# The Neural Basis of Mentalizing Deficits Experienced by Adults with Autism Spectrum Disorder

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Doctor of Philosophy

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Psychology

September 2017

# Abstract

Adults with autism spectrum disorder (ASD) are widely regarded to exhibit difficulties inferring the internal states of others; known as ‘mentalizing’. However, the existing literature is inconsistent regarding the nature of the mentalizing difficulties experienced by adults with ASD. Two neural systems; the mirror neuron system (MNS) and the mentalizing system, are both thought to play important roles in inferring others’ internal states from their actions but the precise roles of these systems and the nature of the interaction between them are unknown. The aim of this thesis was to explore the nature of mentalizing deficits associated with ASD, delineate between existing models of MNS involvement in mentalizing and identify the neural basis of mentalizing difficulties associated with ASD. The first empirical chapter presents two behavioural experiments which found adults with ASD were impaired at explicitly inferring the intentions of others from their actions. The second empirical chapter presents a transcranial magnetic stimulation (TMS) experiment which identified mentalizing-induced modulation of MNS activity, at the end of observed actions, in typically developing participants. The third empirical chapter presents an experiment which used TMS and electroencephalography (EEG) to measure MNS activation in adults with ASD when mentalizing and found higher levels of autistic traits predicted lower right-lateralised MNS activity. The final empirical chapter presents a functional magnetic resonance imaging (fMRI) experiment which identified higher levels of autistic traits predicted reduced functional connectivity between the MNS and the mentalizing system. Collectively, these data suggest that connectivity between the MNS and the mentalizing system is higher when inferring intentions of others from their actions, the interaction between the two systems may be best conceptualised within a predictive coding framework and reduced connectivity between these systems may underlie the mentalizing difficulties adults with ASD experience.

## List of Contents

# List of Tables

# List of Figures

# Acknowledgements

First, I would like to thank A/Prof. Peter Enticott, for his generosity, support and expertise. Without his involvement, I would not have gained the same research skills, attended career-changing workshops or taken a life-changing trip to Australia. This thesis and my post-doctoral position would likely not have materialised without the enthusiasm of A/Prof Enticott and his team at the Cognitive Neuroscience Unit at Deakin University, Melbourne. I cannot thank them enough.

Secondly, I would like to thank both my supervisors, Dr. Nick Barraclough and Prof. Timothy Andrews for their knowledge and guidance. Particularly Prof. Andrews, for selflessly taking me on as a supervisee in the final year of my PhD. Also, Katie Slocombe for her valuable input on the design and analysis of the behavioural experiments.

I would also like to thank the wonderful fellow PhD students and early career research staff in the Psychology Department at the University of York. I believe that the department is one of a kind; so many supportive and generous individuals working under one roof. These colleagues were always there to provide encouraging words, listening ears, big hugs, baked goods, home cooked meals and drinks at the pub, in order to get each other through the tougher times (or celebrate the good ones). I have definitely made friends for life and I will never forget my time at the University of York for that reason.

On a similar note, I need to thank my partner Dr Robert Seed, for his continued support, encouragement and firm belief in me. His efforts in minimising my duties outside of thesis writing did not go unnoticed and I am incredibly lucky to have such a supportive partner.

Finally (and probably most importantly), I would like to thank my parents for their never-ending belief in me and continued support, I hope they never underestimate how much that means to me. As my father likes to point out, they also provided me with the genes in which enabled me undertake a PhD, which is obviously the overriding factor which made this all possible.

# Author’s Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

The research presented was funded by a 1+3 PhD studentship and an overseas fieldwork grant, both from the Economic and Social Research Council (ESRC).

The empirical work presented in this thesis has been published or is currently under review in the following peer-reviewed journals:

Cole, E.J., Barraclough N. E., Enticott. P.G. (2018). Investigating mirror system (MS) activity in adults with ASD when inferring others' intentions using both TMS and EEG. *Journal of Autism and Developmental Disorders*. [in press]

Cole, E.J., Slocombe K.E., Barraclough N. E. (2017). Abilities to explicitly and implicitly infer intentions from actions in adults with autism spectrum disorder. *Journal of Autism and Developmental Disorders*. doi: 10.1007/s10803-017-3425-5.

Cole, E.J., Barraclough N. E., Enticott. P.G. (2017). A simultaneous EEG and TMS study investigating mirror neuron system activity when inferring intentions. *Brain Stimulation.* 10(2): 415-416. [Abstract]

Cole, E.J., Barraclough N. E. (in review). Timing of Mirror Neuron System activation when inferring the intentions of others. *Social, Cognitive & Affective Neuroscience.*

Cole, E.J., Barraclough N. E., Andrews T.J. (in preparation). Reduced connectivity between mentalizing and mirror neuron systems may underlie difficulties inferring social intentions in autism spectrum disorder

Data from multiple empirical chapters have been presented at the following conferences:

Cole, E.J., Barraclough N. E., Enticott. P.G. (2017) A simultaneous EEG, TMS and Eye-tracking study investigating mirror neuron system activity in adults with autism spectrum disorder when inferring intentions, *International Meeting for Autism Research. San Francisco*, USA [poster].

Cole, E.J., Barraclough N. E., Enticott. P.G. (2017). A simultaneous EEG and TMS study investigating mirror neuron system activity when inferring intentions, *International Brain Stimulation Conference*, Barcelona, Spain [poster].

Cole, E.J., Slocombe K.E., Barraclough N. E. (2017) Explicit mentalizing deficits in adults with higher levels of autistic traits in clinical and non-clinical populations, *Experimental Psychology Society (EPS) meeting*, University College London, UK [poster].

# Chapter 1: Introduction

## 1.1 Understanding others' actions

The ability to interpret others' behaviour as well as predict their future actions is both evolutionarily vital and important for successful social interaction. The motor simulation theory states that we understand the actions of others' by internally simulating these actions in our own motor system (Decety & Grèzes, 2006; Landmann et al., 2011; Rizzolatti & Sinigaglia, 2007; Uithol, van Rooij, Bekkering, & Haselager, 2011). It is thought that we simulate observed actions and compare these actions to stored representations of our own actions and their consequences. This allows us to predict upcoming trajectories and goals of observed actions e.g. when observing an arm reaching towards a glass, we can use the initial action kinematics to predict the timing of the upcoming movement and infer that the goal is to pick up the glass to drink from it rather than pick it up to place it elsewhere (deKlerk et al., 2016; Stapel et al., 2012; Messier 1999; Graf et al., 2007). Evidence for the internal simulation of others' actions has been provided by behavioural studies in which irrelevant actions were shown during a task; if observed movements were incongruent to the movements required in order to complete the task, reaction times were significantly slower (Brass et al., 2000; Kilner, Paulignan, & Blakemore, 2003; Liepelt, Cramon, & Brass, 2008). This is termed the 'interference effect'. Additionally, if transcranial magnetic stimulation (TMS) is applied to the primary motor cortex, the induced muscle activation in the recipient's hand is larger if they are observing a hand action compared to when TMS is applied at rest (Luciano Fadiga, Craighero, & Olivier, 2005a; Patuzzo, Fiaschi, & Manganotti, 2003; Strafella & Paus, 2000). The increased muscle activation in the hand of the observer is specific to the muscles involved in the observed action (Fadiga et al., 2005; Fadiga et al., 1995; Urgesi et al., 2006); this provides evidence that observed hand actions are internally simulated by the observer.

Although it is widely accepted that observing others' actions is sufficient in order to predict their immediate goals, it is disputed whether other levels of intention can be inferred from kinematic information. Jacob and Jeannerod have outlined four types of intentions; 'motor intention', 'prior intention', 'social intention' and 'communicative intention' (Jacob & Jeannerod, 2005). A motor intention refers to the actor's immediate goal (e.g. pick up a scalpel). An actor's prior intention refers to the intention of the actor before movement has been initiated (e.g. perform a surgical incision). A social intention refers to the intended impact on others (e.g. to help or to harm). Finally, a communicative intention is the intent to convey information to another person (e.g. point to the clock to signal that the time allocated for the operation is nearly over). Jacob and Jeannerod argue that processing the visual kinematics of others' actions allows motor intentions to be inferred but not other levels of intention. Instead, Jacob and Jeannerod suggest that it is necessary to process the surrounding context (e.g. a surgeon in an operating theatre) in order to infer prior, social and communicative intentions. In contradiction to this argument, the kinematics of hand actions have been shown to differ depending on the actors' prior (Ansuini et al., 2006; Egmose & Koppe, 2017; Johnson-Frey et al., 2004), social (Manera, Becchio, Cavallo, Sartori, & Castiello, 2011; Sartori, Becchio, & Castiello, 2011) and communicative (Peeters et al., 2013; Peeters et al., 2015; Sartori et al., 2009) intentions. Furthermore, when shown videos of hand actions that were prematurely ended so that the action outcomes were not revealed, participants could still determine the actor's social intentions despite the fact that these hand actions were shown without surrounding context (Manera, Becchio, Cavallo, Sartori, &

Castiello, 2011; Sartori, Becchio, & Castiello, 2011). These data suggest that processing kinematic differences via motor simulation may be sufficient in order to infer others' social, prior and communicative intentions.

Motor simulation may also have a role in inferring other aspects of another's internal state such as their emotions, beliefs or desires which can all be vital in order to predict upcoming actions (Wolpert et al., 2003). These internal state inferences are collectively referred to as 'mentalizing' (Chung, Barch, & Strube, 2014; Denny, Kober, Wager, & Ochsner, 2012; Frith & Frith, 2006; Kampe, Frith, & Frith, 2003). Internal state inferences can either be made without being instructed to do so ('implicit mentalizing') or when directly asked to do so ('explicit mentalizing'; Rosenblau, Kliemann, Heekeren, & Dziobek, 2015; Schuwerk, Vuori, & Sodian, 2014; Van Overwalle & Vandekerckhove, 2013). The majority of mentalizing in everyday life is implicit but conversely, the majority of the mentalizing literature has studied explicit mentalizing (Nijhof, Brass, Bardi, & Wiersema, 2016). Level 2 visual perspective taking (VPT-2) is a mentalizing task which involves inferring how another individual perceives elements in their surrounding environment (Hamilton et al., 2009; Kessler & Rutherford, 2010). Response times are longer on VPT-2 tasks if the observer's body position is at an incongruent orientation to the individual they are making inferences about (Kessler, Cao, O'Shea, & Wang, 2014; Kessler & Rutherford, 2010). This suggests that one's own body is mentally rotated to match the other individual's orientation in order to infer how the other person perceives their environment. These data support the importance of internal simulation when inferring others' internal states. The kinematics of actions have also been shown to differ depending on the actor's emotions (Atkinson et al., 2004; Dael et al., 2012; Pollick et al., 2001; Li et al., 2016) and these kinematic differences can be used to successfully infer others’ emotions (Atkinson et al., 2004; Dael et al., 2012; Pollick et al., 2001; Li et al., 2016). Individuals with depression are also more likely to rate actions as more negative, suggesting that errors in emotion attribution arise due to not inhibiting one’s own emotions sufficiently during the internal simulation of others’ emotional actions (Kaletsch et al., 2014). Differences in action kinematics can also be used to identify whether an actor is being truthful or deceptive about the weight of an object (Finisguerra, Amoruso, Makris, & Urgesi, 2016) and whether a movement was made out of free choice or whether the actor was instructed to perform a certain movement (Pesquita, Chapman, & Enns, 2016). Collectively, these data suggest that motor simulation may have a broad role in mentalizing.

Mentalizing can occur in the presence or absence of action information; it is possible that the extent to which motor simulation is involved in mentalizing is related to the amount of action information available. In addition to motor simulation theory, another form of simulation theory is ‘mental simulation’. This theory suggests that predictions about the internal states of others, based on prior knowledge and contextual information, are internally simulated and used to predict their upcoming actions (Hegarty, 2004; Shanton & Goldman, 2010). It is possible that mental simulation and motor simulation are not distinct processes; these processes may inform each other and the degree to which mentalizing relies on one of these processes over the other may depend on the amount of action information available. Alternatively, it is possible that mentalizing only relies on mental simulation, not motor simulation, regardless of the presence or absence of action information. A limitation of the theory that motor simulation alone, not mental simulation, is the basis for mentalizing is that motor simulation can only account for abilities to infer others’ internal states which are directly related to observable action information (Shanton & Goldman, 2010). Humans are able to infer intentions, beliefs, emotions and desires that are not directly related to observed actions and it has been suggested that motor simulation alone may be inadequate to infer these more abstract internal states (Csibra & Gergely., 2007; Jacob & Jeannerod, 2005). Mental simulation allows for internal states to be inferred in the absence of action information and for internal states that are far removed from observable actions to be inferred. Csibra extends motor simulation theory by suggesting that predictions about an actor's internal state are first inferred based contextual information before motor simulation. This allows more abstract inferences to be made and upcoming actions to be efficiently predicted. According to Csibra's model, motor simulation is then used to compare the actual kinematics of the ongoing actions to the predicted upcoming actions. Discrepancies between the predicted actions and actual actions are used to update inferences made regarding the individual's internal state. Therefore, Csibra's model suggests that action monitoring via motor simulation is involved in inferring higher-level aspects of others' internal state from their actions (particularly, updating internal state inferences during ongoing actions) but it is not sufficient alone in order to do so.

Some alternative theories for the basis of mentalizing suggest simulation, in any form, is not required in order to infer others' internal states. Theory theory suggests that humans have either an innate or learnt ability to infer the internal states of others using lawlike generalisations (Goldman, 2012). Rationality theory states that contextual information and prior knowledge about the individual are used to infer the internal state underlying someone's actions (Goldman, 2006; Goldman 2012). Both theory theory and rationality theory therefore suggest that humans can mentalize without needing to internally simulate others' actions. However, the existence of poorer mentalizing abilities in individuals whom experience motor inhibition deficits or display hyperimitation (Chasiotis, 2006; Spengler, Bird, & Brass, 2010) as well as reduced empathy and poorer emotion recognition abilities in children with motor coordination disorders (Cummins, Piek, & Dyck, 2005; Efstratopoulo et al., 2016) imply motor simulation plays an important role in mentalizing. These data also raise the possibility that dysfunction or delayed development of the cortical system involved in motor simulation may result in mentalizing difficulties.

### 1.2 The mirror neuron system

The cortical system associated with motor simulation is the mirror neuron system (MNS; Rizzolatti & Craighero, 2004; Rizzolatti & Sinigaglia, 2010). The MNS is a collection of brain regions that contain neurons that show similar activation patterns during both the performance and observation of actions (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). It is thought that by displaying similar activation patterns during the observation of actions as when performing similar actions, this system is the mechanism in which observed actions are simulated in the observer's own motor system (Rizzolatti and Craighero, 2004). The main components of the human MNS are thought to be the inferior frontal gyrus (IFG) and the inferior parietal lobe (IPL; Rizzolatti & Craighero, 2004; Rizzolatti & Sinigaglia, 2010). MNS activation has been reliably found when inferring others' internal states from their actions such as their communicative intentions (Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; Xu et al., 2009), prior intentions (Spunt & Lieberman, 2011), social intentions (Becchio et al., 2012), motor intentions (Buccino et al., 2007; de Lange, Spronk, Willems, Toni, & Bekkering, 2008), emotions (Hadjikhani et al., 2009; Spunt & Lieberman, 2012a) and beliefs (Grezes, Frith & Passingham, 2004) supporting a role of motor simulation in mentalizing in the presence of human action. Temporary lesions to IFG via direct current stimulation (Herbet, Lafargue, Bonnetblanc, et al., 2014) and IFG lesions in brain damaged patients (Besharati et al., 2016; Dal Monte et al., 2014) also result in poorer mentalizing performances, providing further support for a vital role of these regions during mentalizing tasks. However, although MNS activation has been consistently found during mentalizing tasks in the presence of human action, the role of this system in mentalizing is debated.

### 1.3 Proposed models of the role of the MNS in mentalizing

There are a number of different theories regarding the possible role of the MNS in mentalizing in the presence of human action: 1. The motor simulation theory states that MNS activity alone, reflecting motor simulation of observed actions, is sufficient to mentalize (Rizzolatti & Sinigaglia, 2007). The motor simulation theory is based on empirical evidence showing action kinematics differ depending on the internal state of the actor and these kinematic profiles alone can be used to infer  the internal states of others (Manera, Becchio, Cavallo, Sartori, & Castiello, 2011; Sartori, Becchio, & Castiello, 2011). 2. A dual-process hypothesis has been proposed which suggests that pre-reflective processing of others' internal states based on kinematic differences occurs in the MNS but this system is not involved in actively interpreting intentional information (de Lange, Spronk, Willems, Toni, & Bekkering, 2008; Keysers & Gazzola, 2007; Uddin, Iacoboni, Lange, & Keenan, 2007). The dual-process hypothesis was generated based on social psychology theory of social causal attribution which states that when inferring internal states from actions, incoming sensory information must be translated into internal state information (Gilbert, 1998).  fMRI data has provided evidence that IFG encodes action information which indicates the actors' intentions (Iacoboni et al., 2005; Hamilton and Grafton, 2008), making this area a likely candidate for a brain area involved in translating motor information into internal state information. Additionally, fMRI studies have shown increased connectivity between the MNS and the mentalizing system when inferring internal states from actions but only mentalizing system activation increased in response to increased mentalizing demand of experimental tasks (Spunt & Lieberman, 2011; Spunt & Lieberman, 2012a; 2012b). Spunt and Lieberman hypothesised that the MNS translates kinematic information into internal state information at the subconscious level and passes internal state information to the mentalizing system for this to be actively interpreted. 3. The 'mirroring-first' model implies that processing action kinematics in the MNS is a vital prerequisite for mentalizing but this system is not directly involved in processing others' internal states (Hamilton & Marsh, 2013; Spunt, Satpute, & Lieberman, 2011). This model was based on evidence showing that although connectivity between MNS and the mentalizing system increased when mentalizing, viewing actions with unusual intentions increased activation in the mentalizing system, not the MNS (Brass et al., 2007; de Lange et al., 2008). Instead, increased MNS was found when the kinematics of observed actions were unusual (de Lange et al., 2008). These data suggest that action processing in the MNS is important for mentalizing but this system does not process internal state information.

In opposition to all these theories of MNS involvement in mentalizing, the visual inference model suggests that the internal states underlying others' actions are inferred in the mentalizing system immediately after actions are visual processed in the superior temporal sulcus (STS) and medial temporal gyrus (MTG), without requiring the intermediate step of motor simulation in the MNS (Hamilton & Marsh, 2013). Therefore, the visual inference model suggests the MNS is not required in order to infer internal states underlying others' actions. In support of the visual inference theory, MNS activity has not been found during some mentalizing tasks that do not involve human action processing, suggesting the MNS may not play an important role in inferring others' internal states (Castelli, Happé, Frith, & Frith, 2000; Castelli et al., 2002; Gallagher et al., 2000; Spunt, Satpute, & Lieberman, 2011; Van Overwalle et al., 2009; White, Frith, Rellecke, Al-Noor, & Gilbert, 2014). Additionally, some studies have found equivalent levels of MNS activity when inferring others' internal states from their actions compared to when viewing the same actions and not actively mentalizing (de Lange et al., 2008; Spunt & Lieberman, 2012b). Finally, equivalent levels of MNS activity have been found during the observation of gestures with communicative intent as during the observation of non-communicative gestures (Mainieri, Heim, Straube, Binkofski, & Kircher, 2013). These data provide evidence against the motor simulation theory, as these data imply that the MNS activation alone is not sufficient in order to infer others' internal states.

