaction times in congruent trials, indicative of a facilitative and automatic mimicry-effect, and slower responses in incongruent trials, in which participants have to inhibit the natural tendency to mimic the actress' hand action. Furthermore, a significant interaction between eye contact and mimicry revealed that, only for congruent trials, direct eye gaze further facilitated automatic mimicry of the observed hand actions, while averted gaze did not, thus showing a rapid and selective modulation of automatic mimicry by eye gaze (Wang, Newport, et al., 2011). Further control experiments adopting nonsocial visual distractors (i.e. a flashing box) or gaze cues towards the acting hand ensured that the enhancement of automatic mimicry by eye contact was not driven by differences in visuospatial or joint attention (Wang & Hamilton, 2014; Wang, Newport, et al., 2011).

The authors discuss these findings in the context of the social top-down response modulation (STORM) theory of mimicry (Wang & Hamilton, 2012). This theory claims that mimicry is employed as an unintentional strategy to facilitate social interaction and enhance liking and affiliation. Furthermore, it posits that mimicry behavior is subtly and sophisticatedly controlled by different social signals that guide when and who to mimic, i.e. to make it more cost-efficient and adaptive. However, a prerequisite for the positive affiliative consequences of mimicry is that these copying behaviors have to be detected by the interaction partner (Wang & Hamilton, 2012). In this respect, eye gaze is a critical signal that conveys the interaction partner's focus of attention, providing a plausible explanation as to why mimicry is controlled by different gaze cues (Wang, Newport, et al., 2011).

3.2.2 Neural social top-down control

As previously described in section 2, the mirror system encompasses the inferior frontal gyrus (IFG), inferior parietal lobule (IPL) and the premotor cortices (PMC). These regions are engaged by both observation and execution of actions (Cattaneo & Rizzolatti, 2009) and are strongly linked to mimicry, imitation and other visuomotor processes (Iacoboni, 2009). Another important brain area is the superior temporal sulcus (STS), which constitutes as the main visual input region for the mirror system (Grèzes et al., 2001; Grossman & Blake, 2002). The STORM model proposes that these regions of the mirror network are subjected to a strong top-down control, i.e. STORM, from the mentalizing system, which is engaged when participants evaluate the social features of the broader context in which action observation is embedded (Wang & Hamilton, 2012). The relationship between these two brain networks, as posited by the STORM model, is displayed in **figure 3**.

Further evidence for this neural model was provided by another study by the same group, examining the neural mechanism behind the modulation of mimicry by eye contact (Wang, Ramsey & Hamilton, 2011). During fMRI scanning, participants performed a similar SRC task combining movement and eye gaze cues as previously described. In line with the literature, performing the SRC imitation task activated several regions of the mirror system (i.e. the IFG, IPL and STS), whereas the perception of direct gaze engaged the medial prefrontal cortex (mPFC), a region consistently associated with the mentalizing system. More importantly, Wang, Ramsey et al. (2011) revealed an interaction between mimicry and eye contact in area mPFC, STS and IFG, suggesting that these regions are critical in the control of mimicry by eye contact.

A subsequent neural modelling analysis (i.e. dynamic causal modelling or DCM) suggested a model in which three particular features stand out. First, there was strong intrinsic connectivity from mPFC to both IFG and to STS, suggesting that mPFC constantly exerts a top-down control over the mirror system. Second, when participants performed the SRC task, the connection strength between STS and IFG increased, confirming that these regions are implicated in direct action-perception mapping processes. Third, the interaction between mimicry and eye contact enhanced the influence of mPFC over the STS, indicating that the mPFC may be the originator of the gaze-mimicry interaction by – indirectly via the STS – modulating the visual input to the mirror system (Wang, Ramsey, et al., 2011). However, since this was the only study to explicitly investigate STORM-related processes, it is possible that top-down modulation could also originate from other parts of the frontal cortex or subcortical areas (see also General Discussion).

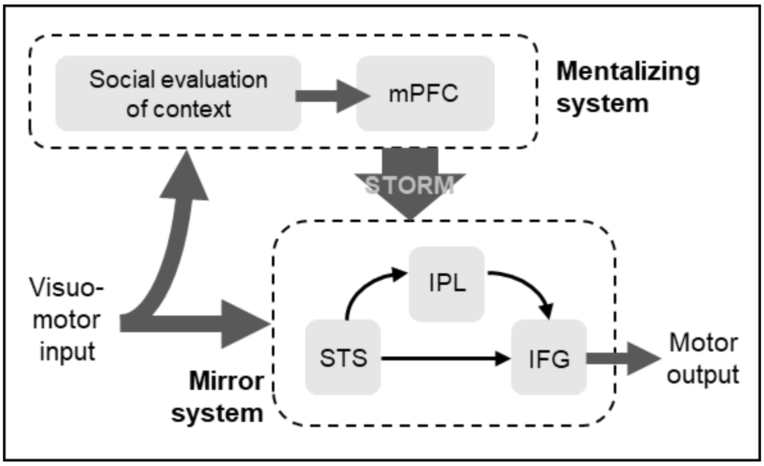

Figure 3. Social top-down response modulation (STORM) model for mimicry. The model shows how the mirror system, the neural substrate of mimicry, interacts with the mentalizing system during social information processing. Figure adapted from Wang and Hamilton, 2012, *Frontiers in Human Neuroscience*.

3.2.3 *“STORMy” mimicry in ASD*

The STORM model proposed by Wang and Hamilton (2012) can also be extended towards individuals diagnosed with an autism spectrum disorder (ASD). Indeed, mimicry and imitation deficits have been studied extensively in ASD. Systematic reviews indicate that a putative imitation deficit may not be uniform in ASD, but that some types of imitation are more difficult than others (Kana, Wadsworth & Travers, 2011). Although this pattern of mixed results is compatible with the view that the social modulation of imitation by social cues is abnormal in ASD, thereby indirectly supporting STORM, only one study to date has explicitly investigated the social modulation of mimicry in ASD from a STORM perspective (Forbes, Wang & Hamilton, 2017).

Using the previously established SRC paradigm to measure the tendency to mimic the interaction pattern in the presence or absence of direct gaze, Forbes et al. (2017) provided further evidence for the STORM account of mimicry in ASD. First, participants with ASD demonstrated a reliable congruency effect, suggesting that the imitation skills in ASD may be intact. However, in contrast with earlier results in neurotypical participants (Wang & Hamilton, 2014; Wang, Newport, et al., 2011; Wang, Ramsey, et al., 2011), a consistent main effect of gaze, but no gaze-mimicry interaction was encountered in ASD; i.e. all mimicry responses were faster after direct compared to averted gaze in ASD. In neurotypical participants, direct gaze enhanced only congruent motor responses (i.e. mimicry), but slowed incongruent motor responses, resulting in no overall effect of observed gaze. Thus, the overall gaze effect in people with ASD suggests that although they may be sensitive to direct gaze as a *general* alerting signal, they do not use it to specifically modulate their mimicry behavior in order to reap the most cost-effective social benefits (Forbes et al., 2017).

3.2.4 Gaps in the STORM model identified

Taken together, the studies by the Hamilton group (Wang & Hamilton, 2012, 2014; Wang, Newport, et al., 2011) suggest a STORM model of mimicry, in which mimicry is carefully but automatically controlled to maximize one’s social advantage. Individuals with ASD can automatically mimic the actions of others, but do not use important social cues, such as eye gaze, to determine when and what to mimic (Forbes et al., 2017). Recent fMRI data in neurotypical participants appoints the mPFC as the originator of this top-down control process (Wang, Ramsey, et al., 2011).

However, mimicry is a complex socio-cognitive function, that relies heavily on other cognitive and motor skills, including self-other mapping, basic motor functioning, body schema, spatiotemporal representation, visuospatial attention and motor execution (Hamilton, 2013; Kana et al., 2011). As such, a major difficulty in interpreting behavioral mimicry results concerns their specificity, as well as their ability to pinpoint particular brain systems as the underlying cause of this behavior. Similarly, poor performance on imitation/mimicry tasks in ASD could be caused by a failure of the underlying neural structures (i.e. the mirror system), or deficits in visual processing, or poor motor functioning, or both (Hamilton, 2013). Indeed, ASD participants have been known to display deficits in motor functioning due to difficulties with kinesthesia (i.e. the ability to precisely reproduce given motions) and apraxia (i.e. the ability to plan, execute and perform skilled gestures; Kana et al., 2011). Furthermore, automatic mimicry of observed actions is but one functional marker of the human mirror system. Remarkably, no research to date has directly investigated STORM-related modulations in the neurophysiological correlates of the human mirror system that are less impacted by other cognitive or motor processes, such as TMS-induced motor resonance and/or EEG-based mu rhythm suppression, neither in neurotypical nor in ASD participants. Lastly, responses to socio-emotional stimuli, particularly eye-region related signals, may vary greatly from individual to individual, even within the healthy population (Grossmann, 2017; Skuse, Morris & Lawrence 2003). These knowledge gaps will be addressed in the current doctoral project, as further specified in **section 5**.

3.3 Sniffing around oxytocin

Oxytocin is a hypothalamic neuropeptide that is projected to different areas in the brain, where it has a key role in the regulation of complex social behaviors and cognition. Due to the discovery that exogenous oxytocin can be non-invasively delivered to the human brain using intranasal administration, oxytocin has gained increasing interest as a modulator of social behavior and cognition, including interpersonal interactions (e.g. trust, cooperative behavior, attachment, eye contact, generosity, empathic behavior), social stress and social perception (i.e. emotion and face recognition, social memory; summarized in Graustella & Macleod, 2012 and Meyer-Lindenberg, Domes, Kirsch & Heinrichs, 2011). Furthermore, oxytocin was shown a modulator of activity in social brain areas and systems, including the amygdala, the reward system and mPFC (for reviews, see Bethlehem, van Honk, Auyeung & Baron-Cohen, 2013 and Wigton et al., 2015). In relation to its clear behavioral and neural effects, the translational potential for oxytocin as an effective treatment of severe social impairments, such as those encountered in ASD, is an active topic of investigation (for reviews, see Anagnostou et al., 2014 and Guastella & Hickie, 2016).

Several theories have been proposed concerning the mechanisms by which oxytocin affects social behavior, namely (i) by reducing (social) anxiety and stress reactivity (Maroun & Wagner, 2016; Neumann & Slattery, 2015); (ii) by enhancing attentional resources towards salient social cues in the environment (Shamay-Tsoory & Abu-Akel, 2016); or (iii) by increasing the desire to affiliate with others (Bartz, 2016). Note however that these theories are not necessarily mutually exclusive, and that the same research findings can often be interpreted in the light of different mechanisms. In general, the overall effect of oxytocin seems to comprise an increase of approach-related behaviors, and an inhibition of avoidance-related behaviors (Kemp & Guastella, 2011). Furthermore, a comprehensive review by Bartz, Zaki, Bolger and Ochsner (2011) has signaled the importance of taking inter-individual differences in oxytocin-related responding into account, as the assumed prosocial effects of oxytocin can be mediated (or even reversed) by for example the participant's dominant attachment style.

3.3.1 Oxytocin and motor simulation

Of particular interest for the current doctoral project is the notion that oxytocin can impact several functional markers of the human mirror system. First evidence for this notion was provided by Kéri and Benedek (2009), who investigated the effect of oxytocin on the detection sensitivity (*d'*) for biological (a walking human character) and non-biological (a rotating shape) motion perception from masked point light displays (i.e. embedded in a cloud of noise dots). In this study, oxytocin, relative to placebo, selectively increased detection sensitivity of biological, but not of non-biological motion from moving dots.

More direct evidence for a link between oxytocin and motor simulation was demonstrated by De Coster, Mueller, T'Sjoen, De Saedeleer and Brass (2014), who adopted a SRC task to investigate automatic imitation of simple finger movements. As explained previously, pre-specified finger movements can either be congruent (i.e. match) or incongruent (i.e. no match) with the observed movements in a SCR imitation task. To rule out general effects of oxytocin on cognitive control, a Stroop color-word interference task was also included. Oxytocin selectively enhanced the congruency effect in the SCR task, but not the congruency effect in the Stroop task. Interestingly, reaction times for incongruent trials were significantly longer after intranasal oxytocin administration, but reaction times for congruent trials were not facilitated. The authors speculated that this decreased inhibitory control over automatic imitation tendencies during incongruent trials caused by oxytocin reflects a decreased self-other distinction, which therefore leads to larger interference costs (De Coster et al., 2014).

Although these behavioral studies provide first indications for a role of oxytocin in mediating mirror system activation, only one study to date has provided direct neurophysiological evidence for a link between the action of this neuropeptide and the mirror system. Showing participants a point light display of continuous biological motion (a walking human character) or non-biological motion (a rolling circle), Perry et al. (2010) replicated the previous behavioral results by Kéri and Benedek (2009), and demonstrated that suppression of the EEG mu rhythm was selectively enhanced by oxytocin compared to placebo for biological motion only, indicative of an enhanced mirror system activation.

The authors interpret their findings in light of the saliency account of oxytocin; stating that oxytocin enhances attentional orienting towards salient stimuli.

3.3.2 Oxytocin and eye contact

Of importance for the current project are studies showing that oxytocin promotes gaze behavior towards the eye region of the interaction partner (Auyeung et al., 2015; Guastella, Mitchell & Dadds, 2008), which in turn might contribute to other socio-cognitive processes (e.g. emotion recognition; Hubble et al., 2017a). Also within the ASD literature, there is evidence that oxytocin-related improvements in facial emotion recognition are, at least in part, mediated by participants' increased fixation time of the eye region of faces (Andari et al., 2010). Nevertheless, other studies in healthy participants show mixed evidence, with one study showing that the differential impact of oxytocin on gaze behavior to the eye region is valence-based (i.e. increased gaze for positive faces, but decreased gaze for negative faces; Domes, Steiner, Porges & Heinrichs, 2013). Other studies suggest that oxytocin-related improvements in facial recognition tasks are not driven by changes in gaze behavior (Domes et al., 2010; Hubble et al., 2017b; Lischke et al., 2012), but may instead be related to increased autonomic arousal and affiliative-motivational processes (as indexed by pupil dilation; Prehn et al., 2013).

3.3.3 Gaps in the effect of oxytocin on motor simulation identified

Although the prosocial neuropeptide oxytocin is increasingly investigated in modulating social brain areas, including limited but promising effects on mirror system correlates (De Coster et al., 2014; Kéri & Benedek, 2009; Perry et al., 2010), its effect on both TMS-based markers of mirror system engagement and STORM-related modulations of motor resonance remains unexplored. The current doctoral project aims to address this issue further, as outlined in the next sections. This may be of particular importance in light of the saliency hypothesis of oxytocin (Shamay-Tsoory & Abu-Akel, 2016), positing that oxytocin increases attentional resources for salient social cues, such as eye contact.

4 Methodology: transcranial magnetic stimulation

The present doctoral project is centered around the technique of transcranial magnetic stimulation (TMS). TMS provides a non-invasive, safe and painless method for stimulating the human cortex through the skull (i.e. ‘transcranial’) by using a magnetic field, and has the capacity to both investigate and modulate the functionality of specific cortical areas (Rossi, Hallett, Rossini & Pascual-Leone, 2012). Since its introduction by Anthony Barker (Barker, Jalinous & Freeston, 1985) at the University of Sheffield (UK), the use of TMS in neuroscience research has spread widely due to its many applications.

Briefly, TMS can be applied one pulse at a time (single-pulse TMS), in pairs of pulses separated by a variable interval (paired-pulse TMS), or in trains of repeated pulses (repetitive TMS). Single-pulse TMS is mainly used to map cortical outputs and localize brain function. It was first applied in the motor system, where it produces muscle activity, but can also be used to map sensory processes (e.g. the perception of phosphenes after stimulation of the visual cortex) and cognitive functions. Paired-pulse TMS protocols probe cortico-cortical interactions, such as intracortical inhibitory and facilitatory processes. Repetitive trains of TMS pulses (rTMS) are able to produce long-lasting modifications of activity in the targeted brain region, which can outlast the period of stimulation itself. As such, rTMS protocols can be used in both investigational and therapeutic applications (see Machado et al., 2013 for an overview).

In the current thesis, single-pulse TMS is used as an investigational tool to explore modulation of corticospinal excitability under various experimental conditions. In the following paragraphs, this application of TMS will be addressed in more detail.

4.1 Single-pulse TMS

4.1.1 Basic mechanisms and principles

Neuron-to-neuron communication in the brain relies heavily on electric signaling. Depolarization of a neuron, i.e. rapid increases and decreases in the voltage of a neuron’s membrane, elicits an action potential. This action potential then activates nearby neurons and enables the transfer of information throughout the brain. In this respect, TMS can be considered an artificial method for inducing neuronal activation.

TMS relies on the principles of electromagnetic induction. An electric pulse generator (i.e. the stimulator) is connected to a magnetic field generator (i.e. the magnetic coil), which is placed near the participant's scalp. The stimulator generates a pulse of electric current through the magnetic coil, which in turn produces a rapidly changing (~100 μs) but powerful (~2T) magnetic field that passes painlessly through skin and bone. Because the strength of the magnetic field falls off very rapidly with distance from the TMS coil, one can target specific regions of the cortex in a focal manner. Perpendicular to the magnetic field, a secondary electric current is induced in the underlying cortical tissue (but not in the skull as bones cannot conduct electricity). This ‘artificially’ induced electric current then causes a depolarization of the underlying neurons (i.e. neural activation), which sets in motion a chain of action potentials and the execution of the neuron’s function.

Neurons in the primary motor cortex (M1) fulfill a pivotal role in the execution of voluntary movements, as they control the muscles of the body according to a somatotopic organization (**figure 4**). Axons extending from these M1 neurons carry movement-related information down through the brainstem into the spinal cord, where they synapse on spinal motoneurons that innervate and control the respective muscles. Together, this system is also termed the corticospinal tract. When single-pulse TMS is applied to M1 at appropriate stimulation intensity, a descending volley is sent through the corticospinal tract, which causes an involuntary muscle contraction in the targeted muscle. Single-pulse TMS-induced muscle activity is commonly denoted as ‘motor evoked potentials’ or MEPs, and can be recorded on electromyography (EMG) by using surface electrodes applied over the muscle belly. In practice, the peak-to-peak amplitude of the MEP and the motor threshold (MT), defined by the minimum TMS intensity necessary to evoke MEPs of > 50 mV in about 50% of successive trials in the target muscle, are considered useful biomarkers to estimate the excitability of M1 and the corticospinal tract (Wasserman et al., 2008).

4.1.2 Application during action observation

Single-pulse TMS in combination with EMG recordings has proved a valuable tool for assessing activity within the motor system. Importantly, Fadiga et al. (1995) were the first to demonstrate the potential of single-pulse TMS in the context of action observation research. In their now seminal study, they applied single-pulse TMS to participants’ left M1, and recorded MEPs from the right hand and arm muscles. During stimulation, participants observed an experimenter grasping different objects or performing meaningless arm gestures. Their results showed that MEP amplitudes were significantly enhanced during both the observation and execution of these actions compared to several control conditions (i.e. the dimming of a light and the perception of the 3D objects only). Importantly, this enhancement happened in a muscle specific way, i.e. this increase was selective for only those muscles that are intrinsically recruited when producing the observed movements. They theorized that observation-induced facilitation of MEPs must therefore rely on the involvement of the mirror system. Although single-pulse TMS delivered to M1 provides an index about the efficacy of a chain of synapses along the different levels of the corticospinal tract (i.e. from cortical neurons to muscles), it is generally assumed that the facilitation of MEPs induced by action observation is indeed a consequence of excitatory cortico-cortical connections between M1 and mirror regions in the brain (Fadiga et al., 2005; see also General Discussion).

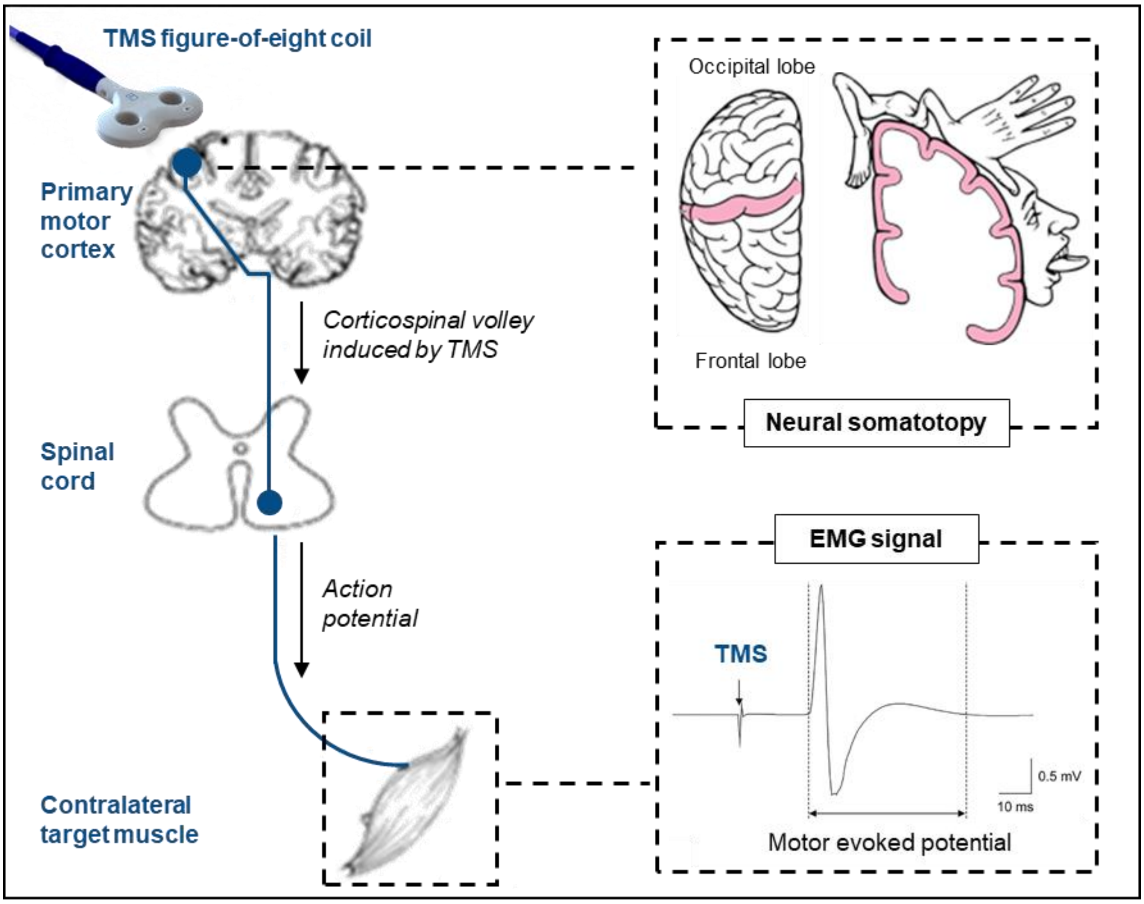

Figure 4. Schematic representation of TMS over the motor cortex. TMS over the somatotopically organized primary motor cortex (upper inset) leads to a corticospinal volley that activates the spinal motoneurons and elicits a contraction in the target muscle (i.e. motor evoked potential) that can be recorded using EMG (lower inset).

4.2 Current single-pulse TMS paradigm

In the current studies, single-pulse TMS was applied to the hand representation area of participants' left M1. Participants were instructed to spontaneously observe (videoclips of) a model performing simple, intransitive whole-hand opening or index finger abduction movements while gazing either towards or away from the participant. Since observation-induced facilitation of MEPs is always muscle specific, MEPs were collected from the contralateral (i.e. right) hand and/or index finger muscles that are naturally implicated in the to-be-observed movements. Only right-handed participants were included.

5 Aims, outline and potential impact of the thesis

The overall aim of this doctoral project was to thoroughly investigate the effect of observed eye gaze on interpersonal motor resonance by adopting the TMS technique, and by extension to further develop the social top-down response modulation (STORM) account on mimicry. To address this general aim, six different chapters are described, which target three broad objectives:

1. To investigate the effect of observed eye gaze on different neurophysiological markers of the human mirror system in healthy adult participants (chapter 1-3).
2. To assess to what extent variability in gaze-related modulations of interpersonal motor resonance can be explained by inter-individual variability in the social domain (chapter 4-5).
3. To explore the effect of a single dose of oxytocin on gaze-related STORM dynamics of motor resonance (chapter 6).

Part 1. Taking a look at motor simulation

The first part of the doctoral thesis includes three studies investigating gaze-related modulations in mirror system activation from a neurophysiological perspective. **Chapter 1** includes our ‘proof of principle’ study, in which we collaborated with Wang and Hamilton and adopted their stimulus set to confirm that the mirror system forms the neurophysiological substrate for the previously encountered interaction between behavioral mimicry and perceived eye gaze. In this study, we also incorporated a control eye tracking experiment to address the role of visuospatial attention herein. In **chapter 2**, we designed and validated our own set of experimental stimuli, optimized for the TMS protocol, and addressed the value of adopting naturalistic stimuli in social neuroscience research. In **chapter 3**, we investigated gaze-related modulations in another functional marker of the human mirror system, i.e. EEG-based mu rhythm suppression over the sensorimotor strip. Additionally, since the relationship between TMS-induced interpersonal motor resonance and EEG mu suppression remains unclear, this study also addressed this issue further.

Part 2. In the eye of the beholder

In line with recent considerations to adopt a dimensional approach to investigate socio-cognitive processes (Zaki & Ochsner, 2009), the second part of the dissertation focused on inter-individual variability in the social domain and its association with interpersonal motor resonance. This dimensional framework will be assessed in a relatively large sample of healthy adult participants (**chapter 4**), and in adult participants with a clinical autism spectrum disorder (ASD) diagnosis (**chapter 5**), who are known to demonstrate particular socio-interactive deficits. Furthermore, in line with a more categorical point of view, chapter 5 also examines the feasibility of the Broken Mirror Theory and the ASD-adaptation of the STORM account in explaining putative mirror system dysfunction in participants with ASD compared to neurotypical control participants.

Part 3. Sniffing around oxytocin

Lastly, the objective of the third part of this doctoral thesis was to investigate the effect of a single dose of the prosocial neuropeptide oxytocin on the social top-down response modulation of motor resonance upon perceived eye contact (**chapter 6**). This is particularly relevant in light of the saliency hypothesis of oxytocin (Shamay-Tsoory & Abu-Akel, 2016), positing that oxytocin increases attentional resources for salient social cues, such as eye contact, and may provide first indications for effective treatment options to help individuals with simulation deficits (e.g. ASD) effectively interact with the social world.

This doctoral dissertation will finalize with a **general discussion**, in which the main findings of this PhD project will be summarized and debated in the context of the available literature. The anticipated impact of the project will be discussed, and directions for future research will be proposed.

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PART I.

Taking a look at motor resonance

Chapter 1

Direct eye contact enhances mirroring of others' movements.

A transcranial magnetic stimulation study.

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Abstract

Direct eye contact is a powerful social cue to regulate interpersonal interactions. Previous behavioral studies showed a link between eye contact and motor mimicry, indicating that the automatic mimicry of observed hand movements is significantly enhanced when direct eye contact exists between the observer and the observed model. In the present study, we aim to investigate the neurophysiological basis of the previously reported behavioral enhancements. Here, transcranial magnetic stimulation (TMS) was applied to assess changes in cortico-motor excitability at the level of the primary motor cortex (M1) to explore whether and how the motor system is facilitated from observing others' hand movements and, in particular, how this process is modulated by eye contact. To do so, motor evoked potentials (MEPs) were collected from two hand muscles while participants received single-pulse TMS and naturally observed video clips of an actor showing hand opening movements or static hands. During the observation, either direct or averted eye gaze was established between the subject and the observed actor. Our findings show a clear effect of eye gaze on observation-induced motor facilitation. This indicates that the mapping or 'mirroring' of others' movements is significantly enhanced when movement observation is accompanied by direct eye gaze compared to averted eye gaze. Our results support the notion that eye contact is a powerful social signal with the ability to direct human non-verbal social behavior. Furthermore, our findings are important for understanding the role of the mirror motor system in the mapping of socially relevant actions.

1 Introduction

Human social interaction is a complex behavior between two or more individuals to communicate thoughts, intentions, emotional states and actions to one another. Ever since their discovery, ‘mirror neurons’ have been suggested to form an integral part of the neural circuitry that mediates our capacity to understand the meaning of the actions and behaviors of others (Gallese, 2009).

Neurons with mirror properties were first discovered using single-cell recordings in the ventral premotor cortex of macaque monkeys (Rizzolatti et al., 1988), and were shown to have the ability to fire not only when the monkey executes a certain motor action, but also when the monkey observes another individual performing the motor action (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996). Using movement observation paradigms in combination with functional neuroimaging techniques such as fMRI (Buccino et al., 2001; Iacoboni et al., 1999) and PET (Grafton et al., 1996; Rizzolatti et al., 1996) a homologous action observation–execution matching system or ‘mirror system’ has been localized in the human brain. Particularly, both frontal (inferior frontal gyrus; IFG) and parietal (inferior parietal lobule; IPL) areas have been shown to become increasingly activated during the mere observation of others’ actions (Chong et al., 2008; Kilner et al., 2009).

Overall, and according to the notion of ‘embodied cognition’, this process of ‘mapping’ observed actions onto the corresponding sensorimotor representations has been hypothesized to form the core neural mechanism by which others’ actions and emotional states can be simulated, recognized and understood (Iacoboni, 2009; Iacoboni et al., 2005; Rizzolatti and Craighero, 2004; Rizzolatti & Fabbri-Destro, 2008). However, note that also weaker accounts of ‘embodied cognition’ have been put forward, arguing that conceptual ‘understanding of actions’ may not be represented exclusively in terms of sensorimotor processes, but may additionally involve an abstract or modality-independent representation (Caramazza et al., 2014; Mahon, 2015).

In the past decade, the non-invasive brain stimulation technique transcranial magnetic stimulation (TMS) has been used extensively to measure resonant ‘mirror motor’ activity in the observer’s motor system. By applying TMS over the primary motor cortex (M1), a motor evoked potential (MEP) can be elicited from the contralateral muscles to obtain a measure of cortico-motor excitability (Fadiga et al., 1995). Interestingly, a number of studies (for a review, see Fadiga, Craighero & Olivier, 2005) have shown that during the mere observation of others’ actions, cortico-motor excitability within parts of M1 becomes increasingly facilitated, as indicated by significant enhancements in MEP amplitudes. Furthermore, this process has been shown to be highly muscle-specific, such that modulations in M1 cortico-motor excitability are predominantly observed in the muscles that are used in the observed action (Alaerts, Heremans, Swinnen & Wenderoth, 2009; Alaerts, Swinnen & Wenderoth, 2009; Strafella & Paus, 2000). Besides muscular involvement, a number of studies used the TMS technique to explore how different kinematic features of the observed actions are encoded by the observer’s motor system, such as temporal dynamics (Gangitano, Mottaghy & Pascual-Leone, 2001), grip force (Alaerts, de Beukelaar, Swinnen & Wenderoth, 2011; Alaerts et al., 2010; Alaerts, Swinnen & Wenderoth, 2010), orientation (Maeda, Kleiner-Fisman & Pascual-Leone, 2002) and predictability (Maeda, Chang, Mazziotta & Iacoboni, 2001).

The mapping mechanism for conveying information from others’ behaviors is not only affected by kinematic features, but may also be influenced by the processing of socially relevant cues from the observed environment (Wang & Hamilton, 2012). One such powerful social cue is perceived eye contact. The role of eye gaze in social behavior has been investigated extensively, with several neuroimaging studies showing that observed eye contact is a strong modulator of activity in regions of the ‘social brain’, a network of structures that is specialized to process social information such as faces, theory of mind and empathy, but also biological motion, action and goal direction (for a review, see Senju & Johnson, 2009). Particularly within the superior temporal sulcus (STS), brain activity has been shown to be specifically enhanced when direct eye contact is perceived (Pageler et al., 2003; Pelphrey, Viola & McCarthy, 2004). Furthermore, the STS region has also been hypothesized to form an integral part of the ‘extended’ mirror system network by providing the main visual input to upstream fronto-parietal mirror-motor regions (Grèzes et al., 2001; Grossman & Blake, 2002; Grossman et al., 2000).

To date however, only a handful of studies have explored the effect of perceived eye contact on the processing of the actions and movements of others. In terms of movement mimicry, a recent behavioral study by Wang, Newport and Hamilton (2011) provided first indications that the tendency of an observer to mimic others’ actions is enhanced when eye contact exists between the observer and the model. As a form of unconscious imitation, mimicry is strongly associated with the mirror neuron system (Iacoboni, 2009). In particular, reaction times for mimicking a hand closing or opening movement were shown to be faster when direct eye contact was established, rather than when eye gaze was averted (Wang, Newport, et al., 2011). Also a magnetoencephalographic (MEG) study by Kilner, Marchant and Frith (2006) provided evidence that the social relevance of a stimulus (modulated in terms of the observer’s viewpoint) can enhance putative mirror neuron activity. Together, these observations provide first indications that activity within the human mirror system can be influenced by distinct socially relevant cues from the observed environment.

To the best of our knowledge, no studies to date have directly investigated the neurophysiological basis of the effect of eye contact on motor resonance, as research has mainly focused on mimicry (Wang, Newport, et al., 2011; Wang, Ramsey, et al., 2011) or the influence of higher-order cognitive processes such as social relevance observation (Kilner et al., 2006). However, since eye contact is a powerful social cue, it would be interesting to directly explore whether direct eye gaze can modulate the mapping of others' actions in the observer's motor system. In the present study, the TMS technique was used to assess the effect of eye gaze on motor facilitation of M1 during movement observation. In particular, single-pulse TMS was applied over left M1 to measure the level of cortico-motor excitability of two hand muscles (right abductor pollicis brevis (APB) and first dorsal interossei (FDI)) during the observation of an actor performing simple hand movements involving those muscles. During the movement observation trials, the actor looked either directly towards or away from the observing participant to assess the effect of direct versus averted eye gaze on observation-induced motor facilitation at the level of M1. If eye gaze forms a salient social cue for modulating the process of mirror-motor mapping at the level of M1, we expected TMS-evoked MEPs to be higher when accompanied by direct gaze compared to averted gaze.

2 Materials and methods

2.1 Main experiment: Measurements of cortico-motor excitability during movement observation

2.1.1 Participants

Thirty-three right-handed individuals (16 males and 17 females) aged between 19 and 26 years old (mean ± SD: 22;7 ± 1;8 years;months) participated in this study. Handedness was assessed with the Edinburgh Handedness Questionnaire (EHQ; Oldfield, 1971). All participants provided signed written informed consents prior to the experiment, reported no history of neurological/psychiatric illness or motor dysfunctions of the hands/arms and met safety criteria for TMS. Ethical approval for the experiment was granted by the local Ethics Committee for Biomedical Research at the Katholieke Universiteit Leuven and conformed to the Code of Ethics of the World Medical Association (Helsinki, 1964). One female subject was excluded due to technical problems during the experiment.

To explore whether modulating effects of eye gaze were related to inter-individual differences in social responsiveness, subjects completed the Dutch self-report version of the Social Responsiveness Scale for adults (SRS-A; Constantino & Todd, 2005). The SRS-A (64 items) is a widely used screening tool to identify the presence and extent of any social impairments in the typical population using a four-point Likert-scale. It encompasses four subscales, including social awareness (19 items; $\alpha = .80$), social communication (22 items; $\alpha = .88$), social motivation (11 items; $\alpha = .83$) and rigidity/repetitiveness (12 items; $\alpha = .79$). Lower scores indicate higher social responsiveness. For raw SRS scores, a cut-off point of 54 is suggested for signaling impairments in social responsiveness (Noens et al., 2012).

2.1.2 General procedure

Participants were seated in a comfortable chair approximately 80 cm in front of a widescreen DELL monitor (resolution: 1920 × 1080 pixels, refresh frequency: 60 Hz) on which video stimuli of hand movements were displayed with a frame rate of 29 Hz. The right hand was placed palm-down on a soft cushion on their lap and participants were asked to relax their hand muscles while spontaneously viewing the presented video clips. During the experiment subjects' vision of their own hands was obstructed by another cushion placed on top of their hands.

2.1.3 Electromyography recordings and TMS

Dependent measures of cortico-motor excitability, i.e. motor evoked potentials (MEPs), were recorded via electromyography (EMG). To do so, disposable self-adhesive electrodes were attached to the muscle bellies of the right hand abductor pollicis brevis (APB) and first dorsal interossei (FDI), with two referential electrodes attached at the wrist. Both muscles were shown to be involved in the to-be-observed hand movement (hand opening), although activations were more pronounced for the APB compared to the FDI muscle (see supplementary methods). Single-pulse transcranial magnetic stimulation (TMS), using a Magstim 200 stimulator (Magstim Company Ltd, UK) with a hand-held 70 mm figure-of-eight coil, was administered to locate the optimal scalp site for stimulating the primary motor cortex (M1) ("hotspotting"). The coil was positioned over the left hemisphere, tangentially to the scalp and 45° away from the midsagittal line, such that the induced current flow was in a posterior anterior direction, i.e. approximately perpendicular to the central sulcus.

Optimal coil location for the experimental TMS-stimulation of M1 was determined as the site that produced maximal responses in the contralateral APB muscle while at rest. Although parameter setting procedures were prioritized for the APB, MEPs were simultaneously obtained from the APB and FDI muscles. Due to the overlap of hand muscle representations in M1, stimulation parameter settings are assumed to be satisfactorily effective for assessing condition-specific modulations simultaneously from both muscles (Facchini et al., 2002; Gertner & Classen, 2006; Krings et al., 1998; Scheiber, 1990). Next, the resting motor threshold (rMT) was defined for each participant as the lowest stimulation intensity that produced a peak-to-peak MEP of at least 50 µV in five out of ten consecutive trials (Rossini et al., 1994). During the experimental procedure, stimulation intensity was set at a supra-threshold of 130% of the subject's rMT (Alaerts, Swinnen & Wenderoth, 2009). Signal Software (version 2.02, Cambridge Electronic Design, UK) was used for EMG-recordings and triggering of the TMS-stimulator. EMG recordings were sampled at 2000 Hz via a CED Power 1401 unit (Cambridge Electronic Design, UK), amplified, band-pass filtered (5-1000 Hz) and stored on a PC.

2.1.4 Video stimuli

During TMS, video clips were presented to the observing participants. Video stimuli were identical to those used in a previous study by Wang, Newport, et al. (2011) and Wang, Ramsey and Hamilton (2011) in which an actor performed a head movement followed by a simple intransitive (i.e. not directed towards an object) hand movement (**figure 1**). At the onset of each clip, the actor was facing away from the camera with her eyes closed and her left hand static in front of her face. Then, the actor opened her eyes and turned her head either towards the camera, which resulted in direct gaze towards the observer, or away from the camera, providing averted gaze. Her hand remained static during the duration of the head movement. Subsequently, the actor performed a hand movement (i.e. opening of the hand) or the hand remained static. This resulted in a 2 × 2 factorial design with the factors ‘observed hand movement’ (opening or static) and ‘gaze direction’ (direct or averted). An illustration of the different conditions is provided in **figure 1**.

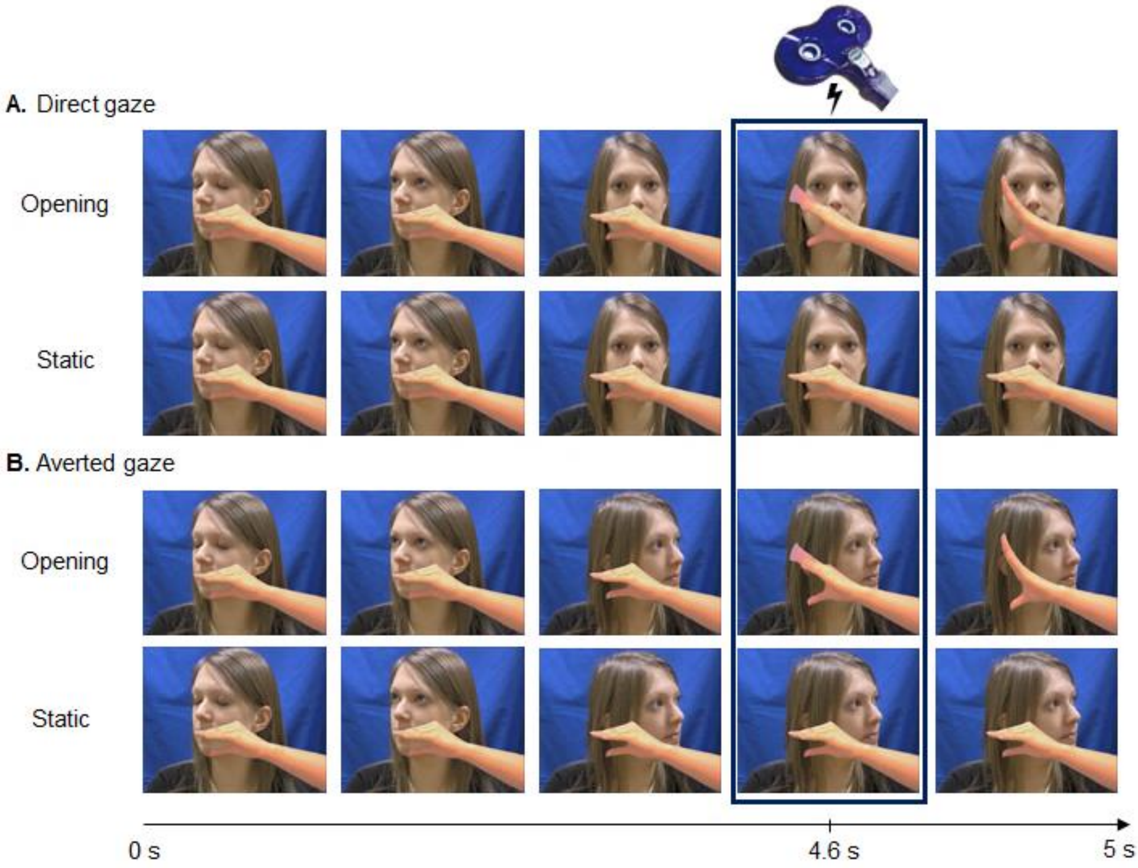

Figure 1. Illustration of the experimental video clips. Participants were presented with a series of video clips of an actor performing a head movement to establish direct or averted gaze towards the observer, followed by a hand opening movement or no movement (static hand). This resulted in a 2 × 2 factorial design with the factors ‘observed hand movement’ (opening or static hand) and ‘gaze direction’ (direct or averted gaze).

Each of the four conditions was presented five times in blocks of four five-second video clips (i.e. total of 20 trials per condition). Block presentation order was randomized across subjects and experimental blocks were randomly interleaved with four ‘baseline’ blocks in which only a blue background was shown. During movement observation, TMS pulses were delivered approximately 4.6 seconds after the start of the video clip which corresponded to the execution phase of the observed hand opening movement (see **figure 1**). Subjects’ attention to the presented videos was randomly assessed between blocks by asking the subject to report the type of hand movement and gaze direction that was previously observed. In 92.5% of the assessments, subjects gave a correct response, ensuring attention to the presented videos. Video presentation timing was controlled by LabVIEW software (version 14.0, National Instruments, UK) and was triggered by the Signal Software for TMS-stimulation and EMG-recording.

2.1.5 Data analysis and statistics

Based on the recorded EMG data, peak-to-peak amplitudes of the TMS-evoked MEPs were determined. Additionally, background EMG was quantified by calculating the root mean square (RMS) across the 110 to 10 millisecond interval prior to TMS-stimulation. Since background EMG is known to modulate the size of MEP amplitudes (Devanne et al., 1997; Hess et al., 1987), peak-to-peak MEP amplitudes from trials with excessive background EMG (exceeding 2.5 standard deviations away from the mean) were discarded (2.42% of the trials for the APB, and 1.94% of the trials for the FDI). Further, MEP peak-to-peak amplitudes were considered as outliers and were removed from the analysis when they exceeded Q3 ± 1.5*(Q3-Q1), with Q1 and Q3 denoting the first and third quartile computed for each condition in each subject (Electronic Statistics Textbook, 2008, StatSoft). This resulted in an additional omission of 8.48% of the trials for the APB and 8.45% of the trials for the FDI. Note that the total number of discarded trials was similar across conditions ($F(4,124) = 1.67$, $p = .16$) and muscles ($F(1,31) = 0.16$, $p = .70$).

MEP peak-to-peak amplitudes and RMS-scores were averaged separately for each condition. Due to high inter-individual differences in raw MEP responses, MEPs recorded during the four experimental conditions were normalized relative to baseline MEP responses separately for each subject (Halaki & Ginn, 2012). Shapiro-Wilk’s *W* tests ensured a normal distribution of the MEP data for each condition. Normalized MEP amplitudes were entered in two repeated measures analyses of variance (ANOVAs), one for each muscle separately (APB, FDI), with the within-subjects factors ‘observed hand movement’ (opening hand, static hand) and ‘gaze direction’ (direct gaze, averted gaze) to explore whether cortico-motor excitability of M1 is modulated by movement observation and/or eye contact.

Fisher’s least significant difference (LSD) contrasts were used for post-hoc between-condition analyses. All statistics were calculated with Statistica 10 (StatSoft, USA) and results were considered significant with a *p*-value lower than .05.

2.2 Control experiment: gaze behavior during movement observation

To explore whether gaze behavior and/or attention towards the presented hand movement was similar for the direct and averted eye gaze conditions, an additional eye tracking experiment was conducted while participants observed the four video clips of the main experiment: observed hand movement (opening or static) × gaze direction (direct or averted). Twenty-eight new subjects (20 males and 8 females), between the ages of 18 and 29 years participated in the additional eye tracking experiment to measure gaze behavior during observation of the video clips adopted in the main experiment. All participants provided signed written informed consents prior to the experiment and had normal or corrected-to-normal eye vision. Participants of the eye tracking experiment were not the same as those participating in the main movement observation TMS experiment.

2.2.1 Procedure

During the eye tracking session, the four video clips as described above were presented on a Tobii T120 binocular eye tracking device. The Tobii eye tracking system consists of a high-resolution camera embedded in a 17 inch TFT monitor (resolution: 1280 × 1024 pixels, sampling rate: 120 Hz, average precision: 0.5° of visual angle). Subjects were seated approximately 60 cm from the device. After a five-point calibration procedure, participants were instructed to naturally view the videos that were shown on the screen. Each of the four conditions was presented in one block of four five-second video clips (i.e. total of four trials per condition). An inter-block interval consisting of a black screen was shown for one second between blocks. The order of block presentation was randomized across subjects.

2.2.2 Data analysis and statistics

Two areas of interest (AOI) were defined for each video: the hand region and the eye region. These AOIs were defined using rectangular definition tools to mark the corresponding regions. Dependent measures included (i) the total fixation duration (TFD), which measures the sum of the duration for all fixations within an AOI; and (ii) fixation count (FC), which was calculated as the number of times the participant fixates the AOI (i.e. the number of times the participant's eye gaze enters and leaves the AOI). The gaze data was checked for outliers (none) and normality was assessed by means of Shapiro-Wilk's W tests (gaze data was sufficiently normally distributed). For each dependent variable (TFD, FC) and AOI (hand region, eye region), a repeated measures ANOVA with the within-subject factors 'observed hand movement' (opening hand, static hand) and 'gaze direction' (direct gaze, averted gaze) was conducted to examine gaze behavior for each condition.

3 Results

3.1 M1 facilitation during movement observation

A repeated measures ANOVA with the within-subject factors ‘observed hand movement’ (opening hand, static hand) and ‘gaze direction’ (direct gaze, averted gaze) was conducted on the normalized MEP data separately for each muscle (APB, FDI) to explore the effect of eye gaze on observation-induced facilitation of the primary motor cortex (M1). **Figure 2** displays the MEP amplitude data separately for each muscle and condition.

In both muscles, a two-way interaction between ‘observed hand movement’ and ‘gaze direction’ was revealed (tentatively in the APB: $F(1,31) = 2.89$; $p = 0.06$; $\eta^2 = .09$; significantly in the FDI: $F(1,31) = 7.07$; $p < .05$, $\eta^2 = .19$), indicating a differential impact of eye gaze on observation-induced M1 facilitation. Direct exploration of the difference in MEP response between direct and averted eye gaze showed that for observing the hand opening movement, MEP responses of the APB and FDI were significantly higher for the direct eye gaze condition compared to the averted eye gaze condition (Fisher LSD: both $p < .05$; **figure 2**, left panel). During observation of the static hand condition, MEP responses in the APB muscle were not significantly different between the direct and averted eye gaze conditions ($p = .75$), whereas in the FDI muscle, M1 facilitation was reversibly modulated, indicating significantly *lower* MEP responses when the static hand observation was accompanied by direct versus averted eye gaze ($p < .001$; **figure 2**, right panel). No main effects of ‘gaze direction’ (all $p > .29$) or ‘observed hand movement’ (all $p > .48$) were revealed in either muscle.

MEP scores were not confounded by modulations in background EMG scores. This was tested by conducting similar ANOVAs to the corresponding background EMG data (i.e. normalized RMS-scores). Background EMG was generally small and condition-specific modulations were minimal, as no significant main or interaction effects were revealed. For all conditions and muscles, background EMG scores are listed in **supplementary table 1**.

3.2 Link with social responsiveness

Participants reported a mean total SRS-A score of 36.15 (SD = 19.74), which, as a group, is well below the cut-off score of 54 signaling impairments in social responsiveness. However, for five out of the 32 participants a total score higher than the cut-off point was reported, indicative of impairments in terms of social responsiveness. To explore whether inter-individual differences in social responsiveness were related to the extent by which direct gaze elicited higher MEP responses compared to averted gaze during movement observation, a regression analysis was conducted with ‘gaze effect’ (difference in MEP response between direct and averted eye gaze conditions) as dependent variable and ‘social responsiveness (sub)score’ as predictor (across muscles). Overall, *beta*-values were generally small and none of the relationships reached significance (all $p > .25$, see **supplementary table 2**) indicating that social responsiveness was not predictive for the extent of M1 facilitation.

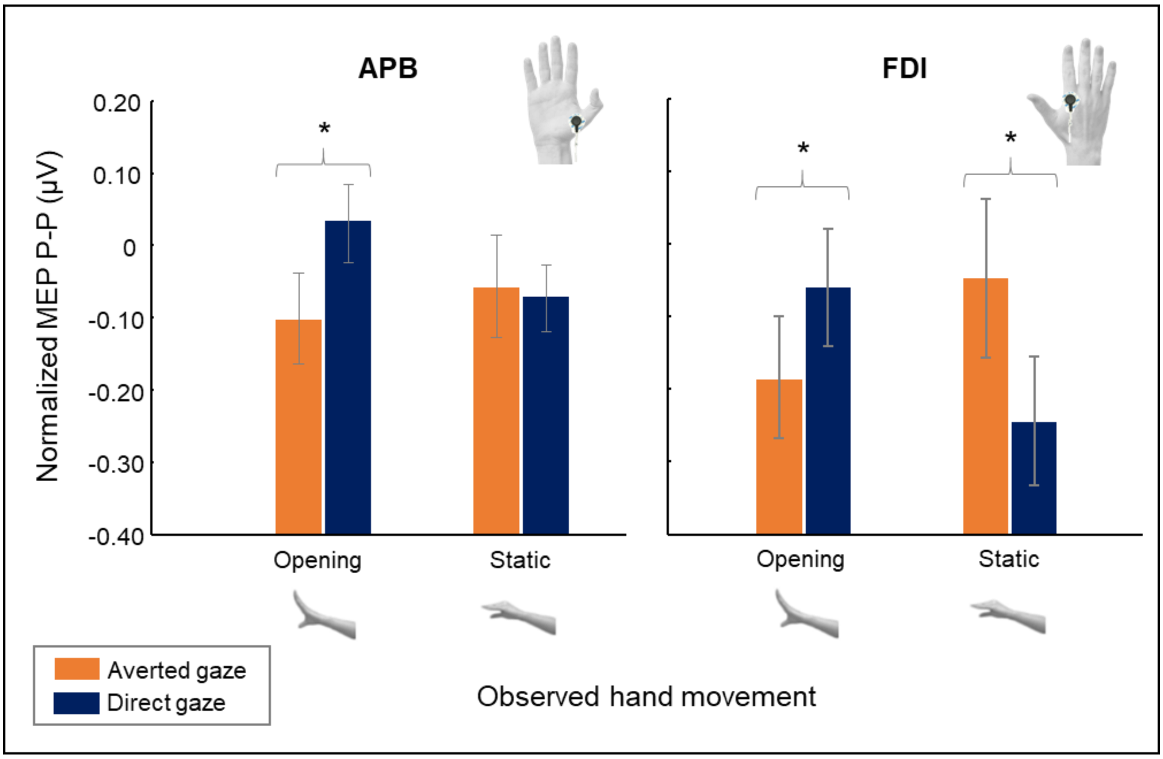

Figure 2. Normalized MEP peak-to-peak amplitude data for the APB and FDI during action observation, separately for each condition. Vertical bars denote standard errors of the mean. * Denotes a significant difference between conditions ($p < .05$).

3.3 Gaze behavior during movement observation

An additional eye tracking experiment was conducted to explore whether the observer’s gaze behavior was different when the movement observation conditions (opening hand, static hand) were accompanied by direct versus averted gaze. To do so, eye tracking was performed and the total fixation duration (TFD) and fixation count (FC) were determined in an area-of-interest (AOI) centered over the hand and eye region of the presented video clip.

For the TFD, a repeated-measures ANOVA with the within-subject factors ‘observed hand movement’ (opening hand, static hand) and ‘gaze direction’ (direct, averted) revealed no significant main effect of ‘gaze direction’, indicating that across movement observation conditions, participants fixated an equal amount of time towards the hand region during direct as during averted gaze conditions (*F*(1,27) = 0.79, $p = .38$, $\eta^2 = .03$). Similarly, no significant effect of 'gaze direction' was revealed for the FC data, indicating that participants made a comparable amount of saccades towards the hand region for the direct and averted gaze conditions (*F*(1,27) = 0.75, $p = .39$, $\eta^2 = .03$). Note however, that the ANOVA revealed significant main effects of 'observed hand movement', indicating that irrespective of gaze direction (direct, averted), subjects looked significantly more (FC: *F*(1,27) = 21.24, $p < .0001$, $\eta^2 = .44$) and longer (TFD: *F*(1,27) = 26.62, $p < .0001$, $\eta^2 = .50$) towards the hand region when the actor performed the opening hand movement, compared to when the actor's hand remained static.

The eye tracking data were additionally used to explore whether direct versus averted eye gaze differentially modulated the observer's gaze behavior towards the eye region of the actor. Not surprisingly, for the eye region AOI, a significant main effect of 'gaze direction' was revealed, indicating that across hand movements, subjects looked longer towards the eye region during direct gaze than during averted gaze (i.e. indicative of the establishment of eye contact between the actor and the observer during direct gaze conditions; TFD: *F*(1,27) = 8.73, $p < .01$, $\eta^2 = .24$). In terms of FC, participants made a comparable amount of saccades toward the eye region during direct gaze as during averted gaze (FC: *F*(1,27) = 0.06, $p = .81$, $\eta^2 = .002$). Mean TFD and FC values are displayed separately for each AOI and condition in **supplementary table 3**.

4 Discussion

In the present study, transcranial magnetic stimulation (TMS) was used to assess cortico-motor excitability at the level of the primary motor cortex (M1) during movement observation, and, in particular, whether observation-induced facilitation of M1 is altered when accompanied by direct or averted eye gaze. Overall, our results show that observation-induced M1 facilitation was most pronounced when direct eye gaze was observed, indicating that eye gaze forms a salient social cue that can modulate the extent by which others' actions are 'mapped' onto the observer's motor system.

As such, the present TMS study extends previous findings from behavioral studies by Wang, Newport, et al. (2011) and Wang, Ramsey, et al. (2011) studying the effect of eye gaze on automatic motor mimicry using similar video clips as those adopted in the present study. In these studies, a stimulus–response compatibility paradigm was adopted where participants were asked to perform the same movement or the opposite movement as viewed in the video clip, and a clear congruency effect was found indicating that responses were significantly faster when the same movement was performed (e.g. hand opening observed – hand opening performed), compared to trials in which the opposite hand movement was performed (e.g. hand closing observed – hand opening performed). Interestingly, Wang, Newport, et al. (2011) demonstrated that this mimicry congruency effect was even more enlarged when direct eye contact was established between the observer and the observed actor, indicating a rapid modulation of mimicry by eye contact. Our study provides insights into the neurophysiological mechanism underlying this modulating effect of eye gaze on automatic motor simulation, by showing that direct eye gaze can significantly enhance the extent by which the observed movement is mirrored onto the observer's motor system.

Our findings are also in agreement with previous results from an MEG study by Kilner et al. (2006). In this study, MEG was used to record cortico-motor activity whilst participants observed upper limb movements of an actor that was facing away or towards them. Results from this study showed that cortico-motor responses to movement observation (oscillatory activity in the 7–12 Hz frequency range) are dependent on the relative perspective of the observed model towards the observer, such that cortico-motor modulations to movement observation were only present when the actor was facing towards the observer, not when the actor was facing away from the observer. The authors suggested that distinct social signals accompanying the observed movements and actions of other people (e.g. the perspective relative to the observer) can modulate visuospatial attention, such that only specific visual information of the most salient and most socially relevant actions is allowed to enter the mirror system for further processing. Our study extends these findings by showing that not the perspective per se, but the establishment of direct eye gaze may be a highly salient cue in determining the extent by which an observed action will be mapped onto the observer's motor system. In addition to perceived eye contact, also other social signals may sophistically direct motor resonance (Wang & Hamilton, 2012). Indeed, previous studies have shown that also social cues such as self-construal (Obhi et al., 2011), social interaction (Hogeveen & Obhi, 2012) and power (Hogeveen et al., 2014) can influence motor resonance. It has therefore been argued that the control of motor resonance may involve a 'social top-down response modulation' (STORM) that is dependent on the social context in which others' actions are observed (Wang & Hamilton, 2012).

Previous fMRI studies in humans (Kampe et al., 2003; Nummenmaa and Calder, 2009; Senju & Johnson, 2009b) and single-cell recordings in monkeys (Emery, 2000; Perrett et al., 1992) consistently showed that the medial prefrontal cortex (mPFC) and the superior temporal sulcus (STS) are increasingly activated during direct versus averted gaze, highlighting the importance of these two regions in gaze processing. Wang, Ramsey, et al. (2011) replicated these findings and additionally suggested that the mPFC may well be the originator of the effect of eye gaze on motor mimicry by modulating functional connectivity with the STS, i.e. the main visual input region to the fronto-parietal mirror system. In other words, the model by Wang, Ramsey, et al. (2011) suggested that gaze-related activations at the level of mPFC may impose a top-down control over the processing of visuo-motor information at the level of the STS, which in turn may impact the extent by which observed actions are processed in down-stream mirror regions in the inferior frontal gyrus, ventral premotor regions and inferior parietal lobule. In this view, M1 may be conceived as the end-state region of a chain of cortico-cortical connections signaling on whether or not the cortico-spinal tract and the corresponding peripheral muscles are to be recruited for initiating overt motor simulation. Correspondingly, by receiving direct input from upstream premotor and mirror regions, modulations in cortico-motor excitability at the level of M1 may reflect an end-state cortical measure of how the brain 'evaluated' the social relevance or saliency of the observed visual scene. Our results therefore provide additional support to the notion that direct eye gaze from the actor forms a strong mediator for evaluating whether or not visuo-motor information of the observed action is sufficiently relevant to be processed up to the level of M1. Instead of simulating all possible movement-related information perceived in a visual scene, eye contact may direct the motor system to give preference to processing visuo-motor input originating from the most socially relevant person.

Note that in the FDI muscle, but not in the APB, we found an inverse effect of eye gaze on M1 facilitation during the observation of the static hand, indicating *increased* M1 excitability for the averted compared to the direct gaze condition. One potential interpretation could be that during the trials in which *no* actual hand opening movement was observed (only a static hand), direct eye gaze might have induced an increased inhibitory effect on M1 excitability to encode more efficiently that *no* movement is observed during these trials. From this perspective, it can be hypothesized that direct eye primes the observation-to-execution mapping system by increasing the signal-to-noise ratio when perceiving motion stimuli (i.e., by effectively heightening M1 excitability during actual movement observation, and inhibiting M1 excitability when no movement is observed). This interpretation remains speculative however as it is unclear why this effect was then only significantly observed in the FDI muscle and not in the APB muscle.

Nummenmaa and Calder (2009) showed that observing another person’s gaze can automatically induce gaze following, thereby shifting spatial attention toward the scene observed by the model. In this view, an alternative explanation for the observed gaze effect of the present study can be put forward, suggesting that the averted gaze conditions induced a shift of spatial attention away from the observed hand movement, thereby reducing the observation-induced M1 facilitation. We explicitly addressed this alternative explanation in a control eye-tracking experiment, in which the same video clips were displayed while the participants’ gaze behavior was recorded. Overall, we found no indications that participants spend less time fixating on the to-be-observed hand movement in video clips with averted gaze compared to direct gaze, which makes it implausible that differences in visual spatial attention are responsible for the encountered gaze-related modulations in observation-induced M1 excitability. Also Wang, Newport, et al. (2011) explicitly addressed this issue in their control “flashbox” experiment in which distracting stimuli (i.e. flashing white squares) were displayed in the periphery during the movie clip to draw participant’s attention. These manipulations did however not alter the effects of eye gaze on motor mimicry, indicating the robustness of the eye gaze effect relative to the presence of distracters competing for attention. Furthermore, previous studies have shown that eye gaze can have a direct effect on several physiological measures such as skin conductance, indicating a heightened response of the observer’s autonomic nervous system during direct versus averted eye gaze (Hietanen et al., 2008; Pönkänen et al., 2011). While direct eye gaze conditions may have induced a similar heightening of arousal in the present study, it is unlikely that these arousal effects directly affected the modulation of MEP responses, since enhancements in M1 facilitation were specifically observed during the movement observation condition (i.e. hand opening) and not during the observation of the static hand. Also background EMG scores – which were measured before the TMS pulse – did not show any condition specific modulations.

Nevertheless, future research on the effect of eye gaze on motor facilitation would benefit from the inclusion of parallel assessments of skin conductance to explore the possibility of arousal-related effects further. Also in terms of the assessment of viewing behavior and attention to the presented stimuli, future studies would benefit from the inclusion of online eye tracking. In the present study, assessments of viewing behavior were only assessed in a separate eye tracking experiment in which the participants were different from those participating in the main experiment. While this additional eye tracking experiment already provided relevant information related to viewing behavior towards the presented stimuli, online eye tracking would have allowed a direct assessment of viewing behavior and attention on a trial-by-trial basis and its potential relationship to the evoked MEP responses. In the present TMS experiment, attention to the presented stimuli was only assessed randomly, by asking the participants to verbally report the type of hand movement and gaze direction that was observed in the previous video clip. Since this assessment might have affected the subjects' explicit awareness of the presented experimental manipulations, it should be beneficial for future experiments to adopt other strategies to assess the subjects' online attention to the presented stimuli, such as the inclusion of eye tracking.

Overall, mirror motor mapping is hypothesized to form the basic neural mechanism by which others' actions and emotional states can be simulated, recognized and imitated. Considering the hypothesized link between motor simulation and these high-level social skills, the 'broken mirror theory of autism' has been put forward, postulating that behavioral deficits in action understanding, imitation and empathy seen in Autism Spectrum Disorders (ASD) may result from aberrant functioning of the fronto-parietal mirror motor mapping (Ramachandran & Oberman, 2016). While a number of neurophysiological studies provided support for aberrant 'mirroring' in ASD (Dapretto et al., 2006; Enticott et al., 2012; Hadjikhani et al., 2006; Oberman et al., 2005), also several studies found no evidence to support this account (for a review, see Hamilton, 2013). Related to these controversies, and considering that eye contact may form a highly salient cue and perhaps even a prerequisite for the initiation of motor simulation, it would be interesting for future research to evaluate whether and how gaze-related effects on motor mirroring are affected in patients with ASD, who are well-known to display particular difficulties with engaging mutual eye contact (Kaartinen et al., 2012; Kylliäinen & Hietanen, 2006; Kylliäinen et al., 2012; Senju & Johnson, 2009a). Note that in the present study, we found no significant relationships between scores on the social responsiveness scale (SRS) and the extent of the ‘gaze effect’. However, considering that inter-individual differences in SRS scores were generally small in our rather homogenous sample of neurotypical students, it should be interesting for future studies to explore the relationship between the eye gaze effect and social responsiveness in more heterogeneous samples including individuals with particular implications in the social domain, such as ASD or social anxiety disorders (Myllyneva et al., 2015; Wieser et al., 2009).

Further, considering that based on the present sample, uncertainty exists with regard to the behavioral correlates of the observed gaze effect on motor mirroring, it should be interesting for future studies to explore whether - in addition to social competence - potentially also other personality traits may be informative in predicting inter-individual variations in the observed gaze effect. For example, based on the work by Hietanen et al. (2008), several links have been suggested between seeing direct or averted eye gaze and inter-individual differences in the motivational system towards approach and avoidance. In particular, neuroticism and scales assessing social phobia have been suggested to form important predictors of behavioral direct gaze avoidance and subjective averted gaze preference (Myllyneva et al., 2015; Uusberg et al., 2015). In this view, it should also be interesting for future research to discern whether inter-individual differences in these motivational preferences towards eye contact may be important in determining the facilitative effect of eye gaze on motor mirroring.

To sum up, the present results provide evidence that the mapping of others’ movements onto the observer’s motor system is enhanced when direct compared to averted eye gaze is established between the observer and the observed model. These findings support the notion that eye contact is a powerful and highly salient social signal with the ability to modify activity in the human mirror-motor system, thereby directing human social interactions.

5 Supplementary materials

5.1 *Supplementary methods: measurement of muscle activity during movement execution*

Participants. Eight subjects (4 male, age range 18 – 24) participated in an additional movement execution experiment measuring electromyography (EMG) during the actual execution of a hand opening movement. All participants were right-handed and provided written informed consents. Participants of this movement execution experiment were not the same as those participating in the main movement observation TMS experiment.

Task. In twelve trials, participants were instructed to observe and simultaneously execute the hand opening movement adopted in the main movement observation experiment. Additionally, twelve trials were recorded during which the hand was held static (not performing a movement).

EMG. During the execution of the movement, a surface electromyogram (EMG) was simultaneously recorded from the APB and FDI hand muscles. EMG was quantified by calculating the root mean square (RMS)of the signal across an interval of 100 milliseconds which overlapped the time point of TMS stimulation in the main movement observation experiment (see main **figure 1**).

Data analysis. For each participant, EMG scores were averaged across trials, separately for each muscle and movement condition. A total of 13.02% of all trials were identified as outliers and were removed from the analysis (see main manuscript for outlier detection procedure). A repeated measures ANOVA design on the EMG data was performed with the within-subjects factors muscle (APB, FDI) and hand movement (opening, static).

Results. The ANOVA analysis revealed a significant main effect of muscle ($F(1,7) = 31.99$, $p < .001$), indicating significantly higher muscle activation for the APB compared to the FDI. Also a significant main effect of hand movement ($F(1,7) = 37.10$, $p < .001$) was revealed, indicating significantly larger muscle activity during the execution of the hand opening movement, compared to static hand condition in both muscles. However, a significant muscle by hand movement interaction ($F(1,7) = 23.74$, $p < .001$), indicated that the differential muscle activation was significantly more pronounced in the APB compared to the FDI.

5.2 Supplementary tables

Table S.1. Recorded background EMG data (RMSE-scores) separately for each muscle (APB, FDI) and condition. Data are represented as mean ± standard deviation (*N* = 32).

Muscle	Gaze	Movement	EMG (µV)
APB	Averted	Opening	0.0080 ± 0.0095
		Static	0.0072 ± 0.0066
	Direct	Opening	0.0079 ± 0.0107
		Static	0.0076 ± 0.0088
FDI	Averted	Opening	0.0086 ± 0.0076
		Static	0.0085 ± 0.0085
	Direct	Opening	0.0084 ± 0.0072
		Static	0.0084 ± 0.0075

Table S.2. Linear regression results for assessing the relationship between the SRS-A (subscale) scores and the gaze effect (differential MEP response for direct vs. averted gaze) for observation of the hand opening movement (separately for APB and FDI muscle)

SRS-A subscale	ABP (*n* = 32)			FDI (*n* = 32)		
	β	***t*(30)**	***p***	***β***	***t*(30)**	***p***
Total score	- .10	- 0.56	.57	.11	0.62	.54
Social awareness	- .21	- 1.18	.25	.11	0.63	.54
Social communication	.04	0.22	.83	.18	0.99	.33
Social motivation	- .05	- 0.28	.78	.11	0.59	.56
Rigidity/repetitiveness	- .19	- 1.05	.30	- .08	- 0.46	.65

Table S.3. Recorded gaze behavior (total fixation duration (TFD) and fixation count (FC)), separately for each dependent measure, area-of-interest (AOI: hand, eyes) and observation condition. Data are represented as mean ± standard deviation (*N* = 28).

AOI	Gaze	Movement	TFD	FC
Hand	Averted	Opening	5.57 ± 3.73	12.64 ± 7.89
		Static	4.37 ± 3.92	10.32 ± 8.52
	Direct	Opening	5.85 ± 4.35	12.82 ± 10.14
		Static	3.37 ± 3.30	8.29 ± 8.37
Eyes	Averted	Opening	7.24 ± 3.98	13.68 ± 7.18
		Static	9.38 ± 5.15	13.32 ± 7.66
	Direct	Opening	9.81 ± 6.14	13.54 ± 8.57
		Static	9.65 ± 4.30	13.46 ± 7.04

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Chapter 2

Eye contact enhances interpersonal motor resonance.

Comparing video stimuli to a live two-person action context.

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Abstract

Previous research has shown a link between eye contact and interpersonal motor resonance, indicating that the mirroring of observed movements is enhanced when accompanied with mutual eye contact between actor and observer. Here, we further explored the role of eye contact within a naturalistic two-person action context. Twenty-two participants observed simple hand movements combined with direct or averted gaze presented via a live model in a two-person setting or via video recordings, while transcranial magnetic stimulation (TMS) was applied over the primary motor cortex (M1) to measure changes in M1 excitability. Skin conductance responses and gaze behavior were also measured to investigate the role of arousal and visual attention herein. Eye contact significantly enhanced excitability of the observer's M1 during movement observation, but was most pronounced within a two-person setting. Notably, participants with higher social responsiveness (Social Communication subscale of the SRS) displayed a more pronounced modulation of M1 excitability by eye gaze. Gaze-related modulations in M1 excitability were however not associated with differences in visual attention or autonomic arousal. In sum, the current study highlights the effectiveness and feasibility of adopting paradigms with high ecological validity for studying the modulation of mirror system processes by subtle social cues, such as eye gaze.

1 Introduction

In humans, observation of others' actions has been shown to activate similar brain regions as those involved when executing that action. At the basis of this mechanism are neural cells, known as 'mirror neurons', which respond to both action observation, imagination and execution (Cattaneo & Rizzolatti, 2009). By means of several neuroimaging techniques, distinct frontal and parietal regions in the human brain (i.e. the inferior frontal gyrus, inferior parietal lobule and the ventral premotor cortex) have been identified with mirror-like properties (Caspers, Zilles, Laird & Eickhoff, 2010; Molenberghs, Cunnington & Mattingley, 2012), together constituting the action observation network or 'mirror system'.

According to the embodied simulation account (Gallese & Sinigaglia, 2011), the automatic simulation of observed actions in the mind of the observer, a process also known as 'mirror-motor mapping' or 'interpersonal motor resonance', is anticipated to constitute the core neural mechanism for recognizing and understanding others' actions, intentions and emotional states (but see De Bruin & Gallagher, 2012; Press & Cook, 2015; Schilbach, 2010 for critical appraisals of a pure mirror neuron account of social cognition). In this view, the human mirror system plays an important role in facilitating everyday social functioning in general, and interpersonal reciprocity during social interactions in specific, by synchronizing the own behavior to others' actions (Feldman, 2017).

One of the most salient cues to initiate interpersonal synchrony is eye contact. Indeed, previous research showed that observed gaze cues form an important modulator of automatic mimicry and interpersonal motor resonance. Wang, Newport, and Hamilton (2011) studied the effect of perceived eye contact on automatic motor mimicry using a stimulus-response compatibility paradigm, in which participants were asked to perform the same movement or the opposite movement as viewed in a video clip. A clear congruency effect was found, indicating that responses were significantly faster when the same movement was performed, compared to trials in which the opposite hand movement was performed. Notably, it was shown that this mimicry congruency effect was further enlarged when direct eye contact was established between the observer and the observed actor.

These initial behavioral observations were recently extended by our lab by investigating the neurophysiological mechanism underlying the enhancing effect of eye gaze on automatic motor simulation, using transcranial magnetic stimulation (TMS) (Prinsen et al., 2017; Prinsen, Brams & Alaerts, 2018). TMS is a non-invasive method for stimulating cortical neurons via the administration of a brief magnetic pulse to the scalp. A single TMS pulse delivered to the somatotopically organized primary motor cortex (M1) produces a muscle contraction or motor-evoked potential (MEP) in the corresponding peripheral muscles, as measured with electromyography (EMG). Importantly, Fadiga et al. (1995) demonstrated that when TMS is applied to M1 during the mere observation of others' actions, MEP amplitudes within the stimulated muscles are enhanced, indicating a muscle-specific and observation-induced facilitation of corticospinal excitability at the level of M1. Adopting this TMS technique, we have demonstrated that MEP amplitudes are significantly enhanced when participants observe actions accompanied with direct versus averted gaze from the model, indicating increased observation-induced M1 excitability upon mutual eye contact (Prinsen et al., 2017, 2018).

Previous mirror system research predominantly adopted video presentations of dynamic (i.e. involving biological motion to recruit mirror system regions) but simplified action contexts, such as point light displays (e.g. Ulloa & Pineda, 2007) or videos of isolated limb movements (e.g. Alaerts, Swinnen & Wenderoth, 2009). This allows for a strict control over the stimuli and accurate time-locking of behavioral (e.g. mimicry) or neurophysiological (e.g. M1 excitability) responses. While these screen-based paradigms have been highly instrumental in uncovering several important properties of the human action observation network, the gap between the highly-controlled lab-setting and everyday social interactions remains high. Indeed, screen-based presentations have been argued to lack the 'richness' of real life aspects of social interactions, which may be of particular relevance when examining the effect of salient social cues such as eye contact (Reader & Holmes, 2016; Risko, Laidlaw, Freeth, Foulsham & Kingstone, 2012). Consequently, researchers have advocated for a more naturalistic, second-person approach to investigate social cognition and its neurobiological bases (Schilbach et al., 2013).

To date however, studies investigating interpersonal motor resonance upon observation of real life versus videotaped motor actions are sparse. One previous magnetoencephalography (MEG) study by Järveläinen et al. (2001) reported significantly stronger M1 activity upon the observation of live versus videotaped hand movements, thereby underlining the relevance of adopting naturalistic action contexts in mirror system research. With the present study, we specifically aim to further address these concerns about ecological validity by re-exploring the enhancing effect of perceived eye contact on M1 excitability (as conveyed with video recordings in previous studies) when gaze and movement cues are presented in a naturalistic two-person action context (as suggested by Reader & Holmes, 2016). To do so, TMS was applied and MEPs were recorded while participants observed a live or videotaped model performing a simple index finger movement accompanied by direct or averted gaze. In line with Järveläinen et al. (2001), we hypothesized naturalistic gaze cues to be more salient for facilitating M1 excitability compared to video presentations. Furthermore, and in accordance to previous mirror system research adopting TMS (Strafella & Paus 2000; Fadiga et al. 1995), we expected the facilitatory effect of eye gaze on M1 excitability to be specific to the muscle implicated in the observed movement.

In addition to the TMS-based assessments, the current study also performed measurements of skin conductance responses (SCRs) to assess whether gaze-related effects on interpersonal motor resonance are potentially modulated by variations in autonomic arousal. Indeed, research has shown that - especially within ecologically salient social contexts - the experienced level of arousal can influence action readiness and motor flexibility (for a review, see Frijda, 2010). Furthermore, a series of previous studies robustly demonstrated that perceived direct gaze from a live model induces significantly higher states of arousal (Helminen, Kaasinen & Hietanen, 2011; Hietanen, Leppänen, Peltola, Linna-Aho & Ruuhiala, 2008; Pönkänen, Peltola & Hietanen, 2011). Considering that a similar live action context is adopted in the current study, the combined assessment of SCRs allowed to re-explore the effect of eye contact on autonomic arousal; as well as to examine for the first time whether eye contact-induced changes in M1 excitability are related to eye contact-induced changes in autonomic arousal. Finally, considering the hypothesized link between motor resonance and high-level social skills (Iacoboni & Dapretto, 2006), we aimed to explore the association between gaze-related modulations in the recorded measures and a self-report measure of social responsiveness (SRS; Constantino & Todd, 2005).

2 Materials and methods

2.1 Participants

A total of 23 right-handed individuals (8 men and 15 women) aged between 21 and 30 years old (mean ± SD: 25;3 ± 2;5 years;months) participated in this study. Right hand dominance was assessed with the Edinburgh Handedness Questionnaire (EHQ; Oldfield, 1971). Exclusion criteria comprised medication use, any diagnosed psychiatric (e.g. autism, ADHD) or neurological disorder (e.g., stroke, epilepsy, concussion), left handedness or any contraindication for TMS (Rossi, Hallett, Rossini & Pascual-Leone, 2012). Written informed consent was obtained from all participants. Consent forms and study design were approved by the Ethics Committee for Biomedical Research at the University of Leuven in accordance to the Declaration of Helsinki (World Medical Association, 2013). One female subject did not complete the full experimental procedure due to intolerability to TMS and was excluded from the analyses.

2.2 Experimental procedure and stimuli

Prior to the experimental procedure, subjects completed the Dutch self-report version of the Social Responsiveness Scale for adults (SRS; Constantino & Todd, 2005). The SRS is a widely used screening tool to identify the presence and extent of any social impairments using a four-point Likert-scale. The Dutch version (Noens, De la Marche & Scholte, 2012) consists of 64 items encompassing four subscales; Social Awareness, Social Communication, Social Motivation and Rigidity/Repetitiveness. Higher scores indicate more social impairments.

Afterwards, participants were seated at a distance of approximately 80 cm from a computer screen or panel and were instructed to observe and pay close attention to the presented stimuli and to stay as motionless and relaxed as possible. The presented stimuli comprised the face of a female experimenter (J.P.) gazing either towards the participant (i.e. showing direct gaze and engaging in mutual eye contact) or gazing 30° to the right (i.e. showing averted gaze). In half of the trials, the model performed a simple index finger abduction movement (movement observation condition). In the other half of the trials, only the gaze cues were conveyed, without hand movements (control condition).

Stimuli were presented to the observing participant in two separate modes: by means of a live model or by means of videos of the same model presented on a computer screen (**figure 1A**). In the live condition, the stimulus person’s face was presented through a 20 × 30 cm voltage-sensitive liquid crystal (LC) shutter screen (DreamGlass Group, Spain) attached to a black frame between the stimulus person and the participant (similar setup as used by Hietanen et al., 2008). The stimulus person was sitting at a distance of 15 cm from the panel. Signal software (version 6.02, Cambridge Electronic Design, UK) was used to trigger the LC window to shift from an opaque to a transparent state within milliseconds. In the video condition, videos (frame rate: 29 Hz) of the previously described stimuli were displayed on a widescreen computer screen (resolution: 1920 × 1080 pixels, refresh frequency: 60 Hz). Video presentation timing was controlled by LabVIEW software (version 14.0, National Instruments, UK) and triggered by Signal software. The order of the presentation mode (live, video) was counterbalanced across participants. An illustration of the stimuli is provided in **figure 1B**.

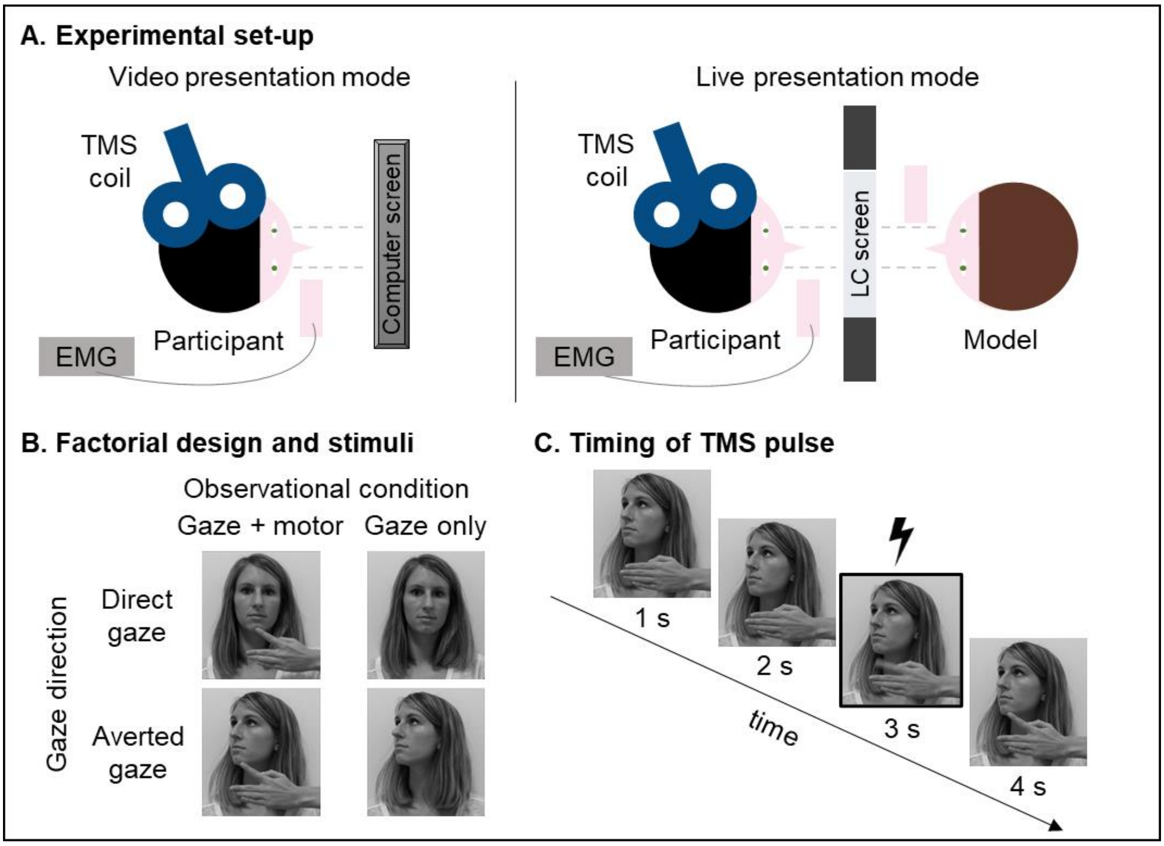

Figure 1. (A) Schematic overview of the experimental set-up in the video (left panel) and live (right panel) presentation mode. **(B)** Factorial design and stimuli showing the stimulus person (experimenter J.P.) engaging in mutual eye contact (direct gaze) or not (averted gaze) while performing either a simple finger abduction movement or no movement. The last still of each condition is depicted. (**C)** Single-pulse TMS was delivered 3 seconds after the onset of each video clip (in the video presentation mode) or after the opening of the LC screen (in the live presentation mode), which corresponded to the execution phase of the observed movement.

Per presentation mode, each of the four conditions was presented five times in blocks of four (i.e. total of 20 trials per condition for each presentation mode). This resulted in a 2 × 2 × 2 factorial design with the factors ‘presentation mode’ (live or video), ‘observed movement' (present or not) and 'observed gaze direction' (direct or averted). Presentation order of blocks was pseudo-random (no more than three consecutive blocks of the same type). The duration of a single trial in each presentation format was 4 seconds. The inter-stimulus-interval between trials was 2 seconds, during which the shutter remained opaque and the computer screen had a black background.

During all trials, single-pulse TMS was administered, and dependent measures of M1 excitability (i.e. motor-evoked potentials or MEPs) were recorded via electromyography (EMG). Simultaneous recordings of autonomic arousal were performed by assessing skin conductance responses (SCRs). Additionally, eye tracking was performed to ascertain that participants were attentive towards the presented stimuli.

2.3 Electromyography recordings and TMS

During observation of the experimental stimuli, single-pulse TMS (Magstim-200 stimulator, Magstim Company Ltd., UK) was applied over the left primary motor cortex (M1) using a hand-held 70 mm figure-of-eight coil. The coil was positioned over left M1, tangentially to the scalp and 45° away from the midsagittal line. Optimal coil location for TMS-stimulation was determined as the site that produced maximal responses while at rest ("hotspotting") in the contralateral first dorsal interosseous (FDI) muscle, a muscle implicated in the to-be-observed finger movement. MEPs were also collected from the abductor minimi digiti (ADM), which is not implicated in the movement and therefore serves as a control muscle. Although parameter setting procedures were prioritized for the FDI, they are assumed to be effective for assessing condition-specific modulations simultaneously for both muscles due to overlapping hand muscle representations in M1 (Gentner & Classen, 2006; Krings, Naujokat & Graf v. Keyserlingk, 1998). MEPs were measured by means of surface EMG recorded from electrodes attached to the muscle bellies of the investigated muscles, with referential electrodes attached at the wrist.

Experimental stimulation intensity was defined according to the resting motor threshold (rMT) for each participant, i.e. the lowest stimulation intensity that produced a peak-to-peak MEP of at least 50 µV in five out of ten consecutive trials (Rossini et al., 1994), and set at a supra-threshold of 130% of the subject's rMT. In both presentation modes, a single TMS pulse was delivered on the third second of stimulus presentation (with 6 s inter-pulse interval), which coincided with the execution of the index finger abduction movement of the model (**figure 1C**; for the live presentation mode, the live model was trained to perform the index finger movement at exactly 3s to coincide with the TMS pulse). EMG recordings were sampled (2000 Hz), amplified and band-pass filtered (5-1000 Hz) via a CED Power

1401 analog-to-digital converting unit (Cambridge Electronic Design, UK) and stored on a PC for offline analysis. Signal software was used for EMG-recordings and triggering of the TMS-stimulator.