However, the studies providing evidence against MNS involvement in mentalizing are not without their limitations; it is possible that when not actively mentalizing, observing the same actions shown during mentalizing tasks induces implicit mentalizing. Equivalent levels of MNS activity across both mentalizing and non-mentalizing conditions would therefore reflect involvement of MNS in both implicitly and explicitly inferring internal states from others' actions. The stimuli used in the mentalizing tasks that did not observe MNS activation were simplistic cartoon still images or animations and therefore, inferring the internal states of these characters may not have required the same complex information to be processed as when inferring the internal states of others in naturalistic situations e.g. contextual information, prior knowledge about the individual, facial expression and eye gaze (Castelli, Happé, Frith, & Frith, 2000; Castelli et al., 2002; Gallagher et al., 2000; Spunt, Satpute, & Lieberman, 2011; Van Overwalle et al., 2009; White, Frith, Rellecke, Al-Noor, & Gilbert, 2014). Finally, when observing 'non-communicative intentions' it is possible that participants were attempting to infer communicative intent from the unusual actions shown and therefore MNS activation may reflect attempts to mentalize.

Data from other previous studies show MNS activity is modulated by the intentions of observed actions (Canessa et al., 2012; Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; de Lange et al., 2008) and MNS activity is higher if observed actions are social rather than non-social (Bucchioni et al., 2013; Centelles et al., 2011; Enticott et al., 2013). These results suggest that the MNS does encode others' internal states, which contradicts the both the visual inference model and the mirroring-first hypothesis. However, different levels of MNS activation during actions with different intentions could reflect the different kinematic profiles or increased complexity associated with social compared to non-social actions. Despite this, individuals with MNS lesions (Besharati et al., 2016; Dal Monte et al., 2014), atypical MNS functioning (Baron-Cohen et al., 1999; Holt et al., 2014; Kana et al., 2014) or delayed MNS development (Bastiaansen et al., 2011) also display poorer mentalizing abilities, supporting the importance of the MNS in inferring others' internal states.

Despite evidence that the MNS has a role in processing others' internal states, equivalent MNS activity has been found when actively inferring others' intentions compared to when passively viewing actions which portray the actor's intention (de Lange et al., 2008; Spunt & Lieberman, 2012b). These data suggest that although the MNS may process internal state information, this system is not likely to be involved in actively interpreting this information; this supports the dual-process hypothesis. However, the dual-process hypothesis cannot account for data from different studies which have shown higher levels of MNS activity when explicitly inferring the internal states of others' compared to passively observing the same actions (Buccino et al., 2007; Schippers et al., 2009). Consequently, the role of the MNS in inferring others' internal states is still debated.

## 1.4 Methods of measuring MNS activity and their limitations

The most common neuroscience techniques used to measure activity in the human MNS are functional magnetic resonance imaging (fMRI), electroencephalography (EEG) and transcranial magnetic stimulation (TMS; Hobson & Bishop., 2016; Kilner & Lemon, 2013; Maeda et al., 2002). FMRI has the highest spatial resolution of these techniques (Crosson et al., 2010). In fMRI studies, the location of MNS areas are either determined using a functional localiser task (Sperduti et al., 2014; Spunt and Lieberman, 2012c) or anatomically defined using coordinates of areas traditionally associated with the MNS from previous studies, meta-analyses, or brain atlases as a reference (Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; Fishman, Keown, Lincoln, Pineda, & Müller, 2014; Liew et al., 2011; Mainieri, Heim, Straube, Binkofski, & Kircher, 2013). Functional localiser tasks usually involve participants both moving their right hand and also observing another person move their right hand in the same way; brain areas which are active during both of these tasks are considered to exhibit mirror properties. Defining MNS areas anatomically potentially elicits a problem known as 'the reverse inference' (Hutzler, 2014; Poldrack., 2011). This is when certain cognitive processes are assumed based on the neuroimaging data. Brain areas have multiple functions and therefore it is not accurate to assume certain cognitive processes (e.g. ‘mirroring’) based on neuroimaging data (Poldrack, 2006). Even when MNS areas are functionally defined in fMRI studies using a localiser task; it cannot be determined whether the same neurons in the identified areas are activated during both tasks. It is possible that neighbouring populations of neurons respond to either the performance or observation of a movement and therefore the neurons do not display mirror properties. Consequently, researchers should be cautious when drawing conclusions regarding mirroring properties of brain areas.

EEG studies quantify levels of MNS activity by measuring the degree to which brain activity in the alpha frequency band (8-12Hz) is suppressed when observing the actions of others compared to rest. This is known as *mu* suppression (Fox et al., 2016; Frenkel-Toledo, Bentin, Perry, Liebermann, & Soroker, 2014; Oberman, Pineda, & Ramachandran, 2007). When performing or observing actions, large amplitude oscillations in the 8-12Hz frequency range over sensorimotor cortex (mu rhythm) are suppressed (Fox et al., 2016; Frenkel-Toledo, Bentin, Perry, Liebermann, & Soroker, 2014; Oberman, Pineda, & Ramachandran, 2007). Therefore, mu suppression is taken as an index of MNS activity. However, the source of mu suppression is unknown; a study which combined both EEG and fMRI showed that mu suppression correlated with activity in multiple cortical areas, not just areas associated with the mirror neuron system (Yin et al., 2016) and magnetoencephalography (MEG) studies have suggested that the source of mu suppression may be the somatosensory cortex, rather than core MNS areas (Cheyne et al., 2003; Jones et al., 2009). Therefore, the reliability of mu suppression as a measure of core MNS activity has been questioned (Hobson & Bishop, 2016).

Finally, TMS indirectly measures MNS activity by applying single-pulses to the primary motor cortex (M1) and measuring changes in corticospinal activity in contralateral hand muscles via electromyography ( EMG; Aziz-Zadeh, Maeda, Zaidel, Mazziotta, & Iacoboni, 2002; Fadiga, Craighero, & Olivier, 2005). Applying single TMS pulses to M1 induces increased activity in contralateral hand muscles (known as motor evoked potentials; MEPs), which are larger when viewing hand actions than when at rest. These increased MEP sizes when viewing hand actions are thought to reflect increased excitability in M1 as the result of excitatory cortico-cortical connections from prefrontal MNS areas (IFG/vPMC). The degree to which MEP sizes increase during action observation is therefore regarded as an index of MNS activity (Luciano Fadiga et al., 2005a; Patuzzo et al., 2003; Strafella & Paus, 2000). Although this technique provides high temporal resolution, it only generates a transient measure of MNS activation. This means that if an experimental task modulates MNS activity in a temporally specific manner, MNS activity must be measured at precisely the time of MNS modulation. MEP sizes also show a high degree of variability (Kiers et al., 1993; Ellaway et al., 1998) which could result from experimental error e.g. slight changes to coil location and orientation (Barker et al., 1987; Hashemirad et al., 2017), However, as this index of MNS activity is an indirect measure of MNS activation, changes to the state of the corticospinal tract or the muscles recording from could also influence MEP sizes (Gruet et al., 2013). Consequently, changes in motor cortex excitability signalled by changes in MEP sizes are confounded by slight changes in stimulation administration and subcortical excitability.

Despite all three of these techniques providing useful indices of MNS activity, without direct neural recordings, MNS activity cannot be conclusively identified in humans (Hickok, 2009; Kilner & Lemon., 2013). Therefore, researchers should be cautious when making conclusions regarding MNS activity and consider the spatial and temporal limitations of these techniques.

## 1.5 The mentalizing system and connectivity with the MNS

In addition to the MNS, another cortical system referred to as the 'mentalizing system' is activated when inferring the internal states of others (Denny et al., 2012; Schurz, Radua, Aichhorn, Richlan, & Perner, 2014; Van Overwalle & Baetens, 2009). The core regions of the mentalizing system are considered to be the temporoparietal junction (TPJ), the medial prefrontal cortex (mPFC) and the temporal poles (Frith & Frith, 2006). These two systems are thought to have distinct roles during mentalizing tasks; in comparison to motor simulation in the MNS, the mentalizing system is thought to use action context and prior experience to infer others' internal states (Chambon et al., 2017b; de Lange et al., 2008; Keysers & Gazzola, 2007; Spunt & Lieberman, 2012b). The more unusual, abstract or further removed the internal states being inferred are from the observed actions or the more limited the action information available, the greater the considered reliance on the mentalizing system (Brass, Schmitt, Spengler, & Gergely, 2007; Chambon et al., 2017a; Liepelt, Von Cramon, & Brass, 2008).

The mPFC can be separated into distinct subregions and review papers have noted that activity in different mPFC subregions is associated with different aspects of mentalizing (Amodio & Frith, 2006; Frith & Frith, 2006; Sallet et al., 2007; Wilson, Gaffan, Browning, & Baxter, 2010). Methods used to separate mPFC into different subregions have differed across tasks but the mPFC has most commonly been divided into dorsal and ventral subregions (Bzdok et al., 2013). Activity in dorsal mPFC (dmPFC) is specifically associated with mentalizing in the presence of human action (Amodio & Frith, 2006; Frith & Frith, 2006; Sallet et al., 2013). Additionally, the dmPFC shows greater connectivity with areas of the MNS than ventral mPFC (vmPFC; Bzdok et al., 2013). Therefore, it is likely that connectivity between the MNS and this region of the mPFC is particularly important when mentalizing in the presence of human action.

Despite reliable activation in both mirror neuron and mentalizing systems during mentalizing tasks, the nature of the interaction between these two systems is uncertain. Numerous studies have shown increased connectivity between areas of the MNS and areas of the mentalizing system when inferring the internal states of others from their actions (Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; Lombardo et al., 2010; Marsh et al., 2010; Sperduti, Guionnet, Fossati, & Nadel, 2014; Spunt & Lieberman, 2012b; Trapp et al., 2014; Xu, Gannon, Emmorey, Smith, & Braun, 2009) and when not directly engaged in mentalizing tasks but when observing social actions compared to non-social actions (Ciaramidaro et al., 2014; Xu et al., 2009). However, the areas that have been reported to display increased functional connectivity differ across studies. Increased connectivity has been found between right IFG and dmPFC when inferring prior intentions from actions (Spunt & Lieberman, 2012b), increased connectivity between left IFG and dlPFC has been reported in one study (Xu et al., 2009) and between dmPFC and bilateral premotor cortex and aIPS in another study (Ciaramidaro et al., 2014) when inferring communicative intentions from hand actions. Right IFG shows increased connectivity with dmPFC as well as bilateral TPJ when inferring the underlying cause of displays of emotion in movie clips (Spunt & Lieberman, 2012a). Finally, increased connectivity has been found between right IFG and the anterior cingulate cortex (ACC) when passively viewing emotional facial expressions (Rudie et al., 2012). It is likely that different areas of the MNS and the mentalizing system are functionally connected depending on the mentalizing task being performed and therefore connectivity between these two systems cannot be generalised across mentalizing tasks. The limited existing data regarding functional connectivity between the MNS and the mentalizing system during mentalizing tasks mean it is not possible to characterise the connections which are important for different tasks. However, connectivity between the right IFG and the mPFC seems to be the most commonly reported connection when inferring the internal states of others' from their actions (Rudie et al., 2012; Spunt & Lieberman, 2012a; Spunt & Lieberman, 2012b; Tettamanti et al., 2017).

Only one study to date has investigated functional connectivity when inferring others' social intentions from their actions (Chambon et al., 2017). This study did not find increased connectivity between areas of the MNS and the mentalizing system which was unexpected as increased functional connectivity between these systems has been consistently reported when inferring internal states of others' from their actions (Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; Lombardo et al., 2010; Marsh et al., 2010; Sperduti, Guionnet, Fossati, & Nadel, 2014; Spunt & Lieberman, 2012b; Trapp et al., 2014; Xu, Gannon, Emmorey, Smith, & Braun, 2009) and increased activity in both systems was reported in this study when inferring social intentions (Chambon, Domenech, et al., 2017a). The lack of increased functional connectivity between the MNS and the mentalizing system in this study may have been due to the area of the mentalizing system which was seeded for the functional connectivity analysis. The authors seeded a region of the vmPFC rather than dmPFC. An area of dmPFC showed higher activation when inferring social intentions compared to more ventral activation when inferring non-social intentions but the authors decided to seed an area of the vmPFC for their connectivity analysis which was activated across all tasks in their study (Chambon, Domenech, et al., 2017a). The dmPFC is specifically associated with inferring internal states of others from their actions (Amodio & Frith, 2006; Frith & Frith, 2006; Sallet et al., 2013), processing social information (Apps & Ramnani, 2017; Chambon et al., 2017b; Lotze, Veit, Anders, & Birbaumer, 2007; Sallet et al., 2013) and perspective taking (Bzdok et al., 2013). In comparison, the ventral mPFC is associated with judgements about non-social actions, self-relevant information and the value of action outcomes (Apps & Ramnani, 2017; Bzdok et al., 2013; Chambon et al., 2017a; Glacher, Hampton, & O 'doherty, 2009). Therefore, if the dmPFC had been seeded then this region may have shown increased functional connectivity with areas of the MNS when inferring social intentions.

In addition to the inconsistency regarding which areas of the MNS and the mentalizing system display increased functional connectivity when mentalizing, the direction of connectivity between these two systems is uncertain. The mirroring-first theory and the dual-process hypothesis propose exclusively 'bottom-up' connectivity from the MNS to the mentalizing system when mentalizing in the presence of human action (de Lange, Spronk, Willems, Toni, & Bekkering, 2008; Hamilton & Marash, 2013). In support of this, Spunt and colleagues found that MNS activity preceded mentalizing system activity when participants inferred others' emotions from their facial expressions (Spunt & Lieberman, 2012a). Additionally, increased functional connectivity from IFG to mPFC has been shown when inferring the communicative intentions of others (Tettamanti et al., 2017) and inferring superordinate compared to basic intentions has been found to increase connectivity from supplementary motor area (SMA; part of the extended MNS) to the mPFC (Chambon, Domenech, et al., 2017b). These data support the bottom-up up flow of information from the MNS to the mentalizing system when inferring internal of others from their actions.

In contrast to the theory of exclusively bottom-up connectivity between the MNS and mentalizing system when mentalizing, the predictive coding model of MNS functioning in combination with the predicted response outcome model of mPFC function, propose the importance of top-down connectivity from mPFC to the MNS. The predictive coding theory of MNS functioning states that action context is signalled to the MNS prior to motor simulation, in order for predictions to be made about the outcome of upcoming actions (Kilner et al., 2007). This model of MNS functioning was based on empirical evidence showing that when observing actions, EEG signals reflecting motor preparation are generated (Kilner et al., 2004), suggesting the intended outcome of observed actions are predicted in order to prepare the appropriate response. Additionally, increased muscle activation in observer's mouths has been found when observing grasp-to-eat actions but not grasp-to –place actions before the outcome of the actions are shown (Cattaneo et al., 2007). This suggests that predicted intentions of observed actions are encoded in the observer's MNS. The predictive coding model would suggest that predictions regarding upcoming actions are formed based on action context outside of the MNSand this information is signalled to the MNS via top-down connectivity (Kilner, Friston, & Frith, 2007). As the action unfolds, the kinematics of the observed action are compared to the predictions made and discrepancies, known as 'prediction errors', are formed in the MNS and signalled to higher areas (Kilner et al., 2007; Urgen & Miller, 2015). The predicted response outcome model by Alexander and Brown suggests that the mPFC makes predictions about the outcomes of upcoming actions based on action context and prior experience (Alexander & Brown, 2011).

Additionally, neuroimaging studies have provided evidence that action context and prior expectations about an upcoming action are processed in the mPFC (Alexander & Brown, 2011; Becchio et al., 2012; Chambon et al., 2017b; Cooper, Kreps, Wiebe, Pirkl, & Knutson, 2010; Ferdinand & Opitz, 2014; Fogelson, Shah, Scabini, & Knight, 2009; Forster & Brown, 2011; Jahn, Nee, Alexander, & Brown, 2014; Leue, Cano Rodilla, & Beauducel, 2015; Schiffer, Krause, & Schubotz, 2014). Therefore, the mPFC is a likely candidate for the area signalling predicted intentions of upcoming actions to the MNS, within the predictive coding framework, in order for comparisons to be made between actual and predicted intentions (Alexander & Brown, 2011). Top-down signalling of predictions regarding the upcoming action are suggested to be important in order to react sufficiently quickly in certain contexts e.g. potentially dangerous situations (Alexander & Brown, 2011). Often, it is necessary to respond before another's action has been completed, both for successful social interaction and survival. MNS activation is associated with action preparation (Fabbri-Destro, Cattaneo, Boria, & Rizzolatti, 2009; Newman-Norlund, van Schie, van Zuijlen, & Bekkering, 2007). Therefore, if predicted intentions formed in the mPFC suggest a potential risk then signalling from mPFC to the MNS at an early stage during action observation may allow a sufficiently quick response.

Previous neuroimaging studies have provided evidence to support this predictive coding model; first, MNS activity has been shown to be modulated by action context (Amoruso & Urgesi, 2016; Iacoboni, Molnar-Szakacs, Gallese, Buccino, & Mazziotta, 2005), prior expectation (Fontana et al., 2012) and whether participants are explicitly told to mentalize (Buccino et al., 2007; Schippers, Gazzola, Goebel, & Keysers, 2009). These data collectively imply the presence of top-down connectivity to the MNS. Second, a functional magnetic resonance imaging (fMRI) study found that when observing hand actions of others, activation levels in the mPFC and IFG were different depending on whether the actions had individual intent or social intent, even though the outcome of the actions were not seen (Becchio et al., 2012). This suggests that predictions about upcoming actions based on inferred social intentions, which are likely formed in the mPFC, are signalled to the IFG.

The evidence implying the presence of top-down connectivity from the mentalizing system suggest that neither the mirroring-first theory nor the dual-process hypothesis are likely to be full accounts of information processing during mentalizing tasks. It is possible that an extended version of the dual-process model which incorporates aspects of the predictive coding hypothesis (e.g. top-down signalling of action predictions based on contextual information prior to motor simulation) could provide a more complete picture of the interaction between the MNS and the mentalizing system when mentalizing in the presence of human action. Therefore, the direction of mentalizing-induced increased functional connectivity is likely to be temporally specific.

## 1.6 Autism spectrum disorder

Autism spectrum disorder (ASD) is a neurodevelopmental disorder which is strongly associated with mentalizing impairments (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Chung, Barch, & Strube, 2014; Frith, 2001; Holt et al., 2014; Jolliffe & Baron-Cohen, 1999). Mentalizing abilities are even assessed in some diagnostic and screening tools for ASD (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Lord et al., 2000). There are two diagnostic classification systems used in the UK: the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V) and the International Classification of Diseases, version 10

(ICD-10). The DSM-V uses the term ASD to describe a number of neurodevelopmental disorders, classified according to core deficits in social communication as well as restricted and repetitive interests (Wing, Gould, & Gillberg, 2011). The disorders previously referred to as autistic disorder, Asperger's disorder and persuasive disorder not otherwise specified (PDD-NOS) in the preceding DSM edition are now all classified as ASD (Lee, Thomas, & Lee, 2015; Tsai & Ghaziuddin, 2014). The term Asperger's is still widely used and is included in the ICD-10 despite being removed from the DSM-V (Tsai, 2013). A diagnosis of Asperger's reflects typical language and cognitive abilities without developmental delays (Scottish Intercollegiate Guidelines Network, 2007; Volkmar, Klin, Schultz, Rubin, & Bronen, 2000). In this thesis, the term 'ASD' will typically be used but 'Asperger's' will be used if researchers have specifically recruited individuals with this diagnosis in order to control for differences in cognitive abilities and language development.

As ASD is a spectrum disorder, individuals with and without a diagnosis display varying degrees of autistic traits. Individuals with relatively high but not clinically significant levels of autistic traits have been shown to display subtler versions of the behavioural and neurological characteristics associated with ASD (Best, Arora, Porter, & Doherty, 2015; Di Martino et al., 2009; Lindell, Notice, & Withers, 2009; Ridley, Homewood, & Walters, 2011; van Boxtel & Lu, 2013) including mentalizing deficits (Baron-Cohen, Wheelwright, Skinner, et al., 2001; Chung et al., 2014b; Happé, 1994; Kana, Libero, Hu, Deshpande, & Colburn, 2014; Moran et al., 2011). There are a number of psychological assessments that can be used to measure the level of autistic traits that individuals display, either using questionnaires or observational measures (Baron-Cohen, Wheelwright, Skinner, et al., 2001; Constantino et al., 2003; Lord et al., 2000; Lord, Rutter, & Couteur, 1994; McDonald et al., 2006; Wigham, McConachie, Tandos, & Le Couteur, 2012). Therefore, researchers investigating the nature of mentalizing impairments associated with ASD have sometimes assessed the relationship between mentalizing abilities and levels of autistic traits rather than examining potential group differences between clinical and non-clinical populations (Gökçen, Petrides, Hudry, Frederickson, & Smillie, 2014; Gökçen, Frederickson, & Petrides, 2016; Nijhof, Brass, Bardi, & Wiersema, 2016). This method allows behavioural or neurological characteristics associated with ASD to be examined across the spectrum and avoids the possibility of the high variability in autistic traits within clinical and non-clinical populations masking potential group differences.

### 1.7 Experimental evidence for mentalizing deficits in ASD and reasons for inconsistencies in the current literature

Despite the strong association between ASD and mentalizing deficits, experimental evidence regarding the nature of these deficits is inconsistent; adults with ASD have been shown to display poorer performances on tasks involving inferring the intentions of characters in stories and comic strips (Happé, 1994; Kana et al., 2014; Moran et al., 2011), reduced abilities to infer the mental states of others' from still images of their eyes (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001), from their actions (Senju, Southgate, White, & Frith, 2009) and infer the mental states elicited from animated shapes (Castelli et al., 2002). Additionally, adults without an ASD diagnosis but high levels of autistic traits have shown reduced abilities to infer the mental states of others' from still images of their eyes (Gökçen, Petrides, Hudry, Frederickson, & Smillie, 2014; Gökçen, Frederickson, & Petrides, 2016). However, a number of studies have found adults with ASD are not impaired at inferring others' intentions or mental states (Hubert et al., 2007; Kana, Keller, Cherkassky, Minshew,

& Just, 2009; Murphy, Brady, Fitzgerald, & Troje, 2009; Saygin, Cook, & Blakemore, 2010; Schuwerk et al., 2014; Spek, Scholte, & Van Berckelaer-Onnes, 2010) and abilities to infer others' false beliefs are not related to the level of autistic traits displayed (Nijhof et al., 2016). Differences in the explicitness of the instructions provided, the particular aspects of mentalizing being assessed, the stimuli used and methods used to measure mentalizing abilities have all likely contributed to the inconsistency in the behavioural data.

The majority of tasks that have been used to investigate the mentalizing abilities of adults with ASD have explicitly instructed the participants to do so (Baron-Cohen et al., 2001; Castelli et al., 2002; Happé, 1994; Kana, Libero, Hu, Deshpande, & Colburn, 2014). The instructions given in lab settings regarding which elements should be attended to and when to attend to these elements, may allow some high functioning adults with ASD to perform at a typical level. Only a small number of studies have examined the capabilities of adults with ASD to infer the internal states of others when not specifically told to do so; known as 'implicit mentalizing'(Castelli et al., 2002; Rosenblau et al., 2015; Schuwerk et al., 2014; Senju et al., 2009). These studies have consistently found that adults with ASD exhibit poorer performances. This has led to the suggestion that ASD is associated with implicit but not explicit mentalizing deficits (Frith, 2001b; Rosenblau, Kliemann, Heekeren, & Dziobek, 2015; Schuwerk et al., 2014)

The apparent difference between implicit and explicit mentalizing abilities may be, at least in part, due to differences in the stimuli and methods used to measure mentalizing abilities across explicit and implicit tasks. The tasks that have been most commonly used to assess explicit mentalizing abilities in adults with ASD have been Happé's strange stories test which involves inferring the intentions of characters in short passages of text (Happé, 1994), the Reading the Mind in the Eyes (RME) task in which participants are asked to infer the others' mental states from still images of their eyes (Baron-Cohen et al., 1997) and a task which requires participants to infer the intentions of characters in comic strips (Kana et al., 2014). The stimuli used in all of these tasks are simplistic and do not require complex factors such as body posture, action kinematics, face and eye movements to be processed which are vital in order to successfully interpret others' internal states in real life social interactions. In contrast, the stimuli that have been used to measure implicit mentalizing capabilities have been videos or animations which were more complex and naturalistic than the stimuli used in the majority of explicit tasks (Rosenbalu et al., 2015; Schuwerk et al., 2014; Senju et al., 2009).

Two previous studies (Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Roeyers, Buysse, Ponnet, & Pichal, 2001) investigated the abilities of adults with Pervasive Development Disorders (PDD; including ASD) to explicitly infer the mental states of others using both simplistic stimuli (images of people's eyes and short passages of text) as well as naturalistic videos of social interactions (Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Roeyers et al., 2001). The adults with PDD did not display impaired mentalizing abilities when simplistic stimuli were used but did show impairments on the tasks that used more complex, naturalistic stimuli. Additionally, the only study to date that measured both implicit and explicit mentalizing abilities using complex, naturalistic stimuli found that adults with ASD displayed equivalent impairments on both implicit and explicit tasks (Rosenblau et al., 2015). Therefore, differences in the stimuli used between studies that investigated explicit mentalizing abilities compared to implicit mentalizing abilities may have contributed to the consistent implicit mentalizing deficits reported for adults with ASD but the inconsistent literature regarding the explicit mentalizing abilities of adults with ASD.

In addition to stimuli differences across studies investigating explicit and implicit mentalizing abilities, there have also been differences in the way in which mentalizing abilities were assessed. Studies investigating implicit mentalizing abilities have tended to use eye-tracking data to measure implicit mentalizing (Schuwerk et al., 2014; Senju et al., 2009). In these studies, false belief tasks were used which involved a character who was unaware that another character had changed the location of an object, either because they were out of the room at the time or were looking elsewhere. The character therefore falsely believed that the object was still in the original location. When the character came back into the room or diverted their attention back to the social scene, adults with ASD tended to show reduced durations of fixation on the location in which the character falsely believed the object was located. Shorter durations of visual fixation on the locations of false belief in adults with ASD were interpreted as impaired implicit mentalizing (Schuwerk et al., 2014; Senju et al., 2009). However, shorter durations of fixation on the location of interest do not necessarily reflect a lack of processing; not only are unusual patterns of eye gaze widely reported for autistic individuals when processing social stimuli (Kliemann, Dziobek, Hatri, Steimke, & Heekeren, 2010; Pelphrey et al., 2002) but unusual visual fixation patterns have been found in studies that have found no behavioural differences (Rutherford & Towns, 2008; Spezio, Adolphs, Hurley, & Piven, 2007). Therefore, eye-tracking data alone may not be an accurate measurement of mentalizing abilities. In comparison, explicit mentalizing abilities have always been assessed using measurable behavioural outcomes rather than eye-tracking data alone.

Differences in the specific mentalizing abilities assessed across studies may have also contributed to inconsistencies in the literature. The term 'mentalizing' covers a range of internal state inferences which may involve different processes (Call & Tomasello, 2008; Pineda & Hecht, 2009). This is supported by neuroimaging studies which have shown that different brain areas are active during mentalizing tasks depending on the inferences being made (Pineda & Hecht, 2009; Schurz et al., 2014). Adults with ASD have shown more consistent impairments in inferring others' intentions (Kana et al., 2014; Murdaugh, Nadendla, & Kana, 2014; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001) and emotions (Atkinson, 2009; Cassidy, Ropar, Mitchell, & Chapman, 2013; Peter G Enticott, Kennedy, Johnston, et al., 2013; Hubert et al., 2007; Nackaerts et al., 2012) but less consistent difficulties in inferring mental states (Baron-Cohen et al., 1997; Kana et al., 2009; Kirkovski et al., 2015; Kleinman, Marciano, & Ault, 2001; Roeyers et al., 2001; Spek et al., 2010) or false beliefs (Frith & Happé, 1994; Schuwerk et al., 2014; Senju et al., 2009). Studies which have assessed abilities to infer intentions have differed in the types of intentions participants were required to infer; consistent deficits have been found in inferring social (Chambon, Farrer, et al., 2017; Happé, 1994; Moran et al., 2011) and prior intentions (Kana et al., 2014; Moran et al., 2011; Sivaratnam, Cornish, Gray, Howlin, & Rinehart, 2012) but not motor intentions (Aldridge, Stone, Sweeney, & Bower, 2000; Baron-Cohen, Leslie, & Frith, 1986; Broekhof et al., 2015; Carpenter, Pennington, & Rogers, 2001). These data suggest that adults with ASD may be particularly impaired in inferring certain aspects of another's internal state. Therefore, differences in the aspects of mentalizing assessed, explicitness of the instructions, stimuli used and the methods used to measure mentalizing abilities, may have all contributed to the heterogeneity in the literature. Adults with ASD seem to display particular impairments when inferring more abstract intentions or emotions from realistic action stimuli. Difficulties inferring others' social intentions is of particular importance as this deficit can result in individuals with ASD making inappropriate social decisions and consequently being vulnerable to mistreatment (Fisher, Moskowitz, & Hodapp, 2013; The National Autistic Society, 2014).

## 1.8 The broken mirror neuron hypothesis of ASD

One of the neurobiological theories for the social impairments associated with ASD has been the 'broken mirror' theory. The broken mirror theory states that atypical functioning of the MNS underlies the social impairments associated with ASD (Iacoboni & Dapretto, 2006; Oberman & Ramachandran, 2007). It is theorised that ineffective internal representations of others' actions are formed as a result of dysfunctional MNS activation which causes impaired interpretation of others' actions and ultimately leads to deficits in social communication. The broken mirror theory was formed as a result of the high incidence of comorbid motor deficits in individuals with ASD (Gowen & Hamilton, 2013; Larson & Mostofsky, 2006; Ming et al., 2007) and a number of neuroimaging studies showing that children with ASD display atypical MNS activity during tasks typically associated with MNS functioning such as imitation (Dapretto et al., 2005; Hobson & Hobson, 2008; Rogers et al., 2003; Williams, Whiten, & Singh, 2004), action planning (Cattaneo et al., 2007; Dowd et al., 2012; Fabbri-Destro, Cattaneo, Boria, & Rizzolatti, 2009) and gestural performance (Dewey et al., 2007). However, despite the empirical evidence for MNS dysfunction in children with ASD, neuroimaging data to support atypical MNS activity during tasks traditionally associated with MNS functioning in adults with ASD is limited.

A small number of studies have provided evidence to suggest that MNS activation is atypical during action observation in adults with ASD (Bernier, Dawson, Webb, & Murias, 2007; Enticott et al., 2012; Honaga et al., 2010; Martineau, Andersson, Barthélémy, Cottier, & Destrieux, 2010) and adults with high levels of autistic traits (Puzzo, Cooper, Vetter, Russo, & Fitzgerald, 2009). However, these results are limited: Martineau and colleagues found higher IFG activation in adults with ASD when observing hand actions (Martineau et al., 2010) but this result was due the absence of IFG activation in the control group which is very unusual (Buccino et al., 2001; Nelissen, Luppino, Vanduffel, Rizzolatti, & Orban, 2005; Plata Bello, Modroño, Marcano, & González-Mora, 2013). One transcranial magnetic stimulation (TMS) study by Enticott and colleagues found reduced MNS activity in adults with ASD when observing hand movements (Enticott et al., 2012) but a subsequent TMS study conducted by the same research group did not replicate this result (Enticott et al., 2013). In addition, a study by Honaga and colleagues found dysfunction in the MNS when memorising observed actions in order to imitate them at a later stage but mentalizing system dysfunction was also reported during this task (Honaga et al., 2010). It is possible that recruitment of the mentalizing system due to the demands of the task resulted in atypical MNS activity in adults with ASD. The majority of neuroimaging studies investigating MNS activity during tasks traditionally associated with MNS activation e.g. observing and performing actions, in adults with ASD have shown typical levels of MNS activity (Avikainen, Kulomäki, & Hari, 1999; Bastiaansen et al., 2011; Dinstein et al., 2010; Enticott et al., 2013). Despite the limited functional neuroimaging data to support the broken mirror theory, lower grey matter volumes and cortical thinning have been discovered in the IPL and IFG in adults with ASD (Hadjikhani, Joseph, Snyder, & Tager-

Flusberg, 2006; Yamasaki et al., 2010) and these anatomical differences were shown to correlate with the degree of social impairment. Levels of grey matter volume in left IFG have also shown to correlate with autistic traits in individuals without an ASD diagnosis (Geurts, Ridderinkhof, & Scholte, 2013). However, adults with ASD generally display typical behavioural performances on tasks traditionally associated with MNS functioning (Bird, Leighton, Press, & Heyes, 2007; Sari Avikainen, Wohlschläger, Liuhanen, Hänninen, & Hari, 2003). Therefore, evidence to support general dysfunction of the MNS in adults with ASD is limited (Hamilton, 2013).

Despite the limited evidence supporting general dysfunction of the MNS during action observation in adults with ASD, reduced MNS activation has been found in adults with ASD during mentalizing tasks. Lower MNS activity has been shown in adults with ASD when viewing emotional body movements depicted by point-light displays (Hadjikhani et al., 2009) and making emotional judgements about faces (Wicker et al., 2008). Mixed samples of adults and adolescents with ASD have shown atypical MNS activation when inferring motor intentions from hand actions (Libero et al., 2014), inferring prior intentions of characters in comic strips (Kana et al., 2014) and inferring the mental states of others from their facial expressions (Baron-Cohen et al., 2001; Holt et al, 2014). Reduced MNS activity has also been shown to correlate with poorer performances on mentalizing tasks in adults and adolescents with ASD (Baron-Cohen, Wheelwright, Hill, et al., 2001; Holt et al., 2014). Children with ASD and adults with high levels of autistic traits have been shown to display difficulties on VPT-2 tasks (Brunyé et al., 2012; Hamilton, Brindley, & Frith, 2009) which are mentalizing tasks that been shown to elicit MNS activation (Arora, Schurz, & Perner, 2017; Mazzarella, Ramsey, Conson, & Hamilton, 2013; Schurz et al., 2015). The degree of MNS activation during VPT-2 tasks has also been shown to be positively correlated with task performance (Mazzarella et al., 2013). These data provide evidence to suggest that reduced MNS during mentalizing tasks may underlie mentalizing difficulties experienced by adults with ASD.

### 1.9 Mentalizing system activity in ASD

An alternative hypothesis for the neural basis of mentalizing deficits associated with ASD is reduced mentalizing system activation (Frith, 2001; Hamilton, 2009). Reduced mentalizing system activation has been reported in individuals with ASD during a range of mentalizing tasks (Castelli et al., 2002; Happé et al., 1996; Holt et al., 2014a; Kana et al., 2009, 2014). Adults and adolescents with ASD have shown impaired performances on mentalizing tasks which only elicit mentalizing system activation not MNS activation due to the absence of human action (Castelli et al., 2002; White et al., 2014) and atypical mentalizing system activation has been shown during these tasks (Castelli et al., 2002; White et al., 2014). This has led some researchers to conclude that reduced mentalizing system activation is the neural basis for mentalizing difficulties associated with ASD, regardless of whether mentalizing tasks involve interpreting human action (Frith, 2001; Frith, Morton, & Leslie, 1991; Hamilton, 2009). In support of this theory, reduced mentalizing system activation during mentalizing tasks has been shown to correlate with degree of social impairment (Lombardo, Chakrabarti, Bullmore, & Baron-Cohen, 2011; O'Nions et al., 2014; Wang, Lee, Sigman, & Dapretto, 2006). Autistic adults have been shown to exhibit reduced utilisation of prior expectations when inferring the social intentions of hand actions (Chambon, Farrer, et al., 2017) and increased mPFC activity has been found when reliance on prior expectations is higher when inferring social intentions (Chambon, Domenech, et al., 2017a). These data suggest reduced mPFC activation in adults with ASD when inferring the social intentions of others from their actions. Additionally, reduced grey matter has also been found in areas of the mentalizing system in individuals with ASD (Duerden, Mak-Fan, Taylor, & Roberts, 2012; Greimel et al., 2013; McAlonan et al., 2008) further supporting atypical functioning of this system in ASD.

In dispute of this theory, adults with ASD have shown typical performances on mentalizing tasks during which they have exhibited reduced mentalizing system activity (Kana et al., 2009; Kirkovski et al., 2015). A number of studies that have reported atypical mentalizing system activation during mentalizing tasks have shown higher mentalizing system activation in individuals with ASD rather than reduced activation (Libero et al., 2014; Marsh & Hamilton, 2011; Mason, Williams, Kana, Minshew, & Just, 2008; White et al., 2014). Finally, individuals with ASD have shown more consistent deficits on mentalizing tasks which involve inferring internal states from actions and therefore elicit MNS activity as well as mentalizing system activation rather than tasks that do not involve human action (Kana et al., 2009; Kirkovski et al., 2015; Lombardo et al., 2011; Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Roeyers et al., 2001; Rosenblau et al., 2015).