Based on the recorded EMG data, peak-to-peak amplitudes of the TMS-evoked MEPs were determined to assess condition-induced changes in M1 excitability. Additionally, background EMG was quantified by calculating the root mean square (RMS) across the 110 to 10 millisecond interval prior to TMS-stimulation to ensure that subjects were completely relaxed during stimulation. Trials with excessive tonic muscle activity prior to TMS stimulation (i.e. background EMG > mean + 2.5 SD) were not included in the final analysis. Further, extreme MEP-amplitudes (exceeding 1.5 interquartile distance from mean) were removed from the analysis. This procedure omitted 11.42% of all trials for FDI and 10.43% of all trials for the ADM.

2.4 Skin conductance recordings

The Nexus-32 multimodal acquisition system and BioTrace+ software (version 2015a, Mind Media, The Netherlands) were used to collect stimuli-specific skin conductance responses (SCRs) with a sampling rate of 128 Hz. Two Ag/AgCl Velcro snap-on electrodes were attached to the palmar surface of the distal phalanxes of the index and middle fingers on the participant's non-dominant hand. Before electrode attachment, the skin was prepared with TD-246 Skin Conductance Electrode Paste (0.5% saline in a neutral base). Typically, stimuli-specific SCRs are characterized by a rise from initial level to a peak within 1-4 s after stimulus onset, followed by a relatively long recovery period of 20 – 30 s (Boucsein et al. 2012). Since our experimental design was optimized to measure TMS-evoked MEP responses, incorporating a short ISI of 2 s, superimposed SCRs were likely to be observed within blocks. Therefore, only SCRs to the first stimulus of a block were analyzed. A standard peak-detection method was used; defining the SCR as the maximum change in amplitude relative to baseline during the four seconds after the start of stimulus onset. The baseline was determined as the average of the two seconds right before stimulus onset. Maximum change from baseline amplitude scores below 0 µS were set to zero. The SCR data of one participant was not recorded (video session only) due to the malfunctioning of the acquisition hardware.

2.5 Eye tracking

Gaze behavior was recorded with a sampling rate of 30 Hz by means of head-mounted SMI eye tracking glasses and SMI iView acquisition software (SensoMotoric Instruments, Germany). The glasses were adjusted to the participant's comfort and a three-point calibration procedure was performed before recording. The ‘semantic gaze mapping’ procedure incorporated in SMI BeGaze analysis software (version 3.0, SensoMotoric Instruments, Germany) was used, whereby fixations were mapped into a pre-specified areas of interest (AOI) positioned over the eye region of the stimulus person. For each condition, the number of fixations and the average gaze time (in ms) per fixation towards the eye AOI was assessed across trials.

2.6 Data analysis and statistics

All statistics were calculated with Statistica 10 (StatSoft, USA) and results were considered significant with a *p*-value lower than .05. To investigate condition-specific modulations of M1 excitability, a four-way repeated measures analysis of variance (RM-ANOVA) with the within-subject factors ‘muscle’ (experimental FDI, control ADM), ‘presentation mode’ (video, live), ‘observed gaze’ (averted, direct) and ‘observation condition’ (movement, no movement) was performed on the recorded MEPs. Significant effects were further investigated by means of Fisher Least Significant Difference (LSD) post-hoc tests. The partial Eta square (η^2) value was calculated as an estimate of effect size. Similar RM-ANOVAs where conducted for the other outcome measures (SCRs and gaze time) and were adapted when necessary (for example, no inclusion of the ‘muscle’ factor in these analyses).

In order to explore the potential relationship between the effect of eye contact on M1 excitability (MEPs) and autonomic arousal (SCRs), a Pearson correlation analysis was performed. To capture the ‘eye contact effect’, the difference between responses recorded during the direct and averted gaze conditions was calculated (i.e. MEP amplitude $_{\textit{direct gaze}}$ – MEP amplitude $_{\textit{averted gaze}}$ and SCR amplitude $_{\textit{direct gaze}}$ – SCR amplitude $_{\textit{averted gaze}}$ difference score), with positive scores indicating a higher MEP or SC response during the direct versus the averted gaze condition. Pearson correlation coefficients were also calculated between on the one hand the eye contact effect on MEPs/SCRs, and on the other hand the (sub)scores of the SRS questionnaire. However, to restrict the number of performed correlations, difference scores and Pearson correlation coefficients were only computed for those conditions in which a significant eye contact effect was encountered. For all performed correlations, the Cook’s distance metric was used to identify influential outliers, but none were detected (all Cook’s $D < 0.86$).

3 Results

3.1 The effect of eye contact on M1 excitability (TMS-induced MEPs)

The four-way RM-ANOVA demonstrated a significant main effect of observed eye gaze ($F(1,21) = 10.97$, $p = .003$, $\eta^2 = .34$), indicating that, on average, MEP responses were higher during conditions with direct eye gaze, compared to conditions with averted gaze. However, the identification of a significant four-way interaction of perceived eye gaze with the factors ‘muscle’, ‘presentation mode’, and ‘movement condition’ indicated that the effect was modulated by these factors ($F(1,21) = 4.33$, $p = .04$, $\eta^2 = .17$). Indeed, post-hoc investigations of this four-way interaction, directly assessing the effect of eye gaze separately for each muscle and movement condition, showed that, for MEPs recorded from the target FDI muscle, the effect of eye gaze was most pronounced during the observation of movements performed by the live stimulus person (Fisher LSD: $p < .001$). The effect was only evident at trend-level during the video presentation mode ($p = .07$), and absent when no movement was observed (live: $p = .11$; video: $p = .41$). As expected, further post-hoc explorations showed no significant effects of observed eye gaze for MEPs recorded from the control ADM muscle, for any of the four observational conditions (Fisher LSD: all $p > .19$).

Also a significant main effect of presentation mode ($F(1,21) = 5.77$, $p = .03$, $\eta^2 = .22$) as well as a significant presentation mode by muscle interaction ($F(1,21) = 7.48$, $p = .01$, $\eta^2 = .27$) were encountered. Post-hoc investigation of this two-way interaction showed that, for the FDI muscle, MEPs were generally higher during presentation of the live model than during the video presentations (irrespective of eye gaze or movement observation condition; Fisher LSD: $p < .001$), whereas for the ADM muscle, MEPs were on average not significantly modulated by presentation mode ($p = .70$). The overall ANOVA model also revealed a significant main effect of muscle ($F(1, 21) = 13.98$, $p = .001$, $\eta^2 = .40$) indicating that on average, MEP responses recorded from the FDI muscle were larger compared to MEP responses recorded from the ADM muscle. The main effect of observed movement was however not significant ($F(1,21) = 0.17$, $p = .68$, $\eta^2 = .008$), nor was any of the two- or three-way interactions with this factor (all $p > .42$).

Together, these results indicate that observed eye contact significantly augmented M1 excitability upon movement observation, especially when eye contact was conveyed by a live model. Furthermore, these effects were specific for the muscle that was implicated in the observed index finger movement (i.e. the FDI muscle of the index finger). The mean MEP peak-to-peak amplitudes for each condition and muscle are presented in **figure 2**.

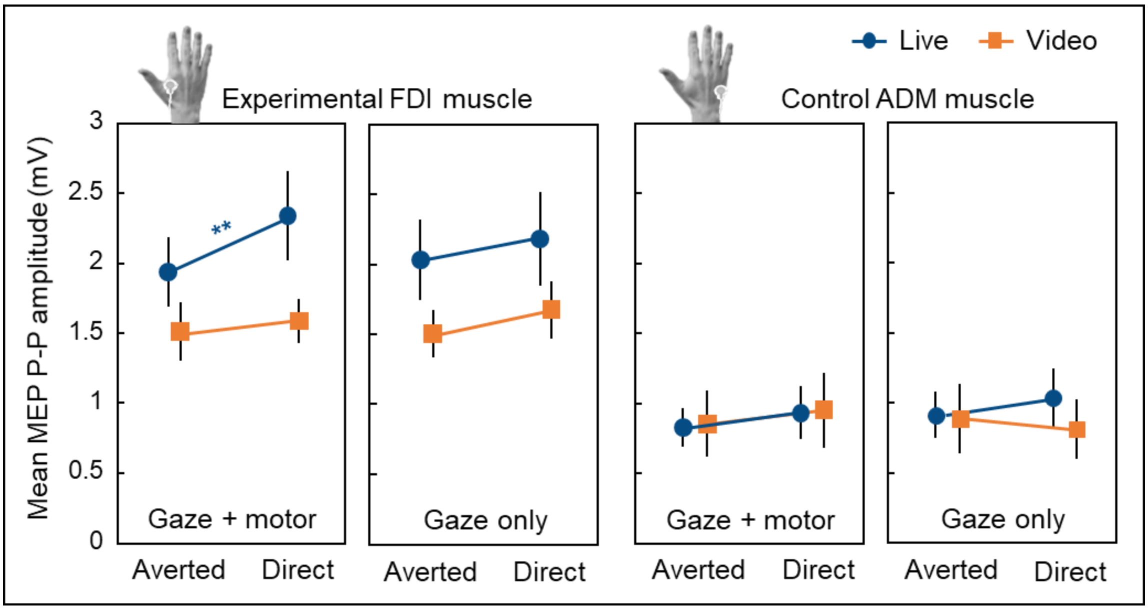

Figure 2. The effect of perceived eye gaze (direct, averted) and presentation mode (live, video) on MEP peak-to-peak amplitude, per observational condition (upper panels: gaze and motor cues; lower panels: gaze cues only) and muscle (left panels: experimental FDI muscle; right panels: control ADM muscle). ***p* < .001, error bars denote mean ± SE.

3.2 *The effect of eye contact on autonomic arousal (SCRs)*

Similar to the analysis on TMS-induced MEPs, we performed a three-way RM-ANOVA on the recorded skin conductance responses (SCRs) (note that there is no muscle factor for the SC data). The analysis revealed a significant main effect of eye gaze ($F(1,20) = 16.39$, $p < .001$, $\eta^2 = .45$) and presentation mode ($F(1,20) = 7.94$, $p = .01$, $\eta^2 = .28$), as well as a two-way interaction between these two factors ($F(1,20) = 5.24$, $p = .03$, $\eta^2 = .21$). Post-hoc tests showed that although direct eye contact generally yielded higher SCRs, the differential response was more pronounced when gaze cues were conveyed by the live model (Fisher LSD: $p < .001$), compared to video presentations ($p = .23$). The main and interaction effects of the ‘observed movement’ factor were not significant (all $p > .17$). **Figure 3A** shows the average SC response magnitude as a function of gaze direction and presentation mode.

3.3 *Relationship between M1 excitability, autonomic arousal and person-dependent factors*

As outlined in the previous sections, direct gaze was shown to significantly enhance M1 excitability (MEP responses) as well as autonomic arousal (SC responses) upon movement observation, especially when conveyed by a live model. In order to explore the possibility that the observed eye contact-induced increases in autonomic arousal were potentially related to the eye contact-induced enhancements of M1 excitability during the observation of movements in a live two-person setting, a Pearson correlation analysis was performed taking into account the ‘eye contact effect’ score for each measure (i.e. direct minus averted difference scores, see Methods section). No significant relationship was revealed ($r = -.14$, $p = .53$; **figure 3B**).

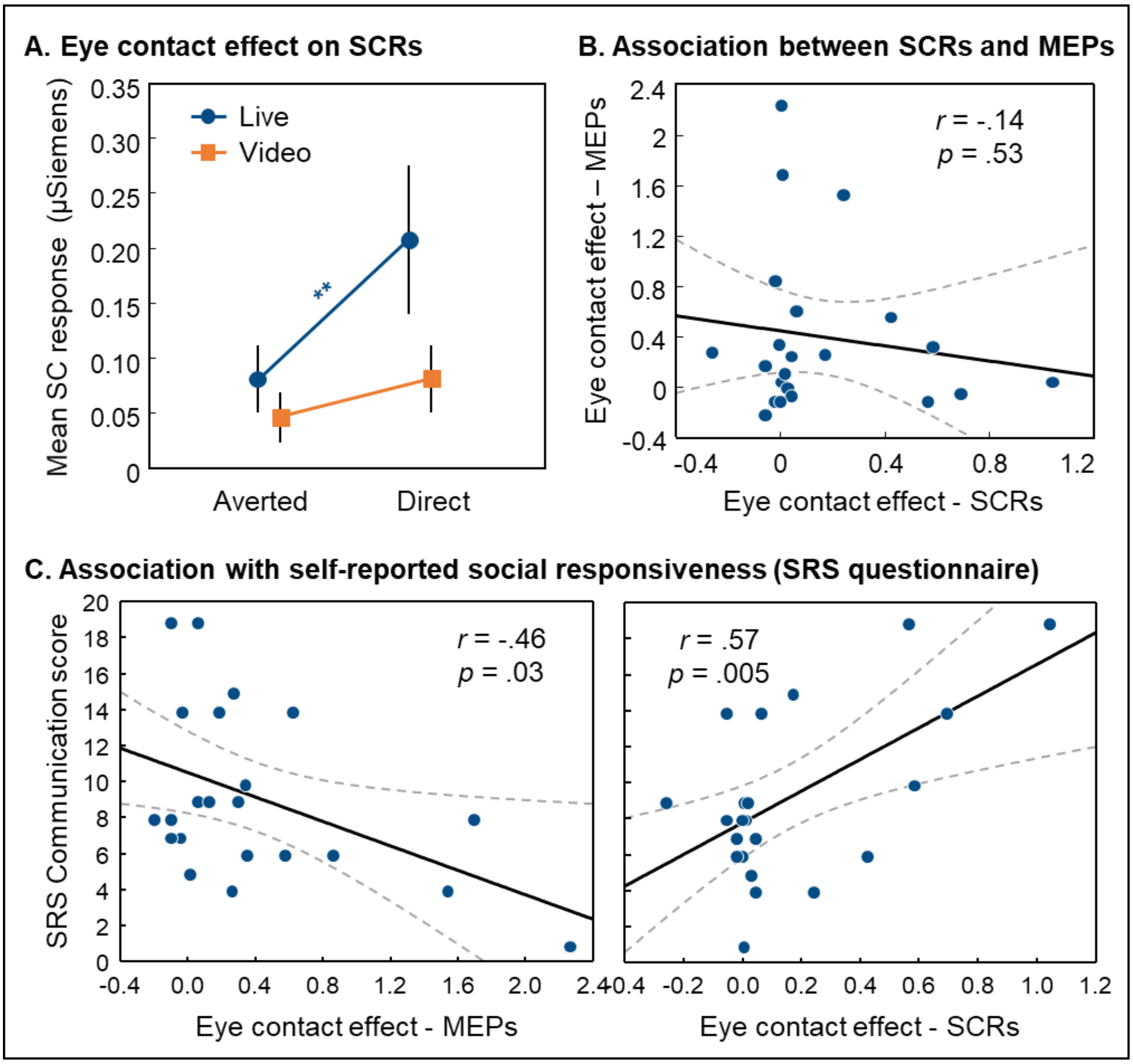

Figure 3. (A) Magnitude of the average skin conductance response per presentation mode and observed gaze direction (averaged across movement observation conditions). $^{**}p < .001$, error bars denote mean ± SE. **(B)** The relationship between the eye contact effect on M1 excitability and the eye contact effect on autonomic arousal was not significant. **(C)** Opposite modulatory effects were noted for the association between the SRS Social Communication score (higher scores denote more impairments) and the eye contact effect on M1 excitability (left panel; negative correlation) and autonomic arousal (right panel; positive correlation). Dotted lines denote 95% CI.

It was also explored whether the eye contact effect on M1 excitability and/or autonomic arousal (i.e. direct-averted difference scores for MEPs and SCRs) was associated with self-reported social responsiveness (as measured by the SRS). The analysis identified a trend towards a negative correlation between the eye contact effect on M1 excitability and the SRS total score (*r* = -.38, *p* = .08). Correlation analyses performed separately for each SRS subscale revealed that the relationship was significant for the subscale assessing Social Communication (*r* = -.46, *p* = .03; **figure 3C**) and at trend-level for the subscales assessing Social Awareness and Social Motivation (both *p* < .1; see **supplementary table 1**). Notably, opposite modulatory effects were evident for autonomic arousal; i.e. a positive association between the eye contact effect on autonomic arousal and SRS Total Score was found (*r* = .43, *p* = .04; see **supplementary table 1**). Also here, the association was most pronounced for the SRS subscale assessing Social Communication (*r* = .57, *p* = .006; **figure 3C**).

3.4 Gaze behavior

Eye tracking was performed to ascertain that participants were attentive towards the presented stimuli. A RM-ANOVA with within-subject factors presentation mode, observed gaze direction and observation condition showed no significant main or interaction effects (all *p* > .06) for the average fixation duration data, indicating that the average gaze time per fixation towards the eye region of the stimulus person's face was overall similar across conditions. A similar RM-ANOVA on the fixation count data revealed a significant main effect of observed gaze ($F(1,21) = 9.27$, $p = .006$, $\eta^2 = .31$) and movement condition ($F(1,21) = 19.79$, $p < .001$, $\eta^2 = .49$), indicating that participants made more eye movements towards the eyes AOI during direct gaze conditions and during conditions in which only the gaze cues were conveyed (without the inclusion of a hand movement) (see **figure 4**).

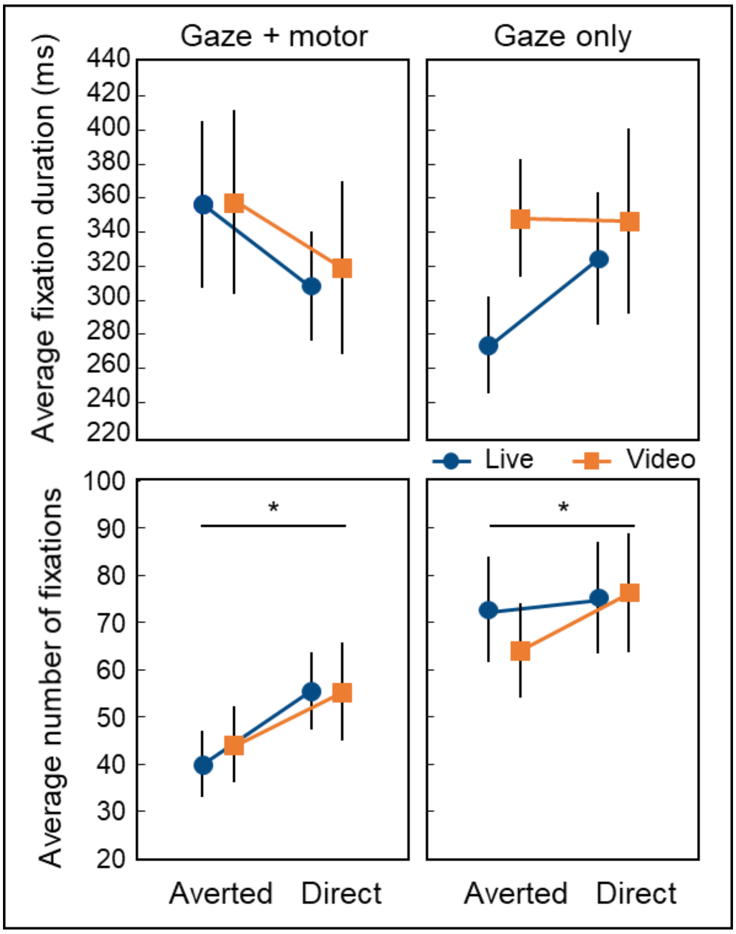

Figure 4. Effect of observed gaze direction and presentation mode on average fixation duration (upper panels) and number of fixations (lower panels) to the 'eyes' area of interest (AOI). * *p* < .05, error bars denote mean ± SE

4 Discussion

In the present study, single-pulse TMS was applied over the primary motor cortex (M1) to assess changes in M1 excitability (interpersonal motor resonance) while participants observed a live or videotaped model performing simple hand movements accompanied by direct or averted gaze. Additionally, stimuli-specific skin conductance responses (SCRs) and gaze behavior was recorded to obtain a measure of autonomic arousal and visual attention.

Compared to averted gaze, direct eye gaze conveyed within a live two-person action context was shown to enhance M1 excitability during movement observation, but not during non-movement related trials. For the screen-based video presentations, the eye contact effect was only evident at trend-level. Furthermore, and in accordance to previous mirror system research adopting TMS (Strafella & Paus 2000; Fadiga et al. 1995), the facilitatory effect of eye gaze on M1 excitability was shown to be specific to the muscle implicated in the observed movement. These findings extend results from previous behavioral studies (Wang & Hamilton, 2014; Wang, Newport, et al., 2011) and TMS studies from our lab (Prinsen et al., 2017, 2018) showing that eye contact conveyed by video stimuli facilitates automatic mirror-motor mapping of others’ actions.

While previous studies have shown that impoverished motion stimuli such as point light displays (Ulloa & Pineda, 2007) or ‘pictorial’ movement features such as seen in shadow motions (Alaerts, Van Aggelpoel, Swinnen & Wenderoth, 2009) are *sufficient* to trigger mirror-motor system activation, findings from the present study, as well as a previous MEG study (Järveläinen et al., 2001), highlight that the use of ecological valid stimuli may provide a more salient context for inducing interpersonal motor resonance. In this context, Järveläinen et al. (2001) argued that real life action paradigms are more likely to increase participant interest, attention or motivation, as they are more representative of the way in which actions are observed in daily life. This notion was also discussed in a recent review by Reader and Holmes (2016), who identified the visual fidelity or quality of the observed stimuli as one potential source of variability that might drive encountered differences between naturalistic and experimental responses. Relating to the present study, it can be envisaged that – although great care was taken to ensure that the visual information during both presentation formats was similar – larger variability in head positioning, hand and wrist stabilization movements and eye blinks might have occurred in the live presentation format compared to the video presentations. Although these sources of variability can be regarded as ‘nuisance’ factors, they are actually in favor of the ecological appearance of the action.

Another critical factor outlined by Reader and Holmes (2016), but also by Risko et al. (2012), is the social potential of the stimuli, i.e. the ability of the stimuli to provide actual interactions. They argued that whereas the use of video stimuli may reduce social interaction to the level of observation only, the mere potential for social interaction in a live two-person paradigm may already increase its social validity (see for example the study by Laidlaw, Foulsham, Kuhn & Kingstone, 2011). In the particular case of eye contact research, it has initially been suggested by Conty, George, and Hietanen (2016) and more recently also by Hietanen (2018) that responses to directly looking eyes might additionally reflect the awareness of another individual’s attention directed to the self, rather than the basic processing of visual information from the eyes of the sender (also known as the ‘watching eyes’ effect). Or, as elegantly phrased by Hietanen (2018): “images do not look back”.

Indeed, while in the current study the purely visuo-motor information in both presentation formats was overall similar, the knowledge that the self is attended to by the other person in the live presentation format might have been the decisive component for driving the enhancement of M1 excitability. Evidence in support of this view was provided by a study from Myllyneva and Hietanen (2015) investigating autonomic arousal in response to a live individual’s gaze direction in two conditions. Either the participant believed that he/she and the model were able to see each other normally, or the participant was led to believe that a half-silvered mirror was placed between him/her and the model and that the model could not see the participant. Crucially, greater arousal responses to direct compared to averted gaze were observed *only* when the participants believed that the model was able to see them.

Taken together, the current results are in line with the recent proposal of a ‘second-person neuroscience’ (Reader & Holmes, 2016; Risko et al., 2012; Schilbach et al., 2013), and promote the use of ecologically valid stimuli for investigating naturalistic social cognition. Here, the employment of realistic, contextually embedded motor acts demonstrated that mirror system engagement can be modulated by observed gaze cues. According to the ‘social top-down response modulation’ (STORM) framework by Wang & Hamilton (2012), this adjusting property of motor resonance at the level of M1 is grounded in a top-down control presumably originating from the mentalizing system and driven by an integrative evaluation of all social features in the current interaction (Wang, Ramsey & Hamilton, 2011). This framework also fits recent theoretical proposals (Vogeley, 2017; Yang et al., 2015) and meta-analytic findings (Arioli & Canessa, 2019) suggesting the joint involvement of two neural systems when processing interpersonal actions: the mirror system, responsible for the automatic processing of biological motion, and the mentalizing system, involved in inferring others’ mental states and intentions.

For the first time, the present study combined a TMS-based assessment of M1 excitability with recordings of autonomic arousal based on skin conductance responses (SCRs). Similar to previous studies, direct gaze was shown to elicit significantly higher autonomic arousal responses (Helminen et al., 2011; Hietanen et al., 2008; Pönkänen et al., 2011). Also in the present study, the eye contact effect on autonomic arousal was shown to be more salient when eye gaze was conveyed by a live model, compared to video recordings of gaze cues. Critically however, while both SCRs and MEP responses were shown to be significantly enhanced during the direct compared to the averted gaze condition, no apparent association was revealed between the two measures. This result implies that the eye-contact induced enhancements in M1 excitability were not driven by or dependent on eye contact-induced increments in autonomic arousal, a finding that is also supported by the observation that eye contact-induced changes in MEPs were only evident in trials in which the live model performed a movement, but not during trials in which the model was presented without performing a movement or in the video presentation conditions. Lastly, the absence of any significant modulatory effects in the control ADM muscle also precludes a non-specific arousal effect affecting all muscles. Broadening this notion to other measures of arousal, one other study has investigated whether the modulation of corticospinal excitability by a salient social context (moral vs. immoral actions) could be explained by non-specific pupil dilation responses. In sum, pupillary responses showed a different pattern of results, and did not correlate with MEP amplitudes (Liuzza, Candidi, Sforza & Aglioti, 2014).

While no direct relationship was evident between MEPs and SCRs, opposite associations were revealed with inter-individual differences in social responsiveness (assessed with the Social Responsiveness Scale or SRS). Specifically, participants with higher self-reported social responsiveness (lower scores on the Social Communication subscale) were shown to display stronger enhancements in M1 excitability (interpersonal motor resonance), but fewer changes in autonomic arousal upon direct eye gaze. Together, these observations suggest a complex interplay between person- and context-dependent factors, indicating that the presented social context (i.e., mutual gaze) may be experienced differently depending on individual characteristics. In line with this notion, a series of studies has linked self-report measures of empathy to mirror system activation, suggesting that participants with greater empathy may show greater motor resonance during observation of others’ actions (for a review, see Baird, Scheffer & Wilson, 2011, specific studies by Borgomaneri, Gazzola & Avenanti, 2015; Cheng et al., 2008; Gazzola, Aziz-Zadeh &

Keysers, 2006). Furthermore, previous studies adopting naturalistic gaze cues conveyed by a live model identified similar associations between heightened autonomic arousal and person-dependent factors, such as the level of social impairments in children with ASD (Kaartinen et al. 2012), and the extent of social anxiety in individuals with social anxiety disorders (Myllyneva, Ranta & Hietanen, 2015). Considering these associations, we encourage future investigations to explore how these person-dependent factors shape the effect of contextual (social) factors on adaptive mirror system functioning.

Limitations of the study should be noted. First, we did not reveal a significant main effect of ‘observation condition’ (movement, no movement), indicating that recorded MEP responses were not significantly higher during trials in which the movement was displayed, compared to trials in which only the model was presented without performing a movement. Since movement and non-movement trials were presented in a random order, and since participants were unaware of the nature of the upcoming trials, the possibility has to be considered that the use of a within-subjects protocol induced a ‘carryover’ or ‘priming’ effect, leading to similar (anticipatory) motor resonance processes during both movement and non-movement trials. One way to avoid this anticipation of movement is to adopt a between-subjects design, testing condition-specific modulations of M1 excitability in separate groups of participants (as adopted in Lagravinese et al., 2017). Nonetheless, the observation that the effect of eye gaze was only evident in terms of M1 excitability of the FDI muscle (not for the control ADM muscle) and only during movement observation trials (not for non-movement trials), affirms that gaze-related modulations of M1 excitability are condition- and muscle-specific. Further, the same female model was presented to every participant, but previous studies have shown that both the participant’s and the model’s gender may influence gaze processing (Jones et al., 2010; Slepian, Weisbuch, Adams & Ambady, 2011). Also in terms of autonomic arousal, Pönkänen et al. (2011) showed that for female participants, a significant effect of eye contact was evident for viewing female, but not male faces. Since the investigation of gender differences was beyond the scope of the present study, future research is warranted to systematically assess whether gender impacts the eye contact effect.

5 Conclusion

With the present study, we show that interpersonal motor resonance is modulated by the broader social context in which movement observation is embedded. More specifically, using a naturalistic two-person action context, we revealed that mutual eye contact significantly augmented M1 excitability during movement observation, particularly in individuals with higher self-reported social responsiveness. Importantly, the eye contact effect encompassed a muscle-specific increase in M1 excitability and was not driven by or dependent on differences in autonomic arousal or visual attention. The current findings highlight the importance and feasibility of employing stimuli with high ecological validity to investigate modulations of interpersonal motor resonance processes by subtle social cues, such as eye contact.

6 Supplements

Supplementary table 1. Means, standard deviations and Pearson correlations coefficients of self-report scores on the Social Responsiveness Scale or SRS (n = 22). Correlation coefficients assess the relationship between the SRS (subscale) scores and the ‘eye contact effect’ for M1 exitability (MEP difference score: MEP_{direct} minus $MEP_{averted}$) and autonomic arousal (SCR difference score: SCR_{direct} minus $SCR_{averted}$) in the live movement observation condition.

SRS subscale	**Mean**	**SD**	**MEP eye contact effect *Pearson r* (*p*-value)**	**SCR eye contact effect *Pearson r* (*p*-value)**
Total Score	30.86	13.48	-.38 (.08)	.43 (.04)
Social Awareness	8.82	4.87	-.39 (.07)	.27 (.27)
Social Communication	9.18	4.78	**-.46 (.03)**	**.57 (.006)**
Social Motivation	5.82	2.75	-.37 (.09)	.40 (.07)
Rigidity and Repetitiveness	7.09	3.60	-.008 (.97)	.19 (.40)

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Chapter 3

The mirror system is modulated by eye contact.

Multimodal evidence from TMS-induced motor resonance and EEG mu rhythm suppression.

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Abstract

Eye-to-eye contact is a salient cue for regulating everyday social interaction and communication. Previous research has demonstrated that eye contact between actor and observer specifically enhances the ‘mirroring’ of others’ actions in the observer, as measured by transcranial magnetic stimulation (TMS)-induced motor evoked potentials (MEPs; an index of motor cortex excitability during action observation). However, it remains unknown whether other markers of mirror system activation, such as suppression of the EEG mu rhythm suppression (i.e. attenuation of neural oscillations in the 8-13 Hz frequency band over the sensorimotor strip), are also susceptible to perceived eye contact. In the current study, a multimodal approach was adopted to assess both TMS-induced MEPs and EEG mu suppression (in separate sessions), while 32 participants (20 men; mean age: 24;8 years) observed a simple hand movement in combination with direct or averted gaze from a live stimulus person. Both indices of mirror system functioning were significantly modulated by perceived eye gaze; showing a significant increase in MEP amplitude and a significant attenuation of the mu rhythm when movement observation was accompanied with direct compared to averted gaze. Importantly, while inter-individual differences in absolute MEP and mu suppression scores were not significantly related, a significant association was identified between gaze-related changes in MEP responses and mu suppression. As such, it appears that while the neurophysiological substrates underlying mu suppression and TMS-induced MEP responses differ, both are similarly affected by the modulatory impact of gaze-related cues. In sum, our results suggest that both EEG mu rhythm and TMS-induced MEPs are sensitive to the social relevance of the observed actions, and that a similar neural substrate may drive gaze-related changes in these distinct markers of mirror system functioning.

1 Introduction

Ever since the discovery of ‘mirror neurons’ in the macaque brain, firing not only when the monkey executes a motor action, but also when the monkey merely observes another individual performing that action (di Pellegrino, Fadiga, Fogassi, Gallese & Rizzolatti, 1992), the description of a homologous action observation-execution matching or ‘mirror’ system and its properties in humans has been a topic of increasing interest. Accordingly, a variety of neuroimaging and electrophysiological techniques – including functional magnetic resonance imaging (fMRI), magneto- or electroencephalography (M/EEG), and transcranial magnetic stimulation (TMS) – have been adopted to identify patterns of ‘mirror system’ activity during action observation.

One commonly used method is TMS, a non-invasive brain stimulation technique that activates cortical neurons via the administration of a brief magnetic pulse to the scalp. When TMS is administered over the somatotopically organized primary motor cortex (M1), it induces an involuntary muscle contraction or motor-evoked potential (MEP) in the corresponding peripheral muscles (measured with electromyography; EMG), of which the peak-to-peak amplitude reflects variations in M1 excitability. In a seminal study, Fadiga, Fogassi, Pavesi, and Rizzolatti (1995) showed that TMS-evoked MEP amplitudes within the stimulated muscles are specifically enhanced during the observation of others’ movements. Subsequent TMS studies have confirmed these observations (for a review, see Fadiga, Craighero & Olivier, 2005), and provided evidence that the human observation-to-execution matching mechanism is specific to the muscles recruited in the observed actions (Alaerts, Heremans, Swinnen & Wenderoth, 2009; Alaerts, Swinnen & Wenderoth, 2009; Fadiga et al., 1995; Strafella & Paus, 2000), with a close temporal coupling (Gangitano, Mottaghy & Pascual-Leone, 2001).

Another commonly adopted method for investigating mirror system activity relates to the assessment of oscillations in the EEG-based mu rhythm, usually defined in the 8-13 Hz frequency band and topographically centered over the sensorimotor regions of the brain (i.e. electrode positions C3, Cz, and C4 according to the 10-20 international system of electrode placement). At rest, sensorimotor neurons fire in synchrony, leading to high mu power. When a person performs, observes or imagines themselves performing an action, the firing of these neurons has been shown to become increasingly desynchronized, leading to a task-induced suppression of the mu rhythm (Muthukumaraswamy, Johnson & McNair, 2004; Pfurtscheller, Brunner, Schlögl & Lopes da Silva, 2006, see Fox et al., 2016 for a meta-analysis). The notion that decreased mu power is related to sensorimotor activation received overall support from EEG-fMRI studies, showing a negative relationship between mu power and the BOLD signal in brain areas considered part of the mirror system (Arnstein, Cui, Keysers, Maurits & Gazzola, 2011; Braadbaart, Williams & Waiter, 2013; Perry & Bentin, 2009). Also several MEG studies, having superior spatial resolution compared to EEG, have shown that sensorimotor cortices are significantly modulated by action observation and execution (Hari et al., 1998; Järveläinen, Schürmann, Avikainen & Hari, 2001).

While the exact role of the mirror system in human social cognition is still a matter of debate, it is generally assumed that the simulation of observed actions in the observer's own motor system contributes to action recognition and understanding, including related socio-cognitive processes that are important for everyday social interaction such as imitation/mimicry, motor planning and gestural performance (Rizzolatti & Craighero, 2004). The mirror system has also been implicated to be involved in higher-order mentalizing processes, such as inferring others' intentions (for a review, see Rizzolatti & Fabbri-Destro, 2008; and specific studies by Becchio et al., 2012; Iacoboni et al., 2005), as well as empathy (a form of 'emotional' imitation; Iacoboni, 2009). One highly powerful cue for driving interpersonal communications and for conveying (social) intentions is eye contact. Whereas perceived direct gaze from others is indicative of their communicative intent, observed averted gaze signals that the attention of others is directed elsewhere. Accordingly, perceiving the gaze of others has been shown to influence several socio-cognitive processes and behavioral responses in the observer (for relevant reviews, see Conty, George & Hietanen, 2016; Hietanen, 2018; Senju & Johnson, 2009). In terms of mirror system activity, several TMS studies have shown that under various experimental conditions, perceived communicative intent from the actor (conveyed by different gaze cues) significantly modulates M1 excitability (MEP responses) in the observer (Betti et al., 2019; Prinsen & Alaerts, 2019; Prinsen et al., 2017; Prinsen, Brams & Alaerts, 2018). To date however it remains unexplored whether suppression of the EEG mu rhythm upon action observation is similarly modulated by social context or communicative intent (i.e. as conveyed by eye contact).

In this respect, it is worth noting that while both TMS and EEG techniques have been widely adopted to investigate observation-to-execution mapping processes, the direct relationship between facilitation of M1 excitability (as assessed with TMS) and suppression of mu rhythm (assessed with EEG) upon action observation is not well established. In one study, Andrews, Enticott, Hoy, Thomson, and Fitzgerald (2015) revealed a significant positive relationship between concurrent recordings of observation-induced mu suppression and M1 excitability in schizophrenia patients and healthy controls. In contrast however, Lepage, Saint-Amour and Théoret (2008) simultaneously recorded M1 excitability and mu suppression during action observation, imagination and execution of simple hand actions in healthy adult participants and showed that while both measures were significantly modulated by the experimental conditions (increased M1 excitability, increased mu suppression), both measures were not significantly correlated at the inter-individual subject level. Similarly, in two recent studies assessing observation-induced changes in M1 excitability and mu suppression, either simultaneously (Cole, Barraclough & Enticott, 2018) or in different recording sessions (Lapenta, Ferrari, Boggio, Fadiga & D'Ausilio, 2018), no direct relationship was revealed between the two measures. Accordingly, it has been suggested that M1 excitability and mu suppression may represent different aspects of the mirror system, presumably due to the different spatial and temporal characteristics of the two techniques. Indeed, while EEG mu suppression indexes the sum of post-synaptic neuronal activity over a large area (not restricted to M1) over a relatively long time period (typically > 1 second), TMS assesses changes in M1 excitability by stimulating a relatively small population of neurons (at the level of M1) at a discrete time point (Andrews et al., 2015; Pineda, 2005; Rossini et al., 1994).

Within the present study we adopted a multi-modal approach for assessing gaze-related modulations of mirror system activity, by recording both TMS-induced MEP responses and EEG mu rhythm suppression upon movement observation with variable communicative intent. In particular, the two mirror system indices were recorded in separate sessions while participants observed simple intransitive hand actions accompanied by either direct gaze (signaling communicative intent) or averted gaze from the actor. Based on the findings of Prinsen and Alaerts (2019), a naturalistic two-person paradigm was adopted, incorporating a 'live' stimulus person to convey the gaze and movement cues. In line with previous TMS studies demonstrating an effect of observed eye contact on the mirroring of others' actions (Prinsen & Alaerts, 2019; Prinsen et al., 2017, 2018), it was hypothesized that TMS-induced MEPs are enhanced upon movement observation accompanied with direct eye gaze, compared to movement observation accompanied with averted gaze. A key question was to assess whether suppression of the mu rhythm is also susceptible to a top-down response modulation by perceived communicative intent (i.e. eye contact); and whether eye contact-induced changes in M1 excitability are associated with changes in EEG mu suppression. Lastly, since the central mu rhythm oscillates in the same 8-13 Hz frequency band and displays similar response properties as occipital alpha rhythms (i.e. dominant when at rest, suppressed by perceptual events and attentional processing), an important issue in EEG action observation studies is the potential contamination of the mu rhythm by changes in alpha. In line with recent guidelines by Fox et al. (2016) and Hobson and Bishop (2017), occipital alpha suppression was also taken into account.

2 Method and Materials

2.1. Participants

A total of 32 individuals (20 men and 12 women) aged between 18 and 36 years old (mean ± SD: 22;9 ± 3;7 years; months) participated in this study. All participants were right-handed, which was confirmed with the Edinburgh Handedness Questionnaire (EHQ; Oldfield, 1971). Exclusion criteria comprised medication use, any diagnosed psychiatric (e.g. ASD, ADHD) or neurological disorder (e.g., stroke, epilepsy, concussion), left handedness or any contraindication for TMS (Rossi, Hallett, Rossini & Pascual-Leone, 2012). Written informed consent was obtained from all participants prior to the experimental procedure. Ethical approval for the experimental protocol was granted by the local Ethics Committee for Biomedical Research at the University of Leuven in accordance to the Declaration of Helsinki (World Medical Association, 2013).

2.2. Experimental protocol and stimuli

Participants were seated at a distance of approximately 80 cm from a 20 × 30 cm voltage-sensitive liquid crystal (LC) shutter screen (DreamGlass Group, Spain) attached to a black frame and were instructed to observe and pay close attention to the presented stimuli. A 'live' female stimulus person (experimenter J.P.) was seated behind the panel (similar set-up as Prinsen & Alaerts, 2019). During the experimental conditions, the stimulus person's face was presented through the LC shutter screen for 4 seconds. Importantly, the stimulus person was unknown to the participants and only briefly interacted with them before the experimental procedure. While the LC screen was transparent, the stimulus person either gazed directly towards the observing participant (i.e. engaging in mutual eye contact) or displayed a gaze 30° to the right (i.e. showing averted gaze). During both gaze conditions, the stimulus person held her right hand horizontally beneath her face with the dorsal side directed to the participants and performed a simple index finger abduction movement. The stimulus person bore a neutral expression and tried to avoid eye blinks during the duration of the trial. An illustration of the experimental conditions is provided in **figure 1A**.

Each gaze condition was presented 20 times in 4 second trials with an inter-stimulus-interval of 2 seconds, during which the shutter remained opaque (similar set-up as Prinsen & Alaerts, 2019). The same stimulus protocol was adopted for the TMS and EEG assessment. In order to ensure that all participants viewed and attended the stimuli properly, participants were asked once at a random time point during each neurophysiological assessment (in-between trials) to verbally report the stimulus that was presented in the previous trial. Participants were able to correctly report the presented stimulus in 98.5% of the assessments, indicating that the participants attended the stimuli properly.

Mirror system activity was investigated in two assessment sessions conducted on the same day, with a fifteen minute break between sessions. In one session, stimulus presentation was accompanied with transcranial magnetic stimulation (TMS) in order to assess excitability at the level of the primary motor cortex. In the other session, electroencephalography (EEG) assessments were performed in order to measure mu rhythm suppression. The order of assessment method (TMS or EEG) was counterbalanced across participants.