### 1.10 Connectivity between mirror neuron and mentalizing systems in ASD

Rather than reduced activation in either the MNS or the mentalizing system individually, it is possible that the neural basis of mentalizing deficits associated with ASD is dysfunctional connectivity between the MNS and the mentalizing system. Dysfunctional connectivity between the MNS and the mentalizing system could explain the findings of typical MNS activation during tasks traditionally associated with MNS function but atypical MNS activity reported during mentalizing tasks in adults with ASD (Avikainen et al., 1999; Dinstein et al., 2010;

Enticott, Kennedy, Rinehart, et al., 2013; Kana et al., 2014; Libero et al., 2014; Wicker et al., 2008). Dysfunctional connectivity between the MNS and the mentalizing system could also explain the consistent impairments found for adults with ASD when mentalizing in the presence human action but not in the absence of action (Kana et al., 2009; Kirkovski, Enticott, Hughes, Rossell, & Fitzgerald, 2016; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001; Rosenblau et al., 2015; Spek et al., 2010). Mentalizing in the absence of action information is not thought to require kinematic information to be signalled from the MNS to the mentalizing system (Van Overwalle & Baetens, 2009). A number of studies have provided evidence that mentalizing system activation alone is sufficient in order to infer internal states in the absence of human action (Castelli et al., 2000; Castelli et al., 2002; Gallagher et al., 2000; White et al., 2014). Therefore, if mentalizing system activation is typical but connectivity between the MNS and the mentalizing system is dysfunctional in adults with ASD then this could explain greater impairments on mentalizing tasks involving human action. Similarly, MNS activation without mentalizing system activation may be sufficient in order to infer motor intentions (Catmur, 2015; Jacob & Jeannerod, 2005); motor intentions are directly related to observed actions, meaning that motor simulation in the MNS may be sufficient in order to infer these intentions and connectivity with the mentalizing system may not be required. Social, prior and communicative intentions are more abstract and further removed from observed actions and therefore inferring these more abstract intentions is thought to require integration of action kinematics with contextual information through connectivity between the MNS and the mentalizing system (Liew et al., 2011; Mainieri et al., 2013; Spunt & Leiberman, 2012c; de Lange et al., 2008). Dysfunctional connectivity between these two systems but typical activation in the MNS could therefore explain the typical performances reported for adults with ASD when inferring motor intentions but consistent impairments in inferring more abstract intentions (Aldridge et al., 2000; Broekhof et al., 2015; Carpenter et al., 2001; Kana et al., 2014; Moran et al., 2011; Sivaratnam et al., 2012). Collectively these data suggest that impairments in ASD are more pronounced when reliance on connectivity between the MNS and the mentalizing system is higher.

Only a limited number of studies have investigated functional connectivity between the MNS and the mentalizing system in individuals with ASD when mentalizing (Ciaramidaro et al., 2015; Kana et al., 2014; Libero et al., 2014; Mason et al., 2008; Von Dem Hagen, Stoyanova, Rowe, Baron-Cohen, & Calder, 2014) and even fewer have investigated connectivity in adults exclusively (Mason et al., 2008; Von Dem Hagen et al., 2014). Developmental changes in brain activation and functional connectivity patterns in individuals with ASD (Long, Duan, Mantini, & Chen, 2016; Nomi & Uddin, 2015) mean that data from children with ASD cannot necessarily be generalised to adults. Some studies have investigated functional connectivity in adults with ASD when mentalizing but not included areas of the MNS in the connectivity analyses (Ciaramidaro et al., 2015; Libero et al., 2014; Von Dem Hagen et al., 2014; Wicker et al., 2008). The limited studies that have investigated functional connectivity between the MNS and the mentalizing system have reported atypical connectivity in adults with ASD when inferring prior intentions from passages of text (Mason et al., 2008), in adults and adolescents with ASD when inferring prior intentions from comic strips (Ciaramidaro et al., 2015; Kana et al., 2014) and in children with ASD when viewing emotional facial expressions (Rudie et al., 2012a). Atypical connectivity between the MNS and the mentalizing system has also been reported during rest in adults (Kennedy & Courchesne, 2008), adolescents (Fishman, Keown, Lincoln, Pineda, & Müller, 2014) and mixed samples of adults and adolescents (Assaf et al., 2010; Shih et al., 2010) with ASD. These data support the existence of atypical functional connectivity between these two systems in ASD but the simplistic stimuli used as well as the inclusion of children and adolescents, mean it is unclear whether dysfunctional connectivity between the MNS and the mentalizing system underlies the mentalizing difficulties experienced by adults with ASD. No studies to date have investigated functional connectivity between the MNS and the mentalizing system when inferring others' internal states from their actions which is important in terms of understanding the neural basis of the deficits associated with ASD. The behavioural data show adults with ASD exhibit consistent impairments in inferring internal states from naturalistic videos of others' actions but not when simplistic stimuli, such as the stimuli used in the existing functional connectivity studies, are used (Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Roeyers et al., 2001).

It is possible that connectivity is more dysfunctional between certain areas of the MNS and the mentalizing system in ASD than between other areas. More consistent impairments have been reported for adults with ASD in inferring intentions and emotions (Atkinson, 2009; Kana et al., 2014; Murdaugh et al., 2014; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001) compared to inferring mental states and beliefs (Kana et al., 2009; Kirkovski et al., 2015; Schuwerk et al., 2014; Spek et al., 2010). Neuroimaging studies have shown that areas of brain activation and patterns of functional connectivity differ depending on the aspects of another's internal state being inferred (Ciaramidaro et al., 2014; Pineda & Hecht, 2009; Rudie et al., 2012b; Schurz et al., 2014; Spunt &

Lieberman, 2012a; 2012b). Therefore, connections which are particularly important for inferring intentions and emotions of others may show higher levels of dysfunction. Currently, the literature is too limited to make any firm predictions regarding the specific areas that may display particularly dysfunctional connectivity in adults with ASD. Further characterisation of the patterns of activity and functional connectivity when making different internal state inferences is required.

### 1.11 Mentalizing deficits as a result of more generalised deficit

Theories of ASD exist which attempt to explain ASD characteristics more broadly and could account for the mentalizing deficits associated with ASD. The executive deficit hypothesis of ASD suggests that individuals with ASD exhibit a more generalised dysfunction in executive functioning (such as planning, inhibition, action monitoring and working memory) rather than mentalizing-specific deficits (Hill, 2004; White 2013). A number of studies have provided evidence for executive functioning deficits in individuals with ASD (Chen et al., 2016; Robinson et al., 2009; Zimmerman et al., 2016). Executive functioning is thought to rely on frontal cortical areas which largely overlap with areas associated with the mentalizing system (Ybarra & Winkielman, 2012). Therefore, it is possible that atypical functioning in areas of the mentalizing system and poorer performances on mentalizing tasks could result from a more generalised deficit in executive dysfunction. However, not all individuals with ASD display executive functioning deficits (Demetriou et al., 2017; Pellicano et al., 2006) and individuals with ASD who do not display executive functioning deficits have shown poorer performances on mentalizing tasks (Pellicano, 2007) suggesting mentalizing deficits associated with ASD can exist independently of executive functioning deficits.

The predictive coding theory of ASD implies that characteristics associated with ASD result from a generalised deficit in prediction (van Boxtel & Lu, 2013, van de Cruys et al., 2014). This theory states that individuals with ASD have reduced abilities to predict upcoming events and cannot appropriately update future predictions as a consequence of prediction errors made, resulting in striving for sameness (de Cruys et al., 2014). Empirical evidence has been provided to show adults with ASD display reduced abilities to predict the outcome of others' actions (Zalla, Labruyère, Clément, & Georgieff, 2010) and do not display typical improved visual discrimination of a gesture when shown a complimentary responsive gesture (von der Lune et al., 2016) suggesting reduced ability to predict actions based on contextual information. Repetitive behaviours associated with ASD are hypothesised to reflect a desire for predictable sensory consequences (Chambon et al., 2017). Kilner and colleagues have hypothesised that MNS functioning can be explained within the predictive coding framework (Kilner et al., 2007). It is possible that MNS dysfunction, and perhaps difficulties inferring internal states from actions, may be the result of a wider predictive coding deficit.

### 1.12 Summary

In summary, both the MNS and the mentalizing system are thought to be involved in inferring others' internal states from their actions but the precise roles of these systems and the nature of the interaction between them are unknown. Adults with ASD are widely regarded to exhibit difficulties inferring the internal states of others (known as 'mentalizing'). However, despite the strong association between ASD and mentalizing difficulties, the existing literature is inconsistent regarding the nature of the mentalizing difficulties experienced by adults with ASD. Differences across studies in the explicitness of the instructions, stimuli used, particular internal state inferences being made, as well as the methods used to measure mentalizing abilities, are all likely to have contributed to inconsistencies in the literature. Only a very limited number of neuroimaging studies have investigated patterns of brain activation and functional connectivity in adults with ASD when inferring the internal states of others from their actions. Therefore, it is uncertain as to the exact nature of mentalizing deficits experienced by adults with ASD and the neural basis of these difficulties.

### 1.13 Aims of the current thesis

The aims of this thesis were to examine the nature of mentalizing difficulties associated with ASD, delineate between existing models of MNS involvement in mentalizing, and identify the neural basis of mentalizing difficulties associated with ASD. The review of the current literature highlights that adults with ASD appear to show consistent implicit mentalizing deficits but inconsistent explicit mentalizing deficits. However, this comparison is confounded by differences in the methods used to measure mentalizing abilities, as well as the stimuli and particular internal states being inferred across studies. Chapter 2 aimed to address these issues by examining the implicit and explicit mentalizing abilities of adults with ASD using the same video stimuli. Abilities to both implicitly and explicitly infer the social intentions of others' from their actions were assessed using measurable behavioural outcomes. Eye-tracking data was also collected to explore the relationship between possible atypical fixation patterns and behavioural performance.

We then sort to investigate the neural basis of the mentalizing difficulties associated with ASD through brain stimulation and neuroimaging experiments. Firstly, the TMS experiment presented in Chapter 3 was designed in attempt to delineate between existing models of MNS involvement in mentalizing; the motor simulation theory, the mirroring-first hypothesis, the dual-process hypothesis and the predictive coding theory. These models differ in the anticipated timing of MNS activation during mentalizing tasks as well as the predicted relationship between MNS activity and behavioural performance. Therefore, the TMS experiment in Chapter 3 investigated the timing of TMS activation as well as the relationship between MNS activation and performance in typically developing individuals whilst inferring the social intentions of others from their actions. The high temporal resolution of TMS and the well-established TMS index of MNS activity meant TMS was the ideal technique to examine the timing of MNS involvement in mentalizing.

The timing of MNS involvement identified in Chapter 3 was then used to investigate mentalizing-specific MNS activity in adults with ASD. The broken mirror hypothesis proposes that MNS activity is reduced in individuals with ASD and this underlies some of the social deficits they experience. Evidence to support reduced MNS activation as the neural basis for mentalizing difficulties in adults with ASD is limited but only a small number of studies have investigated MNS activity during mentalizing tasks in exclusively adult samples and those that have, have used simplistic mentalizing tasks on which adults with ASD do not show consistent impairments. The study presented in Chapter 4 aimed to investigate whether reduced MNS activity may underlie the mentalizing difficulties associated with ASD by examining levels of MNS activity during a mentalizing task which used complex action stimuli. Both TMS and electroencephalography (EEG) techniques were used to measure MNS activity.

The final empirical chapter, Chapter 5, presents an fMRI experiment which aimed to identify whether reduced functional connectivity between the MNS and the mentalizing system in adults with ASD may underlie the mentalizing difficulties they experience. The existing neuroimaging literature suggest that connectivity between these two systems is important for inferring internal states of others from their actions. Behavioural data from adults with ASD imply that mentalizing deficits experienced by these individuals are more pronounced on tasks which are considered to involve increased reliance on connectivity between the MNS and the mentalizing system. However, no study to date has investigated connectivity between the MNS and the mentalizing system in adults with ASD when inferring internal states of others from their actions.

Chapter 6 outlines the key findings from all the experiments presented in this thesis and discusses how the results contribute to our knowledge of MNS involvement in mentalizing, the nature of connectivity between the MNS and the mentalizing system, the mentalizing deficits experienced by adults with ASD and the neural basis of these deficits.

Chapters 2, 3, 4 and 5 correspond to individual papers. The research presented in Chapters 2, 3, and 4 have been submitted for publication, Chapter 5 corresponds to a paper currently in preparation. Because the chapters are based upon research papers, there is a degree of overlap in the content of the introductions in these individual chapters and presented here in the introduction. I apologise for the repetitive nature of the introductions and I hope each introduction helps frame the relevant literature to the specific nature of the questions that are addressed in each chapter.

# Chapter 2: Abilities to Explicitly and Implicitly Infer Intentions from Actions in Adults with Autism Spectrum Disorder

This chapter is adapted from: Cole, E.J., Slocombe K.E., Barraclough N. E. (accepted). Abilities to explicitly and implicitly infer intentions from actions in adults with autism spectrum disorder. *Journal of Autism and Developmental Disorders*.[1]

## 2.1 Abstract

Previous research suggests that Autism Spectrum Disorder (ASD) might be associated with impairments on implicit but not explicit mentalizing tasks. However, such comparisons are made difficult by the heterogeneity of stimuli and the techniques used to measure mentalizing capabilities. We tested the abilities of 34 individuals (17 with ASD) to derive intentions from others' actions during both explicit and implicit tasks and tracked their eye-movements. Adults with ASD displayed explicit but not implicit mentalizing deficits. Adults with ASD displayed typical fixation patterns during both implicit and explicit tasks. These results

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[1] The author, Eleanor Cole, designed the experiment, collected the data, analysed the results and wrote the manuscript under the supervision of Dr Nick Barraclough. Katie Slocombe provided assistance with the experimental design and some of the statistical analysis.

illustrate an explicit mentalizing deficit in adults with ASD, which cannot be attributed to differences in fixation patterns.

## 2.2 Introduction

Autism Spectrum Disorder (ASD) is the term used in the most recent edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) to describe a range of neurodevelopmental disorders, classified according to core deficits in social communication and interaction as well as restricted and repetitive interests (American Psychiatric Association, 2013). One of the social communication difficulties associated with ASD is difficulty inferring the internal states of others including their intentions, mental states and beliefs (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Chung, Barch, & Strube, 2014; Frith, 2001; Holt et al., 2014; Jolliffe & Baron-Cohen, 1999), collectively referred to as mentalizing deficits. Mentalizing deficits are so strongly associated with ASD that mentalizing abilities are even assessed in diagnostic and screening tools such as the Autism Diagnostic Observation Schedule (ADOS-2) and the Autism Quotient (AQ) scale (S Baron-Cohen, Wheelwright, Skinner, et al., 2001; Lord et al., 2000). Due to the spectral nature of ASD, individuals without a diagnosis also display varying degrees of autistic traits. Individuals with relatively high but not clinically significant levels of autistic traits have been shown to display subtler versions of the behavioural and neurological characteristics associated with ASD (Best, Arora, Porter, & Doherty, 2015; Di Martino et al., 2009; Lindell, Notice, & Withers, 2009; Ridley, Homewood, & Walters, 2011; van Boxtel & Lu, 2013) including mentalizing deficits (S Baron-Cohen, Wheelwright, Skinner, et al., 2001; Chung et al., 2014b; F. G. Happé, 1994; Kana et al., 2014; Moran et al., 2011)

Despite the strong association between ASD and mentalizing deficits, experimental evidence regarding the nature of these deficits is inconsistent, with some studies finding that adults with ASD are impaired at inferring intentions, emotions and mental states of others (Baron-Cohen, Wheelwright, Skinner, et al., 2001; Castelli, Frith, Happé, & Frith, 2002; Happé, 1994; Kana et al., 2014; Moran et al., 2011; Senju, Southgate, White, & Frith, 2009) and others reporting adults with ASD (Kana et al., 2009; Kirkovski et al., 2015; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001; Spek et al., 2010) and high levels of autistic traits (Nijhof et al., 2016) show typical performances on mentalizing tasks. A number of factors may have contributed to these inconsistent findings, including whether task instructions explicitly stated that participants should mentalize, the stimuli used, the type of mentalizing assessed and the method used to measure mentalizing abilities.

The majority of previous studies have explicitly asked participants to make inferences about the internal states of others (e.g. Baron-Cohen, Wheelwright, Hill, et al., 2001; Gallagher et al., 2000; Happé, 1994; Holt et al., 2014; Jolliffe & Baron-Cohen, 1999; Kana, Keller, Cherkassky, Minshew, & Just, 2009; Kana et al., 2014; McAleer, Kay, Pollick, & Rutherford, 2011; Roeyers et al., 2001). Only a small number of studies have examined the capabilities of adults with ASD to infer the internal states of others when not specifically told to do so; this is known as ‘implicit mentalizing’. The existing adult literature shows consistent implicit mentalizing deficits associated with ASD (Castelli et al., 2002; Rosenblau et al., 2015; Schuwerk et al., 2014; Senju et al., 2009) but the explicit mentalizing data are inconsistent (Baron-Cohen et al., 1997; Castelli et al., 2002; Kana et al., 2009; Kirkovski et al., 2015; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004). It may be that the instructions given concerning which elements should be attended to during explicit tasks, allow some high functioning adults with ASD to perform at a typical level, which they would be unable to do without the explicit instructions.

The apparent existence of a consistent implicit mentalizing deficit but lack of a consistent explicit mentalizing deficit in adults with ASD in the existing literature may, however, be attributable to other confounding factors, including stimuli differences. Most studies that have reported implicit mentalizing deficits in adults with ASD have used movie stimuli (e.g. Rosenbalu et al., 2015; Schuwerk et al., 2014; Senju et al., 2009), which were more complex and naturalistic than stimuli used in the majority of explicit tasks. The stimuli used in the majority of explicit mentalizing tasks were passages of text, still images or cartoon strips which provide very simplistic representations of social interactions and a number of these studies found no mentalizing deficits in adults with ASD (e.g. Kana et al., 2009; Kirkovski et al., 2015; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers, Buysse, Ponnet, & Pichal, 2001; Spek, Scholte, & Van Berckelaer-Onnes, 2010). In support of this argument, two previous studies (Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Roeyers et al., 2001) investigated the abilities of adults with Pervasive Development Disorders (PDD; including ASD) to explicitly infer the mental states of others using both simple stimuli (images of people's eyes and short passages of text) and naturalistic videos of social interactions. The adults with PDD were not impaired on the explicit mentalizing tasks that used the simple stimuli but did show impairments with the more complex naturalistic stimuli (Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Roeyers et al., 2001). Additionally, the only previous study that has investigated both implicit and explicit mentalizing abilities using complex, naturalistic stimuli found that adults with ASD displayed equivalent impairments on both implicit and explicit tasks (Rosenblau et al., 2015).

Differences in the way mentalizing performances have been measured may have also contributed to existence of consistent implicit mentalizing deficits but inconsistent data regarding explicit mentalizing abilities in the previous adult literature. Some studies have measured implicit mentalizing abilities using eye-tracking data alone (Schuwerk et al., 2014; Senju et al., 2009). In these studies, participants watched animations in which a character wrongly believed an object was in a certain location. Adults with ASD spent shorter periods fixating on the place in which the character wrongly believed the object was located. This was interpreted as impaired implicit mentalizing. However, a number of studies have reported that adults with ASD have unusual patterns of eye gaze when processing social stimuli (Kliemann et al., 2010; Pelphrey et al., 2002) and unusual fixation patterns have been found during face processing tasks in the absence of behavioural differences (Rutherford & Towns, 2008; Spezio, Adolphs, Hurley, & Piven, 2007). Therefore, adults with ASD may be able to deduce the internal states of others despite atypical eye movements. In contrast, explicit mentalizing studies have always used measurable behavioural outcomes to assess mentalizing abilities.