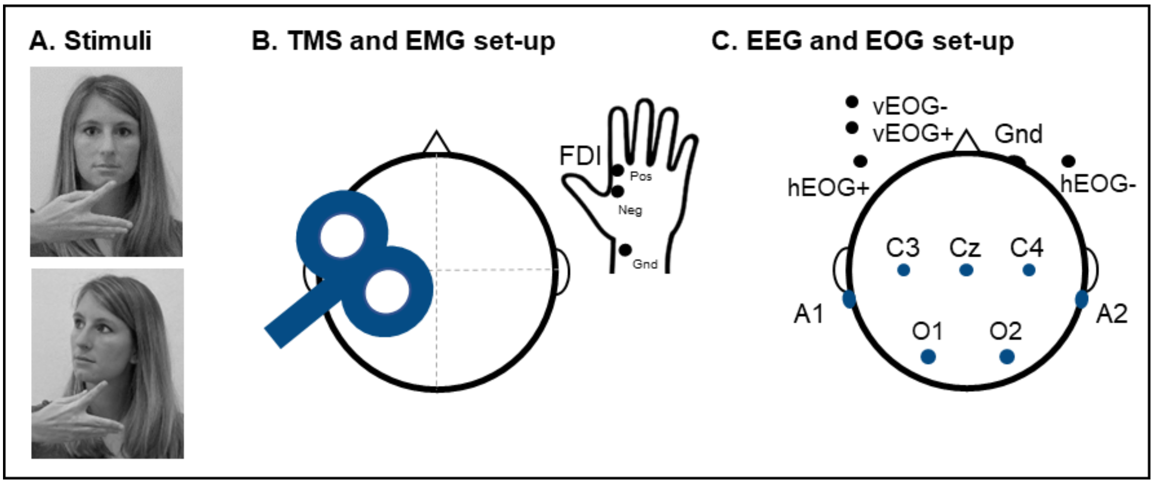

Figure 1. (A) Illustration of the stimuli, showing a live stimulus person conveying direct or averted gaze while performing a simple finger abduction movement. **(B)** MEPs induced by TMS over the left primary motor cortex were recorded from EMG electrodes located on the FDI index finger muscle of the right hand. **(C)** Continuous EEG was acquired from electrode sites C3, Cz and C4 to calculate mu suppression, and sites O1 and O2 for alpha suppression.

2.3. Neurophysiological assessment

2.3.1. TMS and EMG recordings

The TMS and EMG electrode set-up is illustrated in **figure 1B**. During observation of the stimuli, single-pulse TMS was administered over the primary motor cortex (M1) with a hand-held 70 mm figure-of-eight coil (oriented approximately 45° relative to the mid-sagittal line) and a Magstim-200 stimulator (Magstim Company Ltd., UK). Optimal coil location for the experimental TMS-stimulation was determined as the site that produced maximal responses (i.e. MEPs) while at rest (“hotspotting”) in the contralateral first dorsal interosseous (FDI) muscle, a muscle implicated in the to-be-observed index finger opening movement. Resting motor thresholds (rMT) were individually defined as the lowest stimulation intensity that produced a peak-to-peak MEP of at least 50 µV in five out of ten consecutive trials (Rossini et al., 1994). Experimental stimulation intensity was set at a supra-threshold of 130% of the subject's rMT. In each trial, a single TMS pulse was delivered on the third second of stimulus presentation, which coincided with the execution of the index finger opening movement of the model.

Surface electromyographic (EMG) recordings were obtained using disposable adhesive electrodes arranged in a tendon-belly montage. The EMG signal was sampled at 2 kHz, band-pass filtered (5-1000 Hz) and analyzed offline. Signal software (version 6.02, Cambridge Electronic Design, UK) and a CED Power 1401 analog-to-digital converting unit (Cambridge Electronic Design, UK) were used for EMG-recordings, triggering of the TMS-stimulator and shifting of the LC window from an opaque to transparent state.

2.3.2. EEG data acquisition

The NeXus-32 multimodal acquisition system and BioTrace+ software (version 2015a, Mind Media, The Netherlands) were used to collect electroencephalography (EEG) recordings. Continuous EEG was recorded with a cap with 22 sintered Ag/AgCL embedded electrodes (MediFactory, The Netherlands), incorporating 19 EEG channels configured according to the international 10-20 system of electrode placement, two reference electrodes located on the left and right mastoid bones behind the ear (A1 and A2), and a AFz ground electrode. The EEG signal was amplified using a unipolar amplifier and mathematically referenced offline to linked mastoids. Gentle skin abrasion and electrode paste (combination of electrolytic NuPrep gel and conductive 10-20 paste) were used to reduce electrode impedances below 10 kΩ. Eye movements as well as eye blinks were monitored using two pairs of bipolar electro-oculogram (EOG) electrodes, one pair attached to the external canthi of each eye (horizontal eye movements; hEOG) and one pair attached below and above the left eye (vertical eye movements; vEOG). The sampling rate of the recordings was 256 Hz. E-Prime 2.0 software (Psychology Software Tools Inc., USA) and the NeXus Trigger Interface (NTI, 2048 Hz sample rate; Mind Media, The Netherlands) were used to synchronize stimulus events with the NeXus-32 EEG recordings and the triggering of the LC window.

2.4. Data handling and preprocessing

2.4.1. TMS-induced MEPs

Based on the recorded EMG data, peak-to-peak amplitudes of the TMS-induced MEPs were determined using in-house MATLAB scripts (version R2015a, MathWorks Inc., USA). Additionally, background EMG was quantified by calculating the root mean square (RMS) across the 110 to 10 millisecond interval prior to TMS-stimulation. For a given subject, trials with excessive pre-TMS tonic muscle activity (background EMG exceeding 2.5

standard deviations from the mean) were excluded from analysis (1.95% for the FDI). Trials with extreme MEP-amplitudes (exceeding 1.5 interquartile distances from the mean) were also discarded. This resulted in an additional omission of 8.98% of all trials. The total number of discarded trials was similar across gaze conditions (all $p > .11$). MEP peak-to-peak amplitudes were log-transformed to conform to normality.

2.4.2. EEG mu/alpha suppression calculation

Two participants were excluded from the final analysis due to technical malfunctions of the NeXus Trigger Interface, used for time-locking EEG data with the stimulus presentation. EEG data of the remaining participants ($n = 30$) were preprocessed and analyzed offline using BrainVision Analyzer 2 software (version 2.2, Brain Products GmbH, Germany). The raw EEG signal was filtered using a 0.5 - 40 Hz IIR band-pass filter with zero phase shift (Butterworth, 24 dB). Taking into account the vEOG and hEOG channels, deflections resulting from eye blinks and horizontal eye movements were removed by the implemented Independent Component Analysis (ICA) module in BrainVision Analyzer 2 (Jung et al., 2000). Cleaned EEG data were segmented separately for each condition into segments of 4 seconds, corresponding to the duration of the trial. Segments with residual artifacts exceeding ± 100 µV in amplitude were rejected. Note that one additional participant was removed from the final analyses due to excessive artifacts (> 60% of artifactual segments). For each segment, the spectral power (μV^2) in the 8-13 Hz range was computed using the Fast Fourier Transform (FFT) with a resolution of 0.25 Hz (including a Hanning window with an attenuation domain of 25%). Obtained power values were then averaged separately for each experimental gaze condition and electrode.

Suppression indices were computed at three central sites (C3, Cz and C4) located over the sensorimotor strip where mu rhythm modulations are expected. To assess the spatial specificity of the gaze-dependent modulations in the mu rhythm, alpha suppression indices were also calculated for occipital electrodes O1 and O2 (**figure 1C**). Mu and alpha suppression indices for each electrode were calculated as the log-transformed ratio of the 8-13 Hz band power during the 4-second trials relative to the power of a 1-second interval prior to the start of the trial (baseline). Log ratios lower than zero indicate suppression.

2.5. Data analysis and statistics

In order to investigate eye contact-induced changes in M1 excitability, a repeated measures analysis of variance (RM-ANOVA) with within-subject factor ‘observed gaze’ (averted, direct) was performed on the MEP peak-to-peak amplitude data. For the EEG data; it was first tested whether all action observation conditions elicited a significant suppression relative to the pre-trial baseline segments (as recommended by Hobson & Bishop, 2017); i.e. ratio values were tested using single-sample *t* tests against a value of 0, separately for each gaze condition. The mu and alpha suppression indices were analyzed separately using a RM-ANOVA with the within-subject factors 'observed gaze' (averted, direct) and 'electrode' (mu: C3, Cz and C4; alpha: O1 and O2). For the RM-ANOVAs, the categorical factor 'session' was included as effect-of-no-interest to account for potential effects of counter-balancing the two assessment sessions (i.e. TMS or EEG first). Significant interaction effects were further investigated by means of Fisher Least Significant Differences (LSD) post-hoc tests, the partial Eta square (η^2_p) value was calculated as an estimate of effect size.

In order to directly investigate the relationship between the effects of eye contact on the different measures, the 'eye contact effect' was quantified for each subject as the percentage change (%change) in the direct gaze condition relative to the averted gaze condition (similar approach as Enticott, Kennedy, Bradshaw, Rinehart & Fitzgerald, 2011):

$$\%\text{change} = \left[\frac{\text{response}_{\,direct\ gaze} - \text{response}_{\,averted\ gaze}}{\text{response}_{\,averted\ gaze}}\right] \times 100$$

Higher mirror responses during the direct versus averted gaze condition are indicated by a positive %change score for MEPs, and a negative %change score for mu and alpha suppression indices. Pearson correlation analyses were performed to assess the association between the measures. For all performed correlations, the Cook's distance metric was used to identify influential data points (defined as Cook's $D > 1$), but none were detected. The coefficient of determination (R^2) is reported as an estimate of effect size. All statistics were calculated with Statistica 10 (StatSoft, USA). Results were considered significant with a *p*-value lower than .05.

3 Results

3.1. MEP results

A RM-ANOVA with within-subject factor observed gaze (averted, direct) was performed on the MEP data to investigate the effect of observed gaze on M1 excitability. The mean (log-transformed) MEP peak-to-peak amplitudes for each gaze condition is presented in **figure 2A**. In line with our hypothesis, a significant main effect of perceived gaze direction ($F(1,31) = 9.53$, $p = .005$, $\eta^2_p = .24$) was revealed. Thus, in accordance with previous TMS studies investigating the eye contact effect on M1 excitability (Prinsen & Alaerts, 2019; Prinsen et al., 2017, 2018), MEPs recorded from the FDI muscle were significantly higher when movement observation was accompanied with direct eye gaze from the stimulus person (mean: 0.231, SD: 0.356), compared to averted gaze (mean: 0.161, SD: 0.389). Note that no significant main or interaction effects were found for the categorical ‘session’ factor-of-no-interest (all $p > .42$).

3.2. EEG results

Significant decreases in mu power with respect to the included rest condition were encountered for each electrode and gaze condition (single sample *t*-tests against 0: all $p < .001$; see **table 1**), signaling that all gaze conditions induced an overall significant suppression of the mu rhythm during movement observation in the central electrodes.

A RM-ANOVA with observed gaze condition (averted, direct) and electrode (C3, Cz, C4) as within-subject factors revealed a significant main effect of observed eye gaze ($F(1,26) = 6.97$, $p = .01$, $\eta^2_p = .21$), but no gaze × electrode interaction ($F(2,52) = 2.72$, $p = .07$, $\eta^2_p = .09$). This shows that, irrespective of electrode, mu rhythm suppression upon movement observation was more pronounced during the direct versus the averted gaze condition (see **figure 2B**). Also a significant main effect of electrode was revealed ($F(2,52) = 6.81$, $p = .002$, $\eta^2_p = .21$), indicating that, irrespective of gaze condition, mu rhythm suppression was slightly more pronounced in electrode Cz (see **figure 2B** and **table 1**). Although one would expect mu suppression to be strongest for electrode C3, as participants were required to observe a right-hand movement, findings regarding hemispheric lateralization of mu suppression during action observation are quite inconsistent (see e.g. Angelini et al., 2018).

Also alpha activity from occipital electrodes O1 and O2 was significantly suppressed during all gaze conditions compared to rest (all $p < .001$; see **table 1**). Importantly however, a comparable RM-ANOVA as described above did not indicate a significant main effect of observed eye gaze (*F*(1,25) = 1.90, *p* = .18, η^2_p = .07) or electrode (*F*(1,25) = 1.53, *p* = .23, η^2_p = .06) for these occipital electrodes, nor an electrode × gaze interaction (*F*(1,25) = 0.08, *p* = .78, , η^2_p = .003; **figure 2C**). During movement observation, occipital alpha suppression was thus not significantly modulated by the observed gaze cues.

Table 1. Mean central mu and occipital alpha suppression (in 8-13 Hz frequency band) for each gaze condition (averted, direct) and electrode. *******p* < .001

	Averted gaze		**Direct gaze**	
	Mean (SD)	***t* against 0**	**Mean (SD)**	***t* against 0**
Central electrodes				
C3	-0.661 (0.131)	-26.71**	-0.684 (0.106)	-33.98**
Cz	-0.711 (0.128)	-29.78**	-0.758 (0.134)	-30.33**
C4	-0.687 (0.121)	-30.69**	-0.711 (0.091)	-42.02**
Occipital electrodes				
O1	-0.762 (0.176)	-22.91**	-0.781 (0.177)	-23.32**
O2	-0.744 (0.177)	-22.32**	-0.748 (0186)	-21.19**

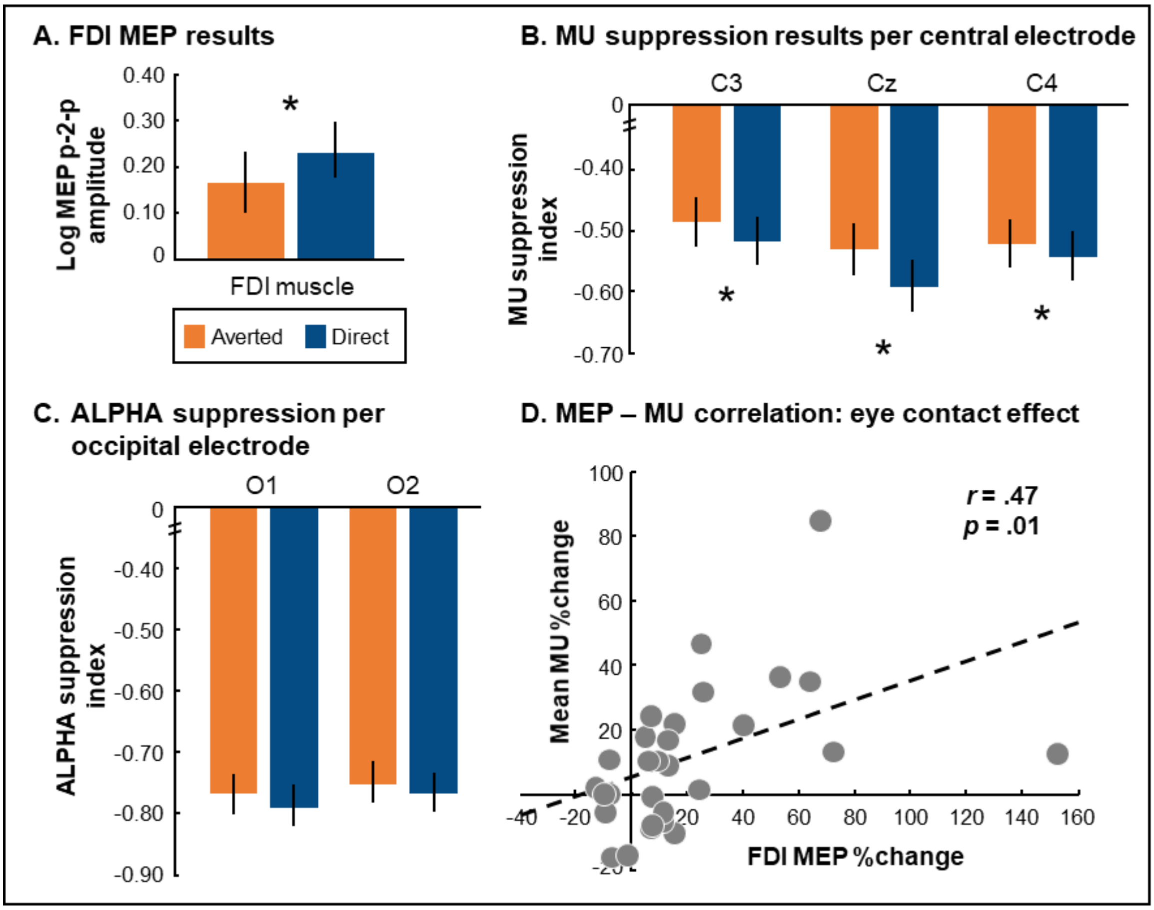

Figure 2. (A) Significant effect of perceived eye gaze (direct, averted) on log-transformed MEP peak-to-peak amplitude in the FDI **(B)** and mu suppression indices per central electrode**.** **p* < .05, vertical error bars denote mean ± SE. **(C)** The effect of perceived eye gaze on alpha suppression indices was not significant, for any occipital electrode. **(D)** A significant positive correlation was found between the eye contact effect on M1 excitability and the eye contact effect on mu suppression.

3.3. TMS-EEG correlations

The relationship between FDI MEP amplitudes and mu suppression over the central electrodes (averaged score across electrodes) was evaluated by means of Pearson correlations, separately for each gaze condition. No significant correlation was revealed between mu suppression over central electrodes and MEP amplitude in terms of absolute responses for either the direct gaze condition ($r(29) = -.18$, $p = .36$, $R^2 = .03$), nor for the averted gaze condition ($r(29) = -.15$, $p = .43$, $R^2 = .03$). Note that the absence of a significant association persisted when only mu suppression over electrode C3 (contralateral to right-hand MEPs and corresponding to the site of TMS stimulation) was considered (averted gaze: $r(28) = -.06$, $p = .73$, $R^2 = .005$; direct gaze: $r(28) = .01$, $p = .95$, $R^2 < .01$)

Interestingly however, it was shown that for the experimental ‘eye contact effect’ (see section 2.5 in Method and Materials), 22% of the variance was shared between the TMS and EEG measures, indicating that increments in MEP amplitude in response to direct gaze were significantly associated with similar enhancements of mu suppression ($r(29) = .47$, $p = .01$, $R^2 = .22$; **figure 2D**). Importantly, this association was specific to the central electrodes, as MEPs were not significantly correlated to alpha suppression indices over occipital electrodes, either in terms of absolute responses (averted gaze: $r(29) = -.21$, $p = .26$, $R^2 = .04$; direct gaze: $r(29) = -.19$, $p = .32$, $R^2 = .04$), or in terms of the experimental ‘eye contact effect’ ($r(29) = .15$, $p = .43$, $R^2 = .02$).

4 Discussion

This study aimed to investigate the impact of observed gaze cues on TMS- and EEG-based measures of mirror system activity. In agreement with previous studies (Prinsen & Alaerts, 2019; Prinsen et al., 2017, 2018), we showed that motor cortex (M1) excitability assessed as TMS-induced MEPs upon action observation was significantly impacted by observed gaze direction from the actor. Furthermore, we demonstrated – for the first time – that also EEG-based mu suppression (in the 8-13 Hz frequency band) over the sensorimotor strip (electrodes C3, Cz, C4) was enhanced when observing direct, compared to averted eye gaze from the actor. Importantly, while absolute MEP and mu suppression scores were not significantly related, a significant association was identified between gaze-related changes in M1 excitability and mu suppression.

The observation that both TMS-induced MEPs and EEG-based mu suppression are modulated by observed gaze cues is in line with the recent notion that interpersonal motor resonance is not a static process, but is adapted depending on the social context in which the observed movements are embedded. According to these theoretical proposals, the social modulation of motor resonance processes is thought to arise from a top-down control by the mentalizing system (Vogeley, 2017; Wang & Hamilton, 2012; Yang et al., 2015). Other TMS studies provide further evidence for this social modulation, indicating that M1 excitability is also modulated by other social factors, such as, amongst others, emotional body language of the actor (Borgomaneri, Vitale, Gazzola & Avenanti, 2015), social reciprocity (Sartori, Cavallo, Bucchioni & Castiello, 2011, 2012), and the level of observed social interaction (Donne, Enticott, Rinehart & Fitzgerald, 2011; Hogeveen & Obhi, 2012). Similarly, EEG activity in the mu frequency range has been demonstrated to depend on the extent by which participants are engaged in a social game (Perry, Stein & Bentin, 2011), the perception of social information such as intentions and emotions (Perry, Troje & Bentin, 2010) and empathic processes (Hoenen, Schain & Pause, 2013). Taken together, this subtle and sophisticated adjustment of motor resonance according to the demands of the social context forms an essential competence of humans for flexibly engaging in interpersonal social interactions (Wang & Hamilton, 2012).

A second objective of the current research was to further disentangle the relationship between TMS- and EEG-based measures of mirror system functioning at the inter-individual subject level, as previous studies (Andrews et al., 2015; Cole et al., 2018; Lapenta et al., 2018; Lepage et al., 2008) provided an unclear pattern of results. In terms of absolute responses, we were unable to establish an association between these two measures. This is in accordance with several previous studies who have directly compared mu suppression and M1 excitability in healthy participants, either by adopting passive observation of simple hand actions (Lepage et al., 2008) or goal-directed grasping movements (Lapenta et al., 2018). One additional study, incorporating a mentalizing task to infer others' intentions in adults with and without an autism spectrum disorder also failed to demonstrate a relationship between these measures (Cole et al., 2018).

On the one hand, this lack of a significant association between absolute mu suppression scores and MEP responses may relate to the substantial differences in neurophysiological underpinnings and temporo-spatial properties between these measures (i.e. induced activation of a small population of M1 neurons recorded at peripheral muscles at a discrete time point versus summed post-synaptic electrical activity from a broad population of neurons over a relatively long time period). In other words; MEP recordings of mirror system functioning during TMS are obtained at the level of the muscle, reflecting corticospinal processes, whereas the EEG mu rhythm mainly reflects central cortical activity. Although both techniques have been shown to reliably capture mirror system activation (see reviews by Fadiga, Carighero & Olivier, 2005; Hobson & Bishop, 2017), it has been suggested that - considering these substantial differences in neurophysiological underpinnings - both techniques might target different aspects of the mirror system. In this respect, the neural processes triggered by action observation have been proposed to be layered in several hierarchically organized functional levels (Grafton & Hamilton, 2007; Kilner, Friston, & Frith, 2007). These proposed levels are (i) the muscular level (decoding the pattern of muscle activity necessary to perform the action); (ii) the kinematic level (mapping the effector movement in time and space); (iii) the aim level (including transitive or intransitive short-term goals); and (iv) the intention level (regarding the long-term purpose of the action).

Without explicitly framing their design or results within this theoretical structure, Cole et al. (2018) demonstrated that higher mu suppression was associated with superior mentalizing performances, whereas TMS-induced MEPs showed no differences associated with mentalizing. These findings might suggest that the EEG mu rhythm is able to capture higher-order processes such as intentions, but MEPs are not. Note however that Cole et al. (2018) opted to deliver the TMS pulse *after* the completion of the video clips conveying the intentions of the actor (i.e. not taking the strict temporal coupling for M1 excitability into account). Furthermore, their results contrast a previous study by the same group that investigated mirror system activation during observation of intransitive, transitive and interacting hand movement stimuli in adults with and without schizophrenia (Andrews et al., 2015). This work revealed a positive association between absolute mu suppression and M1 exitability when averaged across all conditions with biological movement. In sum, future work is necessary to obtain complementary information with respect to this hierarchical organization in terms of absolute responses.

Interestingly, while no direct associations were evident between absolute mu suppression scores and MEPs, it was shown that direct gaze-related increments in MEP amplitude were paralleled by similar enhancements of mu suppression, as indicated by a significant positive relationship of moderate strength between the ‘eye contact’ effect in the EEG and TMS measures. The relationship between gaze-related changes in both measures is an important finding, since it provides initial indications that the two methods do capture similar flexible changes of these underlying neural processes in response to condition-specific manipulations or contexts (e.g. such as presentation of socio-communicative cues). These flexible changes across neurophysiological markers can be considered to reflect a similar gating mechanism, whereby the processing of irrelevant stimuli is inhibited in order to better process socially relevant stimuli (Anderson & Ding, 2011; Kilner, Marchant & Frith, 2006). As such, while the neural correlates underlying absolute MEP and mu suppression scores may not be the same, it appears that the neural regions involved in processing gaze related cues, i.e. superior temporal sulcus (Pelphrey, Viola, & McCarthy, 2004) or associated regions of the mentalizing network (Kampe, Frith, & Frith, 2003), exert a similar modulating impact on the (distinct) neurophysiological substrates that drive mu suppression or TMS-induced MEP responses upon movement observation.

There are several methodological considerations to be taken into account when evaluating the EEG mu rhythm. For an in- depth discussion, the interested reader is referred to reviews by Hobson and Bishop (2017) and Cuevas, Cannon, Yoo, and Fox (2014). Here, we briefly touch upon some relevant issues relevant that motivated our adopted design.

First, given the fact that the mu and alpha rhythms oscillate in the same frequency band and show similar response properties (Hobson & Bishop, 2016, 2017), we also inspected alpha suppression at the occipital electrodes (O1 and O2). Significant alpha suppression was present during action observation, suggesting that an attentional component might have been at play during the observation of the different stimuli. It is however important to note that, in contrast to the central mu rhythm, the occipital alpha rhythm was not subjected to gaze-related modulations (i.e. alpha suppression was not significantly stronger during direct vs. averted gaze at occipital electrodes). Furthermore, only eye-contact induced changes in mu suppression indices, but not alpha suppression indices, were significantly associated with eye-contact induced changes in MEPs. In this respect we believe that the aforementioned observations highlight the specificity of the mu rhythm in reflecting action-specific mirroring processes, as opposed to reflecting contamination or volume conduction from occipital sites. In line with this notion, Debnath, Salo, Buzzell, Yoo and Fox (2019) recently showed that while both central mu and occipital alpha rhythms are indeed similarly suppressed during action observation, phase synchrony was only evident between central-occipital areas, but not between neighboring occipital-parietal and central-parietal electrodes. These results exclude the possibility of a general spread of occipital alpha activity due to volume conduction, but suggest that visuospatial attention (indexed by occipital alpha) and sensorimotor mirroring (indexed by central mu) are functionally distinct but highly coordinated processes during action observation (see Debnath et al., 2019 and Fox et al., 2016 for more detailed hypotheses).

Secondly, similar to Lapenta et al. (2018), the current study assessed TMS and EEG related mirror system activity within two separate sessions, whereas the majority of previous studies have used TMS and EEG simultaneously (Andrews et al., 2015; Cole et al., 2018; Lepage et al., 2008). While concurrent recording may allow for a more direct comparison between both indices, the application of magnetic pulses induces artifacts in the simultaneously recorded EEG signals. It is therefore necessary to specifically exclude the time window that overlaps with the deliverance of the TMS pulse, which is preferably optimized for the action observation scene. As such, some crucial time windows for eliciting mu suppression may be removed.

Lastly, as the key design feature of mu suppression studies is the comparison of an experimental condition to a baseline condition in which one would not expect mirror system activity, the choice of baseline condition has a substantial impact. Ideally, one collects a baseline period just prior to the period of interest (the onset of action), that is identical to the experimental condition, except for this event of interest (Hobson & Bishop, 2016; Tangwiriyasakul, Verhagen, Van Putten & Rutten, 2013). However, the associative property of the mirror system might pose difficulties for establishing an optimal baseline condition (note that this is not limited to EEG, but also applies to other modalities in action observation research). Although theoretically speaking mirror system activity would be greatest during action observation, the mere presence of an interactive agent (or object) may elicit early anticipatory reactivity, especially in a design with multiple repetitions (Cuevas et al., 2014). For example Southgate, Johnson, Osborne and Csibra (2009) have demonstrated anticipatory mu suppression prior to action observation. As few studies to date have taken advantage of the temporal resolution of EEG to examine the temporal dynamics of mu suppression, it is important to take into account that changes in mu might take place before, during or after observation of an action (Fox et al., 2016).

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PART II.

In the eye of the beholder

Chapter 4

The modulatory impact of social responsiveness, anxiety and attachment on mirror-motor mapping during social gaze.

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In preparation

Abstract

Previous studies have demonstrated that eye contact between the performer and the observer of an action readily increases the observer's propensity to internally 'mirror' or resonate with these actions. However, the social meaning embedded in eye contact may be different for individuals with differing social proficiency. Here, we investigated whether variability in social traits is related to one's ability to show enhanced interpersonal motor resonance upon eye-to-eye contact between performer and observer in a large dataset (n = 141) of neurotypical participants. Motor evoked potentials (MEPs) induced by transcranial magnetic stimulation (TMS) were collected while participants observed a person performing simple hand actions combined with direct or averted gaze. Variability in the onlooker's social traits was investigated by means of the Social Responsiveness Scale (SRS), the Social Phobia Inventory (SPIN) and the State Adult Attachment Measurement (SAAM). Two analytical approaches, one experimenter- and one data-driven, were adopted. The experimenter-driven analysis demonstrated that less social impairments and more secure attachment strategies were associated with more adaptive gaze-related modulations. The data-driven *k*-means clustering technique identified three different subgroups (with high, low and average social skills), showing a clear differentiation in MEP response pattern. Overall, participants with high social skills were shown to display enhanced motor resonance upon direct eye gaze cues, indicating enhanced socially adaptive mirror responses. Together, these findings indicate that adaptive interpersonal motor resonance upon perceived eye contact is not a uniform phenomenon, but is modulated by normal variations in social proficiency.

1 Introduction

The action observation network or ‘mirror system’ has been proposed to support a variety of socio-cognitive functions important for everyday life, as it enables us to internally simulate and understand each other’s actions, emotions and intentions (Iacoboni, 2009; Rizzolatti & Fabbri-Destro, 2008). Although this internal simulation (also known as ‘interpersonal motor resonance’) is presumed to be an automatic process, it does not happen in complete isolation, as it has been demonstrated to be modulated by (a) individual differences such as personality traits and (b) the social context in which the observed actions are embedded. For instance, Liuzza, Candidi, Sforza and Aglioti (2014) showed that mirror system activation was suppressed during observation of immoral actions, but only in individuals who exhibited high scores in harm avoidance, a personality trait characterized by vigilance toward social cues that convey information about potential danger or harm. Furthermore, ample studies have illustrated that mirror system activation is adaptive to the (social) relevance of the perceived movements for the observer, preferentially processing only the most relevant visuo-motor input (Kilner, Marchant & Frith, 2006; see Wang & Hamilton, 2012 for a review).

One of the strongest mediators for determining whether or not observed actions are sufficiently relevant to be simulated is perceived gaze direction from the actor, which carries information about the actor’s focus of attention. Experiencing direct gaze from the actor during movement observation signals his/her communicative intent. Averted gaze on the other hand implies that his/her non-verbal cues are directed elsewhere. Using both behavioral and neurophysiological techniques, it has robustly been demonstrated that mirror responses are typically larger upon the observation of actions accompanied by direct compared to averted gaze from the actor (Prinsen & Alaerts, 2019; Prinsen et al., 2017; Prinsen, Brams & Alaerts, 2018; Wang, Newport & Hamilton, 2011; Wang, Ramsey & Hamilton, 2011).

However, impaired responding to eye contact are common in psychopathologies associated with communication difficulties, such as autism spectrum disorders (ASD) (Kylliäinen & Hietanen, 2006; Kylliäinen et al., 2012; Senju & Johnson, 2009; Tanaka & Sung, 2016) and social anxiety disorder (SAD) (Myllyneva, Ranta & Hietanen, 2015; Schulze, Renneberg & Lobmaier, 2013). Also in the typical population, the social meaning embedded in dyadic eye contact may be different for individuals with different social traits. Indeed, both social responsiveness / autistic traits (Nummenmaa, Engell, Von dem Hagen, Henson & Calder, 2012) and social anxiety (Burra, Massait & Vrtička, 2019; Wieser, Pauli, Alpers & Mühlberger, 2009), have been shown to influence different gaze perception in non-clinical samples. For example, Nummenmaa et al. (2012) showed that the extent of autistic traits in neurotypical individuals was positively associated with brain activity in the neural circuit for social attention perception (including right posterior superior temporal sulcus, intraparietal sulcus and bilateral amygdala) while viewing others' eye gaze. According to the authors, this relationship may reflect compensatory mechanisms, as these individuals require more cognitive resources to process others’ gaze cues.

Social anxiety on the other hand is defined as fear of evaluation from others in social situations. The available evidence in terms of gaze processing suggests that eye contact is perceived as threatening in highly socially anxious individuals, as it provides evidence of being scrutinized by others (Schulze et al., 2013). For instance, Wieser et al. (2009) showed that highly socially anxious individuals responded with a pronounced cardiac acceleration to the presentation of direct gaze stimuli. Burra et al. (2019) revealed an inverse association between social anxiety and the visual detection of direct gaze cues in a visual search task (i.e. the “stare-in-the-crowd” effect). Specifically, higher social anxiety levels were associated with a selective increase in errors for the direct gaze targets (i.e. detection of direct gaze targets among averted gaze distracters). The authors ascribed this effect to a plausible strategy in participants high on social anxiety to avoid prolonged exposure to direct gaze cues, as a possible means to preclude the exposure to social evaluation that is conveyed by direct eye contact.

Furthermore, also the impact of adult attachment styles, which reflect the individual’s cognitive-affective working models towards interpersonal relationships, has recently gained much interest (Burra et al., 2019; Cecchini, Iannoni, Pandolfo, Aceto & Lai, 2015; Prinsen, Deschepper, Maes & Alaerts, 2019). Most adult attachment models differentiate between a secure attachment style (denoting faith in attachment figures, comfort with intimacy and interdependence), attachment anxiety (reflecting insecurity about significant others and constant worrying about being rejected, but also an extreme need for closeness), and attachment avoidance (representing a reluctance to trust others, emphasis on self-reliance and a low tolerance for intimacy). Although initially presumed to only affect close bonds with significant others, attachment strategies are now considered to operate within a wide variety of social situations, including interactions with unknown conspecifics (Feldman, 2017). In terms of eye contact, the “stare-in-the-crowd” study by Burra et al. (2019) revealed that highly anxiously attached participants made more errors on direct gaze targets and less errors on averted gaze targets. Implying that error rate reflects attentional capture of a specific condition, the authors speculated that anxiously attached participants’ attention was drawn more strongly to the averted gaze cues, possibly because they contained a salient cue of social rejection for these participants.

In the current study, we adopted data from a large representative sample (*n* = 141) to explore whether inter-individual differences in these constructs exert a modulatory impact on one's ability to show adaptive mirror system activation related to dyadic eye contact. Three self-report questionnaires were adopted to assess variation in (i) social responsiveness (measured by the Social Responsiveness Scale (SRS); Constantino & Todd, 2005), (ii) social anxiety (indexed by the Social Phobia Inventory (SPIN); Connor et al., 2000) and (iii) attachment style (assessed by the State Adult Attachment Measurement (SAAM); Gillath, Hart, Noftle & Stockdale, 2009). All participants underwent the same single-pulse transcranial magnetic stimulation (TMS) paradigm; comprising passive observation of a stimulus person performing a simple intransitive hand movement combined with either direct or averted gaze from the acting model. Motor evoked potentials (MEPs) were collected as an index of mirror system activation upon action observation (i.e. 'interpersonal motor resonance').

2 Method and Materials

2.1. Participants

A total of 141 typically developing adults (98 men, 43 women; mean age: 23 years, 2 months; age range: 18-36 years) participated in 5 different experiments (Prinsen et al., 2017 (**chapter 1**); Prinsen & Alaerts, 2019 (**chapter 2**), **chapter 3** and **5** (in preparation) and Prinsen et al., 2018 (**chapter 6**); see **table 1**). Exclusion criteria comprised left-handedness, a history of neurological or psychiatric illness, any motor dysfunction of the hands/arms and any contra-indication for TMS research (Rossi, Hallett, Rossini & Pascual-Leone, 2012). The protocols for each study were approved by the Ethics Committee for Biomedical Research at the University of Leuven. Participants' written informed consent was obtained according to the Declaration of Helsinki (World Medical Association, 2013).

Table 1. Experiment details.

Chapter	Experiment	Number of subjects (M; F)	Mean age (range)	Format	Observed movement	Target muscle	TMS coil location	TMS stimulation intensity
1	Prinsen et al., 2017	33 (16 M; 17 F)	22;7 (19-26)	Video	Whole-hand opening	Right APB	Left M1 (APB-RA)	130 % rMT
6	Prinsen et al., 2018	26 (all M)	24;4 (19-32)	Video	Whole-hand opening	Right APB	Left M1 (APB-RA)	130 % rMT
2	Prinsen & Alaerts, 2019	22 (8 M; 25 F)	25;3 (21-30)	Live	Index finger abduction	Right FDI	Left M1 (FDI-RA)	130 % rMT
3	Prinsen & Alaerts, in preparation	32 (20 M; 12 F)	22;9 (18-36)	Live	Index finger abduction	Right FDI	Left M1 (FDI-RA)	130 % rMT
5	Prinsen et al., in preparation	28 (all M)	21;6 (18-28)	Live	Index finger abduction	Right FDI	Left M1 (FDI-RA)	130 % rMT

Notes. Age is shown in years; months. M1: primary motor cortex, APB: abductor pollicis brevis, FDI: first dorsal interosseus, RA: representation area, rMT: resting motor threshold.

2.2. Assessment of motor simulation

2.2.1. Stimuli

During TMS, the stimulus person's face was presented to the participant displaying either direct gaze (i.e. engaging in mutual eye contact) or averted gaze (deviance 30° to the right) while performing a simple, intransitive whole-hand opening movement (chapter 1 and 6, see Prinsen et al., 2017, 2018 for more details) or an index finger abduction movement (chapter 2, 3 and 5, see Prinsen & Alaerts, 2019 for more details and pictures of the stimuli). In both instances, the stimulus person held her hand horizontally before her face, bore a neutral expression and avoided eye blinks. In experiment 1 and 6, the stimuli were presented to the observing participants by means of video clips, whereas in experiment 2,3 and 5 the gaze and motor cues were conveyed by means of a 'live' stimulus person seated behind a liquid crystal (LC) shutter screen (see Prinsen & Alaerts, 2019 for set-up details).

2.2.2. Procedure

The experimental procedure comprised a passive action observation task, in which participants were instructed to observe and pay close attention to the presented stimuli while undergoing single-pulse TMS. Each of the two gaze conditions was presented five times in blocks of four (i.e. total of 20 trials per condition), with a duration of 4 s per trial. The inter-stimulus-interval between trials was 2 seconds, during which the shutter remained opaque (live presentation) or a black screen was shown on the computer (video presentation). Presentation order was pseudo-random (no more than three consecutive blocks of the same type).

2.2.3. TMS protocol and EMG recordings

During observation of the experimental stimuli, single-pulse TMS was applied over the left primary motor cortex using a hand-held 70 mm figure-of-eight coil connected to a Magstim 200 stimulator (Magstim Company Ltd., UK). Electromyography (EMG) recordings were performed to measure motor evoked potentials (MEPs) from the contralateral abductor pollicis brevis (APB; thumb muscle) or first dorsal interosseus (FDI; index finger muscle), which are indicated in the observed whole-hand opening (experiment 1 and 6) or index finger abduction movements (experiment 2, 3 and 5), respectively. EMG-recordings were sampled (2000 Hz), amplified and band-pass filtered (5–1000 Hz) and offline analyzed. In each experiment, the TMS pulse was delivered to coincide with the execution of the hand / index finger movement. Coil placement, optimal location for TMS-stimulation and resting motor threshold were defined for each participant as described in Prinsen et al. (2017). Experimental stimulation intensity was set at 130% of the subject’s resting motor threshold. Signal Software (version 6.02, Cambridge Electronic Design, UK) in combination with a CED Power 1401 analog-to-digital converting unit (Cambridge Electronic Design, UK) were used for EMG-recordings and triggering of the TMS-stimulator.

2.3. Assessment of inter-individual differences

Prior to the experimental TMS procedure, all participants completed three self-report questionnaires assessing social responsiveness (Social Responsiveness Scale; SRS), attachment style (State Adult Attachment Measurement; SAAM) and social anxiety (Social Phobia Inventory; SPIN). Complete questionnaire data were available of 116 participants.

First, the **Social Responsiveness Scale** (SRS; Constantino & Todd, 2005) is a widely used questionnaire to identify impairments in **social responsiveness,** both in the typical population and as a screening tool for autism spectrum disorders (ASD). The Dutch version (Noens, De la Marche & Scholte, 2012) consists of 64 items encompassing four subscales: social communication (i.e. reflecting the ability to respond and communicate appropriately with others; 22 items), social awareness (i.e. denoting the ability to correctly perceive and interpret the emotional cues of others; 19 items), social motivation (representing the motivation to be engaged in social interaction with others; 11 items) and rigidity/repetitivity (12 items). A four-point Likert-scale ranging from 1 (completely true) to 4 (not at all true) is used to indicate how well each statement describes the participant's social behavior. Higher scores denote more impairments on each subscale. For raw SRS scores, a cut-off point of 54 is suggested for signaling social impairments.

Secondly, we adopted the **Social Phobia Inventory** (SPIN; Connor et al., 2000), which consists of 17 questions that evaluate fear in social situations (6 items), avoidance of performance or social situations (7 items), and physiological discomfort in social situations (4 items). Participants were asked to score the distress of each symptom according to the frequency during the past week: 0 (not at all), 1 (a little bit), 2 (somewhat), 3 (very much) or 4 (extremely). Higher scores correspond to heavier symptoms of distress in social situations: < 20: none; 21 – 30: mild; 31 – 40: moderate; 41 – 40: severe; >51: very severe.

Lastly, the **State Adult Attachment Measurement** (SAAM; Gillath, Hart, Noftle & Stockdale, 2009) was adopted to measure variation in three dimensions of adult **attachment**: (i) attachment security (e.g. "*I feel like I have someone to rely on*"); (ii) attachment anxiety (e.g. "*I feel a strong need to be unconditionally loved right now*"); and (iii) attachment avoidance (e.g. "*If someone tried to get close to me, I would try to keep my distance*'). Participants were instructed to indicate how much they agreed or disagreed with 21 statements with respect to their current feelings using a 7-point Likert-type scale ranging from 1 (I strongly disagree) to 7 (I strongly agree). Here, higher scores denote higher levels of the specific attachment dimension.

Table 2 shows the average score for each questionnaire (sub)scale, as well as the Pearson correlation coefficients between subscales.

Table 2. Means, standard deviation (SD) and Pearson correlation matrix for the questionnaire (sub)scores (*n* = 116).

	Mean	**SD**	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**
1. SRS Total Score	35.18	16.47	-							
2. SRS Awareness	10.04	5.01	.87*	-						
3. SRS Communication	10.64	6.34	.92*	.76*	-					
4. SRS Motivation	7.35	4.04	.79*	.59*	.70*	-				
5. SRS Repetitivity	7.16	4.20	.73*	.55*	.54*	.41*	-			
6. SAAM Security	5.91	0.86	-.50*	-.40*	-.47*	-.48*	-.32*	-		
7. SAAM Anxiety	3.83	1.24	.05	.12	-.06	-.04	.13	-.11	-	
8. SAAM Avoidance	2.09	0.82	.53*	.42*	.49*	.55*	.31*	-.56*	.15	-
9. SPIN	14.79	7.63	.51*	.41*	.44*	.61*	.27*	-.32*	.14	.48*

Notes. SRS: Social Responsiveness Scale, SAAM: State Adult Attachment Measurement, SPIN: Social Phobia Inventory. * denotes *p* < .001

2.4. Data analysis

To investigate the effect of observed gaze on interpersonal motor resonance, average MEP peak-to-peak amplitudes were log-transformed (to ensure normality) and subjected to a mixed-model analysis of variance (ANOVA) with the within-subject factor Gaze (averted vs. direct) and the between-subject factor Cohort (experiment 1-5; to account for variance potentially induced by the different experiments).