The term ‘mentalizing’ covers a variety of internal state inferences which may involve different processes (Call & Tomasello, 2008; Pineda & Hecht, 2009); it is possible that the different internal state inferences required across studies may have also contributed to the heterogeneity in the literature. Previous studies have reliably found that adults with ASD are impaired at inferring others’ intentions (Kana et al., 2014; Murdaugh, Nadendla, & Kana, 2014; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001) and others’ emotions

(Atkinson, 2009; Cassidy et al., 2013; Peter G Enticott, Kennedy, Johnston, et al., 2013; Hubert et al., 2007; Nackaerts et al., 2012). However, the existing literature is more inconsistent regarding abilities to infer others' mental states (Baron-Cohen et al., 1997; Kana et al., 2009; Kirkovski et al., 2015; Kleinman, Marciano, & Ault, 2001; Roeyers et al., 2001; Spek et al., 2010) or false beliefs (Frith & Happé, 1994; Schuwerk et al., 2014; Senju et al., 2009). The neuroimaging and developmental literature also support the argument that the different subcomponents of mentalizing reflect different processes; the results of a meta-analysis suggest that children develop the ability to infer others' desires before they are able to infer others' beliefs and can detect others' emotions before they can deduce false beliefs (Wellman & Liu, 2004). Additionally, neuroimaging studies have shown that different brain areas are active during mentalizing tasks depending on the inferences being made (Pineda & Hecht, 2009; Schurz et al., 2014). Collectively, these data suggest that the subcomponents of mentalizing are distinct processes associated with different brain areas and developmental trajectories.

In summary, although ASD is associated with mentalizing deficits, the nature of these deficits is unclear. The existing literature suggests that adults with ASD are more likely to show impaired performances on implicit mentalizing tasks using complex naturalistic stimuli that probe understanding of intentions or emotions. To our knowledge, only one study to date has assessed both implicit and explicit mentalizing abilities in adults with ASD using measurable behavioural outcomes (Rosenblau et al., 2015). In this study, a comparison between adults with and without ASD found that participants with ASD showed reduced abilities to both implicitly and explicitly infer the mental states of actors from short movies but there was no difference in the degree of impairment between tasks. However, this study did not use a non-mentalizing control task so it is unclear whether the poorer performances observed in adults with ASD were specifically due to mentalizing deficits or whether poorer performances reflect reduced abilities to perform the experimental tasks in general. Thus the current study aimed to test the abilities of adults with ASD to both implicitly and explicitly mentalize, using complex stimuli, measurable behavioural outcomes and a non-mentalizing control task.

This study specifically investigated the abilities of adults with ASD to both implicitly and explicitly infer the intentions of others from the kinematics of their hand actions using the same naturalistic stimuli. Previous studies have shown that hand actions with different intentions display subtle differences in action kinematics and adults without ASD are able to infer others' intentions from these differences in action kinematics (Ansuini et al., 2015; Sartori, Becchio, Bara, & Castiello, 2009). In the first experiment, participants watched videos of actors playing a poker chip game and had to decide which actor, from a choice of two, they would prefer to play the poker chip game with. Participants were shown one video depicting an actor deliberately not passing a poker chip to another player ('spiteful' action) and a video of another actor accidentally not passing a poker chip to another player ('clumsy' action). In this task, participants were not explicitly asked to infer actors' intentions; rather participants' choice of actor was dependent upon 'covert' mentalizing (implicit mentalizing task). In contrast, during the second experiment, participants watched the same movies and were explicitly asked to infer the intentions of the actors. In addition to contrasting the performance of the ASD and typically developing groups, due to the spectral nature of ASD, we then examined the relationship between the level of autistic traits displayed and abilities to infer others' intentions across all participants. We also tracked participants' eye movements during both experiments in order to test whether any potential behavioural differences associated with autistic traits could be explained by atypical fixation patterns (cf. Schuwerk et al., 2014; Senju et al. 2009). It was predicted that adults with ASD would display reduced abilities to infer the intentions of others compared to matched control participants and across all participants higher levels of autistic traits would predict poorer performances. We also hypothesised that mentalizing deficits associated with ASD would be more evident in the implicit task compared to the explicit task.

## 2.3 Methods

### *2.3.1 Participants*

Twenty-one adults with Autism Spectrum Disorder (ASD; 14 male) were recruited for this study. The majority of the ASD sample were students from the University of York (n=13) and the remaining ASD participants were recruited from a local support group. Four participants were excluded for having scores that were not significantly higher than chance on the control task (see below). This resulted in a final participant sample of 17 adults with ASD (10 male ages 18-56, mean age=23.71, SD=9.24) and 17 individually age, sex and IQ matched control participants (TD – Typically Developing; ages 18-55, mean age=23.71, SD=9.07). See Table 2.1 for participant demographics.

All participants in the ASD group had a clinical diagnosis of Asperger's (n=14) or Autism Spectrum Disorder. All diagnoses were issued by qualified clinicians external to this study. None of the ASD participants had a history of delayed language development or existing learning difficulties. All participants had IQ scores above 100. All neurotypical participants reported that they had no neurological disorders and adults diagnosed with ASD reported no other neurological conditions.

Experiments were approved by the ethics committee of the Department of Psychology, University of York, and were performed in accordance with the ethical standards laid down in the 1990 Declaration of Helsinki.

Table 2.1

*Participant demographic information; group mean (SD) values*

| | ASD | TD | p |
|---|---|---|---|
| AGE | 23.71 (9.24) | 23.71 (9.07) | 0.97 |
| GENDER (male:female) | 10:7 | 10:7 | 1.00 ($X^2$) |
| IQ (WASI)[1] | 120.12 (9.32) | 120.00 (10.09) | 0.93 |
| WASI verbal score[2] | 62.88 (6.66) | 61.61 (7.52) | 0.86 |

[1] The IQ scores were obtained using the two-subtest version of Wechsler Abbreviated Scale of Intelligence (WASI).

[2] The verbal WASI scores given are standardised scores (T-scores).

p values were derived from a one-way MANOVA unless otherwise stated

### *2.3.2 Psychological tests*

The Autism Diagnostic Observation Schedule (ADOS-2; Lord et al., 2000), Social Responsiveness Scale (SRS; Constantino et al., 2003), The Awareness of Social Inference Test (TASIT; McDonald et al., 2006), Autism Quotient (AQ; Baron-Cohen et al. 2001) and Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) were administered to all participants. The ADOS-2 assessments were filmed and then scored by both the experimenter and an independent rater who was blind as to whether participants had a diagnosis or not. Both the experimenter and independent rater were trained to the level of research reliability on the ADOS-2 assessment. If the ADOS-2 scores differed between the experimenter and independent rater, the assessment movies were re-watched and a final score was agreed on. The independent ADOS-2 scores never differed by more than 2 points between the raters. The SRS and TASIT are designed to detect social impairment. The SRS is a self-report measure and TASIT measures abilities to detect sarcasm and lies from movies showing social interactions. The AQ is a self-report measure of autistic traits. The two subtest version of the WASI was used to measure the IQ of participants. All these psychological tests have been shown to have good psychometric properties (Allison, Baron-Cohen, Wheelwright, Stone, & Muncer, 2011; Constantino et al., 2003b; Hurst, Mitchell, Kimbrel, Kwapil, & Nelson-Gray, 2007; Skye McDonald et al., 2006; Oosterling et al., 2010).

### *2.3.3 Stimuli*

The movie stimuli were designed to show different actors playing a poker chip exchange game. The poker chip game involved passing poker chips to another player through slots in a white wooden board (see Figure 2.1). Ten different types of hand actions were filmed (Panasonic TM900 HD-DV camera; 1920 x 1080 pixels at 50Hz progressive scan). Five of the hand actions involved pushing poker chips with the index finger of the right hand through a slot in the board which was level with the surface of the table. The other five hand actions involved grasping poker chips with the index finger and thumb of the right hand and passing them through a slot in the board at head height. Two different types of actions were used to generalise results across different action types. Both pushing and grasping actions were executed by the actor in 5 different ways: 1. clumsily failing to pass one poker chip - here the actor had a positive intention to pass the chip to the other player, but the outcome of the action was unsuccessful (clumsy 1); 2. Clumsily failing to pass five pokers chips; positive intention to pass the chips, but the outcome of the action was unsuccessful (clumsy 5); 3. Spitefully (deliberately) failing to pass one poker chip; no intention to pass the chip to the other player and the outcome of the action was unsuccessful (spiteful 1); 4. Successfully passing one poker chip; the actor intended to pass the poker chip and the action was successful (successful 1); 5. Successfully passing five poker chips; the actor intended to pass the poker chips and the action was successful (successful 5).

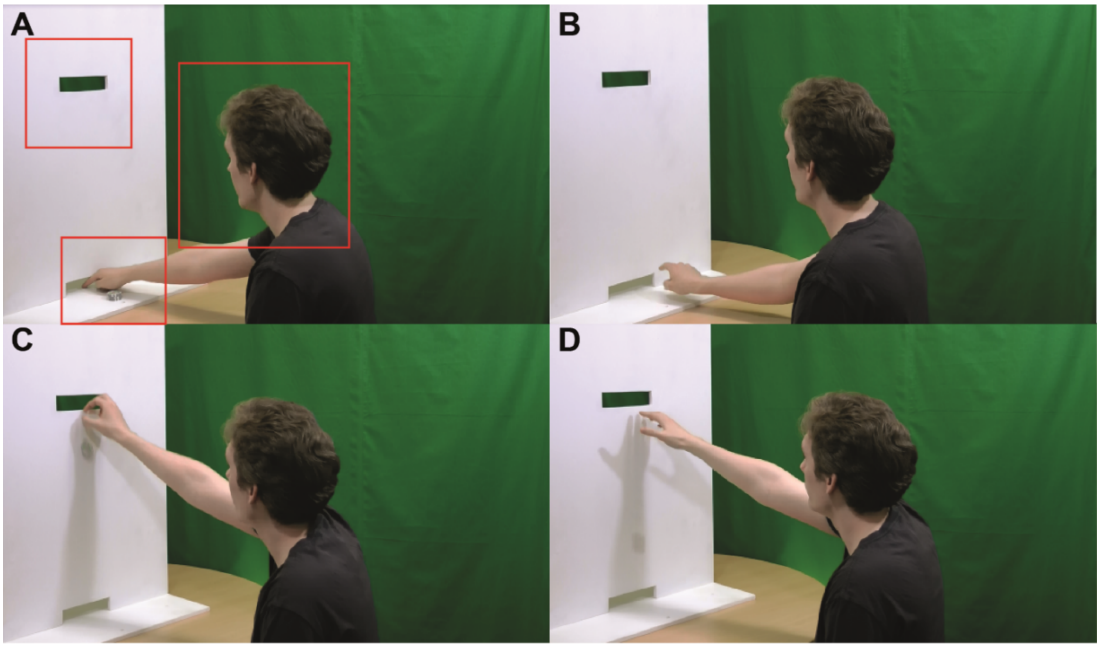

**Figure 2.1** Example screenshots from the hand action movies depicting positive and negative intentions. A. The actor pushes five poker chips with a positive intention (clumsy 5 pushing action). B. The actor deliberately pushes a poker chip away from the slot (spiteful 1 pushing action). C. The actor accidentally drops a poker chip (clumsy 1 grasping action). D. The actor deliberately drops the poker chip (spiteful 1 grasping action). The squares overlaid onto action A illustrate the regions of interest (ROIs) used for the eye-tracking analyses.

Twenty-eight different actors (14 female) were filmed performing all ten actions, from a three-quarters view from behind at an angle that allowed their right hand to be seen in front of them for the entire duration of the hand action but only showed a limited side profile of their face. This prevented participants from using facial information to infer the intentions of actors and required intentions to be inferred from the action kinematics alone (cf. Sartori et al 2009; Ansuini et al. 2015). This was done in order to investigate whether adults with ASD are impaired at inferring others' intentions irrespective of reduced fixation on the eyes, which has been well reported (Bird, Press, & Richardson, 2011; Kliemann et al., 2010; Papagiannopoulou, Chitty, Hermens, Hickie, & Lagopoulos, 2014; Tottenham et al., 2014).

The actors sat in front of a white wooden board measuring 84 x 61cm with two slots (4 x 17cm) cut out of it (see Figure 2.1). Actions started with the actor's right hand resting on a small marker for 3 seconds. In order to ensure all hand actions lasted approximately two seconds, a buzzer indicated to the actors when to move their hand towards the poker chips and signalled again to indicate when the actors should let go of the poker chips. Actors performed each of the 10 different actions at least 3 times; for each actor the action with the best timing and that best depicted the particular intention was selected for the final movie. Movies were edited (Sony Vegas Pro 10) to finish 0.4 seconds after the poker chips left the actors' hands; for grasping actions, this was always before the poker chips hit the table. In addition, the starts of all movies were trimmed such that they lasted exactly 4 seconds. Editing the movies in this way meant that movement onset occurred at slightly different times in each movie (frames 32 - 146).

The chosen movies were rated by 30 independent observers who were students at the University of York. Observers classified each action as either 'clumsy', 'spiteful' or 'neither' by pressing one of three keys on the computer keyboard. Clumsy responses were coded as -1, spiteful responses were coded as 1 and neither responses were coded as 0. For each action, scores were averaged across participants to generate an index of the degree of 'spitefulness' conveyed by each movie where -1 indicates a strong evaluation of the action as clumsy, +1 indicates a strong evaluation of the action as spiteful, and 0 indicates an evaluation of the action as neither clumsy or spiteful. Spiteful videos were required to have spitefulness indexes higher than .4 and clumsy videos were required to have indexes below -.4 to be included in the stimuli set. Three clumsy movies had spitefulness indexes that were higher than -.4 and therefore were deemed to not clearly portray the desired intention (.16, .03 and -.03 spitefulness indexes). These movies were replaced with new stimuli which were rated by another 30 independent observers and these stimuli all obtained ratings lower than -.4. The final stimuli used fell into three significantly ($F(2,165)=1644.94$, $p<.001$, $\eta_p^2=.95$) distinct groups; clumsy ($M=-.68$, $SD=.15$), spiteful ($M=.80$, $SD=.13$) and successful ($M=.01$, $SD=.03$) actions.;

### *2.3.4 Experiment 1 (Implicit mentalizing): Design and procedure*

Experiment 1 tested the participants' abilities to implicitly infer the intentions of others from their hand actions. The task was adapted from one previously used with children (Behne & Carpenter, 2005) and chimpanzees (Call, Hare, Carpenter, & Tomasello, 2004). In these studies, experimenters either deliberately or 'accidentally' did not give the chimpanzees or children rewards (in the form of food or a toy respectively). Both the chimpanzees and the children attempted to interact with the experimenters for longer when experimenters accidentally dropped the reward rather than when they deliberately did not give the reward. This implied the experimenters' intentions had been implicitly inferred and consequently the appropriate social decisions were made.

In our experiment, each participant took part in a poker chip exchange game with the experimenter prior to the main experiment in order to familiarise them with the actions shown during the experiment, and to demonstrate the value of receiving poker chips from a partner. Participants were told that the experimenter would start with 8 poker chips that were each worth one pound. However, in order for the experimenter to receive money for their poker chips at the end of the game, they had to give at least one poker chip to the participant. If the experimenter had all the poker chips on their side of the board at the end of the game, neither the experimenter nor the participant would receive any money. The experimenter then had three chances to make a deal with the participant; they would pass some poker chips through the slots in the wooden board to the participant on the other side. The participant had to accept or reject the number of poker chips that were offered each turn. If the participant accepted then they would receive a pound coin for every chip on their side, if they rejected the number of poker chips offered, then the experimenter would have to offer a different number of chips. If no agreement was reached after three rounds then neither the participant nor the experimenter received any money. The aim of the game for the participant was to end up with as many chips as possible on their side of the board. Every participant played the poker chip game four times to gain a good understanding of the purpose of passing the chips and the value of the chips (three times as the participant and once in the experimenter role). Over the three games in the participant role, each participant experienced (i) a round in which the experimenter acted spitefully (experimenter offered no chips to the participant and explained they were doing so in order to reduce the number of chances to make a deal and increase the chances of the participant accepting a lower offer); and (ii) a round in which the experimenter acted clumsily (experimenter accidentally dropped the poker chips and thus failed to make an offer) so that all participants had practical experience of both clumsy and spiteful actions. Participants also played one game in which they switched roles with the experimenter to ensure they understood the game fully.

A PC running MATLAB R2015a controlled the experiment and recorded participant responses. Participants sat approximately 60cm from an Acer GD245HQ 24” HD monitor on which all stimuli were presented. Participants’ eye movements were recorded during the experiment using an EyeTribe eye tracker (The EyeTribe Abs, Copenhagen). Participants rested their heads in a chin rest and fixation data from both eyes was recorded at 30Hz. A 9-point calibration procedure was carried out before conducting each experiment. Participants for which the eye-tracker could not reach a satisfactory level of accuracy on the calibration (3/5 star rating; indicating <1° accuracy) were excluded from subsequent eye-tracking analysis. Six participants (four from the ASD group, two from the TD group) were removed from the implicit experiment eye-tracking analysis. Eye tracker data recording was controlled using the EyeTribe MATLAB toolbox (Dalmaijer; available on GitHub: https://github.com/esdalmaijer/EyeTribe-Toolbox-for-Matlab).

Participants were told that they would watch movies of individuals playing the poker chip game they had just played themselves. Each movie would show a player’s first attempt to offer poker chips to someone on the other side of the board. The participants watched pairs of movies and had to decide subsequently whether they would rather continue playing the poker chip game with the actor in the first or the second movie. Each trial consisted of two actions of the same type (either both grasping or both pushing) presented sequentially with an inter-stimulus interval (ISI) of 1000ms, during which the screen was black except for a white fixation cross. Following the second movie a response screen was displayed and participants had to indicate whether they would rather interact with the actor in the first or second movie by pressing either 1 or 2 on the keyboard (see Figure 2.2).