Next, the ‘eye gaze effect’ was quantified separately for each participant by calculating MEP percentage change (MEP-PC) scores (see Enticott, Kennedy, Bradshaw, Rinehart & Fitzgerald, 2010) were calculated for each participant. The MEP-PC score for participant *i* captures the percentage difference in MEP peak-to-peak amplitude induced by the observation of direct relative to averted gaze during action perception:

$$MEP - PC_{\,i} = \left[{}^{MEP\ direct\ gaze_{\,i} - \ MEP\ averted\ gaze\ _i}\!\big/_{MEP\ averted\ gaze\ _i} \right] \times 100$$

A MEP-PC score close to zero indicates no modulation of MEPs according to observed gaze. A positive MEP-PC score reflects higher MEPs during action observation combined with direct gaze from the model, whereas negative MEP-PC scores denote higher MEPs when the model displays averted gaze. Thus, positive difference scores can be understood as an index of socially-adaptive interpersonal motor resonance.

To explore the association between social traits and gaze-related modulations of interpersonal motor resonance (MEP-PC scores), we first adopted a split-group (hypothesis-driven) approach, followed by a data-driven clustering approach as outlined in the next sections. All statistical analyses were performed using Statistica 10 (StatSoft Inc., USA) and results were considered significant with a *p*-value lower than .05.

2.4.1. Experimenter-driven split group approach

In a first analysis, MEP-PC scores were used to classify participants in two subgroups consisting of 'low' and 'high' responders (i.e. in terms of displaying the eye gaze effect) to test whether participants classified as high versus low responders differed in terms of their SRS (social proficiency), SPIN (social anxiety) or SAAM (attachment style) (sub)scores by means of independent samples *t*-tests.

Since the distribution of participant's MEP-PC scores is positively skewed, a bootstrapped permutation analysis (*N* = 1000 draws) was ran to generate a benchmark MEP-PC score distribution, which was used to formally classify single participants as high or low responders in response to different gaze cues (see Hernandez-Lallement, van Wingerden, Marx, Srejic, & Kalenscher, 2015; Oberliessen et al., 2016 for other examples of this method). This permutation distribution of MEP-PC scores consisted of *N* = 1000 draws, with the MEP peak-to-peak amplitudes randomly assigned to averted and direct gaze conditions. For each of these draws, the sample MEP-PC score was calculated, generating a distribution of 1000 permuted MEP-PC scores that followed a normal distribution. The upper and lower limit of the 95% confidence interval of this test-statistic was selected as a benchmark score to compare each participant's individual MEP-PC score against.

Participants with a MEP-PC score below the lower limit of the 95% confidence interval were classified as low responders (*N* = 78). Participants with MEP-PC scores exceeding the upper limit of the 95% confidence interval were categorized as high responders (*N* = 38). Participants within the 95% confidence interval of the reference distribution were classified as average responders (*N* = 25; not considered in further analyses). See **figure 1A**; the vertical lines indicate the 95% confidence interval limits. The MEP-PC score of both subgroups were tested against 0 by means of a single sample *t*-test.

2.4.2. Data-driven clustering approach

Next, a data-driven approach was adopted to classify participants into distinct subgroups (clusters) based on their reported questionnaire (sub-scale) scores (SRS, SPIN, SAAM). Importantly, within this data-driven approach, no a priori hypotheses were adopted for categorizing participants into subgroups. To do so, an unsupervised *k*-means clustering algorithm (as implemented in Statistica 10, StatSoft Inc., USA) was adopted to partition our dataset into a set of k clusters (sub-groups). Here, participants were organized in multiple subgroups (i.e. clusters), such that participants within the same cluster are as similar as possible in terms of social traits (i.e. high within-cluster similarity), whereas participants from different clusters are as socially dissimilar as possible (i.e. low between-cluster similarity). Questionnaire (sub)scores were first standardized (mean = 0 and SD = 1) to avoid that arbitrary questionnaire units or ranges impact the clustering algorithm. Euclidian distances were calculated to quantify the distance or dissimilarity (*d*) between each pair of observations:

$$d_{euclidian}(x, y) = \sqrt{\sum_{i=1}^{n} (x_i - y_i)^2}$$

One potential disadvantage of this approach however is that it requires to pre-specify the number of clusters. To determine the optimal number of clusters in the current dataset, the total within-cluster variation (*W*) for *k* from 2 to 7 was calculated and plotted against *k* (‘elbow method’). Total within-cluster variation was defined as:

$$W(C_k) = \sum_{x_i \in C_k} (x_i - \mu_k)^2$$

Where x_i is a data point belonging to cluster C_k and μ_k is the mean value of the points assigned to cluster C_k. Visual inspection of this plot indicated *k* = 3 as the appropriate number of clusters (see **supplementary figure 1**).

3 Results

3.1. Substantial heterogeneity in the eye contact effect

At the group level, log-transformed MEP amplitudes were found to be significantly higher in the direct, compared to the averted gaze condition ($F(1,136) = 25.42$, $p < .001$, $\eta^2_p = .16$), indicating that, on average, mirroring was modulated by observed eye gaze from the actor (**figure 1A**). Importantly, no significant Gaze × Cohort interaction was identified ($F(4,136) = 1.03$, $p = .39$, $\eta^2_p = .03$), indicating that the gaze effect was not significantly modulated depending on cohort. A main effect of experimental cohort ($F(4,136) = 7.69$, $p < .001$, $\eta^2_p = .18$) indicated that on average (i.e. across gaze conditions), MEPs were generally higher in experiments adopting live stimuli, compared to video stimuli (see **supplementary figure 2**).

As visualized in **figure 1A**, MEP percentage change scores (MEP-PC) ranged from -46.99 % to 191.21 %, with positive scores indicating higher MEPs in the direct vs averted gaze condition. As such, while in the overall group a significant main effect of eye gaze was revealed, a substantial degree of variance existed among participants in terms of the magnitude of the eye gaze effect (see dots in **figure 1A**). Participants were classified as high or low responders when their MEP-PC scores exceeded the upper or lower limits of the 95% confidence interval on a reference bootstrapped permutation distribution (see Methods). Based on this procedure, 38 out of 116 participants were classified as high responders, showing positive MEP-PC scores (mean: 60.97 %, SD: 40.08 %) significantly higher than 0 (one-sample $t(37) = 9.38$, $p < .001$). Furthermore, 78 out of 116 participants were classified as low responders, showing significantly negative MEP-PC scores indicative of aversive gaze-related modulations of MEPs (mean: -5.09 %, SD: 12.07 %; one-sample $t(77) = -3.72$, $p < .01$).

3.2. High eye contact responders show more social skills

In a subsequent analysis, split-group analyses were performed to examine whether participants classified as high versus low responders (i.e. high versus low MEP-PC scores) differed in terms of self-reported SRS (social responsiveness), SPIN (social anxiety) and SAAM (attachment style) questionnaire (sub)scores. Full questionnaire data was available for 116 out of 141 participants.

As visualized in **figure 1B**, participants classified as low eye contact responders (i.e. showing aversive gaze-related modulations of MEPs) reported significantly *more* social impairments compared to high responders (SRS Total Score: independent $t(93) = 2.58$, p

= .01). Furthermore, low responders also reported significantly *less* secure attachment strategies compared to high eye contact responders (SAAM Security: *t*(93) = -2.30, *p* = .02) (see **figure 1B**). No significant differences were found for the other SAAM subscales assessing attachment anxiety and avoidance, nor for the SPIN assessing social anxiety (all *p* > .27; see **table 3**).

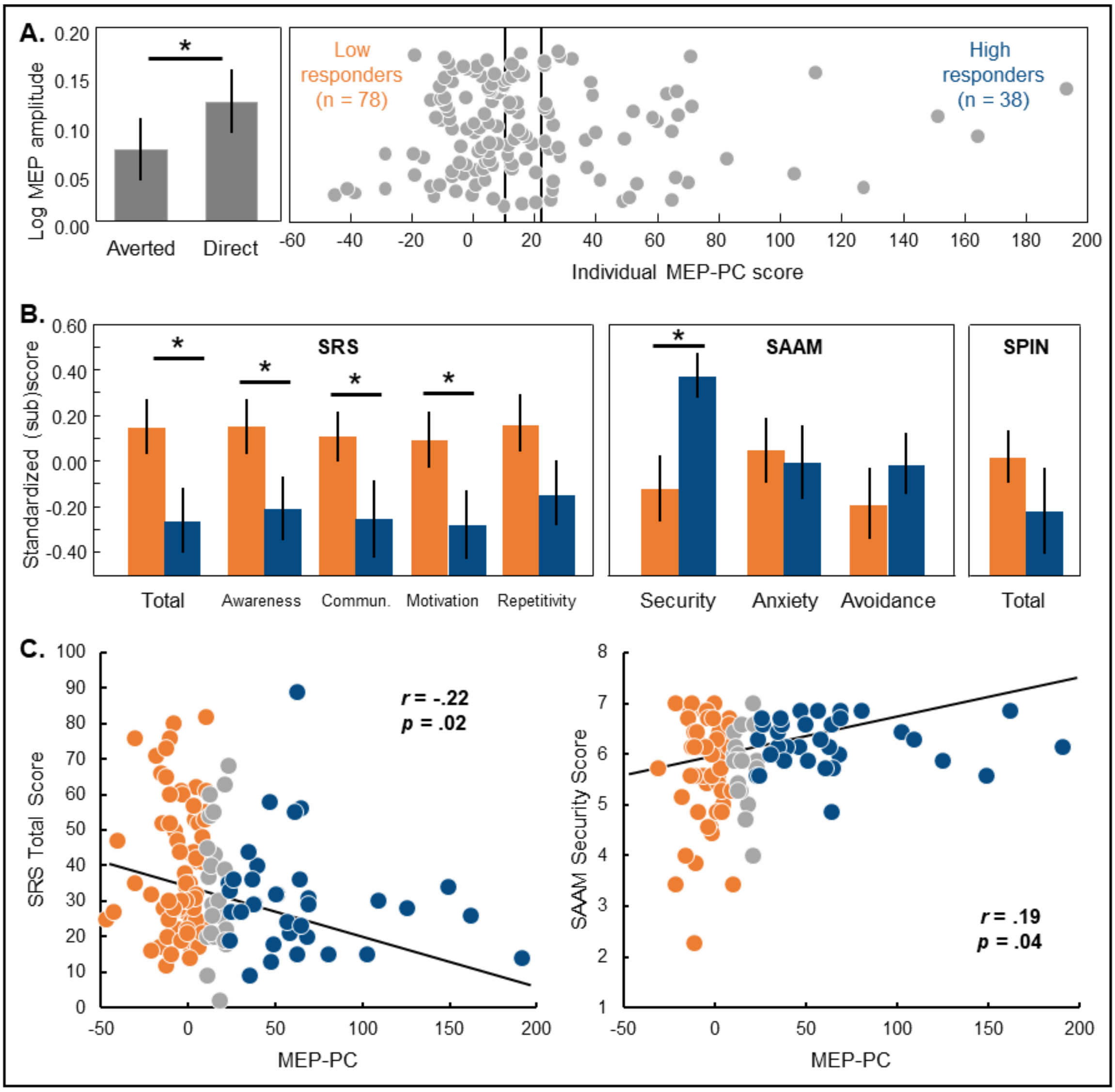


Figure 1. (A) On average, an effect of observed eye gaze on interpersonal motor resonance was revealed, although note that significant inter-individual variability was evident (left panel) (*n* = 141). The vertical lines represent the ± 95% confidence interval for the bootstrapped ‘MEP-PC score’, which were used to classify participants as high or low responders. **(B)** Differences in standardized social responsiveness (SRS), attachment style (SAAM) and social anxiety (SPIN) (sub)scores between low and high responders. **(C)** Significant associations between MEP-PC and SRS Total Score (left) and SAAM Security score (right). SRS: Social Responsiveness Scale, SAAM: State Adult Attachment Measurement, SPIN: Social Phobia Inventory. Vertical error bars denote mean ± SE; * *p* < .05

This association between the magnitude of the ‘eye gaze effect’ of the individual participant (i.e. MEP-PC score) and his/her social responsiveness skills (i.e. SRS total score), as well as with the SAAM subscale assessing attachment security, was also evident when taking the complete spectrum of inter-individual variance into account (**figure 1C**). In particular, a significant negative correlation between SRS total score and the MEP-PC score indicated that the more social impairments were reported (i.e. higher SRS score), the lower the facilitating effect of direct vs. averted gaze on MEP amplitude (i.e. lower MEP-PC score: Pearson $r = -.22$, $p = .02$, $R^2 = .05$). Similarly, the more securely attached (i.e. higher SAAM Security score), the more pronounced the ‘eye gaze effect’ (i.e. higher MEP-PC score: Pearson $r = .19$, $p = .04$, $R^2 = .04$).

Table 3. Differences in social traits between low and high eye contact responders.

	Low responders ($n = 78$) Mean score (SD)	High responders ($n = 38$) Mean score (SD)	t(93)-value
SRS Total Score	38.14 (17.74)	29.24 (12.01)	**2.58***
Awareness	10.81 (5.07)	8.79 (4.25)	**1.95***
Communication	11.61 (6.37)	8.18 (4.99)	**2.68***
Motivation	7.84 (4.27)	5.73 (3.39)	**2.46***
Repetitivity	7.88 (4.62)	6.57 (3.55)	1.42
SAAM Security	5.81 (0.99)	6.23 (0.48)	**-2.30***
SAAM Anxiety	3.89 (1.40)	3.82 (1.15)	0.24
SAAM Avoidance	2.08 (0.86)	1.94 (0.74)	0.80
SPIN	14.95 (7.02)	13.15 (8.27)	1.12

SRS: Social Responsiveness Scale, SAAM: State Adult Attachment Measurement, SPIN: Social Phobia Inventory. * $p < .05$

3.3. Data-driven classification confirms subtypes

Next, a data-driven approach was adopted to classify participants into distinct subgroups (clusters) based on their reported questionnaire (sub-scale) scores (SRS, SPIN, SAAM). The k-means clustering analysis revealed three subgroups of participants (**figure 2A**). Cluster 1 ($n = 59$; shown in blue) represents participants with low levels of social impairments, low social anxiety and highly secure attachment styles. Participants belonging to cluster 2 ($n = 19$; shown in orange) report more social impairments across all subscales of the SRS, more social anxiety and highly avoidant attachment styles. Lastly, participants in the third cluster ($n = 38$; shown in grey) showed a profile of average social skills and traits.

Importantly, these subgroups showed a clear differentiation in terms of socially-adaptive interpersonal motor resonance, as indicated by a significant Gaze × Cluster interaction: *F*(2,113) = 3.90, *p* = .02, η^2_p = .06 (**figure 2B**). Particularly, only for the clusters representing participants with high (cluster 1; Fisher LSD: $p < .001$) or average (cluster 3; Fisher LSD: $p = .006$) self-reported social proficiency, perceived direct gaze facilitated interpersonal motor resonance with the observed actions. For participants who are less socially proficient however, interpersonal motor resonance was not significantly modulated by eye-to-eye contact (Fisher LSD: $p = .63$). The main effect of Cluster was not significant (*F*(2,113) = 0.18, *p* = .83, η^2_p = .006).

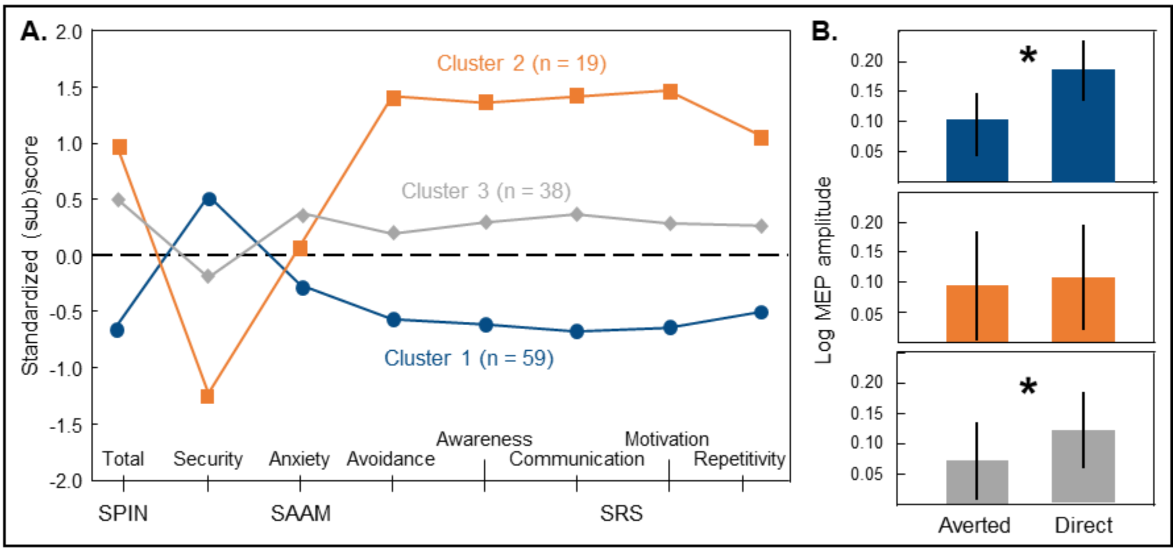


Figure 2. (A) Average standardized questionnaire (sub)scores for each cluster, as obtained by the *k*-means clustering algorithm. SRS: Social Responsiveness Scale, SAAM: State Adult Attachment Measurement, SPIN: Social Phobia Inventory. **(B)** The effect of observed eye gaze on interpersonal motor resonance, separately for each cluster. * $p < .05$

4 Discussion

Previous studies have demonstrated that eye contact between the performer and the observer of an action readily increases the observer's propensity to internally simulate or 'mirror' these actions (i.e. 'interpersonal motor resonance') (Prinsen & Alaerts, 2019; Prinsen et al., 2017, 2018). In this study, we examined whether and how heterogeneity in the onlooker's social traits is associated to his/her ability to show socially adaptive interpersonal motor resonance upon the observation of eye contact. To investigate how individuals resonate with others' actions in different dyadic gaze situations (i.e. direct vs. averted gaze from the actor), motor evoked potentials (MEPS) induced by single-pulse TMS were analyzed. The onlookers' sociality was quantified by adopting the SRS (measuring social responsiveness), the SAAM (indexing attachment style) and the SPIN (measuring social anxiety) self-report questionnaires.

First, we derived a bootstrapped benchmark criterion from the obtained MEPs to partition the participant dataset in three groups of participants: low, average and high eye contact responders. Our data indicated that low eye contact responders, i.e. those who show aversive eye contact-related modulations of interpersonal motor resonance, also report significantly more social impairments and less secure attachment styles, compared to those participants who do show socially adaptive mirroring upon dyadic eye contact (high responders). These results were confirmed by small but significant correlations when taking the full spectrum of inter-individual variability into account. This differential response is in line with previous work demonstrating that social responsiveness traits (Nummenmaa et al., 2012) and attachment styles (Prinsen et al., 2019) modulate gaze-related responses at the level of the brain. Furthermore, our findings are also in accordance with a previous TMS study showing that a complex interplay between individual- and context-dependent factors impacts interpersonal motor resonance (e.g. Liuzza et al., 2014)

No significant differences between high and low gaze responders were encountered in terms of avoidant / anxious attachment styles or in terms of social anxiety. On the one hand, these findings might indicate that inter-individual differences in these social abilities/traits do not impact the facilitative effect of eye contact on interpersonal motor resonance. However, given the fact that other studies did encounter significant associations between eye contact-related modulations and attachment anxiety (Burra et al., 2019), attachment avoidance (Prinsen et al., 2019) and/or social anxiety (Burra et al., 2019; Wieser et al., 2009), it is also possible that the current sample of participants shows too little inter-individual variability in these traits. Indeed, close to 80% of participants (n = 91) scored well below the SPIN cut-off score for signaling mild impairments in social anxiety. Future work is necessary to address this issue further, for example by a priori selecting participants with low vs. high levels on the social trait of interest.

The data-driven *k*-means clustering technique was also included to discover patterns in our data not based on a priori knowledge, which might generate new specific hypotheses (Adolphs, Nummenmaa, Todorov & Haxby, 2016). This analysis complemented the experimenter-driven analysis by finding a partitioning for which all investigated social traits were relevant, and confirmed the existence of three subgroups that differ in social traits as well as their ability to show socially adaptive interpersonal motor resonance. Notably, both analytical approaches indicated that, although neurotypicals usually score well below the established cut-off scores that distinguish between clinically impaired vs. unimpaired participants, different subgroups of participants might exist ‘below the cut-off’. In particular our data highlights that the typical population is quite heterogeneous.

While our study provided important evidence that interpersonal motor resonance of socially salient actions depends on inter-individual differences in social traits, several limitations should be noted. Although the adopted measures were selected to span different social domains (i.e. responsiveness, attachment and anxiety), the full spectrum of social traits will likely not be captured with three self-report questionnaires. Furthermore, there are other ways to stratify the neurotypical population that might be of interest when investigating socio-cognitive processes related to eye contact, such as personality traits and cultural differences. For example the personality trait of Neuroticism has been shown to influence approach/avoidance-related frontal EEG activation in response to observed gaze (Uusberg, Allik & Hietanen, 2015). Similarly, Akechi et al. (2013) demonstrated that although eye contact was processed in a similar way at the physiological level, differences in eye contact behavior between Western European and East Asian participants still emerged in the form of various evaluative ratings of the stimuli (e.g. facial emotion, approachability, pleasantness). Future work is necessary to parse the subtle and specific ways in which the role of observed eye contact on interpersonal motor resonance in specific – and other socio-cognitive processes in general – is impacted by the characteristics of the observer.

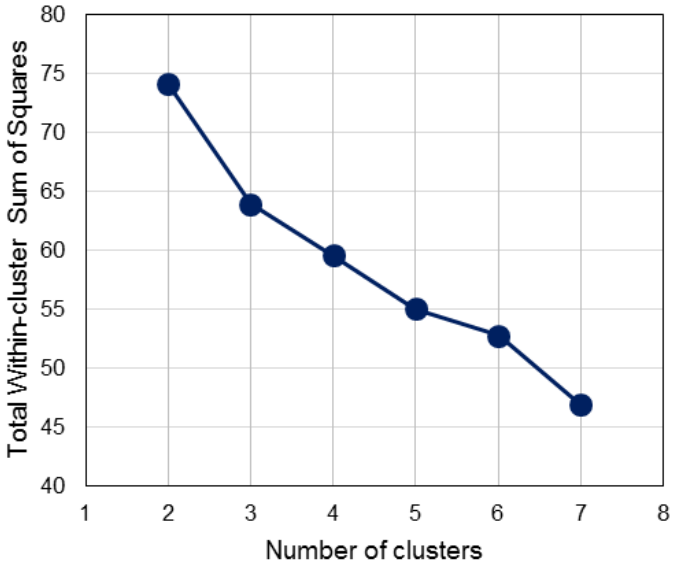
Lastly, it is not clear whether atypical processing of eye contact and its subsequent impact on socio-cognitive processes is associated with specific (sub)clinical traits such as autistic traits and/or social anxiety, or entails a general sign of interpersonal dysfunction. Indeed, besides the well-known aberrant gaze-related neural and sympathetic responses in individuals with a diagnosis of SAD (Myllyneva et al., 2015) or ASD (Kylliäinen et al., 2012), recent studies have also reported altered neurophysiological responding to observed gaze cues in individuals with schizophrenia (Tso et al., 2015) and bipolar disorder (Berchio et al., 2017). Yet, behavioral eye-tracking studies indicate that the associated gaze behaviors by which these aberrant brain-based responses may arise differ across different psychopathologies. Related to the distinction between autistic traits and social anxiety for example, a study by Kleberg et al. (2017) demonstrated that high levels of autistic traits were associated with delayed orienting *towards* the eyes presented among distractors, suggesting a reduced bottom-up driven salience of human eyes. On the other hand, high social anxiety symptoms were related to faster orienting *away* from the eyes once fixated (avoidance), likely reflecting anxiety-driven avoidance. Also in the previously discussed study by Burra et al. (2019), neurotypical variation in social anxiety and attachment anxiety were shown to differently impact gaze behaviors.

To conclude, our study revealed that the facilitative effect of observed eye contact on the onlooker’s propensity to mirror others’ actions is not universal, but strongly depends on his/her social traits. Considering these results, we encourage further investigations to take the interplay between contextual factors and variability in individual characteristics on different socio-cognitive processes further into account.

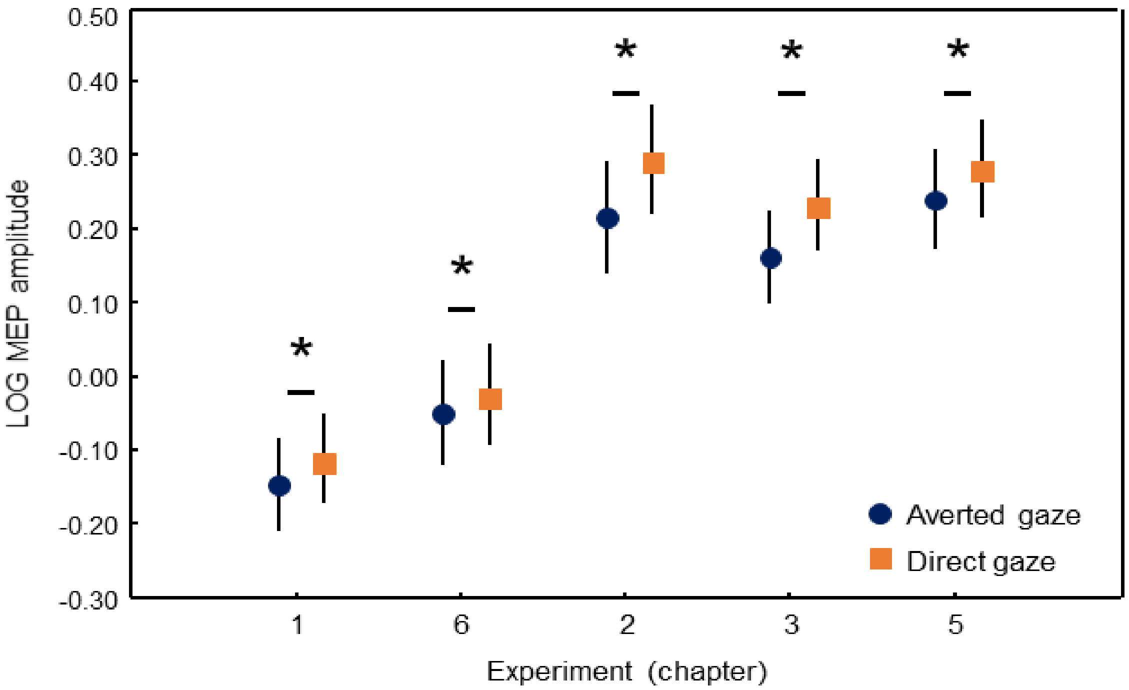
5 Supplementary information

Supplementary figure 1.

Elbow plot showing the total within-cluster variation (i.e. sum of squares) plotted against the number of clusters (*k*), from *k* = 2 to *k* = 7. This plot indicates *k* = 3 as the appropriate number of clusters for the current dataset.



Supplementary figure 2. Gaze effect for every included experimental cohort, with associated chapter number indicated on the x-axis. Although a main effect of experimental cohort was observed, mainly reflecting differences in presentation mode (video clips vs. live presentation), the gaze effect was present in each study (*$p < .05$).



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Chapter 5

Broken mirrors or stormy interactions?

Interpersonal motor resonance in ASD.

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In preparation

Abstract

Individuals with ASD experience persistent difficulties during social interactions and communication. The Broken Mirror Theory advances deficits in the mirror system as an important neural substrate, while others have proposed an impaired social top-down modulation (i.e. STORM) of mirror system activity according to the demands of the social context. In this study, we will investigate the feasibility of both accounts by examining neurophysiological motor resonance in individuals with ASD under various (social) conditions. Individuals with ASD (*n* = 25) and matched neurotypicals (*n* = 28) completed a transcranial magnetic stimulation (TMS) experiment in which the left primary motor cortex was stimulated during observation of simple hand movements (no social context condition) or in combination with direct and averted gaze from the acting model (social context condition). Motor evoked potentials (MEPs) were recorded from the contralateral first dorsal interosseous, a muscle implicated in the to-be-observed movements. In sum, there were no significant group differences between ASD and NT participants during the observation of these actions, providing no evidence for a global mirror system deficit or STORM-related deficits in ASD. However, significant negative associations between putative mirror system activity and self-reported social symptom severity (as measured by the Social Responsiveness Scale) was encountered within the ASD group. Overall, this finding indicates there may be a subgroup of individuals with ASD who show persistent deficits in terms of mirror system function (i.e. across non-social and social conditions), and a subgroup of individuals who show no atypical mirror responses.

1 Introduction

Autism spectrum disorders (ASD) are a set of neurodevelopmental disorders characterized by severe and pervasive impairments in reciprocal social interaction and communication, combined with a reduced and restricted pattern of interests and behaviors (DSM-5; American Psychiatric Association, 2013). Several brain-based mechanistic accounts have been put forward to explain the encountered social difficulties in ASD. One influential theory is the ***Broken Mirror Theory*** (Iacoboni & Dapretto, 2006; Oberman & Ramachandran, 2007), which posits that individuals with ASD have difficulties in communicating with others due to abnormalities in the so-called neural ‘mirror system’. The human mirror system can be defined as a set of brain regions which are activated both when participants perform a particular action and when they observe another person performing the same action (Cattaneo & Rizzolatti, 2009). Although mirror neurons have initially been observed in the macaque monkey, human neuroimaging studies have indicated several brain areas that contain mirror neuron populations, such as the inferior frontal gyrus (IFL) and the inferior parietal cortex (IPL) (for reviews and ALE meta-analyses, see Molenberghs, Cunnington & Mattingley, 2012; Oosterhof, Tipper & Downing, 2013).

By directly mapping or simulating observed actions onto the observers own motor system, the human mirror system is considered to play a key role in the understanding of others’ actions and associated higher-order socio-cognitive processes (e.g. empathy; a form of emotional simulation), several of which are impaired in ASD (Iacoboni, 2009; Rizzolatti & Sinigaglia, 2010). Accordingly, it has been suggested that dysfunction within the mirror system might contribute to poor social cognition in ASD. This is supported by several studies using a range of methods, including fMRI, structural, M/EEG suppression indices of mu waves in the 8-13 Hz band over the sensorimotor strip, and TMS indices of corticospinal excitability during action observation. Together, these studies have shown less activity (Grèzes, Wicker, Berthoz & de Gelder, 2009; Martineau, Andersson, Barthélémy, Cottier & Destrieux, 2010) and reduced cortical thickness (Hadjikhani, Joseph, Snyder & Tager-Flusberg, 2006) in brain areas belonging to the mirror system, and diminished mu suppression (Bernier, Dawson, Webb & Murias, 2007; Dumas, Soussignan, Hugueville, Martinerie & Nadel, 2014; Nishitani, Avikainen & Hari, 2004; Oberman et al., 2005) and corticospinal excitability (Enticott et al., 2012; Théoret et al., 2005) during the observation of movements in adults with ASD compared to healthy controls, respectively. Other studies have however reported no such deficits (fMRI: Marsh & Hamilton, 2011; EEG: Fan, Decety, Yang, Liu & Cheng, 2010; TMS: Enticott et al., 2013), indicating that evidence for the Broken Mirror Theory is mixed (see Hamilton, 2013 for a review). Studies of children with ASD show the same inconsistencies: Dapretto et al. (2006) showed no mirror activity in the IFG while imitating and observing emotional expressions, whereas Raymaekers, Wiersema, and Roeyers (2009) find similar EEG mu suppression in ASD and typical children.

Interestingly, abnormal mirror responses in ASD have mainly been reported when observing social or emotional stimuli (Dapretto et al., 2006; Grèzes et al., 2009), but normal responses when viewing goal-directed actions (e.g. Marsh & Hamilton, 2011). Similarly, Oberman, Ramachandran and Pineda (2008) found that mu suppression in ASD is sensitive to familiarity: children with ASD only show mu suppression when they can identify in some personal way with the observed actions (e.g. when observing own movements or the movements of a familiar person). This pattern of mixed results in ASD is consistent with the ***social-top down response modulation*** (STORM) model advanced by Wang and Hamilton (2012). This model consists of two core components; the mirror system – responsible for basic visual-to-motor mapping processes – and the mentalizing system, which exerts a top-down control over the mirror system to modulate mirror activity in accordance to its evaluation of the encountered social situation. Evidence in favor of this top-down notion has initially been provided by Wang, Newport and Hamilton (2011), who - using a stimulus-response compatibility (SRC) paradigm - found that automatic imitation of simple hand movements is enhanced by eye-to-eye contact between actor and observer. By adopting TMS, recent studies by our lab (Prinsen & Alaerts, 2019; Prinsen et al., 2017; Prinsen, Brams & Alaerts, 2018) showed that the mapping of others' movements into the observer's primary motor cortex (M1) is enhanced when observed movements are accompanied by direct gaze compared to averted gaze, thereby providing important insights into the neurophysiological basis of the eye contact effect on automatic imitation as encountered by Wang, Newport and Hamilton (2011).

However, using the same SRC paradigm in participants with ASD, Forbes, Wang and Hamilton (2017) showed that although participants with ASD were able to adequately imitate the observed hand actions, imitation was not socially modulated by the observed gaze conditions. As such, the STORM framework can be extended to the case of ASD, positing that the mirror system by itself may be intact in persons with ASD, but that they are not able to modulate mirror system responses according to the social demands of the situation (Forbes et al., 2017; Hamilton, 2013). Especially in the case of eye-to-eye contact, hypothesized to be experienced as unpleasant (known as the eye avoidance hypothesis; Tanaka & Sung, 2016) or not socially salient (known as the Social Motivation Theory; Chevallier, Kohls, Troiani, Brodkin & Schultz, 2012) in ASD, these social cues could interfere with an appropriate top-down control and lead to aberrant mirror responses. Since eye contact provides an important foundation for everyday social interaction and communication, understanding the exact nature of this eye contact aversion and its consequences on down-stream information processing is crucial to map deficits in social information processing in ASD.

In sum, previous studies evaluating putative mirror system deficits in ASD have reported mixed results that appear to be dependent on the social or emotional content of the stimulus, which is in line with the STORM framework. In order to directly explore the putatively impaired social top-down response modulation (STORM) of mirror system responses in ASD, the current study principally aimed to extend the behavioral work by Forbes et al. (2017) by adopting the single-pulse TMS technique. Single-pulse TMS is a non-invasive brain stimulation method that, in the context of action observation research, is used to magnetically stimulate M1 in order to assess its excitability under various observational conditions. Importantly, the observation of others' results in an enhancement of corticospinal (M1) excitability, which is considered a product of excitatory mirror system activations (Fadiga, Craighero & Olivier, 2005).

Specifically, to disentangle the role of social context on mirror-motor mapping in ASD, we used TMS to probe group differences in observation-induced modulation of M1 excitability in response to either simple, intransitive hand movements without social context (i.e. **basic mirror-motor mapping**) or in response to an actor performing similar hand movements combined with direct or averted gaze cues (i.e. **socially modulated mirroring**). Importantly, to increase the ecological validity of the observation conditions, movement and gaze cues were conveyed by a live stimulus person in a two-person setting (i.e., as adopted previously in Prinsen & Alaerts, 2019). Note that in addition to the assessment of basic and socially modulated mirroring, **baseline levels of corticospinal excitability** while at rest will also be taken into account to rule out the possibility of a baseline neurophysiological deficit in ASD. If mirror-motor mapping is found to be impaired irrespective of observation condition in individuals with ASD compared to a group of age- and IQ-matched neurotypical control participants, data would be in support of the general Broken Mirror account. On the other hand, and in line with the STORM model, mirror-motor mapping is hypothesized to be overall intact in ASD (i.e. no mirroring differences in conditions without social context), whereas the modulatory effect of eye contact on mirror-motor mapping is anticipated to be absent in ASD (i.e. no social top-down enhancement of mirroring upon direct, compared to averted gaze).

2 Method and Materials

2.1. Participants

Participants were 26 young adult men, aged between 18-35 years, with a clinically established diagnosis of ASD according to DSM-IV or DSM-5 criteria (American Psychiatric Association, 2013) and 28 age- and IQ-matched control subjects (see **Table 1** for participant demographics), recruited between May 2018 and December 2019. All participants were right-handed (confirmed with the Edinburgh Handedness Questionnaire; Oldfield, 1971), met safety criteria for TMS (Rossi, Hallett, Rossini & Pascual-Leone, 2012) and provided written informed consent. Ethical approval was granted by the local Ethics Committee for Biomedical Research at the University of Leuven (S56327) in accordance to the Declaration of Helsinki (World Medical Association, 2013).

Individuals with ASD were recruited via the Expertise Center for Autism at the University Hospital Leuven (n = 5) and advertisements in Flemish support groups and websites for individuals with ASD (n = 21). When a participant was not diagnosed by a clinician from the Expertise Center for Autism, the diagnosis was confirmed by participants providing a copy of their diagnostic report. Reported comorbid disorders included ADHD/ADD (4 participants), depression (3), burnout (1), dyslexia (1), dyscalculia (1), anxiety problems (1) and Gilles de la Tourette (1). Six of the ASD participants reported stable use of psychoactive medication for at least three months at the time of study enrollment (2 atypical antipsychotic, 1 atypical antipsychotic and tricyclic antidepressant, 2 psychostimulant, 1 psychostimulant and atypical antipsychotic medication). Control participants were recruited via advertisements placed at KU Leuven university buildings and reported no history of substance abuse, neurological illness (e.g. stroke, epilepsy, concussion) or psychiatric disorder (e.g. ASD, ADHD).

There were no significant group differences in terms of age or in verbal and/or performance abilities as assessed by four subtests of the Dutch Wechsler Adult Intelligence Scale, fourth edition (WAIS-IV-NL; Wechsler, 2012) (see **table 1**). The participant groups differed significantly on all subtests of two self-report questionnaires assessing ASD symptomatology; the Autism Questionnaire (AQ; Baron-Cohen, Wheelwright, Skinner, Martin & Clubley, 2001) and the Social Responsiveness Scale (SRS; Constantino & Todd, 2005). Both questionnaires are often used as a screening tool for ASD. Note that the current data collection is part of a larger study investigating the effect of a single dose of oxytocin on the mapping of others' actions upon eye contact in individuals with ASD

(ClinicalTrials.gov protocol identifier: NCT03640156; see also **chapter 6** for a similar design), but due to practical constraints will not be discussed here.

Table 1. Participant demographics. Data are shown as mean (SD).

	ASD group	**Control group**	**Independent *t*-test**	
	n = 26	**n = 28**	***t*-value**	***p*-value**
Age in years: months	22:4 (4:1)	21:6 (2:10)	-.84	.40
AQ Total Score (raw)	27.12 (7.22)	12.59 (5.46)	8.47	< .001
Social skills	4.50 (2.59)	1.24 (1.43)	5.85	< .001
Attention switching	6.38 (2.02)	3.24 (1.46)	6.67	< .001
Attention to detail	6.00 (2.26)	3.52 (2.53)	3.82	< .001
Communication	5.19 (2.40)	1.62 (1.32)	6.93	< .001
Imagination	5.04 (2.14)	3.82 (2.14)	3.82	< .001
SRS Total Score (raw)	72.91 (29.61)	34.48 (13.07)	5.92	< .001
Social Awareness	19.54 (9.24)	10.40 (4.26)	4.44	< .001
Social Motivation	24.42 (11.86)	10.08 (5.59)	5.45	< .001
Social Communication	13.88 (5.12)	6.56 (2.86)	6.21	< .001
Repetitive Behavior	15.08 (5.98)	7.44 (3.99)	5.28	< .001
WAIS-IV-NL subtest (scaled)				
Vocabulary	10.60 (3.42)	11.43 (2.63)	.99	.32
Similarities	10.76 (3.44)	11.46 (2.32)	.88	.38
Matrices	11.04 (2.95)	11.32 (2.39)	.38	.70
Block Patterns	13.20 (3.78)	13.36 (2.31)	.18	.85

AQ: Autism Quotient; SRS: Social Responsiveness Scale; WAIS-IV-NL: Wechsler Adult Intelligence Scale, fourth edition (Dutch version).

2.2. TMS and EMG set-up

Participants were seated in a comfortable chair with their right hand placed palm-down on a pillow at a distance of approximately 80 cm from a 20 × 30 cm voltage-sensitive liquid crystal (LC) shutter screen (DreamGlass Group, Spain) attached to a black frame (similar set-up as described in Prinsen & Alaerts, 2019) through which the stimuli were presented. Mirror system activity was assessed by administering single-pulse TMS to the left primary motor cortex (M1) using a Magstim-200 stimulator (Magstim Company Ltd., UK) with a hand-held 70 mm figure-of-eight coil. When applied to the somatotopically organized M1, single-pulse TMS elicits a twitch or motor evoked potential (MEP) in the contralateral muscle that corresponds to the stimulated region in M1.

Optimal coil location for magnetic stimulation of M1 was identified for each participant as the scalp position that produced the largest MEPs in the right first dorsal interosseous (FDI) index finger muscle while at rest. This muscle was selected as it is intrinsically implicated in the to-be-observed hand movements; and the mirror system simulates observed actions in a strictly muscle-congruent fashion (Fadiga, Fogassi, Pavesi & Rizzolatti, 1995). During assessment, MEPs were also collected from the abductor minimi digiti (ADM) pink muscle, which is not implicated in the to-be-observed movements and therefore serves as a control muscle. Resting motor threshold (rMT), expressed as a percentage of the maximum stimulator output, was individually defined as the lowest stimulation intensity that produced MEPs of at least 50 mV in 5 out of 10 consecutive trials. TMS pulses during the experimental procedure were delivered at 130% of the subject's rMT.

MEP amplitudes from the FDI and ADM muscles were recorded via surface electromyography (EMG) using disposable Ag-AgCl adhesive electrodes (Kendall Medi-Trace) arranged in a tendon-belly montage, with the reference electrode attached to the wrist. The EMG signal was sampled at 2000 Hz, amplified and band-pass filtered (5-1000 Hz). Pre-stimulus EMG recordings were used to assess the presence of unwanted background EMG activity in the 110–10 millisecond time interval preceding the magnetic pulse. A CED Power 1401 analog-to-digital converting unit (Cambridge Electronic Design, UK) in combination with Signal software (version 6.02, Cambridge Electronic Design, UK) was used for triggering of the TMS-stimulator and EMG recordings, and the shifting of the LC window from an opaque to transparent state.

2.3. Experimental procedure

To assess baseline corticospinal excitability (CSE), participants were first administered ten TMS pulses while at rest with a 4 second interval between pulses. To investigate observation-induced modulations of CSE, movement observation trials were presented to the participants via a 'live' female stimulus person seated behind the panel. Through the LC screen, participants viewed simple index finger abduction movements of a horizontally held right hand (dorsal view) against a white background (*no social context*), in combination with the stimulus person gazing directly towards the observing participants (*socially salient context*), and in combination with the stimulus person averting her face and gaze 30° to the right (*non-salient social context*) (see **chapter 3**, for illustrations of the stimuli). Each condition was of 4 seconds duration, with an inter-trial interval of 2 seconds, and was presented 20 times (i.e. 20 MEPs per condition) in a pseudo-random sequence. Coinciding with the TMS pulse (130% rMT) on the third second of stimulus presentation, the stimulus person performed the index finger abduction movement.

In order to ensure that all participants viewed and attended the stimuli properly, they were asked once at a random time point during the TMS assessment to verbally report the stimulus that was presented in the previous trial. Furthermore, gaze behavior was recorded by means of head-mounted SMI eye-tracking glasses (sampling rate: 30 Hz) and SMI iView acquisition software (SensoriMotor Instruments, Germany). The glasses were adjusted to the participant’s comfort and a three-point calibration procedure was performed before recording. Note however that the analysis of this data is still in preparation.

2.4. Data analysis

Peak-to-peak amplitudes of the TMS-induced MEPs were determined using in-house MATLAB scripts (version R2015a, MathWorks Inc., USA). Trials in which there was evidence of excessive tonic muscle activity (i.e. exceeding 2.5 standard deviations from the mean) within 110 to 10 milliseconds prior to TMS administration were not included in the analysis (9.23% of all trials). Trials were also removed from further analysis if MEP peak-to-peak amplitudes exceeded 1.5 interquartile distances from the mean (10.87% of all trials). Note that the total number of discarded trials was similar across groups (all $p >$ .13).

To examine whether there was any group difference in baseline corticospinal excitability when at rest and/or resting motor threshold (rMT), independent samples *t*-tests were initially performed. Then, consistent with previous TMS studies (Enticott et al., 2013, 2012), mirror system activity during action observation in the various conditions was putatively measured using an MEP percentage change variable (MEP-PC) relative to the average MEP response while at rest. The formula for calculating this variable is:

$$MEP - PC = {}^{(MEP_{condition} - \ MEP_{rest})}\!/_{MEP_{rest}} \times 100$$

A higher MEP-PC score is indicative of more interpersonal motor resonance. Since Shapiro Wilk’s tests (*W*) indicated non-normal distributions, MEP-PC values were logarithmic transformed. As a log-transformation cannot be performed for negative values (and some MEP-PC values were negative), prior to the transformation we added a constant of 100 to each of the values to ensure that they were all positive (similar procedure as Enticott et al., 2013, 2012). After transformation, normality was ensured for all variables (*W p* > .07). Note that for illustrative purposes, **figures 1 and 2** in the Results section display the untransformed MEP-PC values.

First, it was tested whether log-transformed MEP-PC scores (i.e. indexing % change from rest) during observation of simple hand actions were significantly higher than during rest by means of single-sample *t*-tests against zero, separately for each participant group (ASD and NT) and investigated hand muscle (FDI and ADM). Subsequently, they were analyzed via a 2 (group: ASD vs. NT) × 2 (muscle: FDI vs. ADM) mixed-model analysis of variance (ANOVA). To investigate group differences in socially modulated mirroring, the within-subject factor gaze (averted vs. direct) was additionally included. To specifically examine whether larger MEPs are obtained when participants observe direct as compared to averted gaze from the acting model, four planned comparisons were also conducted, comparing direct vs. averted gaze for each group and muscle. Note that the between-subject factor session (i.e. first or second session to receive the placebo nasal spray) was also included in each ANOVA model as a categorical-factor-of-no-interest to control for variability induced by the cross-over oxytocin manipulation within the larger study. The partial Eta square (η^2_p) value was calculated as an estimate of effect size for each factor.

In order to explore the contribution of several predictor variables (age, SRS total score, AQ total score) on the dependent variables of interest, step-wise multiple regression analyses (with forward selection: *F* to enter = 1, *F* to remove = 0) were conducted. Here, the coefficient of determination (R^2) is given as an estimate of effect size. All statistics were calculated with Statistica 10 (StatSoft, USA). Results were considered significant with a *p*-value lower than .05.

3 Results

3.1. Baseline corticospinal excitability and rMT

Our results show no group difference in **resting motor threshold** (rMT), i.e. the lowest intensity of stimulation, expressed as a percentage of the maximum stimulator output, required to produce MEPs of at least 50 mV in 5 out of 10 consecutive trials in the experimental FDI muscle (independent samples t-test: $t(52) = 0.58$, $p = .56$). Similarly, no differences were encountered in terms of the average peak-to-peak MEP amplitude of the targeted FDI muscle in response to 10 supra-threshold (130% rMT) TMS pulses between individuals with ASD and neurotypical (NT) participants ($t(51) = -0.51$, $p = .61$). Together, these data suggest that baseline corticospinal excitability is not affected in ASD.

3.2. Mapping of simple hand movements

Basic mirror-motor mapping was investigated by presenting simple, intransitive index finger abduction movements without any context (i.e. in front of a white background) to a group of ASD and NT participants. Average MEP-PC data (% change from rest; untransformed) are visualized in **figure 1A**, separately for each group and muscle.

Single-sample t-tests against zero showed that MEP amplitudes of the experimental FDI were significantly enhanced compared to rest (i.e. MEP-PC > 0) when observing index finger actions in both the ASD ($t(25) = 2.33$, $p = .03$) and NT group ($t(27) = 2.06$, $p = .05$), which is indicative of mirror-motor mapping. MEPs were also enhanced compared to rest in the control ADM muscle of the ASD group ($t(25) = 2.40$, $p = .02$), but this was not the case for NT participants ($t(27) = 1.55$, $p = .13$). Yet despite these subtle differences, a 2 (group: ASD vs. NT) × 2 (muscle: FDI vs. ADM) mixed-model ANOVA on the log-transformed MEP-PC data indicated no main effect of group ($F(1,50) = 1.39$, $p = .24$, $\eta^2_p = .03$), no main effect of muscle ($F(1,50) = 0.62$, $p = .43$, $\eta^2_p = .01$), nor a group × muscle interaction effect ($F(1,50) < .01$, $p = .94$, $\eta^2_p < .001$). A similar analysis on the background EMG data, recorded during the 110-10 milliseconds before application of the TMS pulse, yielded no significant results (all $p > .10$).

A step-wise multiple regression analyses with forward selection (F to enter = 1, F to remove = 0) was conducted to investigate the impact of several predictor variables (age, SRS total score, AQ total score) within the ASD group. The regression model indicated a significant effect of SRS total score ($F(1,21) = 9.52$, $p = .006$, $R^2 = .31$). Inspection of the beta coefficient ($\beta = -.56$) revealed a negative association between self-reported social impairments and the extent that simple hand movements are mapped into the observer's own mirror system (**figure 1B**). The other remaining variables (age, AQ total score) were not allowed to enter the model. Note that no significant associations were identified in the NT group (none of the predictor variables were allowed to enter the model; F to enter < 1).

3.3. Social top-down response modulation of mirror responses

Socially modulated mirroring was investigated by showing the ASD and NT participants similar intransitive finger abduction movements as described previously, combined with either direct or averted gaze from the acting model. Untransformed MEP-PC data (% change from rest) for each gaze condition, muscle and group are presented in **figure 1C**.

In accordance with previous studies from our lab (Prinsen & Alaerts, 2019; Prinsen et al., 2017, 2018), the 2 (group: ASD vs. NT) × 2 (muscle: FDI vs. ADM) × 2 (gaze: direct vs. averted) mixed-model ANOVA revealed a significant effect of observed gaze direction ($F(1,50) = 12.29$, $p < .001$, $\eta^2_p = .20$), indicating that across groups and muscles, perceived direct gaze elicited significantly higher MEPs (relative to baseline MEPs) compared to averted gaze. There was no significant main effect of group ($F(1,50) = 1.34$, $p = .26$, $\eta^2_p = .07$) or muscle ($F(1,50) = 1.05$, $p = .31$, $\eta^2_p = .02$), and no interaction effect between gaze × group ($F(1,50) = 1.19$, $p = .28$, $\eta^2_p = .02$) or between muscle × group ($F(1,50) = 0.01$, $p = .92$, $\eta^2_p < .001$). In contrast to the previous studies from our lab (Prinsen & Alaerts, 2019; Prinsen et al., 2017, 2018), there was no muscle × gaze interaction ($F(1,50) = 0.10$, $p = .75$, $\eta^2_p = .002$). Also the three-way interaction between these factors was not significant ($F(1,50) = 0.81$, $p = .37$, $\eta^2_p = .02$). Thus, concerning our STORM hypothesis, there was no evidence that socially modulated mirroring in relation to observed gaze cues is overall impaired in ASD participants.

Further inspection of the mean data by means of planned comparisons suggests the possibility of a less specific muscle activation pattern mainly in the ASD group, as they showed significant gaze-related modulations in both the experimental FDI (planned comparisons: $t(25) = -2.58$, $p = .02$) and control ADM muscle ($t(25) = -2.71$, $p = .009$). NT participants on the other hand only demonstrated a significant gaze effect in the FDI ($t(28) = -2.11$, $p = .04$), but not the ADM muscle ($t(28) = -0.95$, $p = .35$). However, since the overall ANOVA interaction terms were not significant, caution is advised when interpreting these effects. Lastly, a similar ANOVA performed on the background EMG data indicated a main effect of group, showing that across gaze cues and for all recorded muscles, pre-pulse muscle tension was overall higher in the ASD compared to the NT participants ($F(1,50) = 4.23$, $p = .04$, $\eta^2_p = .08$).

In order to quantify gaze-related modulations during action observation, a similar MEP-PC score as described previously was calculated to capture the extent to which observed direct gaze elicits higher MEPs relative to the MEP response during averted gaze. The step-wise forward regression model revealed a significant effect of SRS total score herein ($F(1,21) = 5.72$, $p = .02$, $R^2 = .21$). Examination of the beta coefficient ($\beta = -.46$; **figure 1D**) suggested that the ability to show adaptive gaze-related modulations of interpersonal motor resonance is associated with less self-reported impairments in the social domain within the ASD group. No significant associations were identified in the NT group.

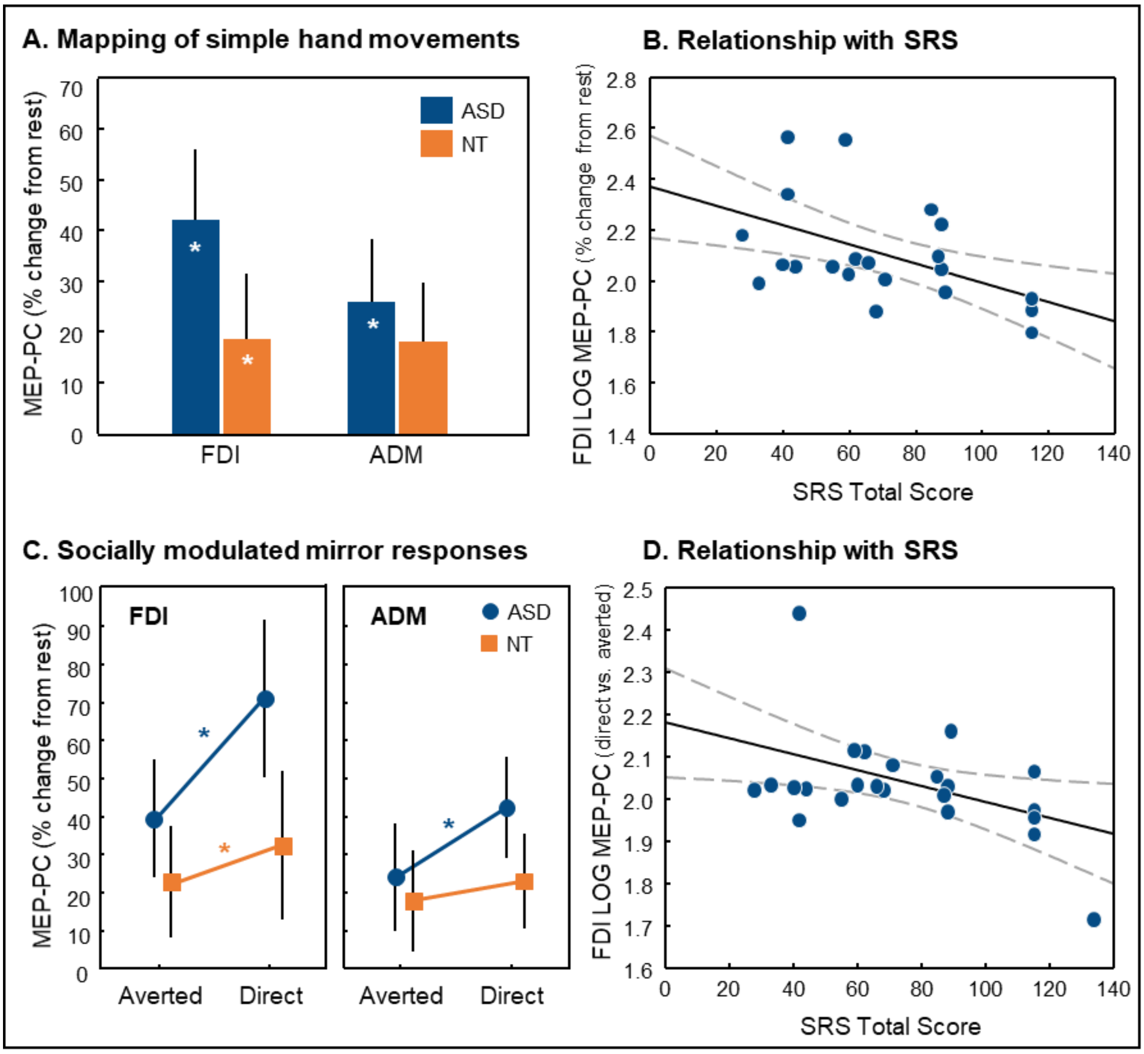

Figure 1. (A) Average (± SE) (untransformed) MEP-PC values (% change from rest) for each group and muscle. A higher value is indicative of enhanced interpersonal motor resonance (*$p < .05$). No significant group differences were encountered. **(B)** Scatterplot demonstrating the significant negative relationship between log-transformed FDI MEP-PC scores during observation of simple finger movements and ASD symptom severity, as measured by the Social Responsiveness Scale (SRS). **(C)** Average (± SE) (untransformed) MEP-PC values (% change from rest) for each muscle, group and gaze condition. No significant effects were revealed by the mixed-model ANOVA. **(D)** Scatterplot demonstrating the significant negative association relationship between ASD symptom severity, as measured by the SRS and log-transformed FDI MEP-PC scores capturing gaze-related modulations (% change direct gaze versus averted gaze). Dotted lines denote 95% CI.

4 Discussion

Individuals with a diagnosis of autism spectrum disorders (ASD) have impairments in social communication and interaction (American Psychiatric Association, 2013). In line with the hypothesized role of the mirror system in several important social abilities (e.g. action perception, intention understanding and empathy), the Broken Mirror Theory (Oberman & Ramachandran, 2007) was put forward, suggesting that a dysfunction of the mirror system could result in some of the social symptoms of ASD. However, another recent theory proposed that these impairments may be due to difficulties in using social cues to adapt interpersonal motor resonance to the demands of the social context (STORM; Wang & Hamilton, 2012). Using the non-invasive brain stimulation technique of transcranial magnetic stimulation (TMS), the current study investigated the feasibility of both accounts by examining neurophysiological motor resonance (quantified as the amplitude of TMS-induced motor evoked potentials; MEPs) in individuals with ASD compared to neurotypical (NT) participants under various observational conditions.

First, we used single-pulse TMS to investigate two parameters of **baseline corticospinal excitability**; i.e. resting motor threshold and average MEP amplitude when at rest. We found no differences in either, suggesting that baseline corticospinal excitability is not affected in participants in ASD, which is in line with previous published TMS studies (Enticott et al., 2013, 2012; Minio-Paluello, Baron-Cohen, Avenanti, Walsh & Aglioti, 2009; Théoret et al., 2005). Although there were some subtle differences in the muscle-specificity of MEPs in response to the **observation of simple hand movements**, no significant group differences in terms of mirror-motor mapping were encountered. This is in accordance with the previously discussed research showing negative results (Enticott et al., 2013; Fan et al., 2010; Marsh & Hamilton, 2011; Raymaekers et al., 2009), but also with more recent studies that report no mirror system impairments in ASD during observation of transitive and/or intransitive hand movements (Pokorny et al., 2015; Ruysschaert, Warreyn, Wiersema, Oostra & Roeyers, 2014), nor upon observation of point light displays depicting biological motion (Sotoodeh, Taheri-Torbati, Sohrabi & Ghoshuni, 2019). Taken together, these findings argue against a global mirror system deficit in ASD, thereby placing considerable limitations on the Broken Mirror Theory of ASD.

Earlier studies evaluating mirror system function in ASD seemed to indicate that putative impairments are dependent on the socio-emotional content of the stimulus (Dapretto et al., 2006; Grèzes et al., 2009) (but this notion is challenged by more recent studies that did not find ASD-NT group differences in emotional tasks; e.g. Bastiaansen et al., 2011; Schulte-Rüther et al., 2017). Here, we combined the observation of simple hand movements with direct and averted gaze cues from the acting model to investigate putative differences in **socially modulated mirroring** between ASD and NT participants.

Overall, enhanced motor resonance with the observed movements during direct compared to averted gaze from the interaction partner was found. Contrary to our expectations however, individuals with ASD showed no evidence of reduced interpersonal motor resonance during the observation of hand movements in combination with eye contact. This finding goes against the STORM account of mirror system dysfunction in ASD (Wang & Hamilton, 2012), positing that motor resonance in ASD is primarily atypical when the mentalizing system is engaged. In contrast with earlier findings from our lab (Prinsen & Alaerts, 2019; Prinsen et al., 2017, 2018), the muscle-specific effect of motor resonance, as initially demonstrated by Fadiga et al. (1995) and later replicated extensively (for a review, see Fadiga et al., 2005), was not demonstrated for the overall group. Further inspection of the mean data suggested the possibility of a less specific muscle activation pattern mainly in the ASD group, as they showed significant gaze-related modulations in both the experimental FDI and control ADM muscle, whereas NT participants only demonstrated a significant gaze effect in the FDI, but not the ADM muscle. One speculative possibility could entail that interpersonal motor resonance in ASD is more driven by unspecific arousal processes. This notion is also supported by the finding that the background EMG data, denoting muscle tension before application of the TMS pulse, was overall significantly higher in this group. However, since the overall interaction effect was not significant in the current study, caution is provided when interpreting this effect. Further examination of these subtle differences in mirror system response patterns between ASD and NT participants are necessary.

It is however important to note that, among the ASD participants included in the study, self-reported social impairments as measured by the Social Responsiveness Scale (SRS) were significantly associated with all dependent variables of interest (i.e. simple mirror-motor mapping and gaze-related mirroring). This association was not present among NT participants. This is in line with previous studies reporting an association with social symptom severity across neuroscientific methods (Enticott et al., 2012; Fan et al., 2010; Wadsworth, Maximo, Donnelly & Kana, 2018), and suggests that various functional markers of the mirror system may be able to reflect symptom heterogeneity in ASD. As such, we do not necessarily argue against any mirror system dysfunction in ASD. Rather, we propose that the ubiquitous heterogeneity in ASD might be the most likely candidate to explain the inconsistent pattern of results encountered in the literature. This notion is further underscored by recent studies by Hudac et al. (2015, 2017), who demonstrated that children and adults with distinct genotypes of ASD were differentially impacted in terms of mu rhythm suppression in response to social vs. non-social stimuli. Partially in line with the Broken Mirror Theory, there may be a subgroup of individuals with ASD who show intrinsic deficits in terms of mirror system function, as has been demonstrated by initial, but also more recent studies (Wadsworth et al., 2018, 2017).

Recently, studies showing aberrant structural (Fishman, Datko, Cabrera, Carper & Müller, 2015) and functional (Fishman, Keown, Lincoln, Pineda & Müller, 2014) connections between the mirror and mentalizing system in participants with ASD. At face value, this might provide further evidence in favor of the STORM account. Note however an emerging body of evidence showing that the neural basis of ASD cannot be pinpointed to specific brain regions, but that symptomatology is instead increasingly linked to atypical connectivity within and between functionally specialized brain networks in ASD (which may or may not include the mirror system; Müller & Fishman, 2018). On a similar note, the encountered associations between ASD social symptom severity and interpersonal motor resonance as assessed by TMS may not only be due to deficits within the mirror system, but may also result from inter-individual differences in visuospatial attention in ASD (further analysis of the participants’ gaze behavior, as collected by eye-tracking technology in this study, may provide further indications herein). Further investigations are necessary to determine the source (e.g. differences in attention, atypical neural connectivity) and the functional significance of these impairments. This will allow us to decide whether the mirror system should be a possible target for diagnosis and/or treatment in ASD, or can be considered an epiphenomenon of other mechanisms.

Taken together, the current findings provide no support for an overall mirror system deficit in all individuals with ASD, as posited by the Broken Mirror Theory. Similarly, no hard evidence in favor of an aberrant social top-down response modulation (STORM) of the mirror system was encountered. However, more mirror system impairments – whether measured in terms of basic motor mapping or socially modulated mirroring – were associated with greater symptom severity in the ASD group. As such, our findings add to the controversy surrounding the role of mirror system dysfunction in ASD. At the same time, they also underline the necessity to discover the different social conditions under which motor resonance impairments are evident in ASD, and how this might relate to ASD (social) symptom severity. As studies that have or have not found impairments in the ASD mirror system seem to be equally distributed across techniques, (broad) types of visual stimuli and age groups, the field could greatly benefit from a formal overview of the available data.

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PART III.

Sniffing around oxytocin

Chapter 6

To mirror or not to mirror upon mutual gaze, oxytocin can pave the way.

A cross-over randomized placebo-controlled trial.

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Abstract

The eyes constitute a highly salient cue to communicate social intent. Previous research showed that direct eye contact between two individuals can readily evoke an increased propensity to ‘mirror’ other peoples’ actions. Considering the implicated role of the prosocial neuropeptide oxytocin (OXT) in enhancing the saliency of social cues and modulating approach/avoidance motivational tendencies, the current study adopted the non-invasive brain stimulation technique transcranial magnetic stimulation (TMS) to explore whether a single dose of intranasal OXT (24 IU) modulated (enhanced) a person’s propensity to show heightened mirroring or motor resonance upon salient social cues, such as eye contact. The study involved a double-blind, placebo-controlled, cross-over trial with twenty-seven healthy adult men (19–32 y). By applying single-pulse TMS over the primary motor cortex during movement observation, it was shown that motor resonance was significantly higher when movement observation was accompanied by direct, compared to averted gaze, but that a single dose of OXT did not uniformly enhance this effect. Significant moderations of the treatment effect were noted however, indicating that participants with high self-reports of attachment avoidance displayed a stronger OXT-treatment effect (enhancement of motor resonance upon direct eye contact), compared to participants with low attachment avoidance. Particularly, while participants with high attachment avoidance initially displayed a reduced propensity to increase their motor resonance upon direct eye contact, a single dose of OXT was able to promote an otherwise avoidant individual’s propensity to engage in motor resonance upon a salient social cue such as eye contact.

1 Introduction

Interpersonal interactions are extremely complex, involving both approach and avoidance behaviors toward other conspecifics. An important feature of successful social interaction and indicator of social approach is biobehavioral synchrony, or the coordination of biological and behavioral processes between interaction partners (Feldman, 2017). At the neural level, the brain's action observation system or mirror system is anticipated to play a key role in establishing interpersonal synchrony or 'resonance'. Several neuroimaging and neurophysiological studies show that distinct motor regions in fronto- and parietal cortices are increasingly activated not only when performing a particular action, but also when merely observing the same action performed by others, thereby providing a direct 'mirror-motor matching' or 'motor resonance' mechanism (Rizzolatti & Craighero, 2004). Overall, this 'mapping' of observed actions onto the observer's own motor system is suggested to form the basic mechanism by which others' actions, facial expressions or emotional states can be recognized, understood and acted upon (Cattaneo & Rizzolatti, 2009; Rizzolatti & Fabbri-Destro, 2008).

Albeit automatic, the propensity to 'synchronize' with conspecifics is anticipated to depend heavily upon the presented social context and prior social experiences of the individual (Wang et al., 2012). Among different social cues from the environment, mutual gaze forms a very powerful signal to express communicative intent and attention, and may therefore constitute a salient cue to evoke interpersonal synchrony or approach-related behavior (Grossman, 2017; Senju & Johnson, 2009). In line with this notion, studies from our and other labs showed that eye contact can rapidly and specifically facilitate automatic mirroring of others' actions, indicative of social approach (Prinsen et al., 2017; Wang et al., 2011a, 2011b). Here, we aim to explore the effect of social context (i.e. eye gaze) on motor resonance further and, in particular, whether administration of the prosocial neuropeptide oxytocin (OXT) can modulate this effect.

Endogenous OXT is synthesized in the hypothalamus where neurons of the paraventricular nuclei project to various cortical and subcortical brain areas involved in social behavior and socio-cognitive processes. Since the discovery that central OXT levels can be pharmacologically manipulated by means of intranasal administration of exogenous OXT (Born et al., 2002; Churchland & Winkielman, 2012), an ever growing body of research has tested the implication of OXT on human sociality. Based on early findings reporting beneficial effects of OXT on social behavior, OXT has gained its prosocial reputation. However, this exclusively prosocial view of OXT has been nuanced by findings showing that the effects of OXT are strongly dependent upon the context in which the social interaction happens (Bos et al., 2012), as it can for example lead to a decrease in social cooperation towards members of an out-group (De Dreu et al., 2010).

Although not mutually exclusive, several mechanisms have been proposed by which OXT affects social behavior, namely (i) by enhancing the saliency of social cues; (ii) by modulating reward sensitivity and approach/avoidance motivational tendencies; and (iii) by reducing (social) anxiety (Bartz, 2016; Neumann & Slattery, 2015; Shamay-Tsoory & Abu-Akel, 2016). In particular interest for this study, eye-tracking studies showed that exogenously administered OXT promotes gaze towards the eye region of the communicator (Guastella et al., 2008) and increases eye contact during naturalistic social interactions (Auyeung et al., 2015). Increasing evidence also suggests that OXT can mediate the processing of the communicator’s body language (Bernaerts et al., 2016; De Coster et al., 2014; Kéri & Benedek, 2009; Perry et al., 2010). For example, in terms of mapping of bodily cues, a handful of behavioral studies showed that a single dose of OXT reduced reaction times in an imitation task (De Coster et al., 2014) and enhanced biological motion perception or emotion recognition from so-called point-light display’s (Bernaerts et al., 2016; Kéri & Benedek, 2009). An initial EEG study showed that OXT induced an increase in mu-rhythm suppression during biological motion perception, which is indicative of mirror-neuron activation (Perry et al., 2010).

With the present study, we adopted a novel paradigm to explore the prosocial effects of OXT-treatment on mirror-motor mapping or interpersonal motor resonance from a neurophysiological perspective. Particularly, by using the non-invasive and widely-used brain transcranial magnetic stimulation (TMS) technique, motor resonance upon movement observation was measured in order to obtain an unbiased neurophysiological measure of an individual’s propensity to ‘synchronize with’ an observed model. In the past decade, single-pulse TMS has been used extensively as an assessment tool to measure resonant mirror activity in the observer's motor system during the observation of others’ actions (see Fadiga et al., 2005 for a review). In particular, by applying a single magnetic pulse over the primary motor cortex, the underlying cortical neurons are activated, which elicits a motor evoked potential (MEP) from the corresponding contralateral muscles. Fadiga et al. (1995) showed that during the mere observation of others’ actions, activity within the primary motor cortex becomes increasingly facilitated, as indicated by significant enhancements in MEP amplitudes elicited by TMS. By measuring the amplitude of the motor evoked potentials (MEPs) elicited by TMS under various experimental conditions, TMS can be used to monitor changes in putative mirror system activity in a relatively high temporal resolution.

As previous research showed that eye gaze provides a salient modulator of motor resonance (Prinsen et al., 2017; Wang et al., 2011a, 2011b), we expected to observe an enhancement of ‘synchronization’ during movement observation accompanied with direct gaze from the model (indicative of communicative intent), compared to averted gaze (indicative of no or even averted communicative intent). A key objective was to examine whether an individual’s propensity to show motor resonance upon direct gaze is modulated from the administration of a single dose of OXT. In line with the implicated role of OXT in enhancing the saliency of social cues and modulating approach/avoidance motivational tendencies, we expected OXT to induce an augmentation of motor resonance or ‘approach behavior’ upon a salient communicative cue such as direct eye contact (i.e., socially adaptive mirroring). Furthermore, since OXT has been shown to impact viewing behavior towards the eye region, we also explored whether changes in viewing behavior were related to changes in interpersonal motor resonance. Finally, considering the emerging relevance of person-dependent factors in modulating the prosocial effects of OXT (Bakermans-Kranenburg & van IJzendoorn, 2013; Bartz et al., 2011a,b), we additionally explored whether the observed treatment effects of OXT on motor resonance were moderated by inter-individual differences in social responsiveness or attachment style.

2 Materials and methods

2.1 General study design

This randomized, double-blind, placebo (PL)-controlled, cross-over trial with a wash-out period of one week was conducted at the Department of Rehabilitation Sciences at the University of Leuven (Belgium) to test single-dose effects of intranasal oxytocin (OXT) administration on interpersonal motor resonance assessed using transcranial magnetic stimulation (TMS) (**figure 1A**). Written informed consent was obtained from all participants. Consent forms and study design were approved by the Ethics Committee for Biomedical Research at the University of Leuven (S56327) in accordance to the Declaration of Helsinki (World Medical Association, 2013). The trial was registered with the ClinicalTrials.gov database of the U.S. National Institutes of Health (NCT03010670).

Table 1. **Participants' characteristics (*n* = 26).**

Measure	Mean ± SD
Age (years; months)	24;4 ± 3;6
Social Responsiveness – SRS-A	
Social Awareness	7.50 ± 4.40
Social Communication	9.96 ± 6.04
Social Motivation	7.27 ± 4.06
Rigidity and Repetitive Behavior	7.81 ± 4.04
State Attachment – SAAM	
Attachment Security	6.05 ± 0.61
Attachment Anxiety	3.43 ± 1.42
Attachment Avoidance	1.96 ± 0.68

SAAM = State Adult Attachment Scale; SRS-A = Social Responsiveness Scale, adult version.

2.2 Sample size and participants

A total of 26 participants (age-range: 19–32 years; participants' characteristics see **table 1**) completed the two sessions of the cross-over trial and were included in the final analyses (see CONSORT flowchart in **Appendix A**). Inclusion criteria comprised gender (male); age (18–35 years old); and handedness (right). Only male participants were recruited to avoid potential sex differences in OXT response as well as the potential interaction with the female hormonal cycle. Other exclusion criteria comprised medication use; any diagnosed psychiatric or neuropsychological disorder (e.g., stroke, epilepsy, concussion) or any contraindication for TMS (Rossi et al., 2012). In one prior clinical trial, a cross-over design was used to assess the effects of single-dose OXT-treatment on a neurophysiological measure of mirror activity (mu rhythm) using EEG. Significant effects (large size) were reported for a total of 24 participants who completed the OXT/PL cross-over treatment (Perry et al., 2010). Considering this prior cross-over study, the current sample size was set at a comparable sample of 26 participants.

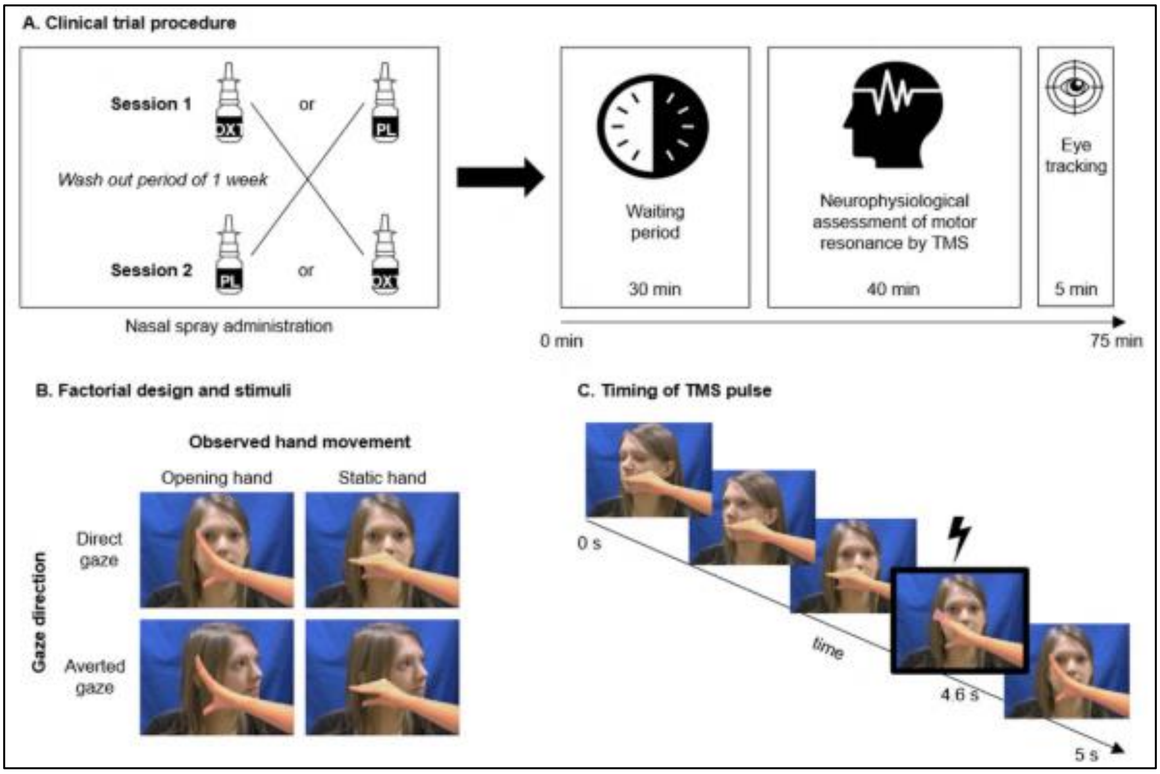

Figure 1. Overview of the experimental design choices. (A) Clinical trial procedure and timing schedule. **(B)** Factorial design: video stimuli showing a model performing a simple intransitive hand movement (hand opening) or no movement (static hand), accompanied with either direct or averted gaze. The last still of each video clip is depicted. **(C)** Example of the timing of the TMS pulse. Single-pulse TMS was delivered approximately 4.6 s after the start of each video clip, which corresponded to the execution phase of the observed hand opening movement.

2.3 *Drug protocol*

Participants were randomly assigned to receive the OXT (Syntocinon®, Sigma Tau) or PL (saline solution of sodium chloride in water) nasal spray on the first/second testing session. Both sprays were prepared by the KU Leuven University Hospital pharmacist and were administered in identical amber 15 ml glass bottles with metered pump, such that all research staff conducting the trial and participants were blind to treatment allocation. According to the golden standard in human OXT research (Graustella & MacLeod, 2012), a single dose of 24 international units (IU), delivered as 3 puffs of 4 IU per nostril, was adopted. Participants received clear instructions about the use of the nasal spray prior to self-administration (Guastella et al., 2013). Studies investigating OXT concentrations in saliva (Daughters et al., 2015) and plasma (Gossen et al., 2012; Striepens et al., 2013) after intranasal administration of a single dose of OXT have indicated that peripheral OXT levels significantly increase approximately half an hour after intranasal administration. The efficacy of this time interval has also been confirmed by animal research (Chang et al., 2012; Neumann et al., 2013). Consequently, in healthy humans, the impact of a single dose of intranasal OXT on social cognition is commonly evaluated using a 30–45 min wait-time before the experimental task (see Graustella & MacLeod, 2012 for a review). Here, a thirty-minute wait-time was incorporated prior to any experimental task in order to test during peak OXT concentrations. All experimental measures were conducted within the assumed 75 min time window in which heightened levels of peripheral OXT can be observed (Daughters et al., 2015; Gossen et al., 2012; Striepens et al., 2013) (see **figure 1A**). Participants were monitored onsite for the full experimental procedure (until approximately 1.5 h after nasal spray administration) and were screened for potential adverse events or side effects. Additionally, the Profile of Mood States questionnaire (POMS; Wald & Mellenbergh, 1990) was used at the beginning and end of each session to monitor transient mood levels of participants within and across sessions.

2.4 Neurophysiological outcome measure: motor resonance

The primary outcome measure was assessed 30 min after nasal spray administration (Daughters et al., 2015), using the non-invasive brain stimulation TMS technique. During the assessment of motor resonance by TMS, participants were seated in a comfortable chair approximately 80 cm in front of a widescreen monitor (resolution: 1920 × 1080 pixels, refresh frequency: 60 Hz) with their hands placed palm-down on a soft cushion on their lap and another cushion placed on top to obstruct vision of the own hands during the experiment. Participants were asked to relax their hand muscles while they spontaneously viewed a random sequence of four different video clips showing a model performing a simple hand movement (hand opening) or no movement (static hand), accompanied with either direct or averted gaze (**figure 1B**). Video clips were identical to those previously adopted in Prinsen et al. (2017) and Wang et al. (2011a). Each condition was presented five times in blocks of four five-second video clips (total of 20 clips per condition). Video presentation timing was controlled by LabVIEW software (version 14.0, National Instruments, UK). During observation of the video clips, single-pulse TMS (Magstim200 stimulator, Magstim Company Ltd., UK) was applied over the left primary motor cortex using a hand-held 70 mm figure-of-eight coil and electromyography (EMG) recordings were performed to measure motor-evoked potentials (MEPs) from the contralateral abductor pollicis brevis (APB) muscle, a muscle implicated in the observed hand opening movement. TMS pulses were delivered to coincide with the hand opening phase, i.e., 4.6

s after the start of the video clip (see **figure 1C** for an example). Coil placement, optimal location for TMS-stimulation and resting motor threshold were defined for each participant as described in Prinsen et al. (2017). Experimental stimulation intensity was set supra-thresholded at 130%. Signal Software (version 2.02, Cambridge Electronic Design, UK) was used for EMG-recordings and triggering of the TMS-stimulator. All EMG-recordings were sampled (2000 Hz), amplified and band-pass filtered (5–1000 Hz) via a CED Power 1401 analog-to-digital converting unit (Cambridge Electronic Design, UK). The neurophysiological assessment with TMS lasted approximately 40 min.

2.5 Secondary outcome measure: eye tracking

After the neurophysiological assessment, a short eye tracking session was conducted to evaluate potential changes in spontaneous viewing behavior of the participants. During this session (duration approximately 5 min, see **figure 1A**), participants sat in front of a Tobii T120 binocular eye tracking device (resolution: 1280 × 1024 pixels, sampling rate: 120 Hz, average precision 0.5° of visual angle) (Tobii AB, Sweden) and were presented with the same experimental video clips as described above. During eye tracking, the total fixation duration (TFD) or the sum of the durations of all fixations towards a predefined area of interest (AOI) centered over the eye region of the model’s face was assessed. Please note that data of two participants was excluded from the final analysis due to technical errors during gaze behavior acquisition.

2.6 Assessment of person-dependent factors

To assess inter-individual differences in treatment-effects related to person-dependent factors, participants completed self-report questionnaires assessing social responsiveness (Social Responsiveness Scale for adults, SRS-A; Constantino & Todd, 2005) and state attachment (State Adult Attachment Measure, SAAM; Gillath et al., 2009). The SRS-A is a 64-item questionnaire to assess variations in social responsiveness in the typical population and autism spectrum disorders using a four-point Likert-scale. It encompasses four subscales: social communication (22 items), social awareness (19 items), social motivation (11 items) and rigidity/repetitiveness (12 items). Higher scores indicate less social responsiveness. The SAAM is a 21-item questionnaire to assess inter-individual differences in state attachment using a seven-point Likert-scale. The questionnaire comprises three subscales of 7 items assessing attachment security (e.g. “*I feel like I have someone to rely on*”); attachment anxiety (e.g. “*I feel a strong need to be unconditionally loved right now*”); and attachment avoidance (e.g. “*If someone tried to get close to me, I would try to keep my distance*”).

2.7 *Data analysis and statistics*

Based on the recorded EMG data, peak-to-peak amplitudes of the TMS-evoked MEPs were determined to assess condition-induced changes in cortico-motor excitability at the level of M1. Additionally, background EMG was quantified by calculating the root mean square error (RMSE) across the 110–10 millisecond interval prior to TMS-stimulation to ensure that subjects were completely relaxed during stimulation. Trials with excessive tonic muscle activity (background EMG exceeding 2.5 standard deviations from the mean) were not included in the final analyses (2.41% of all trials). Further, extreme MEP-amplitudes (exceeding 1.5 interquartile distances from the mean) were removed from the analysis (8.77% of all trials). Note that the number of discarded trials was similar across sessions and observation conditions (all $p > .68$).

As raw MEP amplitude values were not normally distributed, mean MEP amplitudes were log-transformed. To explore whether log-transformed MEPs recorded upon movement observation were modulated by 'gaze condition' or 'treatment', a two-way repeated-measures ANOVA with the within-subject factors 'observed eye gaze' (direct gaze, averted gaze) and 'treatment session' (PL, OXT) was conducted. In subsequent ANCOVA analyses, we explored whether the baseline 'gaze' effect at the placebo session was potentially modulated by variations in person-dependent factors. Two separate ANCOVA models were performed, one model in which the subscales of the SRS ($n = 4$) and one model in which the subscales of the SAAM ($n = 3$) were inserted as continuous regressors. Similarly, the influence of person-dependent factors on the OXT treatment effect was investigated in a similar way, i.e. by repeating the aforementioned two-way ANOVA analysis with the additional inclusion of the person-dependent variations in SAAM or SRS questionnaire scores as continuous regressors.

To visualize significant relationships, Pearson correlation coefficients were calculated when a modulatory effect was detected. In order to quantify the baseline 'gaze' effect, the difference in MEP amplitude between direct and averted gaze was calculated ($MEP_{direct} - MEP_{averted}$ difference score) for each subject. The OXT treatment effect was calculated separately for each subject by subtracting the difference score of the PL session from the difference score of the OXT session, divided by the pooled standard deviation ($(\Delta Gaze_{OXT} - \Delta Gaze_{PL})/\text{sqrt}((SD^2_{OXT} + SD^2_{PL})/2)$) (Cohen's *d* treatment effect; Cohen, 1992).

All statistics were calculated with Statistica 10 (StatSoft, USA) and results were considered significant with a *p*-value lower than .05. The partial Eta square (η^2) value was calculated as an estimate of effect size.

3 Results

3.1 Side effect screening

All participants were screened for potential side effects or changes in mood states related to the OXT treatment. As described in detail in the appendices, only minimal, non-treatment specific side effects (see **supplementary table B.1**) or changes in mood states (**supplementary figure C.1**) were reported.

3.2 The effect of eye contact on motor resonance and its modulation by oxytocin

In **figure 2**, the effect of observed eye gaze on MEP amplitudes (i.e. interpersonal motor resonance) is visualized for the experimental opening hand condition, separately for each session (PL, OXT). The repeated-measures ANOVA analyses on the log-transformed MEP amplitudes with the within-subject factors observed gaze (direct, averted) and treatment session (PL, OXT) revealed a significant effect of gaze ($F(1,25) = 5.79$, $p = .02$, $\eta^2 = .19$), indicating that across treatment sessions, MEP responses were significantly larger for the direct, compared to the averted eye gaze condition. These results are in line with previous reports of an enhancing effect of direct gaze on interpersonal motor resonance during movement observation (Prinsen et al., 2017).