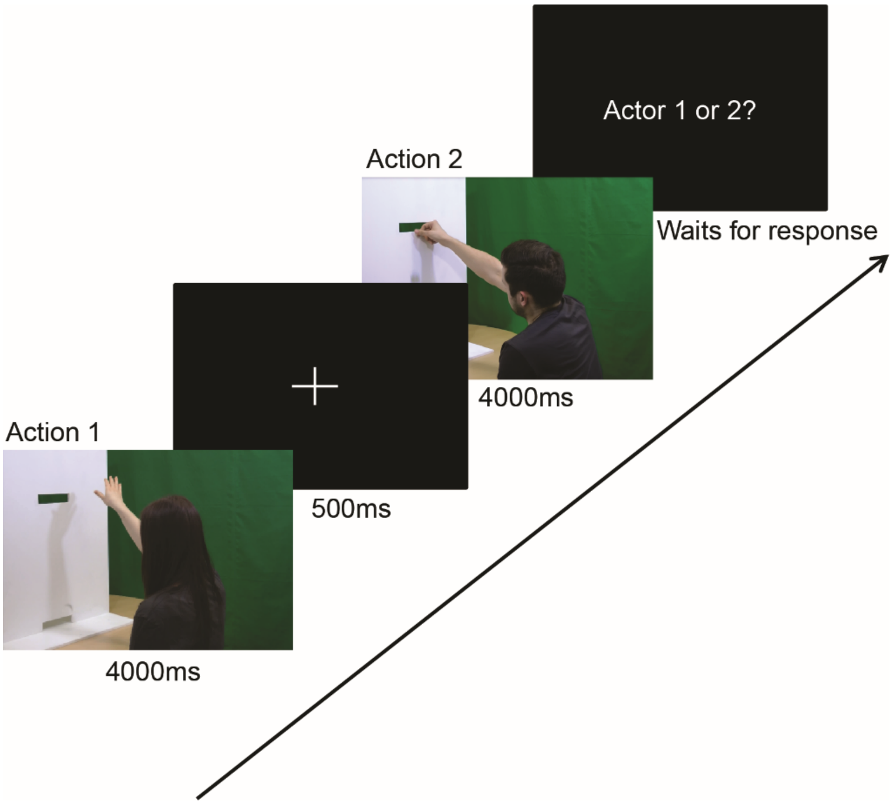

**Figure 2.2** Sequence of stages during a Mentalizing trial in the implicit task. Action 1 shows a female actor deliberately dropping a poker chip (spiteful 1) and action 2 shows a male actor accidentally dropping a poker chip (clumsy 1). In order to decide whether to interact with actor 1 or actor 2 the participant must infer the intentions of the actors from the kinematics of their actions because the outcomes of the two actions are identical.

Different forms of decision making were required to make a choice between the first and second actors in three different conditions; we refer to them as ‘Mentalizing’, ‘Action’ or ‘Either’ conditions. 1. **Mentalizing condition**: correct decisions could be based upon inferences of intention only and consisted of movies of an action with a positive intention (clumsy 1) and an action with a negative intention (spiteful 1). Here, in order to decide between the actors, participants needed to infer the intentions of the actors from the kinematics of their actions. The outcomes of the actions were the same (both actors failed to pass a poker chip to another player) but the intentions of the actors were different. 2. **Action condition**: correct decisions could be based upon action recognition only, consisted of movies of actors successfully passing poker chips (successful 1 and successful 5). Here, in order to decide between the actors, participants needed to recognise whether the actor was passing 1 or 5 poker chips, and did not require participants to mentalize in order to complete the task. 3. **Either condition**: decisions were based upon recognition of the action, or possibly inferences of intention, and consisted of movies of actors attempting to pass poke chips (clumsy 1 and clumsy 5). Here, in order to decide between the actors, participants were expected to focus on the number of chips being offered and choose the actor trying to pass the higher number of poker chips, but participants may have automatically processed the actors' intentions and recognized that both actors have the same positive intention. This condition was included in order to test for the differences in success of the actions between the Mentalizing and Action conditions, given that Mentalizing trials always showed unsuccessful actions and Action trials always showed successful actions; Either trials always showed unsuccessful actions but did not require mentalizing.

At the start of testing, participants completed six practice trials (two of each condition) in order to familiarise them with the experimental procedure. The stimuli used in the practice trials were not included in the main experiments and the actors compared against each other in the implicit practice trials were not compared against each other in the main implicit experiment.

Participants completed 72 trials in total, viewing 144 actions (12 repeats of each action except clumsy 1 which was shown 24 times); trials lasted approximately 12 seconds depending on response times, and testing took approximately 15 minutes. The same actor never performed the same action (e.g. spiteful1 pushing action) twice, such that participants did not learn to associate certain behaviours with specific individuals. Every actor was seen the same number of times and each actor performed a preferable action 50% of the time; actor gender was also counterbalanced. Condition order was randomised and action order was counterbalanced so that the preferred action would occur first in 50% of the trials, e.g. on 50% of the Mentalizing trials the clumsy movies were shown before spiteful movies.

### *2.3.5 Experiment 2 (Explicit mentalizing): Design and procedure*

In the second experiment, participants were asked explicitly to report the intentions of actors presented in movies. Participants returned approximately 3 months (average 112 days) after they completed Experiment 1 to complete Experiment 2. This helped minimalize the possibility of participants' previous implicit judgements influencing their explicit judgements of the actions. Two of the ASD participants were unable to return to complete the explicit experiment, leaving a sample of thirty participants (15 matched pairs) in the explicit experiment.

As with experiment 1, participants first completed six practice trials (two of each stimulus type), in order to familiarise them with the experimental procedure. Participants then viewed all 144 of the movies seen in the Experiment 1. After each movie, participants had to indicate whether they thought the movie showed a ‘spiteful’ (deliberate), clumsy (accidental) or successful action by pressing 1, 2 or 3 respectively on the computer keyboard. The experiment consisted of two blocks of 10 minutes (72 movies shown in each). Each block contained 36 clumsy actions, 12 spiteful actions and 24 successful actions, the order of movies was randomised within each block and no movies were repeated. A response screen was shown after each movie until the participant responded. The PC, display and eye-tracker were all identical to Experiment 1. Two participants from the ASD group were removed from the explicit experiment eye-tracking analysis for not reaching a satisfactory level of accuracy on the calibration (3/5 star rating; indicating <1° accuracy).

### *2.3.6 Behavioural performance analysis*

For Experiment 1, the numbers of correct responses each participant gave in each condition (Mentalizing, Either, Action) were calculated. All 34 participants included in the analyses had scores significantly higher than chance in the Action condition (Binomial test (0.5), $p<.05$, scores >17/24), indicating that all individuals understood the task. We then subtracted the number of correct responses on the Action condition from the number of correct responses on both the Mentalizing and Either condition for each participant. This allowed us to identify any task specific deficits rather than generalised poorer performances on experimental tasks.

For Experiment 2, we calculated the proportion of correct responses for the mentalizing conditions (clumsy and spiteful actions) and non-mentalizing condition (successful actions) for each participant. Similar to Experiment 1, differences between mentalizing and non-mentalizing conditions were calculated to provide a specific measure of the ability of participants to explicitly infer the intentions of others, whilst controlling for ability to do a simple action discrimination task.

Task-specific scores were not normally distributed even after log transformations had been applied. Therefore, non-parametric analyses (Mann-Whitney U tests) were used to investigate group differences in mentalizing abilities. Further, due to the spectral nature of ASD, linear regressions were used to examine the influence of autistic traits (continuous independent variable) on task-specific performances (continuous dependent variables). These linear regressions were conducted in order to identify whether any significant group differences that were found also showed a significant relationship with the continuum of autistic traits across all participants. In order to obtain a single score for each participant that reflected the level of autistic traits that they displayed, we performed a principal components analysis (PCA) on all the psychological test scores (ADOS-2, AQ, SRS and TASIT). The only factor with an eigenvalue higher than Kaiser's criteria of 1 was extracted and used as a measure of autistic traits. Data analysis was carried out using R i386 3.2.3 (The R Foundation for Statistical Computing, Vienna, Austria, http:// www.r-project.org).

### *2.3.7 Eye-tracking analysis*

Eye tracking data was analysed using the EyeMMV MATLAB toolbox (Krassanakis, Filippakopoulou, & Nakos, 2014). Data from the implicit and explicit experiments were analysed in the same way. First, heatmaps were created using the data from all participants in order to identify regions of interest (ROIs); these were: the head of the actor, the initial start position of the hand with the poker chips, and the grasp release point. Three rectangular ROIs were drawn for each movie outlining these areas of interest. Due to the similarity in the spatial extent of the actions on the screen it was then possible to combine the co-ordinates of the ROIs from all 144 movies to make a single set of ROIs that encompassed the ROIs from all movies (see

Figure 2.1A). We then calculated the number and duration of fixations within each ROI during each condition for each participant. We defined the minimum duration for fixation detection as 150ms.

The duration of fixations in each ROI as a percentage of the total number of fixations were calculated for each participant in each condition. As for the behavioural data, for Experiment 1 the duration each participant fixated in each ROI during the Action condition was subtracted from the time spent fixating in each ROI during the Mentalizing and Either conditions. For Experiment 2, the durations of fixation in each ROI during the non-mentalizing condition were subtracted from the durations of the fixation in each ROI during the Mentalizing condition. For Experiment 1, group differences in fixation patterns were tested using separate mixed-model ANOVAs for each ROI (with condition [Mentalizing-Action, Either-Action] as the within subjects variable and diagnosis (ASD/TD) as the between subjects variable). For Experiment 2, the eye-tracking data were found to violate the assumption of normality even after a log transformation had been applied so non-parametric Mann-Whitney U tests were conducted to examine potential group differences in mentalizing-specific fixation patterns. For both experiments, linear regressions were used to examine the influence of autistic traits on changes in the duration of fixations in each ROI across conditions. The data from different ROIs were treated separately because the data were not independent (participants could only fixate in one ROI at a time).

## 2.4 Results

### *2.4.1 Psychological Tests*

All psychological assessment scores were highly correlated with each other except for IQ which did not correlate with the scores on any other psychological tests

(Bivariate Pearson correlations; see Table 2.2). Three female participants with an ASD diagnosis obtained ADOS scores below the clinical cut-off. However, all of these participants had AQ scores above the clinical cut-off as well as SRS scores that indicated either moderate or severe social impairments (see Table 2.3 for group means scores on all psychological assessments).

Table 2.2

*Correlations between psychological test scores*

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1. ADOS | | | | |
| 2. AQ | .74*** | | | |
| 3. SRS | .77*** | .90*** | | |
| 4. TASIT | .54*** | .73*** | .76*** | |
| 5. IQ | .04 | .17 | .09 | .10 |

***p<.001

Table 2.3

*Participants' psychological test scores; group mean (SD) values*

| | ASD | TD | p | $\eta_p^2$ |
|---|---|---|---|---|
| ADOS | 8.47 (2.58) | 2.76 (1.86) | <.001 | .63 |
| AQ | 35.71 (6.47) | 16.47 (6.57) | <.001 | .70 |
| TASIT | 49.24 (8.61) | 57.76 (3.72) | .001 | .31 |
| SRS | 114.12 (24.26) | 42.76 (18.87) | <.001 | .74 |
| Autistic traits | .84 (.63) | - .84 (.42) | <.001 | .73 |

p values were obtained from one-way MANOVA

Given that the psychological test scores assessing autistic traits were highly correlated with each other (all rs>.54) they were suitable for principal component analysis, the Kasier-Meyer-Olkin measure of sampling accuracy was .81 (above .6) and Barlett's test of sphericity was significant $\chi^2(6)=108.82$ $p<.001$. Additionally, the communalities were all above .7 supporting the inclusion of all the psychological tests in the principle components analysis (PCA). PCA with varimax rotation was used. The initial eigenvalues from the PCA analysis showed that one factor (with an eigenvalue of 3.23) explained 80.81% of the variance in psychological test scores. No other factors had eigenvalues higher than Kaiser's criteria of 1 and therefore only one factor was extracted. This factor was labelled 'autistic traits' (see Table 2.3 for group mean values).

### *2.4.2 Experiment 1*

ASD participants displayed poorer performances on the implicit task than matched controls (see Table 2.4) but group differences were not significant (Mentalizing-Action scores: U=112.50, p=.27, r=.19; Either-Action scores: U=90.00, p=.06, r=.33). Linear regression analyses also showed that higher levels of autistic traits were associated with poorer performances on the implicit task but this trend was not significant (see Figure 2.3; Mentalizing-Action scores: F(1,32)=3.11, p=.09, $R^2=0.09$, 95% CI [-5.91 -2.33]; Either-Action scores: F(1,32)=3.54, p=.07, $R^2=0.10$, 95% CI [-3.14, .124]).

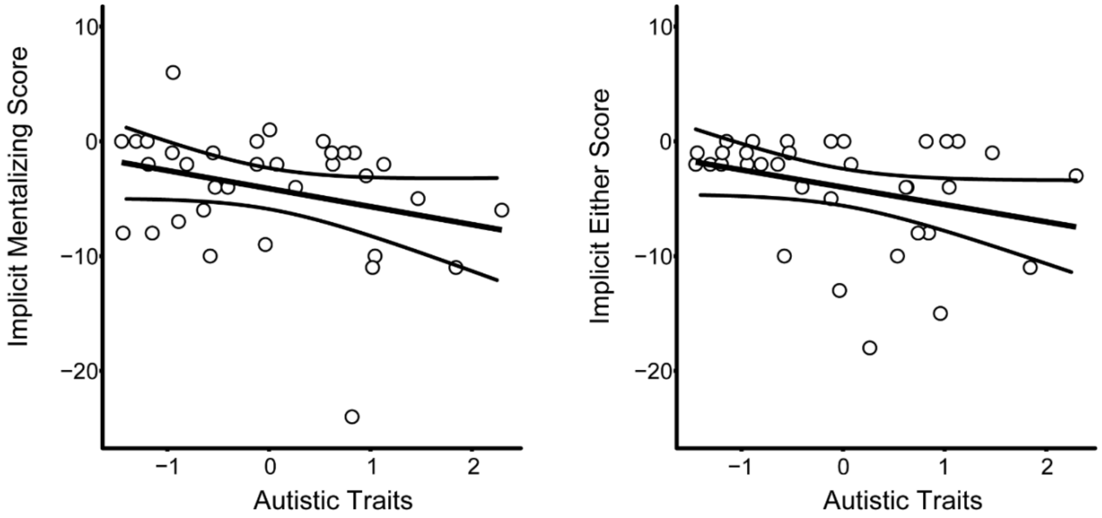

**Figure 2.3** The relationship between the levels of autistic traits displayed and performances on the implicit task in the Mentalizing condition (A) and Either condition (B). Although there was a trend of poorer performances with high levels of autistic traits, linear regression analysis found that the level of autistic traits displayed was not a significant predictor of performance in the Mentalizing (F(1,32)=3.11, p=.09, $R^2$=0.09) or the Either condition (F(1,32)=3.54, p=.07, $R^2$=0.10). The curved lines represent 95% confidence intervals.

Table 2.4

*Group performances; mean (SD) of proportion correct*

| | ASD | TD |
|---|---|---|
| Implicit 'Mentalizing' | .74 (.25) | .84(.14) |
| Implicit 'Either' | .71 (.24) | .88 (.14) |
| Implicit 'Action' | .96 (.04) | .96 (.07) |
| Explicit mentalizing | 0.83 (.15) | 0.93 (.08) |
| Explicit non-mentalizing | 1.00 (.01) | 1.00 (.01) |

In addition, adults with ASD did not show atypical changes in fixation patterns between conditions in the implicit experiment and changes in fixation patterns were not significantly different across Mentalizing and Either conditions for any of the ROIs [head ROI: task [F(1,26)=.45, p=.51, $\eta_p^2$=.02], diagnosis [F(1,26)=.77, p=.39, $\eta_p^2$=.03], task*diagnosis interaction [F(1,26)=.23, p=.63, $\eta_p^2$=.01; Poker chip ROI: task [F(1,26)=2.41, p=.13, $\eta_p^2$=.09], diagnosis

[F(1,26)=.32, p=.57, $\eta_p^2$=.01], task*diagnosis interaction [F(1,26)=.70, p=.41, $\eta_p^2$=.03]; Release point ROI: task [F(1,26)=3.27, p=.08, $\eta_p^2$=.11], diagnosis [F(1,26)=2.99, p=.10, $\eta_p^2$=.10], task*diagnosis interaction [F(1,26)=.55, p=.47, $\eta_p^2$=.02]. Group average values for the percentage of time spent fixating in each ROI can be seen in Table 2.5. The level of autistic traits that participants displayed also did not significantly predict changes in the duration of fixation within any ROI between conditions (see Table 2.6 and Figure 2.4).

Table 2.5

*Percentage duration of fixation in each ROI; Mean (SD) values*

| | ASD | TD |
|---|---|---|
| Head ROI | | |
| Implicit Mentalizing-Action | 4.64 (9.68) | 2.32 (12.01) |
| Implicit Either-Action | 4.32 (8.00) | .37 (11.08) |
| Explicit mentalizing-non-mentalizing* | .06 (18.05) | 1.91 (13.13) |
| Poker chip ROI | | |
| Implicit Mentalizing-Action | 1.16 (6.15) | -1.83 (8.76) |
| Implicit Either-Action | 2.36 (5.58) | 2.12 (11.96) |
| Explicit mentalizing-non-mentalizing* | 4.12 (15.89) | .75 (6.21) |
| Release point ROI | | |
| Implicit Mentalizing-Action | -5.34 (3.98) | -1.99 (5.52) |
| Implicit Either-Action | -6.04 (4.24) | -3.67 (4.76) |
| Explicit mentalizing-non-mentalizing* | -3.53 (6.81) | -3.82 (2.70) |

*Median (IQR) values presented as non-parametric tests were used

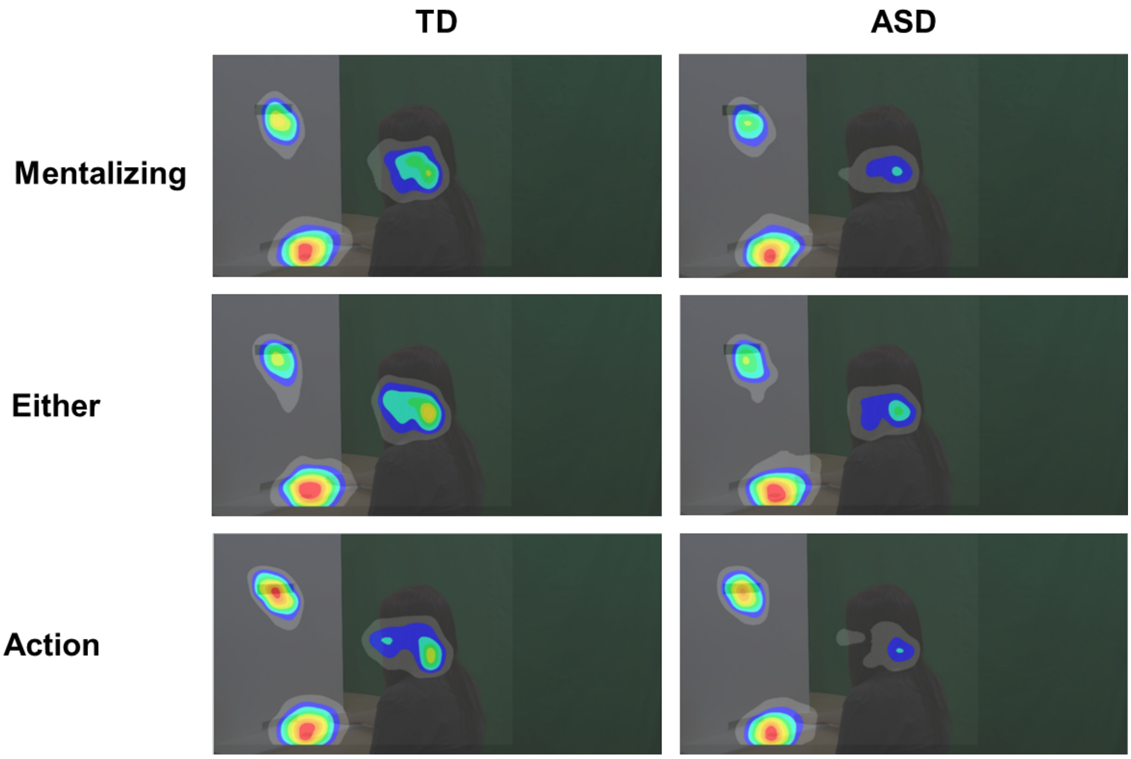

**Figure 2.4** Heatmaps showing fixation patterns for both TD and ASD groups across all three tasks in Experiment 1. Warmer colours indicate longer durations of fixation. There were no significant group differences in fixation patterns.