Although the difference between direct and averted gaze was slightly larger in the OXT session (difference: 0.08 mV, Fisher LSD: $p = .04$), compared to the PL session (difference: 0.05 mV, Fisher LSD: $p = .16$), the interaction between observed gaze and treatment was not significant ($F(1,25) = 0.01$, $p = .92$, $\eta^2 < .001$), indicating that across all participants, the facilitating effect of direct eye contact on interpersonal motor resonance was not significantly augmented by the OXT treatment (**figure 2**). Note that while the mean MEP amplitudes of the direct eye gaze and averted eye gaze conditions were not significantly different between the PL and OXT session, it appeared that the overall dispersion of the data points around the sample mean (standard deviation) was larger in the placebo ($SD_{direct} = 0.90$, $SD_{averted} = 0.92$), compared to the OXT treatment session ($SD_{direct} = 0.68$, $SD_{averted} = 0.67$).

In a subsequent analysis, we explored whether the high variance in interpersonal motor resonance at the PL session was potentially related to inter-individual variance in person-dependent factors (self-reported social responsiveness (SRS) or attachment style (SAAM)). To do so, repeated-measures ANCOVA analyses with the within-subject factor ‘eye gaze’ (direct, averted) were conducted with the person-dependent factors included as continuous regressors (separate models for the SAAM and SRS subscales).

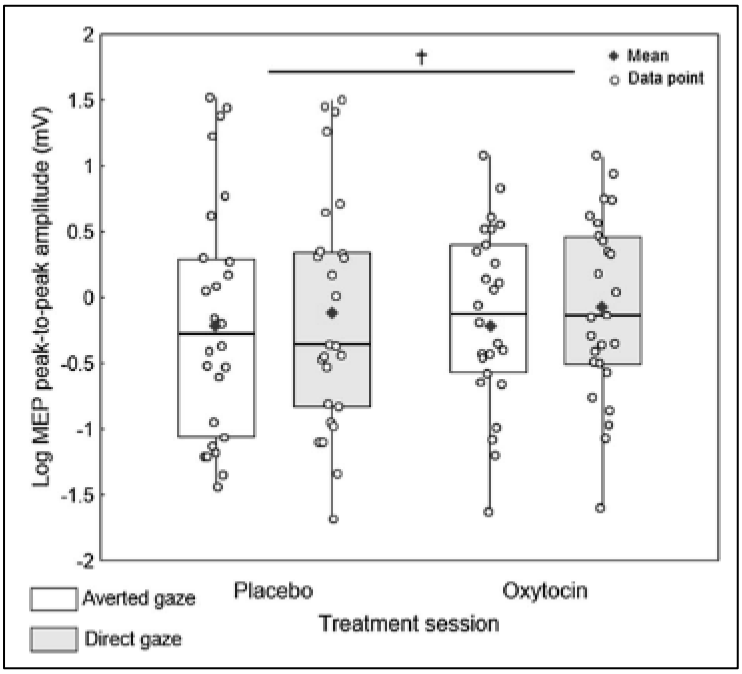

Figure 2. The effect of eye gaze on log-transformed MEP amplitudes, displayed for each gaze condition (averted, direct) and treatment session (PL, OXT). Across treatment sessions, MEPs where higher when action observation was accompanied with direct, compared to averted gaze. Although the difference between direct and averted gaze was more pronounced in the OXT compared to the PL session, primary analysis revealed no significant interaction effect. However, secondary analyses – regressing out variability in treatment responses related to inter-individual differences in reports of attachment avoidance (SAAM) – revealed a significant interaction effect, indicating an augmentation of eye contact induced motor resonance after administration of OXT ($^{\dagger}p < .05$). Horizontal lines show median, boxes denote 25%–75% of data and vertical lines denote non-outlier range.

For the MEP data recorded at the PL session, a significant interaction was revealed between observed gaze and the subscale ‘attachment avoidance’ ($F(1,22) = 6.32$, $p = .02$, $\eta^2 = .22$), indicating that the extent of the eye gaze effect on interpersonal motor resonance was significantly modulated by attachment avoidance. In particular, the modulatory interaction indicated that the facilitating effect of direct gaze on interpersonal motor resonance (higher $\text{MEP}_{\text{direct}} - \text{MEP}_{\text{averted}}$ difference scores) was more pronounced for participants with low attachment avoidance scores, compared to participants with high avoidance scores ($r = -.50$, $p = .009$; **figure 3A**).

No significant modulatory interactions were revealed for the other subscales of the SAAM (gaze x attachment security interaction: $F(1,22) = 0.18$, $p = .67$, $\eta^2 = .008$; gaze × attachment anxiety interaction: $F(1,22) = 0.58$, $p = .45$, $\eta^2 = .03$) or for the model assessing modulatory effects by the subscales of the SRS (all $p > .14$), indicating that the modulation of the eye gaze effect at the PL session was specific for attachment avoidance.

3.3 *Modulation of the oxytocin treatment effect by person-dependent factors*

Considering the modulatory effect of attachment avoidance in the baseline PL session, we further explored the possibility of a modulatory impact of this person-dependent factor on the OXT-treatment response. To do so, the ANCOVA analysis with the within-subject factors ‘observed gaze’ (direct, averted) and ‘treatment session’ (PL, OXT) was repeated with the person-dependent factor ‘attachment avoidance’ inserted as a continuous regressor. Interestingly, a significant tree-way interaction between the factors ‘observed gaze’, ‘treatment session’ and ‘avoidance’ was revealed ($F(1,24) = 8.24$, $p = .008$, $\eta^2 = .26$), indicating that the effect of OXT on the eye gaze effect was significantly modulated by attachment avoidance. In particular, the modulatory interaction with attachment avoidance indicated that while the facilitating effect of direct eye gaze on interpersonal motor resonance was not further augmented by OXT in participants with low attachment avoidance, a single dose of OXT was able to induce a significant augmentation of this eye gaze effect in participants with high attachment avoidance. **Figure 3B** visualizes the significant relationship ($r = .51$, $p = .008$) between attachment avoidance and the individual OXT treatment effect scores (individual Cohen’s *d*, higher *d* scores indicate a stronger facilitation of the eye gaze effect by OXT).

Of note, the aforementioned ANCOVA analysis (with the inclusion of the ‘attachment avoidance’ regressor) also revealed a significant two-way interaction between ‘eye gaze’ and ‘treatment session’ ($F(1,24) = 7.20$, $p = .01$, $\eta^2 = .23$, medium effect). This indicates that − across all individuals − a significant OXT-induced augmentation of the eye gaze effect on interpersonal motor resonance was evident when variations related to inter-individual differences in attachment avoidance are regressed out (**figure 2**).

Note that no significant modulations of the ‘eye gaze × treatment’ interaction were revealed when any of the other SAAM or SRS subscales were inserted as continuous regressors. Accordingly, also no significant correlations were revealed between these person-dependent-factors and the individual OXT treatment effect scores (all $p > .07$), indicating that the modulatory effect was specific for attachment avoidance (see **supplementary table D.1**).

Together, these observations indicate that while participants with high attachment avoidance initially showed a reduced tendency to show enhanced interpersonal motor resonance upon direct eye contact at the PL session, a single dose of OXT was able to induce an augmentation of this effect, particularly for the participants high on attachment avoidance.

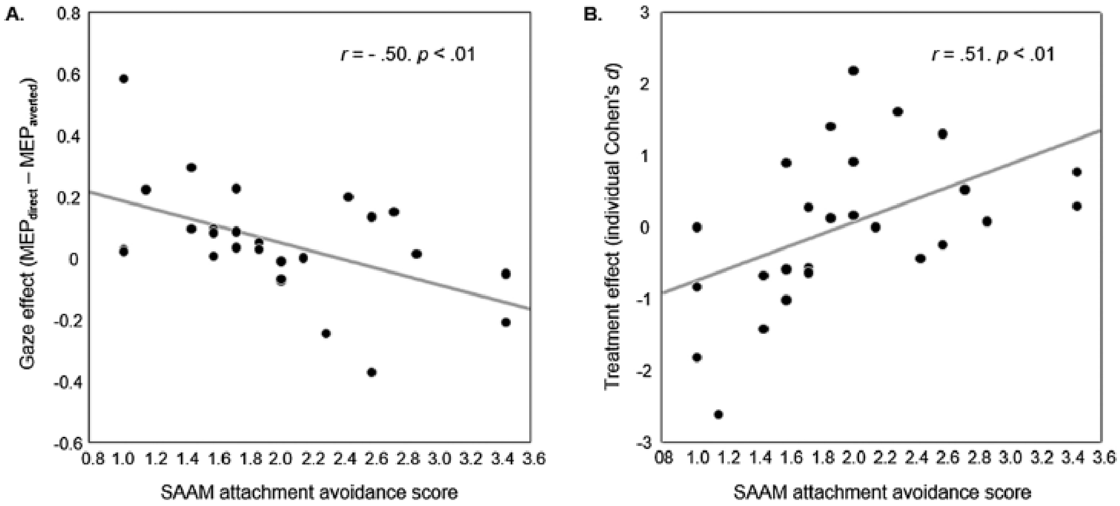

Figure 3. Modulation by person-dependent factors. (A) Relationship between inter-individual variations in self-reported SAAM attachment avoidance and the effect of eye gaze on inter- personal motor resonance at the baseline (PL) session. The facilitating effect of direct gaze on motor resonance (higher MEPdirect – MEPaverted difference scores) was more pronounced for participants with low attachment avoidance scores, compared to participants with high avoidance scores. **(B)** Relationship between inter-individual variations in self-reported SAAM attachment avoidance and the treatment effect of OXT on interpersonal motor resonance (individual Cohen's d scores). The effect of direct eye gaze on motor resonance was further augmented by OXT for participants with high attachment avoidance, not for participants with low attachment avoidance (higher d scores indicate a stronger augmentation of the eye gaze effect by OXT).

3.4 The effect of eye contact on gaze behavior and its modulation by oxytocin

Similarly to the analysis on the MEP data, a two-way repeated-measures ANOVA with the within-subject factors 'observed gaze' (direct gaze, averted gaze) and 'treatment session' (PL, OXT) was conducted on the total fixation time (in sec) towards the eye region of the model's face. A significant main effect of observed gaze was revealed (**figure 4**), indicating that across treatment sessions (PL or OXT) participants fixated significantly longer at the eye region of the face when the presented model displayed direct compared to averted eye gaze ($F(1,23) = 10.45$, $p = .004$, $\eta^2 = .31$).

Although the difference in gaze time between direct and averted gaze was larger in the OXT session (difference: 2.44 s, Fisher LSD: $p = .002$), compared to the PL session (difference: 1.61 s, Fisher LSD: $p = .03$), the interaction between observed gaze and treatment session was not significant ($F(1,23) = 0.67$, $p = .42$, $\eta^2 = .03$), indicating that across participants, the difference in gaze time between the direct and averted eye gaze condition was not significantly enlarged by OXT. Note that, when performing a similar ANCOVA as described for the MEP responses, the gaze × treatment interaction effect on gaze behavior towards the eye region, albeit still not reaching statistical significance, became more pronounced by including attachment avoidance as an additional regressor into the model ($F(1,22) = 2.28$, $p = .14$, $\eta^2 = .09$).

Further, we explored whether the effect of OXT on interpersonal motor resonance upon direct eye gaze (compared to averted gaze) was paralleled by an increase in gaze time towards the eye region during the direct eye gaze condition (compared to the averted gaze condition). Pearson correlation analyses between the individual OXT treatment effect on the MEP data (Cohen's *d* MEP-scores) and the OXT treatment effect on the gaze time data (Cohen's *d* TFD-scores) did not reveal a relationship between these measures (raw correlation: $r = .04$, $p = .84$). The relationship remained insignificant when variance related to attachment avoidance was regressed out (partial correlation: $r = .21$, $p = .34$).

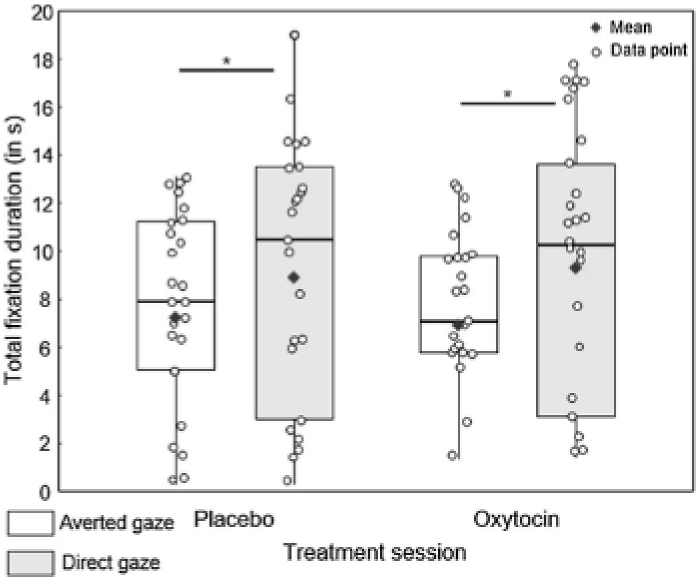

Figure 4. The effect of observed gaze direction on the total fixation duration towards the eye region of the model, for each treatment session. Participants fixated the eye region of the face significantly longer when the presented model displayed direct compared to averted eye gaze. Although OXT enhanced spontaneous gaze behavior towards the eye region of the model's face (in the direct vs. the averted gaze condition), the interaction effect failed to reach statistical significance. Horizontal lines show median, boxes denote 25%–75% of data and vertical lines denote non-outlier range.

3.5 *Control static hand condition and EMG background*

MEP-amplitudes recorded during the observation of the control static hand condition (i.e., no movement observation) were not significantly modulated by eye gaze ($F(1,25) = 0.73$, $p = .40$, $\eta^2 = .03$). The effect of eye gaze during the control condition was also not significantly modulated by the administration of a single dose of OXT ('eye gaze' by 'treatment' interaction effect: $F(1,25) = 0.01$, $p = .91$, $\eta^2 < .01$). No modulations by person-dependent factors (SAAM, SRS) were observed for the eye gaze effect or OXT treatment effect of the MEPs obtained for the control condition. Furthermore, none of the reported effects on MEP responses were modulated by condition- or session-related differences in background EMG scores (all $p > .61$).

4 Discussion

The current study presents results of a double-blind, cross-over, randomized placebo-controlled trial assessing the immediate effects of OXT – a neuropeptide implicated in prosocial behavior − on an individual's tendency to 'synchronize with' or 'approach' an observed model displaying communicative intent (i.e. engaging in direct eye contact) or not (i.e. displaying averted gaze). Particularly, by using the non-invasive brain stimulation tool TMS, an objective neurophysiological index of a person's propensity to show interpersonal motor resonance in different situational contexts was assessed.

4.1 The effect of eye contact on motor resonance and its modulation by oxytocin

Similar to previous research (Prinsen et al., 2017; Wang et al., 2011a, 2011b), this study underlines the notion that the observed model's communicative intent provides a salient modulator of mirror-motor mapping, such that 'synchronization' during movement observation is higher when accompanied with mutual gaze between the observer and observed model. These observations support the notion that interpersonal motor resonance is not an isolated automatic process, but can be controlled by a hierarchical 'social top-down response modulation' mechanism (STORM) that is dependent on the social context in which others' actions are observed (Wang et al., 2012). In this view, instead of automatically simulating all possible movement-related information perceived in a visual scene, salient social cues (such as direct eye contact) may 'direct' the observer's motor system to preferentially process visuo-motor input originating from the most socially salient communicator. With the current study, we also provide first neurophysiological evidence that a single dose of OXT was able to induce an augmentation of eye contact induced interpersonal motor resonance, specifically for participants high on attachment avoidance (as measured by the SAAM). Particularly, our data demonstrated that while participants with high attachment avoidance initially showed a reduced tendency to increase their interpersonal motor resonance upon a salient social cue such as direct eye contact, a single dose of OXT was able to induce an augmentation of this effect.

4.2 Modulation of the treatment effect by person-dependent factors

While in more 'avoidant' individuals the presence of a social cue such as direct gaze did not unanimously result in enhanced interpersonal resonance (indicative of 'approach'), a single dose of OXT was able to promote the propensity of this otherwise 'avoidant' individual to engage in 'approach behavior' upon a communicative cue such as eye contact. All in all, our data are in line with prior reports that the induction of prosocial effects by OXT may be more pronounced for individuals with low baseline levels of social proficiency or approach motivation (e.g. avoidantly attached individuals), whereas for individuals with already high baseline levels of approach motivational tendencies (e.g. securely attached individuals), the additional administration of exogenous OXT may not stimulate prosocial behavior further (Bartz, 2016). In a previous study by our lab, young adult men were administered with a daily dose of OXT for a period of two weeks, and significant improvements in self-reports of attachment avoidance (SAAM) and attachment toward peers (measured by the Inventory of Parent and Peer Attachment (IPPA; Armsden & Greenberg, 1987) were revealed (Bernaerts et al., 2017). Interestingly, and similar to the present study, the treatment-induced changes in the latter study were also found to be most pronounced for participants with less secure attachments. Likewise, Buchheim et al. (2009) found that, in insecurely attached adults, a single dose of intranasal OXT is sufficient to induce a significant increase in the experience of attachment security, as measured by the Adult Attachment Projective Picture System (AAP; George & West, 2001).

Aside the observation that treatment effects may be more pronounced in participants with low social proficiency or high attachment avoidance, recent accounts also highlight the possibility of reversed or anti-social effects of OXT for individuals with a high sensitivity towards rejection (e.g. anxiously attached individuals; Bartz et al., 2015; Bartz et al., 2011a,b). For example, in individuals with borderline personality disorder OXT was shown to induce a reduction in the perception of trust or the likelihood to cooperate (Bartz et al., 2011a,b). In the present study, a measure of inter-individual variation in attachment anxiety was obtained from the SAAM questionnaire, but based on the current sample no moderating effects were revealed. Future studies will however be necessary to address this issue further (e.g., by exploring moderating effects in a priori selected sample of participants with high attachment anxiety).

4.3 The effect of oxytocin on spontaneous gaze behavior

Although an increase in mutual gaze after administration of a single dose of OXT has been observed before (Auyeung et al., 2015; Guastella et al., 2008), we only observed a non-significant trend that OXT enhanced spontaneous gaze behavior towards the eye region of the observed model’s face. Even though we observed that the effect of the OXT treatment on spontaneous gaze behavior was to some extent more pronounced when variance related to inter-individual differences in attachment avoidance was regressed out, we cannot draw any firm conclusions, since none of the effects of OXT on gaze behavior reached significance.

We would like to note however that, since the experimental design was prioritized for assessing the effects of OXT on interpersonal motor resonance as assessed with TMS, the eye tracking assessments of changes in spontaneous gaze behavior were only performed at the end of the experimental session, i.e. around 70 min post-administration. Although uncertainty exists with respect to the pharmacokinetics of OXT, heightened levels of peripheral OXT have repeatedly been observed until 75–90 min post-administration (Daughters et al., 2015; Gossen et al., 2012; Striepens et al., 2013). However, a more recent study suggested the most optimal time window to lie between 45 and 70 min (Spengler et al., 2017). While the timing of the TMS assessment largely overlapped with this time window, the possibility cannot be ruled out that − perhaps within a subset of individuals − the timing of the eye tracking session might have extended beyond the most optimal pharmacokinetic time window to assess the single-dose effect of OXT, hence the observation of only tentative effects. To rule out this possibility of a timing effect on the assessed outcome measures, future studies might envisage adopting a randomized order (instead of a fixed order) for the included experimental assessments.

Despite this methodological consideration in terms of the adopted timing, there have been previous studies that were also not able to show a significant modulation of mutual gaze by OXT (Domes et al., 2010; Hubble et al., 2017; Lischke et al., 2012). Further research may therefore be necessary to establish the robustness of the effect of OXT on increasing spontaneous gaze behavior towards the eye region and the establishment of mutual gaze. Furthermore, considering the current observation of a tentative modulation by attachment avoidance, we recommend these future explorations to continue to take variations in person-dependent factors into account.

4.4 Relationship between motor resonance and gaze behavior

The encountered inter-individual variability in effects raises questions about the mechanism(s) by which OXT modulates approach behavior in general, and interpersonal motor resonance in particular. On the one hand, it can be suggested that OXT exerted these effects by increasing the ‘saliency’ of the presented social cue (eye gaze), which is in line with the social saliency hypothesis of OXT (Shamay-Tsoory & Abu-Akel, 2016). In this view, the demonstrated effect of OXT on enhancing socially adaptive motor resonance in avoidant individuals may have been related to OXT-related enhancements of overt viewing behavior towards the eye region of the model. However, although our study was not specifically designed to test this hypothesis, the obtained pattern of results suggests that the relationship between overt viewing behavior and interpersonal motor resonance may be more complex.

First, since the perception of cues in a presented scene may not be limited to the fixated area, the possibility cannot be ruled out that the modulation of the ‘saliency’ of the presented social cues by OXT may extend beyond the overt fixated area i.e., involving peripheral vision. Furthermore, Myllyneva and Hietanen (2015) have shown that not continued mutual gaze per se, but rather the knowledge of being looked at by another person may be the pivotal factor in modulating responses to social stimuli. In their study, they manipulated participant’s beliefs of whether or not they could be seen by a live person performing direct gaze sitting behind a liquid crystal shutter screen. Notably, only when participants merely believed that the person was able to see him or her through the shutter, enhanced autonomic arousal responses were observed. These results suggest that mental attributions, rather than overt visual attention, are important in modulating the processing of socially relevant information. Of note, other studies that have not showed a significant modulation of mutual gaze by OXT did encounter OXT-induced improvements in a different measured variable of sociality; i.e. facial emotion recognition (Domes et al., 2010; Hubble et al., 2017; Lischke et al., 2012), suggesting that OXT-induced changes in social cognition can occur independently of modulations in overt visual attention. Thus, while overt fixations towards the eye region may be equally high in different participants, it appears that the mental evaluation of the perceived eye contact may be considerably different. For some, direct eye contact may readily trigger an increased tendency to ‘mirror’ the other person, whereas for others, the perceived eye contact may be evaluated as being more unpleasant or intrusive and therefore elicit avoidant related responses (i.e., no increased tendency to mirror). In other words, we speculate that perhaps not the ‘saliency’ of the eye contact per se, but rather the perceived or evaluated ‘approachability’ of the presented social cue may have been modulated by OXT (although note the difficulty in strictly delineating these two constructs on a conceptual level).

To conclude, a single dose of intranasally administered OXT was shown to induce an augmentation of a person’s propensity to engage in interpersonal motor resonance or ‘approach behavior’ upon a salient communicative cue such as direct eye contact, but only in individuals with high reports of attachment avoidance. These results provide neurophysiological support to the implicated role of OXT in modulating approach/avoidance motivational tendencies, and importantly, underscore that inter-individual differences in ‘baseline’ approach/avoidance tendencies can constitute an important moderating factor.

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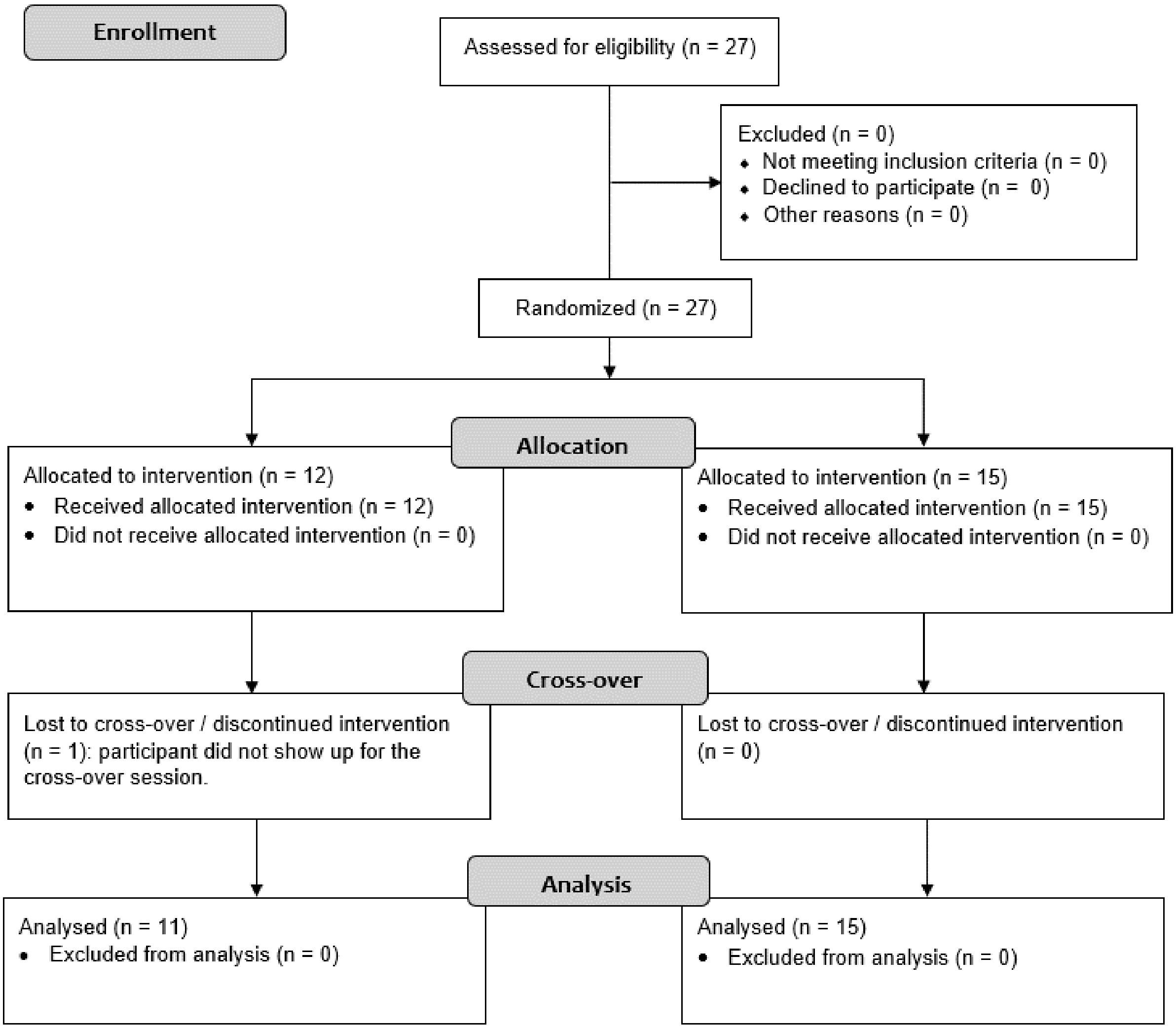
Appendix A. CONSORT flowchart



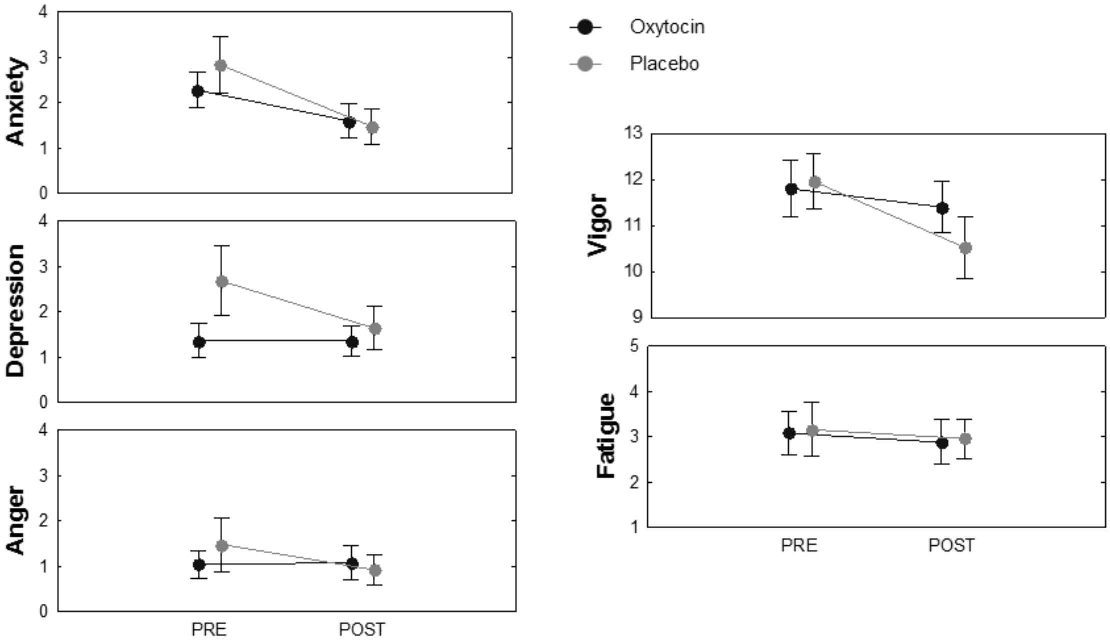
Appendix B. Reported side effects

Supplementary table B.1. Frequency of reported side effects and severity. At the end of each experimental session (OXT = oxytocin; PL = placebo), participants were asked to report whether they experienced any of the listed (or other) side effects and to indicate the severity of the side effect (mild, moderate, or severe). For each session, the number of participants that reported any mild, moderate or severe side effects are listed.

Side effect	Mild		Moderate		Severe	
	OXT	PL	OXT	PL	OXT	PL
Head ache	2	2				
Drowsiness	11	9	2	6		1
Dizziness	1	1			1	
Dry throat/dry mouth	2	1		1		
Congested nose	1	1				
Sneezing	1					
Runny nose	2	3	1			
Muscle pain/cramps		1				
Sweating	1	2				
Blurred vision	1	1				

Appendix C. Results POMS questionnaire

A 32-item short version of the Profile of Mood States (POMS) questionnaire (Wald and Mellenbergh, 1990) was used as a measure of transient affective states in order to assess whether mood levels of participants changed over the course of the trial. This instrument comprises 32 emotional adjectives subdivided in five domains: anxiety (6 items), depression (8 items), vigor (5 items), fatigue (6 items) and anger (7 items) rated on a five-point Likert scale. For all participants, the POMS questionnaire was assessed at the start and end of each experimental session (i.e., pre- and post- administration of a single dose of nasal spray). No significant differences in mood states were revealed between the oxytocin and placebo treatment session (Wilcoxon matched pairs test, all *p* > .18).



Supplementary figure C.1. Mean scores at the start and end of each experimental session (i.e., pre- and post- administration of a single dose of nasal spray) for each subdomain of the POMS questionnaire. Vertical bars denote mean ± SE.

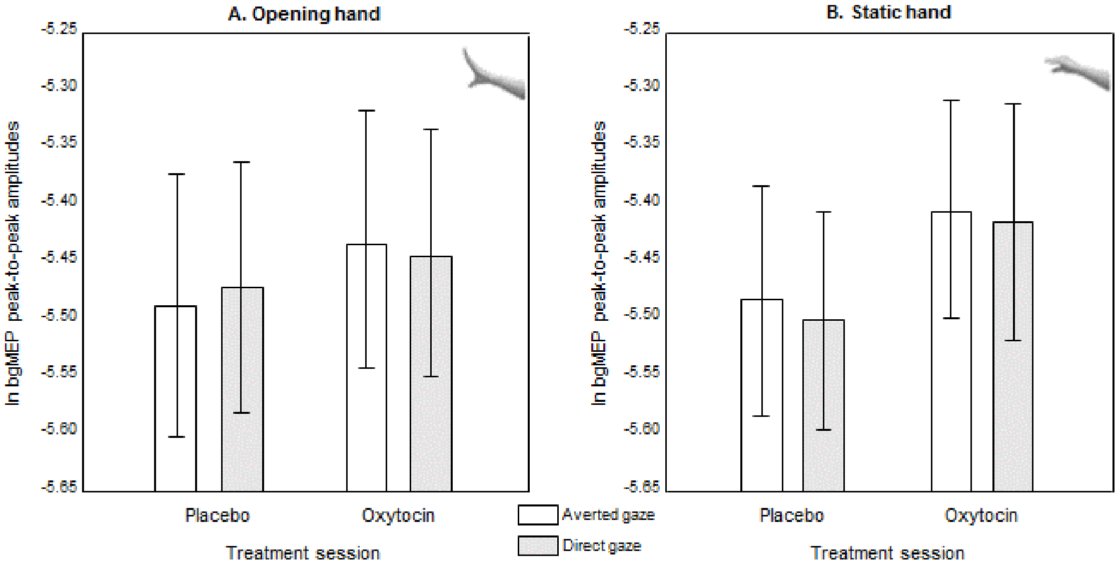
Appendix D. Variability in OXT effect

Supplementary table D.1. Pearson correlation coefficients (N = 26) examining the relationship between self-reported social responsiveness (Social Responsiveness Scale, adult version; SRS-A) and attachment style (State Adult Attachment Scale; SAAM) on the extent by which treatment with a single dose of OXT modulated gaze-dependent interpersonal motor resonance (individual treatment effect scores; Cohen's *d*).

	Individual Cohen's *d*	
Measure	***r***	***p***
Social Responsiveness – SRS-A		
Social Awareness	.07	.72
Social Communication	.31	.13
Social Motivation	.36	.07
Rigidity and Repetitive Behavior	-.14	.49
Attachment Style – SAAM		
Attachment Security	-.27	.19
Attachment Anxiety	-.06	.77
Attachment Avoidance	**.51**	**.008**

Appendix E. Background EMG scores

Background EMG was quantified by calculating the root mean square error (RMSE) across the 110 to 10 millisecond interval prior to TMS-stimulation. Since background EMG is known to modulate the size of MEP amplitudes, similar analysis were performed on the background EMG data. Across treatment sessions, the main effect of gaze was not significant, neither for the opening ($F(1,25) = 0.004$, $p = .95$, $\eta^2 < .001$) nor for the static hand ($F(1,25) = 0.16$, $p = .69$, $\eta^2 = .007$). The 'eye gaze' by 'treatment' interaction effects were also not significant, not for the experimental opening hand ($F(1,25) = 0.26$, $p = .61$, $\eta^2 = .01$), nor for the control static hand ($F(1,25) = 0.01$, $p = .91$, $\eta^2 < .001$) condition.



Supplementary figure E.2. The effect of observed eye gaze (averted gaze, direct gaze) and received treatment (placebo, oxytocin) on the log-transformed background EMG scores, separately for each observed hand (opening hand, static hand). No significant effects were encountered. Vertical bars denote SE ± mean.

General Discussion

1 Summary of the main findings

The main goal of the present dissertation was to provide more insight in the social modulation of the mirror system, both in health and psychopathology, from a neurophysiological perspective (i.e. by means of the single-pulse transcranial magnetic stimulation (TMS) technique). The PhD project's objectives were achieved by conducting six studies, which were reported in the previous chapters and summarized below.

Part I. Taking a look at motor resonance

In our first 'proof of principle' study (**chapter 1**), we developed a sensitive and easily applicable single-pulse TMS protocol for measuring the effect of direct versus averted eye gaze on interpersonal motor resonance, as indexed by corticospinal excitability. A total of 33 healthy, young adult participants (16 men, mean age 22;7 years) participated in this study, undergoing TMS while viewing the same set of stimuli previously adopted by Wang, Newport & Hamilton (2011). Results indicated that only during action observation (i.e. during observation of the opening hand), motor resonance was significantly enhanced when the actress in the video clips displayed direct compared to averted eye gaze. During observation of the static hand however, observed gaze had no effect on motor resonance. An additional control experiment tracking participants' gaze behavior ensured the encountered gaze-related modulations in M1 excitability were not due to differences in visuospatial attention when observing direct vs. averted gaze. Thus, using TMS, we successfully extended the behavioral results from the Hamilton group (Wang, Newport & Hamilton 2011; Wang, Ramsey & Hamilton, 2011) and confirmed that the previously encountered effects of observed eye gaze on mimicry are based on the modulation of the mirror system. Furthermore, we demonstrated that – in healthy participants – **direct eye contact readily elicits an increased propensity to mirror others' actions**. This study is published in *Neuropsychologia* (Prinsen et al., 2017).

One possible limitation in measuring responses to pictorial or videotaped gaze cues is that they may only vaguely approximate the real-life affective significance of eye contact. In **chapter 2**, we explored whether these gaze-related modulations in motor resonance changed as a function of the live presence of the model. Therefore, 22 healthy young adult participants (8 men, mean age 25;3 years) were presented with two set-ups while undergoing TMS: a video presentation set-up in which participants watched videos shown on a computer, and a live presentation set-up with a live model sitting behind a voltage-sensitive liquid crystal shutter screen. The results of this study indicated that **live social stimuli are processed in a similar, but more intense way, than pictured stimuli**, thereby highlighting the importance of using ecologically valid methods for social cognitive neuroscience. Furthermore, the eye contact effect encompassed a muscle-specific increase in M1 excitability, and was not driven by or dependent on differences in autonomic arousal or visual attention. This study is published in *Social Cognitive and Affective Neuroscience* (Prinsen & Alaerts, 2019).

In **chapter 3**, gaze-related modulations were investigated in both TMS-induced MEPs and the EEG-based mu rhythm (neural oscillations in the 8-13 Hz frequency band over the sensorimotor strip) in 32 healthy adult participants (20 men, mean age 24;8 years). Both neurophysiological markers of the mirror system were significantly modulated by perceived eye gaze; i.e. a significant increase in MEP amplitude and a significant attenuation of the mu rhythm when observing direct compared to averted gaze from the model. Interestingly, in terms of the relative gaze effect, a significant correlation between both measuring systems was found at the inter-individual subject level. These results suggest that both the EEG mu rhythm and TMS-induced MEPs are sensitive to the social relevance of the observed actions, and that – despite their very different neural substrates – they may reflect similar gating processes within the mirror system network.

Part II. In the eye of the beholder.

In **chapter 4**, we combined the data from several experiments to obtain a relatively large sample of 141 healthy adult participants to explore how inter-individual variability in social responsiveness, social anxiety and attachment style towards others are related to one's ability to show adaptive gaze-related modulations in terms of interpersonal motor resonance. In sum, both experimenter-driven split group and data-driven cluster analyses demonstrated that **less socially proficient participants also show a reduced tendency to mirror the movements of the interaction partner upon perceived eye contact**. These results suggest that an adequate top-down response modulation (i.e. STORM) of motor resonance in response to observed gaze cues is not universal, but might be linked to the social traits of the beholder.

Individuals with as autism spectrum disorder (ASD) endure lifelong impairments in social and communicative functioning. Both the Broken Mirror Theory and the STORM account propose that the associated socio-interactive deficits in ASD result from impaired mirror system functioning. Whereas the Broken Mirror Theory (Oberman & Ramachandran, 2007) posits a global mirror system dysfunction in ASD, the STORM account (Wang & Hamilton, 2012) postulates that the mirror system itself might not be broken in ASD, but that the communication between the mirror system and other brain regions related to the evaluation of social cues might be altered. In **chapter 5**, we put the feasibility of both the Broken Mirror Theory and the ASD-adaptation of the STORM account to the test by assessing M1 excitability under various observational conditions (i.e. when at rest, during observation of simple hand actions and during observation of similar hand actions in combination with direct and averted gaze from the actor). Based on the STORM account it was expected that, unlike in neurotypical participants, eye contact would not result in enhanced MEP amplitudes during direct compared to averted gaze conditions. However, since we did not encounter significant group differences in our outcome measures, we cannot draw any firm conclusions from this data. Nevertheless, our results provide initial indications that the extent of mirror system (dys)functioning may heavily depend on social symptom severity in ASD.

Part III. Sniffing around oxytocin

Lastly, in **chapter 6**, we explored the effect of oxytocin in targeting the STORM dynamics of motor resonance. As such, a double-blind, placebo-controlled, cross-over trial with 27 healthy young-adult men (mean age: 24;4 years) was conducted to investigate whether a single dose of intranasally administered oxytocin (24 IU) modulated a person's propensity to show increased motor resonance upon the observation of a salient social cues such as eye contact. With this study, we provided first neurophysiological evidence that a single dose of oxytocin is able to selectively augment motor resonance in socially relevant situations, i.e. only when direct gaze from the interaction partner is perceived. However, significant moderations of the treatment effect were noted, indicating that participants with high self-reports of attachment avoidance displayed a stronger oxytocin-related effect (enhancement of motor resonance facilitation by direct gaze), compared to participants with low attachment avoidance. Particularly, while participants with high attachment avoidance initially displayed a reduced propensity to mirror the movements of the interaction partner upon perceived eye contact, a single dose of oxytocin was able to mitigate this reduced mirror system response. Importantly, these results underscore that inter-individual differences in baseline approach/avoidance tendencies can constitute an important moderator of the effectiveness of the oxytocin response. This study was published in *Psychoneuroendocrinology* (Prinsen, Brams & Alaerts, 2018).

2 Placing motor resonance in the gaze context

2.1. STORM of motor resonance

Our data clearly shows that interpersonal motor resonance, as assessed by TMS, is impacted by the extended social context in which action observation occurs. It extends the previously obtained behavioral results obtained by Wang, Newport et al. (2011) showing gaze-related modulations in automatic mimicry, and appoints the mirror system as the neurophysiological substrate for the encountered behavioral effects. Although the TMS technique does not reveal the neural mechanisms that underlies the observed results, this data fits the notion that the human mirror system plays an important role in modulating appropriate responses to the observed actions of others. According to Wang and Hamilton (2012), this adjusting property of motor resonance is grounded in a rapid and sophisticated social top-down response modulation (i.e. STORM) driven by an integrative evaluation of all social features in the current interaction by the mentalizing system, with the main goal to maximize interpersonal affiliation (see **figure 3** in General Introduction, p. 27).

2.1.1. Convergent evidence

Although our studies mainly focused on the observation of simple, intransitive hand actions to delineate the effect of salient social cues (i.e. observed eye contact) on neurophysiological markers of the mirror system (i.e. interpersonal motor resonance as assessed by TMS and EEG mu rhythm suppression), the social nature of other motor-related processes has been examined in several other lines of research.

One line of research has focused on the modulation of action kinematics of reach-to-grasp movements by interactive contexts. Several studies (reviewed by Becchio et al., 2010) report differences in kinematics while *executing* reach-to-grasp movements related to the non-social (reach toward an object, grasp it, and move it from one spatial location to another) vs. social (reach toward and grasp the same object and pass it to a partner) end goal of the movement. Convergently, also when comparing *observation* of social vs. non-social reach-to-grasp movements, increased activation within classic mirror areas (i.e. inferior frontal gyrus (IFG) and inferior parietal lobule (IPL)) has been reported (Becchio et al., 2012). In line with the STORM hypothesis, mentalizing areas such as the temporoparietal junction (TPJ) and the medial prefrontal cortex (mPFC) were increasingly activated when observing the social vs. non-social reach-to-grasp movements (but note that no connectivity or modelling analysis was performed in this study, leaving the notion of a top-down modulation of mirror regions by the mentalizing system in the middle).