Table 2.6

*Results of the linear regression analyses investigating relationships between the eye-tracking data and the level of autistic traits displayed.*

| | Mentalizing-Action | | | | | Mentalizing-Either | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *B* | *SE B* | β | *t* | *p* | *B* | *SE B* | β | *t* | *p* |
| Head ROI | -.72 | 2.05 | -.07 | -.35 | .73 | .26 | 1.86 | .03 | .14 | .89 |
| Poker Chip ROI | 2.68 | 1.36 | .36 | 1.98 | .06 | 1.31 | 1.76 | .14 | .74 | .46 |
| Release Point ROI | -1.42 | .92 | -.29 | -1.54 | .14 | -.99 | 0.85 | -.22 | -1.17 | .26 |

### *2.4.3 Experiment 2*

Participants in the ASD group displayed significant explicit mentalizing deficits (Median difference= -1.88; IQR = 3.50) compared to matched controls (Median difference = -.74; IQR = 1.13; U=61.50, p=.03, r=.39). The participant in the ASD group with the highest level of autistic traits was identified as an outlier in the linear regression analysis for the explicit task (Cook's distance>1 and leverage value >n/4), therefore this participant was removed from the linear regression analysis.

Participants with higher levels of autistic traits displayed poorer performances on the explicit mentalizing condition but this was a non-significant trend (mentalizing-non-mentalizing scores; F(1,27)=3.42, p=.08, $R^2$=.11, 95% CI [-1.15, .06] see Figure 2.5).

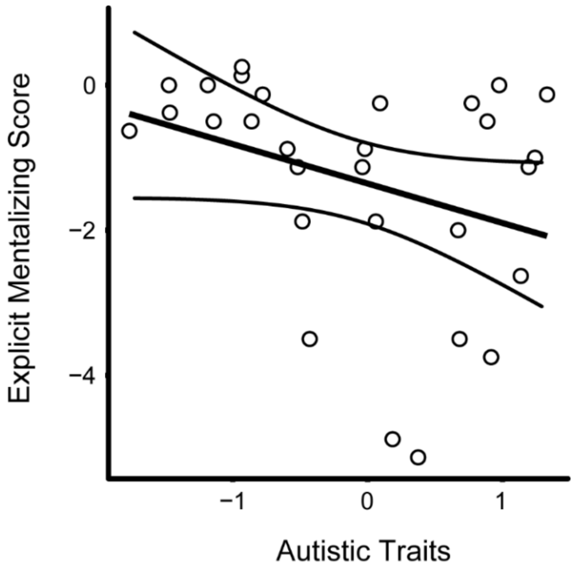

**Figure 2.5** The relationship between the levels of autistic traits displayed and performances on the explicit mentalizing task. Although there was a trend of poorer explicit mentalizing performances with high levels of autistic traits, linear regression analysis found that the level of autistic traits displayed was not a significant predictor of performance F(1,27)=3.42, p=.08, $R^2$=.11. The curved lines represent 95% confidence intervals.

Participants with ASD displayed typical changes in the duration of fixation between mentalizing and non-mentalizing conditions for all ROIs (Head ROI: U=75.00, p=.32, r=.19: Poker chip ROI: U=77.00, p=.36, r=.17: Release point ROI: U=74.00, p=.29, r=.20). See Figure 2.6. The level of autistic traits that participants displayed did not significantly predict changes in the duration of fixation between mentalizing and and non-mentalizing conditions within any ROI (Head ROI: F(1,26)=2.23, p=.15, $R^2$=0.08, 95% CI [-10.77, 1.71]; poker chips ROI: F(1,26)=1.63, p=.21, $R^2$=0.06, 95% CI [-2.04, 8.76]; release point ROI: F(1,26)=1.63, p=.90, $R^2$<0.001, 95% CI [-1.65, 1.47]).

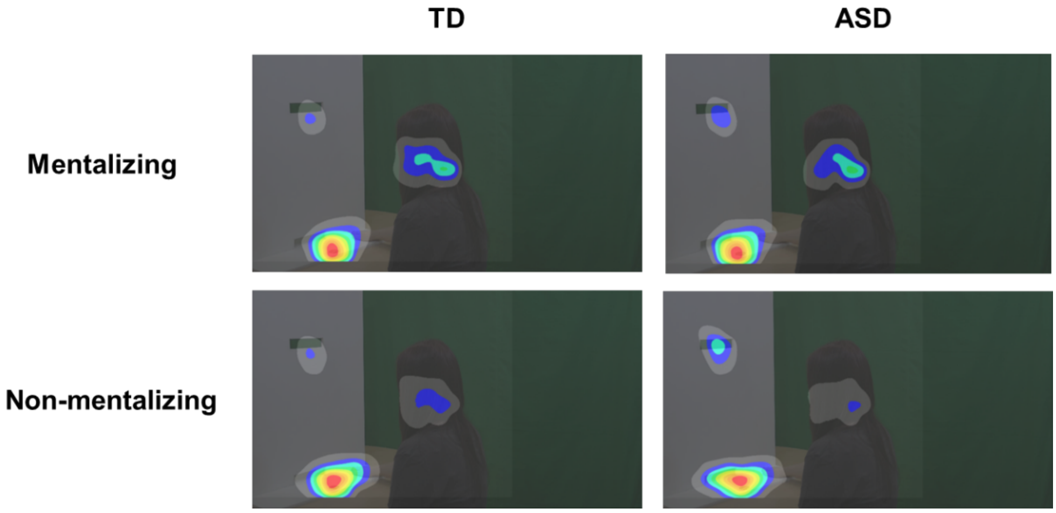

**Figure 2.6** Heatmaps showing fixation patterns for both TD and ASD groups during both mentalizing and non-mentalizing tasks in Experiment 2. Warmer colours indicate longer durations of fixation. There were no significant group differences in fixation patterns.

### 2.5 Discussion

This study aimed to investigate the abilities of adults with ASD to both implicitly and explicitly infer the intentions of others. In the first experiment, participants completed a task where mentalizing was implicit. Here participants were asked to make decisions about who they wanted to interact with between pairs of actors and in some cases these social decisions required the intentions of the actors to be inferred in order to make the appropriate choice. In contrast, during the second experiment, participants were explicitly asked to report the intentions of actors. Our results showed that adults with ASD displayed explicit mentalizing deficits compared to matched controls. Adults with ASD did not display significant implicit mentalizing abilities. Furthermore, ASD participants did not display atypical fixation patterns during both the explicit and implicit experiments. Therefore, the explicit mentalizing deficits exhibited by adults with ASD cannot be explained by differences in fixation.

The explicit mentalizing deficit found with adults with ASD in this study supports a number of previous studies which found adults with ASD were impaired at explicitly inferring others' intentions (Happé, 1994; Kana et al., 2014; Moran et al., 2011). Our data are also consistent with reported difficulties for adults with ASD in everyday life (O'Neal, 2013; The National Autistic Society, 2014). However, some previous studies have not found a connection between ASD and impairments in explicitly inferring the intentions of others (McAleer et al., 2011; Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Roeyers et al., 2001; Schuwerk et al., 2014). This may be due to the simplicity of the stimuli used in these studies, e.g. passages of text and still images (Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Roeyers et al., 2001; Schuwerk et al., 2014). In contrast, our study used a task with complex, naturalistic stimuli more akin to social environments in which individuals are required to make judgements. The use of more simplistic stimuli in previous studies may have allowed some adults with ASD to explicitly infer the intentions of others, perhaps with the help of learned strategies, which are of less help in more complex and natural settings. In support of this argument, two previous studies have investigated the ability of adults with Pervasive Development Disorders (PDDs; including ASD) to infer mental states both using simple stimuli and complex, naturalistic stimuli (Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001). Their results showed that adults with PDDs were only impaired when complex stimuli were used.

In addition to the group analysis, we also investigated the relationship between autistic traits and performance across all participants. This additional analysis was conducted as ASD is a spectrum disorder rather than a dichotomous classification and our results clearly show that participants displayed a range of autistic traits (see Figures 2.3 and 2.4). The linear regression analysis showed that across all participants the wide range of autistic traits shown was negatively associated with performance on both implicit and explicit mentalizing tasks, but these remained non-significant trends. A previous study found no relationship between autistic traits and both explicit and implicit mentalizing abilities (Nijhof et al., 2016). However, this study did not recruit adults with an ASD diagnosis and therefore may have not had the range of autistic traits required to find a relationship between autistic traits and mentalizing performance.

Although a trend was found in our study for poorer implicit mentalizing abilities associated with higher levels of autistic traits, there was not a significant group difference in performance between those with ASD and their matched controls. This lack of clear evidence for a significant implicit mentalizing deficit in adults with ASD was unexpected. We had more participants in this study than in the explicit study, which revealed clear significant results, so it is unlikely the null result is simply due to insufficient statistical power. Additionally, the effect size was much larger for the explicit experiment (r=.39) compared to the implicit experiment (r=.11), further supporting the presence of a significant explicit deficit but no clear implicit mentalizing deficit in these adults with ASD. The existing literature shows consistent implicit mentalizing deficits in adults with ASD (Castelli et al., 2002; Rosenblau et al., 2015; Schuwerk et al., 2014, Senju et al., 2009). The methods we used to measure mentalizing abilities may have contributed to the discrepancy between our findings and the previous literature. Our study measured implicit mentalizing abilities using a measurable behavioural outcome and performances were assessed relative to a control task. Previous implicit mentalizing studies in adults with ASD that used complex stimuli have either used eye-tracking data alone as a measure of mentalizing abilities (Schuwerk et al., 2014; Senju et al., 2009) or not included a control task (Rosenblau et al., 2015). Without the inclusion of a control task, it cannot be determined whether poorer performances linked to ASD are mentalizing-specific or more generalised deficits. Additionally, this study was the first to investigate abilities to implicitly infer intentions in adults with ASD; in contrast previous implicit mentalizing studies in adults have assessed abilities to infer others' mental states and false beliefs (Castelli et al., 2002; Rosenblau et al., 2015; Schuwerk et al., 2014; Senju et al., 2009). Neuroimaging studies have shown that different brain areas are active during different types of mentalizing tasks (Gobbini, Koralek, Bryan, Montgomery, & Haxby, 2007; Pineda & Hecht, 2009; Saxe & Powell, 2006; Schurz et al., 2014), suggesting that the systems used depend on the specific mentalizing task being performed. Therefore, it is possible that ASD is related to more pronounced deficits on certain subcomponents of mentalizing than others.

The lack of clear evidence for a significant implicit mentalizing deficit in adults with ASD in the current study may also be due to the use of action stimuli; implicitly inferring others' intentions from their actions may involve different processes than implicit mentalizing in the absence of action information. Actions with different intentions have been shown to display different kinematic profiles (Manera, Becchio, Cavallo, Sartori, & Castiello, 2011; Sartori, Becchio, & Castiello, 2011). The dual-process model suggests that when intentions are inferred from others' actions, these differences in action kinematics allow automatic, subconscious processing of intentional information in the observer's own motor system before intentions are actively interpreted in a higher-level cortical system (de Lange et al., 2008; Keysers & Gazzola, 2007; Spunt & Lieberman, 2012c; Uddin et al., 2007). Neuroimaging data suggest that in the absence of action information, others' intentions aren't subconsciously processed in the motor system (see a review and meta-analysis; Gallagher et al., 2000; Schurz et al., 2014). Therefore, because intentional information in our study was provided by differences in action kinematics, it is possible that subconscious processing of intentional information in the motor system allowed adults with ASD to select preferable kinematic profiles (required in our implicit task). Whereas, if intentional information was provided by other cues, not solely by differences in action kinematics, then a significant implicit mentalizing deficit may have been found. A larger number of implicit mentalizing studies have been carried out in young children than adults and a number of studies have shown that children with ASD can implicitly infer others' intentions when intention is portrayed using action (Aldridge et al., 2000; Berger & Ingersoll, 2014; Carpenter et al., 2001; Colombi et al., 2009; Liebal, Colombi, Rogers, Warneken, & Tomasello, 2008; Schietecatte, Roeyers, & Warreyn, 2012) but not when intentions are portrayed by social-emotional cues such as eye gaze or facial expression (Berger & Ingersoll, 2014; Vivanti, Hocking, Fanning, & Dissanayake, 2016). These data support the theory that inferring intentions from action kinematics involves different processes than inferring intentions using different cues and that implicitly inferring intentions from action kinematics is not significantly impaired in ASD.

Individuals with ASD and high levels of autistic traits also showed relatively poor performance on Either trials. It seems likely that mentalizing may have influenced the social judgments participants made during the Either condition even though, in principal, mentalizing was not required. The Either condition was included in this study in attempt to control for differences in the success of actions across mentalizing and non-mentalizing (Action) conditions. In the Mentalizing condition, unsuccessful actions were always seen and in the Action condition only successful actions were seen. The Either condition showed unsuccessful actions but did not require mentalizing in order to complete the task, if participants made their decisions based purely on the number of poker chips involved in the hand actions then they would make correct choices. However, previous evidence suggests that the intentionality of observed hand actions is automatically processed (Liepelt, Cramon, et al., 2008), and given participants were blind to the condition, from the participant’s perspective, the relevant feature of the action (number of chips / intention of the actor) only became clear after the second movie had been viewed. Thus, it may have been an effective strategy to pay attention to the intention of the actor in all trials. This may have affected performance in several ways. First, participants with higher levels of autistic traits may have wrongly attributed negative intent to the preferable actions (the actor attempting to pass more poker chips) in the Either condition resulting in incorrect choices. Second, reading actor intentions may have distracted participants from focussing on the number of chips being passed and thus the inclusion of both actor intentions and differential number of chips, may have placed a higher cognitive load on participants, compared to other conditions and this may have contributed to the relatively poor performances in this condition.

Despite the poorer explicit mentalizing abilities found in adults with ASD compared to matched controls in our study, fixation patterns were not different in the ASD group. The typical fixation patterns exhibited by adults with ASD in this study may also be due to the use of action stimuli. The majority of the literature reporting atypical fixation patterns in adults with ASD have found atypical fixation patterns during face processing, in particular, showing reduced fixation on the eyes (Dalton et al., 2005; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Pelphrey et al., 2002; Sterling et al., 2008). In the current study, the actors' faces were not shown and intentional information was portrayed by the kinematics of the actions alone. Adults with ASD may alter their eye movements appropriately according to differences in the mentalizing demand of the task when intentional information is portrayed by action kinematics but not when internal state inferences require face processing. This theory is supported by data from a previous study that showed that when adults with ASD naturalistically viewed videos and pictures of social scenes they displayed reduced fixation on people's faces but showed equivalent fixation on bodies to control participants (Rigby, Stoesz, & Jakobson, 2016). The typical eye-tracking data in conjunction with the explicit mentalizing deficit in the current study suggest that despite receiving the visual cues they needed, adults with ASD could not accurately interpret the social cues embedded within the action kinematics in order to explicitly infer the actors' intentions. This dissociation between the behavioural data and the eye-tracking data has implications for future research assessing mentalizing abilities.

Some previous studies have investigated mentalizing abilities using eye-tracking data alone (Schuwerk et al., 2014; Senju et al. 2009). However, our results demonstrate that poorer mentalizing abilities associated with high levels of autistic traits are not always accompanied by atypical visual fixation patterns. This is supported by previous research which showed that although adults with ASD spent less time fixating on the eyes of others, these atypical fixation patterns did not correlate with poorer mentalizing performances (Cassidy et al., 2013).

In conclusion, we found that adults with ASD were significantly impaired at explicitly but not implicitly inferring the intentions of others from their hand actions. Although there was a trend for adults with high levels of autistic traits to display poorer implicit mentalizing performances, this relationship did not reach significance. The lack of a significant implicit mentalizing deficit may be due to subconscious processing of intentional information when intentions are portrayed by action kinematics. Adults with ASD displayed typical fixation patterns when both implicitly and explicitly inferring the intentions of others. The inconsistency we observed between impaired explicit mentalizing but typical fixation patterns suggests that reduced abilities to explicitly infer intentions from hand actions cannot be attributed to dissimilarities in fixation patterns. Our findings suggest that future research should consider the stimuli used and assess mentalizing abilities with both behavioural and eye-tracking techniques.

# Chapter 3: Timing of Mirror Neuron System Activation when Inferring the Intentions of Others

This chapter is adapted from: Cole, E.J., Barraclough N. E. (in review). Timing of Mirror Neuron System activation when inferring the intentions of others. *European Journal of Neuroscience.*[2]

## 3.1 Abstract

Neuroimaging studies have shown mirror neuron system (MNS) activation when participants infer internal states e.g. emotions, intentions or beliefs (known as 'mentalizing') from others' actions. However, the exact role of the MNS in mentalizing tasks is unknown. The role of the MNS in mentalizing tasks is important for understanding the neurobiological basis of autism spectrum disorders because autistic individuals display mentalizing deficits and MNS dysfunction may underlie these difficulties. This study investigated the timing of MNS activity when participants inferred others' intentions from their actions in order to delineate between existing models of MNS involvement. Single-pulse transcranial magnetic stimulation was applied to the primary motor cortex at different time points during the observation of hand actions whilst participants performed mentalizing and non-mentalizing tasks. Electromyographic activity in the contralateral hand was used as an indirect measure of MNS activity. Greater corticospinal activity was found during

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[2] The author, Eleanor Cole, designed the experiment, collected the data, analysed the results and wrote the manuscript under the supervision of Dr Nick Barraclough.

the mentalizing task but only at the end of observed actions. These results suggest that the MNS is involved in inferring others' intentions but only after prior involvement of another cortical system, which potentially provides feedback to the MNS. The degree to which corticospinal activity was increased when mentalizing was not related to levels of autistic traits participants' displayed or behavioural performance suggesting the MNS has a more automatic role in processing others' intentions, irrespective of mentalizing ability. Our results extend current knowledge of MNS activation when mentalizing, allowing initial delineation between different models of MNS involvement in mentalizing.

## 3.2 Introduction

Inferring others' internal states (e.g. emotions, beliefs or intentions) is referred to as 'mentalizing'. There are a number of theories to explain human mentalizing abilities; 'theory theory' suggests that others' internal states are inferred using lawlike generalisations (Goldman, 2012), rationality theory implies that surrounding context and prior experience are used to rationalise what other individual's internal state 'should' be (Goldman, 2006; Goldman 2012) and simulation theory suggests that another's internal state is inferred by internal simulation (Goldman 2006). There are two subcategories of simulation theory; motor simulation and mental simulation. Motor simulation suggests that others' actions are internally simulated and their internal states are deduced as a result whereas mental simulation theory suggests that another's mental state is simulated based on prior knowledge and contextual information (Hegarty, 2004; Shanton & Goldman, 2010). It is possible that the method used to determine others' internal states depends on the information available; motor simulation may be particularly important when inferring others' internal states from their actions.