Furthermore, although the mirror mechanism is generally associated with a congruent, muscle-specific resonance between performed and observed actions, our everyday interpersonal interactions most often require to not directly imitate the actions of others, but to provide a complementary response, i.e. a *reaction*. For example, if someone is holding a mug by its handle (i.e. performing a precision grip) and hands it to us, we have to select a complementary grip (i.e. whole hand grip) to take it. Indeed, a growing body of work highlights that the mirror mechanism is not only a matter of simple direct-matching between observed and executed actions, but is also involved in motor preparation when complementary responses are appropriate (reviewed by Campbell & Cunnington, 2017). In general, these studies show that the observation of stimuli expressing a social request that involves a complementary action (e.g. handing a mug) induces a shift from symmetrical simulation to reciprocal activation in the participant’s mirror system. This is also reflected in the pattern of muscle activation in the observer as assessed by TMS (e.g. Sartori, Cavallo, Bucchioni & Castiello, 2012).

Related to eye contact processing, only recently TMS studies have taken the interplay between gaze and action cues during interactive requests into account (Betti et al., 2018, 2019), showing that also reciprocal motor preparation is similarly enhanced by observed eye contact. Similar to the notion of a social top-down control, it has been suggested that in task situations where complementary or opposing (e.g. counter-imitative) reactions are necessary, the mirror system is also activated, but subjected to a top-down component originating in the frontal areas underlying executive control (Campbell & Cunnington, 2017). Together, these studies suggest that activity within mirror areas during the observation and execution of movements is shaped in relation to their higher-level social intention, regardless of the specific lower-level muscle pattern, action chain or immediate task goal involved.

2.1.2. Functional and translational potential

The STORM theory as outlined by Wang and Hamilton (2012) claims that the social top-down modulation of mimicry has the overall aim to facilitate social interaction and enhance liking and affiliation. Based on the current results and reviewed literature however, indicating that not only task-dependent automatic mimicry, but also (i) interpersonal motor resonance upon passive action observation (without any associated task) (ii) execution and observation of reach-to-grasp movements and (iii) complementary actions are seemingly top-down modulated by perceived social cues, we additionally propose that the main goal of this modulation is not to maximize interpersonal affiliation per se. Rather, it reflects an increased response saliency and/or the overall tendency in the observer to socially engage with the surrounding environment (see also Becchio et al., 2010). Note that in this view, MEPs and M1 excitability are regarded an end-state cortical measures of how the brain evaluated the social relevance or saliency of the observed visual scene.

As such, the currently employed TMS paradigm may provide a sensitive and relatively easy-to-assess neural marker that captures the individual's propensity to 'approach' or 'avoid' the encountered social scene (see also further, **section 2.4 Integration**). Although perceived eye contact generally triggered an increase in interpersonal motor resonance (presumed to reflect a neural 'approach' reaction in the observer), an interesting association was noted between variability in this propensity to display 'approach' behavior in response to eye-to-eye contact and self-reported inter-individual differences in social proficiency, both in the neurotypical population and in participants with ASD. Specifically, in less socially proficient participants, eye contact-related motor resonance was significantly diminished in comparison to more socially skilled individuals. Importantly, a single dose of intranasally administered oxytocin was shown to enhance the propensity to mirror others' actions upon perceived eye contact, but only in those individuals who initially failed to do so. These findings further underscore the notion that socio-cognitive processes should be considered along a continuum of normal to impaired function. In this respect, and in line with the recent Research Domain Criteria (RDoC) initiative to adopt more objective versus subjective outcome measures for diagnostics and/or treatment outcome evaluation (Cuthbert & Insel, 2013), the findings from this doctoral project indicate that the current neural marker may have promising diagnostic and/or translational potential, especially for investigations situated within the Systems for Social Processes RDoC Domain.

2.1.3. Associated brain regions

Although direct empirical data in favor of this social top-down modulation account is yet rare, Wang, Ramsey and Hamilton (2011) showed that, in the context of the eye contact effect on automatic mimicry, the effective connectivity (as assessed by dynamic causal modelling) between mentalizing region mPFC and the superior temporal sulcus (STS; the main visual input region to the mirror system), is indeed modulated by observed eye gaze. In other words, this data suggests that gaze-related activations at the level of mPFC imposed a top-down control over the processing of visuo-motor information at the level of the STS. In turn, this impacts the extent by which observed actions are processed in upstream mirror regions. In this view, the primary motor cortex (M1) may be conceived as the end-state region of a chain of cortico-cortical connections signaling whether or not the corticospinal tract and the corresponding peripheral muscles are to be recruited for initiating overt motor simulation.

However, more work is needed to further justify this model, since it remains a possibility that a social top-down component over mirror system areas could also emerge from other cortical or subcortical areas. Indeed, in the broader field of social neuroscience, other theoretical proposals that describe how different systems of the social brain work together for eye contact and/or social information processing have underlined the role of other important brain regions, such as the amygdala, herein. Alternatively, others have suggested that increased activity within mirror areas in social situations might as well be bottom-up driven. Those alternative possibilities will be discussed below.

2.2. Neural eye contact models

Functional neuroimaging studies have revealed that perceived eye contact is an important modulator of several social brain regions, including the fusiform gyrus (FG), the anterior and posterior part of the STS, the mPFC and orbitofrontal cortex (OFC) and the amygdala (Senju & Johnson, 2009b). Currently, there are three neuroscientific models that explain the mechanisms underlying the eye contact effect of the social brain.

2.2.1. The affective arousal model

The earliest account, the affective arousal model (**figure 1A**), postulates that the perception of eye contact automatically elicits a strong affective response and directly activates brain areas involved in physiological arousal, particularly the amygdala (Skuse, Morris & Lawrence, 2003). This view is supported by: (i) PET neuroimaging studies reporting the contribution of subcortical structures in direct gaze perception (Kawashima et al., 1999; Wicker, Perrett, Baron-Cohen & Decety, 2003), (ii) evidence from a single-neuron study showing that the monkey amygdala contains neurons that respond selectively to eye contact (Mosher, Zimmerman & Gothard, 2014) and (iii) neurophysiological studies showing that perceiving someone’s direct gaze can readily trigger elevated sympathetic arousal (indexed as skin conductance response or pupil dilation) compared to averted gaze cues or closed eyes (recently summarized in Hietanen, 2018).

However, there is also evidence that autonomic arousal can be dissociated from the effect of direct gaze. For example in the current thesis, the effect of direct gaze on interpersonal motor resonance was shown to be independent of enhancements in sympathetic arousal (Prinsen & Alaerts, 2019). Also the important finding that enhanced arousal responses upon direct gaze require the participant to effectively *believe* that he or she can be seen by the interaction partner (as elegantly illustrated by Myllyneva & Hietanen, 2015, 2016), suggests that other brain areas might also be at play during eye contact perception (see also Cavallo et al., 2015). This suggests that the brain does not encode the mere visual input of two eyes directed to the observer, but specifically encodes the demand of a responsive action in the other's direct gaze.

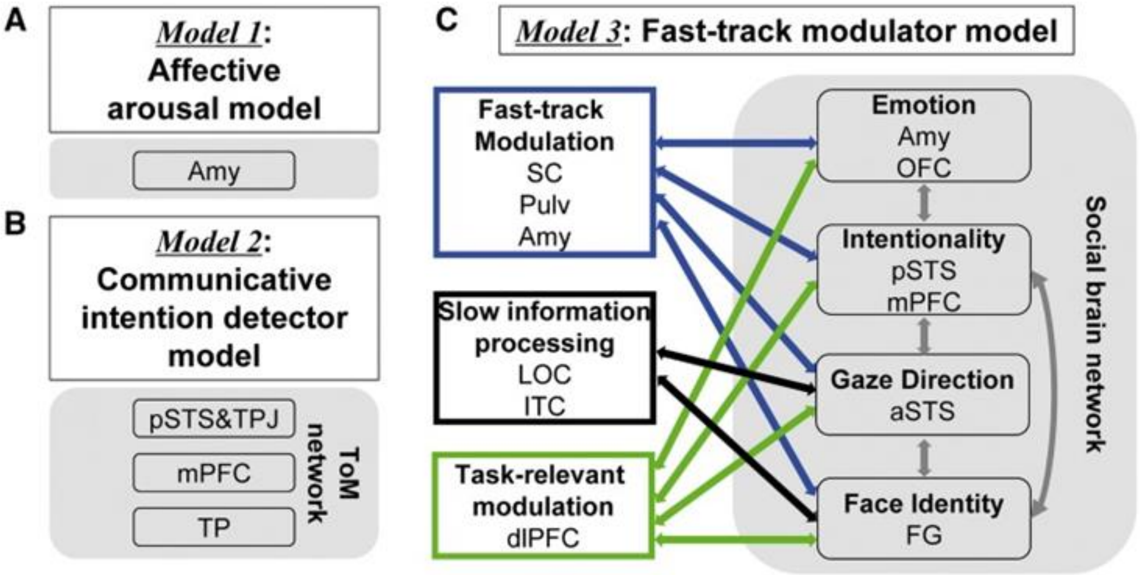


Figure 1. Neuroscientific models of eye contact processing. (A) The affective arousal model postulates that eye contact elicits responses in brain systems responsible for arousal, especially in the amygdala. **(B)** The communicative intention detector model assumes that eye contact signals the intention to communicate with others and involves cortical regions of the ToM network. **(C)** The fast-track modulator model proposes that eye contact is processed via a rapid subcortical (blue arrows) and slow cortical (black arrows) processing route. Information processed in the rapid route modulates processing function-specific regions of the social brain network. At the same time, these regions are modulated via prefrontal regions according to task and context demands (green arrows). For abbreviations, see text. Figure and figure legend adapted from Jiang, Borowiak, Tudge, Otto & von Kriegstein, 2017, *Social Cognitive and Affective Neuroscience*.

2.2.2. The communicative intention detector model

The second account, the communicative intention detector model (**figure 1B**), proposes that eye contact processing directly activates the theory of mind network, since it signals the intention to communicate with the perceiver (Vogeley, 2017). This notion is based on neuroimaging studies (e.g. Kampe, Frith & Frith, 2003) reporting that the neural activation pattern upon eye contact processing substantially overlaps with the regions relevant for theory of mind, i.e. the pSTS, TPJ, mPFC and temporal pole (TP). Of note, in the study by Kampe et al. (2003), activation in the theory of mind network related to eye contact processing was independent of arousal (i.e. changes in pupil diameter).

Also more recent evidence showed that, in the absence of motor acts, the perception of direct gaze by a live person was associated with activity in areas involved in motor execution/observation, including the IFG, the PMC, and the supplementary motor area (SMA) (Cavallo et al., 2015). Moreover, as indicated by PPI analysis, the recruitment of mentalizing area anterior rostral medial prefrontal cortex (arMPFC) during mutual gaze increased in coupling with the IFG. The authors posit that this finding speaks to the integrative role of IFG and arMPFC in social gaze processing and suggests that, during face-to-face interaction with a real partner, an increased coupling between areas concerned with detecting communicative intentions and the preparation of a motor response occurs (Cavallo et al., 2015).

2.2.3. The fast-track modulator model

The most recent account is the fast-track modulator model (**figure 1C**; Senju & Johnson, 2009b), which proposes a dual route to process visual information: a rapid subcortical (i.e. ‘fast-track’) and a slow cortical visual processing route. The rapid subcortical visual processing route – including the superior colliculus (SC), pulvinar (Pulv) and amygdala – operates on low spatial frequency information to detect salient cues, and then subsequently modulates further cortical processing in function-specific areas. Thus, in relation to eye contact and action observation, this model suggests that the fast-track mechanism underlying eye contact processing rapidly occurs before the full and detailed cortical analysis of other socio-environmental cues (such as human action) in the appropriate brain areas (i.e. STS), and then modulates activity in these areas. In an extension of the model, Burra, Mares and Senju (2019) further posit that this bottom-up modulation of the social brain regions can be subjected to other sources of modulation as well, such as a top-down control by the dorsolateral prefrontal cortex (dlPFC) based on task demands or instructions.

Note that the abovementioned neural eye contact models converge with other existing models that clarify atypical eye contact processing in ASD, including the amygdala theory of hyper-arousal (Baron-Cohen et al., 2000), the theory of mind account (Senju, Southgate, White & Frith, 2009) and the ASD-variant of the fast-track modulator model, which suggests that atypical eye contact processing in ASD is based on dysfunctioning of the subcortical detection route (including the amygdala) and/or its aberrant communication with the cortical and subcortical brain areas (Senju & Johnson, 2009a).

2.2.4. Bottom-up mechanisms

Coudé et al. (2016) presented first evidence that there is an integrated representation of others' hand actions and gaze direction at the single-neuron level in the macaque ventral PMC. Specifically, one half of the recorded mirror neurons in this area were modulated by the observed gaze direction of the human target during the observation of grasping actions. The authors discuss their results in light of the alternative view that mirror neurons depend on associative learning (Heyes, 2010). Based on the premise of neural plasticity and (system-level) Hebbian learning, this account posits that mirror neurons arise from the repeated simultaneous presentation of observed and executed movements (Keysers & Perrett, 2004). In this view, the seemingly hardwired representation of eye-hand cues at the single-neuron level in the monkey brain encountered by Coudé et al. (2016) can be explained as a form of mirror system attunement, induced by the fact that coordinated hand and gaze behavior is most frequently observed in daily life.

2.3. General social information processing models

This view of a social top-down modulation (i.e. STORM) of mirror system activation is similar to recent theoretical proposals suggesting that socio-cognitive functioning depends on the **interplay of two social neural systems** during social information processing (Vogeley, 2017; Yang et al., 2015); the mirror system, responsible for the comparably early and automatic detection of potentially salient social information such as bodily signals (confirmed by Spunt & Lieberman, 2013); and the mentalizing or theory of mind system, recruited during comparably late and controlled evaluation of actually socially salient information, including the mental states of persons.

In line with this theoretical notion of joint involvement, recent meta-analytic evidence showed that both systems are concurrently activated during social interaction, with a different weight depending on cues emphasizing either interpersonal motor resonance or mental state reasoning (Arioli & Canessa, 2019). This transition from mirroring to mentalizing has also been illustrated by the previously mentioned fMRI study by Becchio et al. (2012), in which participants were shown grasping movements performed with social versus individual intentions. In this study, activation within classic mirror system areas (IFG and IPL) was stronger during observation of socially intended movements relative to individual movements, whereas areas that form the mentalizing system (TPJ and mPFC) were only active during social grasping movements.

In an attempt to synthesize these key systems for social information processing, Yang et al. (2015) emphasized the central role of the superior temporal sulcus (STS) herein, as it is located at the two system’s intersection. This supposed centrality and inter-connectedness was confirmed via large-scale conjunction analysis and a resting-state functional connectivity analysis (including 1000 subjects) using Neurosynth (Yang et al., 2015). Because of this region’s centrality, the authors also suggest that the STS may be of particular importance for psychiatric disorders characterized by social deficits such as ASD. This notion has also been investigated by prior work from our lab, showing that impaired recognition of biological motion and emotion detection from point light displays implicated STS underactivity and -connectivity with the action observation network in individuals with ASD (Alaerts, Swinnen & Wenderoth, 2017; Alaerts et al., 2014).

2.4. Integration

The abovementioned models generally all converge on a dual system account of implicit and explicit social cognition, proposing that the interplay between two social brain systems supports social information processing: one that is cognitively efficient for detecting and reflexive orienting towards salient social cues, and the other that is more cognitively demanding that allows explicit reasoning about the mental states of others. In this light, specific regional activation depends upon the nature of the employed task; the mirror system is commonly assumed to enable the pre-reflective understanding of others behaviors by internal simulation, whereas the mentalizing or theory of mind system is recruited when people have to actively reflect about others’ behavior (Arioli & Canessa, 2019). While researchers have only just begun to unravel how these two systems interact during naturalistic social information processing, some have proposed a top-down mechanism from mentalizing over mirroring areas (Campbell & Cunnington, 2017; Wang & Hamilton, 2012). This top-down control is hypothesized to emerge from frontal area mPFC, which, via multisensory integration hub STS, impacts the main visuomotor input towards the mirror system (as demonstrated by Wang, Ramsey, et al., 2011).

In addition, and in accordance with the available neuroscientific literature (Kawashima et al., 1999; Mosher et al., 2014; Wicker et al., 2003), a ‘quick and dirty’ bottom-up activation of subcortical structures upon the perception of salient social cues (such as eye contact), particularly within the amygdala, seems evident (Skuse, Morris & Lawrence, 2003). This initial relevance detection mechanism reflects a rapid appraisal of the saliency and/or relevance of a particular stimulus, and plays an important role in arousal regulation and attention allocation (Sander, Grafman & Zalla, 2003). It is assumed that this initial quick and dirty appraisal then guides further information processing (Senju & Johnson, 2009b), but depending on the context of the social encounter, can also be tempered by a neocortical top-down control arising from the frontal areas (Kim et al., 2011, Skuse, Morris

& Lawrence, 2003, see for example Zillekens et al., 2019 for an illustration in the context of biological motion perception). However, as illustrated by the single-neuron findings by Coudé et al. (2016) in the monkey PMC, the possibility of a bottom-up eye contact mechanism in cortical information processing areas cannot be fully excluded.

In context of the results obtained in the current doctoral project, and the (extended) neural STORM model that explains the interplay between gaze and action cues, we propose the following mechanism (outlined in **figure 2**). In healthy adult participants with no particular deficits in the social domain, the perception of direct eye contact during a dynamic social interaction is appraised as 'safe' and/or 'relevant' by the observer. This appraisal is initially subserved by the amygdala in close conjunction with the mPFC, and induces a facilitative top-down control over visuomotor information processing area STS. This control over the STS subsequently impacts the fronto-parietal action observation network, which results in interpersonal motor resonance and is reflected in enhanced TMS-induced MEPs during direct vs. averted gaze cues (indicated in green in **figure 2**).

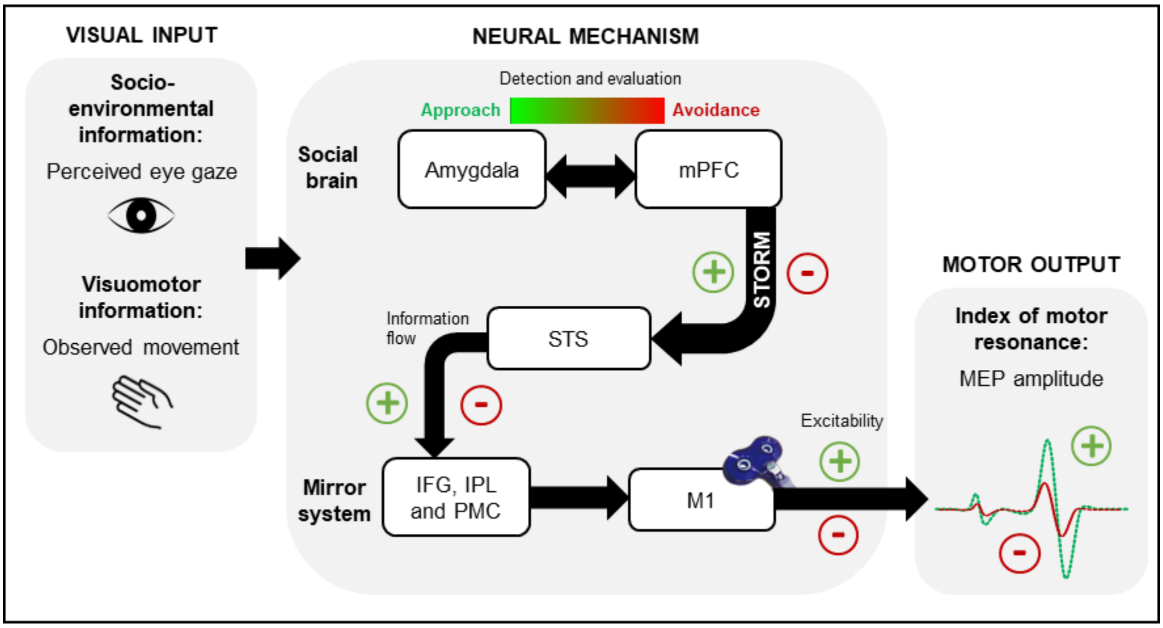


Figure 2. Proposed neural model underlying social information processing. This working model involves a social top-down response modulation (STORM) of social brain areas over sensory integration area STS and, subsequently, the fronto-parietal mirror system. Depending on the social relevance (relevant vs. irrelevant) and/or value (safe vs. threatening) of the observed social cues for the observer, a facilitative (approach) or inhibited (avoidance) top-down control will result in an enhancement (green MEP) or reduction (red MEP) of interpersonal motor resonance, as assessed by TMS.

In individuals with particular deficits in the social domain, which may include participants diagnosed with ASD, prolonged direct eye contact with the experimenter may be experienced as either 'threatening' (known as the eye avoidance hypothesis; Tanaka &

Sung, 2016) or ‘irrelevant’ (reflecting a diminished motivation to interact with others; Chevallier, Kohls, Troiani, Brodkin & Schultz, 2012). In turn, this may lead to an inhibitory top-down control over area STS, resulting in a reduction of interpersonal motor resonance and lower TMS-induced MEPs upon perceiving direct vs. averted gaze cues (indicated in red in **figure 2**).

Via its demonstrated modulatory effect on the amygdala and mPFC however (Wigton et al., 2015), a single dose of intranasally administered oxytocin was shown to enhance the propensity to resonate with others’ actions upon dyadic eye contact in these individuals. This can be attributed to the working mechanism of oxytocin, either by reducing the extent by which eye contact is experienced as socially threatening (Maroun & Wagner, 2016; Neumann & Slattery, 2015), by enhancing the saliency of the observed social cues (Shamay-Tsoory & Abu-Akel, 2016) and/or by increasing the motivation to affiliate with others (Bartz, 2016) (or both, as these possible mechanisms are not necessarily mutually exclusive). As such, the presented model can also be used as a theoretical framework to investigate the therapeutic potential of promising biomedical interventions that act on specific parts of these cooperating neural circuits.

Note that in the case of the currently adopted observational paradigm, in which no effortful task or explicit social reasoning about the mental states of others is involved, we presume that the impact of brain regions related to more cognitive social processing (e.g. the TPJ) is relatively minimal in modulating M1 excitability as assessed by TMS. Secondly, we do not presume that these eye contact-related modulations are limited to interpersonal motor resonance and the processing of visuomotor information, but extend to other domains of social information processing as well (e.g. auditory processing when listening to a speaker talking, as demonstrated by Jiang et al., 2017).

In sum, although it is not possible to delineate the exact neural mechanism underlying the obtained results with the employed TMS technique, the recent literature indicates that the fronto-parietal mirror system should be viewed in the context of a large network that is involved in social information processing. In this view, separate brain areas may mutually inform each other by means of reciprocal connections, with the ultimate goal to adequately adapt the individual’s behavior to the demands of the social situation. We have made an effort to reconcile the obtained TMS results in this doctoral project with one of the dominant views in the literature regarding social information processing, but realize that more empirical evidence is necessary to confirm this proposed neural model (see also **section 4** of the General Discussion for proposed future directions).

3 Methodological considerations

3.1. TMS-induced MEPs

The first evidence that corticospinal (M1) excitability as assessed by TMS is modulated during the observation of an action was given by Fadiga et al. (1995). TMS was applied to the area of M1 that represented the hand and MEPs from the corresponding hand muscles were enhanced during the observation of grasping actions, but not during the observation of arm movements, indicating a muscle-specific mirror mechanism in the human brain that is consistent with that directly observed in monkeys (e.g. di Pellegrino, Fadiga, Fogassi, Gallese & Rizzolatti, 1992). Many studies have since used the singe-pulse TMS technique in the context of action observation (Fadiga, Craighero & Olivier, 2005). However, some critical remarks about TMS need to be addressed.

3.1.1. Cortical or spinal mirror effect?

Since TMS-induced MEPs provide an end-point measure about the efficacy of a chain of synapses across all levels of the corticospinal tract, i.e. from cortical neurons in M1 to the targeted muscles (see **figure 4** in General Introduction, p. 34), it is difficult to distinguish between cortical and spinal contributions to changes in MEP amplitude. It is generally assumed that the facilitation of MEPs by action observation is produced through excitatory cortico-cortical connections between M1 and the premotor cortex, the human homologue of monkey area F5 where mirror neurons were first discovered (i.e. **cortical mechanism**). Yet another possible explanation is that TMS, via the descending volley from the premotor cortex through the corticospinal tract, facilitates the excitability of lower motoneurons in the spinal cord (i.e. **spinal mechanism**).

To test the spinal involvement in action observation-induced MEP facilitation, Baldissera, Cavallari, Craighero and Fadiga (2001) recorded the amplitude of the monosynaptic Hoffmann reflex (H-reflex) from a finger flexor muscle while participants observed grasping hand actions. The H-reflex can be evoked by electrically stimulating afferent peripheral nerves and depends upon spinal motoneuron excitability. In case of a direct influence of the premotor cortex on the spinal cord, a mirror-like facilitation of the H-reflex would already be present. However, their data indicated that the H-reflex varied in accordance with the observed movement, but in the opposite direction to that occurring during actual action execution and cortical TMS stimulation. The authors hypothesized that this spinal ‘inverted mirror’ mechanism allows the motor system to replicate observed actions internally, while also blocking its overt execution of the observed actions (Baldissera et al., 2001; see also monkey literature: Stamos, Savaki & Raos, 2010).

Another technique to probe the cortical origin of MEP facilitation is paired-pulse TMS. With this technique a subthreshold 'conditioning' TMS pulse is applied, followed by a supra-threshold 'test' TMS pulse. Depending upon the delay between the pulses, the subthreshold TMS pulse activates inhibitory or excitatory interneurons within M1, inducing intra-cortical inhibition (ICI; shorter intervals) or intra-cortical facilitation (ICF; longer intervals) of the TMS-induced test response, respectively. As such, ICI and ICF are believed to probe the activity within inhibitory and excitatory circuits at the cortical level. Strafella and Paus (2000) used this approach to investigate ICI and ICF of M1 during action observation. Their results showed that action observation significantly reduced intra-cortical inhibition - but not facilitation - compared to rest, thereby paralleling ICI and ICF results recorded during voluntary movements. The authors conclude that the reduced paired-pulse inhibition observed during both action observation and execution is due to a similar changes in excitability of cortical circuits. Taken together, it is assumed that MEP facilitation during action observation is primarily cortical in origin.

3.1.2. The role of the primary motor cortex

It is still a topic of debate whether M1 should be labelled an actual part of the mirror system or not. In the classic mirror system account, any modulations in M1 activity during action observation are assumed a result of changes in the mirror regions of the brain (i.e. PMC, IPL and IFG). This is supported by anatomical data from connectivity research in monkeys, showing strong reciprocal cortico-cortical connections between area F5 (assumed the homologue of area IFG in humans) and M1 (reviewed by Kilner & Frith, 2007) and several magnetoencephalography (MEG) studies in humans showing that, upon action observation, the IFG is activated first, followed within 100-125 milliseconds by an activation in M1 (summarized by Hari, Levänen & Raij, 2000). Furthermore, as several brain imaging studies in humans failed to show any significant involvement of M1 during action observation only tasks (e.g. Iacoboni, 2009), M1 is traditionally not considered part of the fronto-parietal action observation network.

Recent single-neuron recording studies in macaque monkeys challenge this view, since they clearly demonstrated the existence of pyramidal neurons with mirror-like properties (i.e. responding to viewed actions) in M1 itself. Specifically, approximately half of the M1 neurons that were active when monkeys performed the task were also active when they observed the action being performed by a human experimenter (Dushanova & Donoghue, 2010; Kraskov et al., 2014; Vigneswaran, Philipp, Lemon & Kraskov, 2013). Taken together, although it appears evident that mirror areas (i.e. PMC, IFG and IPL) in the brain exert a modulatory influence over M1 during action observation, at least some of the variation in M1 activity seems attributable to a local motor-mapping process (Kilner & Frith, 2007). These data show that some neurons in M1 are active during both action execution and observation, and would suggest that M1 should be considered part of the mirror system.

3.1.3. The left side of motor resonance

In accordance to most studies in the field, TMS was applied on left M1, and MEPs were recorded from right-hand muscles in right-handed participants while observing right-handed models. This is in line with the known lateralization pattern of M1 facilitation, i.e. each hemisphere is most activated by observation of the contralateral body part (Aziz-Zadeh, Maeda, Zaidel, Mazziotta & Iacoboni, 2002). However, as left-handed participants were initially often excluded from TMS studies, it is not clear whether this notion of lateralization when resonating with others' actions is related to motor dominance. Initial cues are provided by an fMRI study by Cabinio et al. (2010), showing a strong contralateral activation in the mirror system when observing/performing hand actions with the dominant hand, and a more bilateral activation pattern when performing/observing with the non-dominant hand, in both left- and right-handed participants. Ambidextrous left-handed subjects generally show a more bilateral pattern of cortical activation.

A TMS study by Sartori, Begliomini and Castiello (2013), recording MEPs from the dominant and non-dominant hand of left- and right-handed participants while they observed left- and right-handed models grasping an object, confirmed this effect of anatomical correspondence when observing models with similar hand preference. As such, it provided evidence that the classic lateralization pattern of M1 facilitation can be extended to left-handers. When actions performed by a model with a different hand preference were observed, enhanced motor resonance was still noted in the dominant hand, independently from handedness. Taken together, these results seem to indicate that both right- and left-handers tend to translate any observed hand action into their dominant motor program (Cabinio et al., 2010; Sartori et al., 2014).

3.2. Motor resonance and overt visual attention

3.2.1. Indications from the current project

In several studies of the doctoral project (Prinsen & Alaerts, 2019; Prinsen et al., 2017, 2018), eye tracking technology was included to investigate whether gaze behavior and/or visual attention towards the presented stimuli would impact motor resonance with the observed actions. Previous eye tracking studies have shown that observing another person's averted gaze can automatically induce gaze following and shift spatial attention away from the presented stimulus (Nummenmaa & Calder, 2009). In the current studies however, participants equally attended the hand area in both gaze conditions, rendering it unlikely that a shift of visuospatial attention away from the to-be-observed hand movements upon perceiving the model's averted gaze underlies the encountered gaze-related modulations in motor resonance (see also the control experiments by Wang & Hamilton, 2014 and Wang, Newport et al., 2011).

Perhaps surprisingly, in studies assessing motor resonance and gaze behavior concurrently (Prinsen & Alaerts, 2019; **chapter 3**), no linear association between spatial (fixation count) and/or temporal (fixation duration) parameters of gaze behavior and motor resonance was encountered during free viewing, which is in line with other results in the literature (D'Innocenzo, Gonzalez, Nowicky, Williams & Bishop, 2017). Note however that with the adopted mobile eye tracking technology (i.e. wearable glasses), it was not feasible to investigate parameters related to the microstructure of the participants' gaze behavior (e.g. time until first fixation to the eye region). As such, it may be possible that other parameters of gaze behavior than those investigated in the doctoral project may relate to variations in motor resonance. For example, in the study by D'Innocenzo et al. (2017), saccade amplitude (i.e. the angular distance the eye travels during the movement) was negatively correlated with MEP amplitude.

3.2.2. Manipulating attention in the literature

Related to the role of visuospatial attention, the essence of the mirror mechanism is an *automatic* activation of motor representations in the primary motor cortex (M1) upon observing others' actions (Rizzolatti & Sinigaglia, 2010). The term automatic suggests that it is triggered involuntarily upon perceiving actions and relatively immune to manipulations in attention, but this notion is currently under debate (Chong & Mattingley, 2008). Indeed, several studies highlight the crucial role of overt visuospatial attention and its impact on various markers of interpersonal motor resonance. First, researchers showing supraliminal and subliminal presentations of action images demonstrated that the perceptual awareness of the action stimuli is required for motor resonance (as assessed by TMS) to occur (Mattiassi, Mele, Ticini & Urgesi, 2014). Secondly, an EEG study investigating mu rhythm suppression in various task conditions reported mu attenuation in all conditions, but most strongly so when the observed actions were relevant to the task (Schuch, Bayliss, Klein & Tipper, 2010). This was later also confirmed in a study adopting the H-reflex technique in response to rhythmic hand flexion-extension movement to assess excitability of spinal motoneurons (Puglisi et al., 2017). Puglisi and colleagues showed that resonant responses were developed in conditions where the hand was clearly visible but not the focus of subjects' attention for the task at hand. However, the amplitude of these responses was much reduced compared to when participants were asked to explicitly attend the movement. On a similar note, actions viewed in peripheral vision were shown to be effective in activating M1 excitability, but these responses were rough and had lower kinematic specificity than those observed in central vision (Leonetti et al., 2015).

In contrast to these studies (and also contradicting the previous results by Wang & Hamilton, 2014; Wang, Newport et al., 2011), a variant of the previously discussed stimulus-response compatibility (SCR) paradigm to assess automatic mimicry (see General Introduction) showed that congruency effects did no longer occur when participants directed their attention away from the movement-relevant features of the observed actions (Chong, Cunnington, Williams & Mattingley, 2009). Similarly, a recent TMS study indicated that attentional interference induced by distracting stimuli (i.e. brief appearance of a flashing dot in the contralateral space) reduced motor resonance in the corresponding muscles during observation of goal-directed actions, but not motor preparation in the muscles required to perform a complementary response when observing interactive movements (Betti, Castiello, Guerra & Sartori, 2017).

In sum, the reviewed literature suggests that viewing a (supraliminal) action is, in itself, a powerful stimulus to recruit the action observation network, even when it is not the primary focus of subjects' attention or when it is irrelevant to the task, suggesting a certain degree of automaticity. However, the role of selective attention in the mirror system is relatively underexplored (see also Chong & Mattingley, 2008). So far, it seems that for a complete and kinematically fine-tuned motor resonance response to develop, full attention to the action stimuli is required (Leonetti et al., 2015; Puglisi et al., 2017). Other, more salient components of the action scene, such as initiated eye contact (Prinsen & Alaerts, 2019; Prinsen et al., 2017) or the presence of an interactive request (Betti et al., 2017), may on the other hand be impermeable to attentional interferences and appear to be able to automatically engage the mirror system.

4 Future directions

To date, direct empirical evidence in favor of the social top-down response modulation of the mentalizing system over mirror areas as proposed by Wang and Hamilton (2012) is still sparse. The dynamic causal modeling study by Wang, Ramsey and Hamilton (2011) provided first indications that, in the context of the eye contact effect on automatic mimicry, this top-down control by observed eye gaze is implemented in terms of effective connectivity between mPFC and visual input area STS. Further application of state-of-the-art analysis methods that measure effective connectivity between brain regions during social information processing (such as dynamic causal modelling or Graph Theory), will allow us to further **delineate the neural mechanisms and connections** that putatively underlie the TMS-obtained neurophysiological results showing gaze-related enhancements in motor resonance upon action observation. Another plausible option is to adopt repetitive TMS protocols (i.e. trains of magnetic pulses) to inhibit brain regions that are hypothesized to play an important role in STORM-related mechanisms, such as the STS and/or mPFC.

Although Wang and Hamilton (2012) do not make specific claims related to the **temporal dynamics of STORM**, other models – such as the fast-track modulator model (Burra et al., 2019; Senju & Johnson, 2009b) – imply that the detection of eye contact occurs at a very early stage during visual information processing. It might therefore be of interest to evaluate the timing of gaze processing and subsequent modulations with methods with a high temporal resolution (e.g. at the millisecond scale), such as EEG and MEG. However, a clear limitation of these methods is that they not easily allow for the direct measurement of the subcortical structures generally associated with the “fast” detection of direct eye gaze. In terms of cortical responses, gaze perception research employing M/EEG has typically focused on the electric N170 (or magnetic M170), an early face processing component occurring over occipito-temporal sites between 130 and 200 milliseconds after presentation of a face, but results of the M/N170 behavior on gaze direction remain inconsistent (Burra et al., 2019). Other analysis approaches (e.g. source localization) and/or methods (e.g. electrocorticography or intracranial EEG), combined with fMRI connectivity analysis could address this question further.

Secondly, the differentiation in responses to people based on **interaction partner familiarity** is foundational for the organization of social life; continued gaze from a romantic partner is not processed in the same way as a prolonged stare by an unfamiliar person. Yet, whenever a two-person action context is employed to probe aspects of social interaction, the participant-experimenter configuration is often most feasible. This configuration typically denotes a social situation between two fellow humans with an otherwise minimal sense of relatedness (i.e. conspecifics), in which prolonged direct gaze from the experimenter towards the participants can easily be interpreted as ‘awkward’. As such, it remains an open question whether the obtained results (admittedly, including those of the current project) extrapolate to social functioning during other levels of intimacy, including peers, close friendships, romantic partners and/or parental bonds (Feldman, 2017).

Interaction partner familiarity may be of particular importance when investigating social cognition in clinical disorders characterized by deficits in the social domain, such as autism spectrum disorders (ASD). In terms of motor resonance, the EEG study by Oberman, Ramachandran and Pineda (2008) speaks to this importance, as they demonstrated that the mirror system is sensitive to the degree to which the observer identifies with the actor. This study found that the observation of actions performed by familiar individuals (a parent, guardian or sibling) resulted in typical mu wave suppression in individuals with ASD, while the actions of unfamiliar actors did not.

Related to the fact that similar modulations by familiarity have also been obtained for other social brain systems thought to be impaired in ASD, Oberman et al. (2008) suggested that the reported dysfunctions in social brain areas may actually reflect an underlying impairment in identifying with and assigning personal significance to unfamiliar people in ASD. Although more recent studies have also started to bring familiarity into account (e.g. Nuske, Vivanti & Dissanayake, 2014, showed that individuals with ASD are less atypical in their autonomic responses when observing familiar persons), this topic requires further investigation. Again, this brings us back to the notion that the perceived relevance of a social stimulus for the observer shapes his/her motivational tendency to interact, which is reflected in, among others, various markers of mirror system engagement.

Finally, one of the main challenges in social neuroscience is to **approximate naturalistic interpersonal interaction**, as the same methodologies that may promote experimental rigor may also unnaturally constrain the social process-of-interest. Although efforts have been made to increase the ecological validity of the included stimuli and paradigms (e.g. by including live eye contact cues), a further extension could consist in developing experimental paradigms that allow participants to be part of a ‘real’ social interaction, as in the current studies the role of the participant was limited to passive action observation. Furthermore, to mitigate the constrained experimental setting that is inherent to TMS (i.e. limited movement is permitted, receiving magnetic stimulations over the skull etc.), other methodologies that capture interpersonal motor resonance, such as facial electromyography during the perception of others’ facial expressions (e.g. as employed in Sims, Van Reekum, Johnstone & Chakrabarti, 2012), may be adopted. Another possible avenue are the so-called ‘hyper scanning’ methods, that enable the measurement of brain activity in two or more individuals interacting in real-time. This approach might be particularly fruitful when adopting portable devices, such as EEG or functional near-infrared spectroscopy (fNIRS), since they allow dynamic face-to-face investigations beyond the classic single-participant observation setting, thereby further improving the ecological validity of experiments (Reader & Holmes, 2016). In summary, many novel paradigms may help to shed light on the putatively complementary roles of mirror neurons and mentalizing networks in situations that better approximate those encountered in daily life.

5 Conclusion

This doctoral thesis contributed to the research field at three levels. First, we confirmed that observed gaze direction is a highly relevant cue to shape motor resonant processes, and that the influences of visuospatial attention and/or sympathetic arousal are minimal herein. Second, we demonstrated the importance of taking inter-individual differences in the social domain, both in the typical and in the ASD population, on gaze-related modulations of interpersonal motor resonance (and by extension, socio-cognitive processes in general) into account. Third, we provided first evidence that a single dose of intranasally administered oxytocin may provide an effective remedy to enhance the propensity to mirror others’ actions for those individuals who initially fail to do so. In sum, we add to the growing body of literature showing that mirroring processes should not be considered in isolation, but need to be integrated within a broad functional system for optimal adjustment of complex social behaviors.

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Appendices

About the author

Jellina Prinsen was born in Edegem, Belgium on September 27th, 1991. In 2012, she graduated cum laude from the Lessius Hogeschool Antwerpen, Belgium (currently: Thomas Moore Hogeschool) as a Professional Bachelor in Applied Psychology, with a focus on clinical and health psychology. During her Bachelor studies, she spent four months in Paramaribo, Suriname to obtain clinical experience with working with vulnerable populations (e.g. patients, children, ethnic minorities, etc.). After following a one-year abridging program at KU Leuven, she initiated a Research Master in Experimental Psychology in 2013, which she obtained magna cum laude in June 2015.

After graduation, she started her doctoral training at the then newly formed Neuromodulation Laboratory at the KU Leuven Department of Rehabilitation Sciences, under supervision of professor Kaat Alaerts and co-supervision of professor Johan Wagemans. In 2016, she was granted an individual fellowship by the Marguerite-Marie Delacroix Foundation. Her research focusses on the neural circuits that underlie social information processing, both in healthy and clinical populations, with a particular interest for the role of the action observation network or mirror system herein. She was a member of the Council of the Faculty of Movement and Rehabilitation Sciences at KU Leuven.

Apart from her research work, she enjoys travelling, relaxing with a good book, wining and dining, playing the ukulele and spending time with family, friends, her boyfriend Jelle and her cat Chloé.

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Research funding, travel grants and awards

Sep 2016	**PhD fellowship (4 year)** – Marguerite-Marie Delacroix Support Fund
Oct 2016	**Travel grant short stay abroad** – Academische Stichting Leuven Cognitive Neuroscience Lab – University of California, San Diego (USA)
Jun 2017	**Conference grant** – Academische Stichting Leuven
Feb 2018	‘**Broaden Your Horizon’ grant** – Doctoral School for Biomedical Sciences
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May 2018	**Conference grant** – Fonds voor Wetenschappelijk Onderzoek (FWO)
Aug 2018	**School of Advanced Science on Social and Affective Neuroscience** Mackenzie Presbyterian University, Saõ Paulo (Brazil)
Apr 2019	**Best oral presentation award** – ‘NeuroSense & -Sense-ability’ workshop
May 2019	**Conference grant** – Fonds voor Wetenschappelijk Onderzoek (FWO)
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Appositions
Bijstellingen

The scientist’s and scientific publisher’s job is to inform, not to persuade. Reporting the objective truth must always prevail over pursuing a certain narrative.

Het is de taak van de wetenschapper én wetenschappelijk uitgever om te informeren, niet om te overtuigen. Het rapporteren van de objectieve waarheid moet altijd voorgaan op het nastreven van een bepaald verhaal.

Open peer review practices can enhance scientific transparency.

Praktijken die open peer review ondersteunen kunnen ook de transparantie van het wetenschappelijk proces vergroten.

We have to be aware of the effects of our actions as travelers on the environment, local community and local economy of our destinations.

Als frequente reizigers moeten we ons bewust zijn van de gevolgen van onze acties tijdens het reizen voor zowel het milieu, de lokale gemeenschap als de lokale economie van onze bestemming.

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Personal Contribution

The author of this manuscript was – with assistance of supervisor professor Kaat Alaerts – responsible for experiment design, data collection, data analysis, data interpretation and the writing of all chapters included in this doctoral dissertation.

Conflict of Interest

The author of the manuscript declares no potential conflict of interest for any of the contributing authors with respect to the research, authorship and/or publications.

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