The neural system associated with motor simulation is known as the mirror neuron system (MNS). The MNS is a network of brain areas that contain neurons that are active both when an individual performs an action and when they observe others performing similar actions (di Pellegrino et al., 1992; Rizzolatti et al., 1996). The main components of the human MNS are considered to be the inferior frontal gyrus (IFG) and the inferior parietal lobe (IPL; Rizzolatti & Craighero, 2004; Rizzolatti & Sinigaglia, 2010). It has been proposed that the MNS is required to mentalize when action information is available. Indeed, mentalizing tasks that have used stimuli which either include movies of human actions or portray human actions (through sets of still images or point-light displays), have tended to elicit MNS activity (Brunet, Sarfati, Hardy-Baylé, & Decety, 2000; Bucchioni, Cavallo, Ippolito, Marton, & Castiello, 2013; Centelles, Assaiante, Nazarian, Anton, & Schmitz, 2011; Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; Enticott et al., 2013; Iacoboni et al., 2004). Although there is evidence that the MNS is active when inferring others' internal states from their actions, the exact role of the MNS in this task is debated.

There are a number of different theories regarding the involvement of the MNS in inferring other's internal states from their actions: 1. strict motor simulation theory states that MNS activity alone, reflecting simulation of observed actions on the observer's own motor system, is sufficient to encode others' internal states (Rizzolatti & Sinigaglia, 2007); 2. a dual-process hypothesis has been proposed which suggests that internal state information embedded in action kinematics is automatically and subconsciously processed in the MNS and this information is then passed on to a separate cortical system known as the 'mentalizing system' in order for active inferences about the person's internal state to be made (de Lange, Spronk, Willems, Toni, & Bekkering, 2008; Keysers & Gazzola, 2007; Spunt & Lieberman, 2012c; Uddin, Iacoboni, Lange, & Keenan, 2007); 3. the 'mirroring-first' model implies that internally simulating others' actions in the MNS is a vital prerequisite for inferring others' internal states but the MNS is not involved in processing others' internal states itself (Hamilton & Marsh, 2013; Spunt, Satpute, & Lieberman, 2011).

A strict 'mirroring-first' model cannot account for the evidence which shows that MNS activity is modulated by mentalizing (Brunet et al., 2000; Bucchioni et al., 2013; Centelles et al., 2011; Ciaramidaro et al., 2014; Enticott et al., 2013; Iacoboni et al., 2004). One possible source of this MNS modulation could be feedback from the mentalizing system. The predictive coding theory suggests that when inferring aspects of someone's internal state from their actions, contextual information is processed prior to kinematic processing (Csibra & Gergely, 2007; Kilner, Friston, & Frith, 2007). This contextual information is used to infer the internal state of the individual and therefore predict the outcome of upcoming actions. These predictions are then signalled to the MNS and incoming sensory information is compared to predictions made. Discrepancies between predictions made and actual action outcomes are signalled in the form of 'prediction errors' and these are used to inform future predictions (Kilner, Neal, Weiskopf, Friston, & Frith, 2009). The predicted response outcome model by Alexander and Brown suggests that the mPFC, a core area of the mentalizing system, makes predictions about the outcomes of upcoming actions based on action context and prior experience (Alexander & Brown, 2011). Therefore, the predictive coding model, in combination with the predicted response outcome model, suggests a potential fourth model of MNS involvement: information regarding others' internal states is processed by the mentalizing system (mPFC) first based on contextual information before internal states are processed in the MNS. Data from neuroimaging studies have provided evidence to support this model:

action context and prior expectations about an upcoming action modulate activity in the mPFC (Alexander & Brown, 2011; Becchio et al., 2012; Chambon et al., 2017b; Cooper, Kreps, Wiebe, Pirkl, & Knutson, 2010; Ferdinand & Opitz, 2014; Fogelson, Shah, Scabini, & Knight, 2009; Forster & Brown, 2011; Jahn, Nee, Alexander, & Brown, 2014; Leue, Cano Rodilla, & Beauducel, 2015; Schiffer, Krause, & Schubotz, 2014), increased functional connectivity between the mPFC and the MNS has been reported when inferring internal states from actions (Ciaramidaro et al., 2014; Spunt & Lieberman, 2012a; 2012b). Additionally, action context has been shown to modulate MNS activity (Amoruso, Finisguerra, & Urgesi, 2016; Amoruso & Urgesi, 2016; Iacoboni, Molnar-Szakacs, Gallese, Buccino, & Mazziotta, 2005), implying possible top-down connectivity from the mentalizing system to the MNS.

Understanding when MNS activity is modulated by mentalizing may help delineate the possible roles of the MNS in the mentalizing process. Previous studies have shown that the early kinematics of actions vary depending on the actor's underlying intentions and intentions can be predicted from this information before the outcomes of the actions have been revealed (Manera, Becchio, Cavallo, Sartori, & Castiello, 2011; Sartori, Becchio, & Castiello, 2011). Therefore, both the motor simulation theory and dual-process hypothesis suggest that when inferring intentions from actions, MNS activity should be modulated early during action observation whilst kinematic differences reflecting the actor's intention are processed. In contrast, the predictive coding framework suggests that intentions are first processed in the mentalizing system and therefore, mentalizing-induced modulation of MNS activity would be observed later. Finally, the 'mirroring-first' model would suggest that MNS activity was not modulated by mentalizing at all.

Previous studies have used transcranial magnetic stimulation (TMS) to investigate the timing of MNS modulation due to the high temporal resolution of this technique (Amoruso, Finisguerra, & Urgesi, 2016b; Barchiesi & Cattaneo, 2013; Bardi, Schiff, Basso, & Mapelli, 2015; Candidi, Sacheli, Mega, & Aglioti, 2014). Single TMS pulses can be applied at different time points during action observation in order to provide an indirect measure of MNS activity at different stages of an action. For example, Alaerts and colleagues found that the weight of objects being acted upon influenced MNS activity during early stages of action observation even before the objects had been grasped (Alaerts, de Beukelaar, Swinnen, & Wenderoth, 2012). These results suggest that the MNS is involved in forming predictions about upcoming actions based on properties of the objects being acted upon. In contrast, Amoruso and colleagues found that when participants were inferring the goals of observed actions, action context only modulated MNS activity during later stages of the actions (Amoruso et al., 2016). These data show that the MNS is not involved in early processing of action context in order when making predictions about the goals of observed actions. Collectively, these data suggest that MNS activity is modulated at different time points, by different factors during action observation. In the current study, we used single-pulse TMS in order to determine the timing at which mentalizing modulates MNS activity in order to delineate the role of the MNS when inferring the intentions of others' from their actions.

Determining the role of the MNS in inferring others' intentions from actions is of importance in understanding autism spectrum disorder (ASD) which is associated with difficulties inferring the intentions of others (Happé, 1994; Kana, Libero, Hu, Deshpande, & Colburn, 2014; Moran et al., 2011). The 'broken mirror' theory proposes that these individuals exhibit atypical MNS activity (Iacoboni &

Dapretto, 2006; Lindsay M Oberman & Ramachandran, 2007; Ramachandran & Oberman, 2006), and this underlies difficulties these individuals experience in inferring the internal states of others, and consequently contribute to the social interaction deficits associated with ASD (American Psychiatric Association, 2013). ASD is a spectrum disorder, meaning that individuals within the non-clinical population exhibit differing degrees of autistic traits (Robinson et al., 2011). Individuals with relatively high levels of autistic traits without a diagnosis, have been shown to display reduced abilities to infer the internal states of others (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001; Gökçen, Petrides, Hudry, Frederickson, & Smillie, 2014; Gökçen, Frederickson, & Petrides, 2016) and atypical MNS activity (Cooper, Simpson, Till, Simmons, & Puzzo, 2013; Lepage, Tremblay, & Théoret, 2010; Puzzo, Cooper, Vetter, Russo, & Fitzgerald, 2009). Collectively, these data suggest that the level of autistic traits displayed may correlate with MNS functioning and abilities to infer intentions. In this study we, therefore, evaluated the degree of autistic traits displayed by participants in order to examine whether this factor influenced the level of MNS activity displayed when inferring others' intentions.

The present study aimed to investigate MNS activity at different time points during three different tasks in order to delineate between previously proposed models of the role of the MNS in inferring intentions from actions. Single-pulse TMS was applied at different time points during action observation whilst participants inferred others' intentions (Mentalizing task), whilst participants observed actions that did not depict their intentions and were not required to mentalize (Action task), and whilst participants observed actions that reflected the actors' intentions but were not required to mentalize (Either task). If the motor simulation theory explains the role of the MNS in mentalizing, early MNS modulation would be observed during the Mentalizing task, and MNS activity during this task should correlate with ability to infer intentions. If the dual-process hypothesis explains the role of the MNS in mentalizing, early MNS modulation would be observed during both the Mentalizing and the Either task, but MNS activation should not correlate with behavioural performance. If intentional information is processed by an alternative cortical system first (e.g. the mentalizing system) then MNS modulation should only occur at later stages of observed actions during the mentalizing task. Finally, the mirroring-first model would predict equivalent levels of MNS activity across all tasks throughout action observation. Autism quotient (AQ) scores were also measured for all participants in order to examine whether any mentalizing-induced MNS modulation was associated with the level of autistic traits displayed. Individuals with higher levels of autistic traits were expected to exhibit lower levels of MNS modulation.

## 3.3 Method

### *3.3.1 Participants*

Participants were recruited based on their Autism Quotient (AQ) scores (Baron-Cohen et al., 2001) in order to ensure there was a wide range of AQ scores among participants. An email was sent to students at the University of York containing a link to an online version of the 50-item AQ questionnaire. Six hundred and four students responded and completed the AQ assessment. Fifteen participants were recruited (9 female) aged 18-29 (mean=22.13) with AQ scores ranging from 4-40 (mean=18). All participants reported no neurological or psychiatric illnesses, no contradictions for TMS and provided written informed consent. None of the participants had a diagnosis of ASD. Ethical approval was granted by the York

Neuroimaging Centre Ethics Committee. All research was performed in accordance with the ethical standards laid down in the 1990 Declaration of Helsinki.

### *3.3.2 Stimuli*

The stimuli used were a subsample of the movies used in a previous behavioural study (Cole, Slocombe & Barraclough, 2017), and are described in detail there. Briefly, movies were filmed at full HD (1080 x 1920 pixels) and at 50 frames per second using a Panasonic TM900 camera. Actors were filmed sitting in front of a white wooden board and passing or attempting to pass poker chips to another player (out of view) through slots cut out of the board (see Figure 3.1). Actors each performed ten different actions; five hand actions involved pushing poker chips with their right index finger towards a slot level with the surface of the table in front of them. The other five hand actions involved grasping the poker chips with their right index finger and thumb and lifting the chips towards a slot at head height. Both pushing and grasping actions were executed by the actor in one of 5 different ways: 1. clumsily failing to pass one poker chip; positive intention to pass the chip, but the outcome of the action was unsuccessful (clumsy 1), 2. Clumsily failing to pass five pokers chips (clumsy 5), 3. Spitefully failing to pass one poker chip; no intention to pass the chip and the outcome of the action was unsuccessful (spiteful 1), 4. Successfully passing one poker chip (successful 1) and 5.Successfully passing five poker chips (successful 5). Sets of movies of 12 different actors (6 female and 6 male) were used for this experiment.

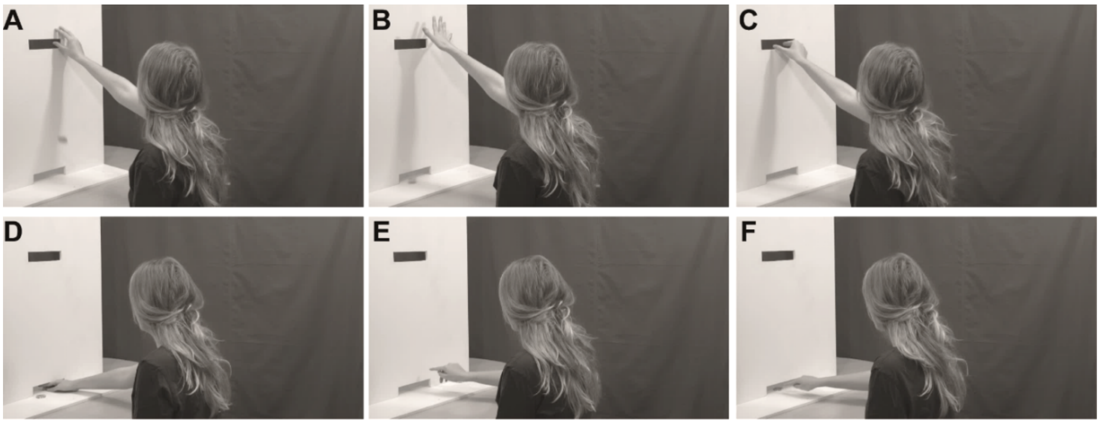

**Figure 3.1** Screenshots depicting the final frame of the video stimuli for one actor. The videos depict an actor: A. Accidently dropping a poker chip (clumsy grasp). B. Deliberately dropping a poker chip (spiteful grasp). C. Passing a poker chip through the higher slot in the board (successful grasp) D. Accidentally not passing a poker chip through the bottom slot (clumsy push) E. Deliberately not passing a poker chip through the bottom slot (spiteful push) F. Passing a poker chip through the bottom slot (successful push).

### *3.3.3 Electromyography and TMS*

Electromyogram (EMG) was recorded from the first dorsal interosseous (FDI) and opponens pollicis (OP) muscles of the left hand using standard surface Ag-AgCl electrodes. Participants' left arms were rested on the table in front of them. EMG signals were band-pass filtered and amplified using a BIOPAC MP150 amplifier (BIOPAC Systems Inc., USA). The top centre point of the head (Cz) was found by measuring half way between the inion and the nasion as well as the half way point between the ears. The position of the hand area of right primary motor cortex (M1) was identified in participants by measuring 5cm lateral and 1cm anterior to Cz. A 3-by-3 grid was created around the estimated position of right M1. Single TMS pulses were delivered using a Magstim Rapid$^2$ and a 70mm figure of eight coil to every point in this grid (Magstim Company Ltd, UK). The point in the grid that produced the largest motor evoked potentials (MEPs) in the left FDI & OP muscles when stimulated was concluded to be the optimal stimulation site in M1. The resting motor threshold was determined for each participant as the minimum stimulation intensity that produced MEPs greater than 50µV amplitude in the FDI and OP muscles in 5/10 cases after single pulse stimulation. The TMS pulses delivered during the experiment were at 120% resting motor threshold.

### *3.3.4 Procedure*

Before starting the experiment, each participant played a poker chip game with the experimenter. This was done in order to familiarise participants with the board and poker chips they would see in the movies and so they understood the aim of the game was to have the highest number of poker chips on your side of the board as possible. For a full description of this poker chip game, see the previous behavioural experiment (Cole, Slocombe & Barraclough, 2017).

During the experiment, MATLAB (MathWorks Inc., Massachusetts, USA) was used to run the experiment, record participants' responses and send TTL pulses to the parallel port in order to trigger the delivery of TMS pulses. Movies were displayed on a 22" flat-screen CRT monitor (Philips 202P40, 1600 x 1200 pixels 100Hz refresh rate) positioned approximately 60cm in front of participants. Each trial consisted of two action movies of the same type (either grasping or pushing) presented sequentially with an inter-stimulus interval of 1000ms. During the inter-stimulus interval, the screen was black except for a white fixation cross. Following the presentation of the second stimulus a response screen was subsequently displayed and participants had to indicate whether they would rather play the poker chip game with the actor in the first or second movie by pressing either '1' or '2' on the keyboard with their right hand. Movies were presented in blocks of 'Mentalizing', 'Either' or 'Action' tasks and all movies were shown in greyscale. During the Mentalizing task, actions with different intentions but the same outcome were shown (clumsy 1 and spiteful 1). Here, in order to make the appropriate decision between the actors, participants were required to infer the intentions of the actors. There were two types of control tasks: Either and Action tasks. During the Action task, pairs of successful actions were presented in which one actor passed one poker chip (successful 1) and another actor passed five poker chips (successful 5). In order to make the appropriate decision during this task, participants simply had to identify which action involved the highest number of poker chips. During the Either task participants were shown two actions with positive intentions but involving different numbers of poker chips (clumsy 1 and clumsy 5). Again, in order to make the appropriate decision, participants simply had to identify which action involved the greatest number of poker chips. However, the movies shown during this task did portray positive intentions of the actors so participants may have automatically inferred the intentions of the actors. This task is referred to as the Either task as it may involve mentalizing or simply action processing. The Either task was included in the experiment in order to control for the fact that the Mentalizing task only showed unsuccessful actions and the Action task showed only successful actions. The Either task showed unsuccessful actions but mentalizing was not required in order to complete the task. Participants completed 12 practice trials, 4 of each task type (Mentalizing, Either and Action), before they took part in the main experiment.

Within each block, every trial showed one action performed by a male actor and one action performed by a female actor. The stimuli were counterbalanced so that gender and identity of the actors were not predictors of the action outcome. There were four blocks of each task, each containing 30 trials (60 movies). Each block contained 15 grasping trials and 15 pushing trials which were randomly presented. Every participant completed all 12 blocks across two sessions. In each session participants completed 6 blocks (2 of each task type). The order in which blocks (n=12) were completed was different for every participant. Additionally, the order in which different tasks (Mentalizing, Either, Action) were completed was counterbalanced across participants and the order in which each participant completed the different task types in the first session was reversed in the second session. This was done to reduce the possibility of order effects. Each block lasted approximately 8 minutes and participants had short breaks in between each block.

Single TMS pulses were delivered at one of six different time points during each movie. The movies were made in a controlled way so that TMS pulses could be applied during specific stages of every action. In all movies, the poker chips were released 20 frames before the end of the movie, the actors were stationary for at least the first 25 frames of every movie and the frame in which the poker chip was first grasped/pushed was recorded for each movie. Therefore, the pulse timings were defined in the following way: Pulse time 1 was at the start of the movie before movement occurred (frame 20), pulse time 2 was after movement onset but before contact had been made with the poker chip(s) (20 frames before the frame in which the poker chip(s) were first grasped/pushed), pulse time 3 was when the poker chip(s) were first grasped/pushed (between frames 73-161), pulse time 4 was during the action before the poker chip(/s) left the actor's hand (15 frames after the frame in which poker chip(s) were first grasped/pushed), pulse time 5 was at the moment at which poker chip(/s) left the actor's hand (frame 180) and pulse timing 6 was at the end of the action after the poker chip(/s) had been released (frame 200; see Figure 3.2). There were 20 repeats of each of the 36 conditions [pulse time (1-6), task (Mentalizing, Either, Action) and action type (grasping or pushing)].The time at which TMS pulses were delivered was counterbalanced across task and action types.

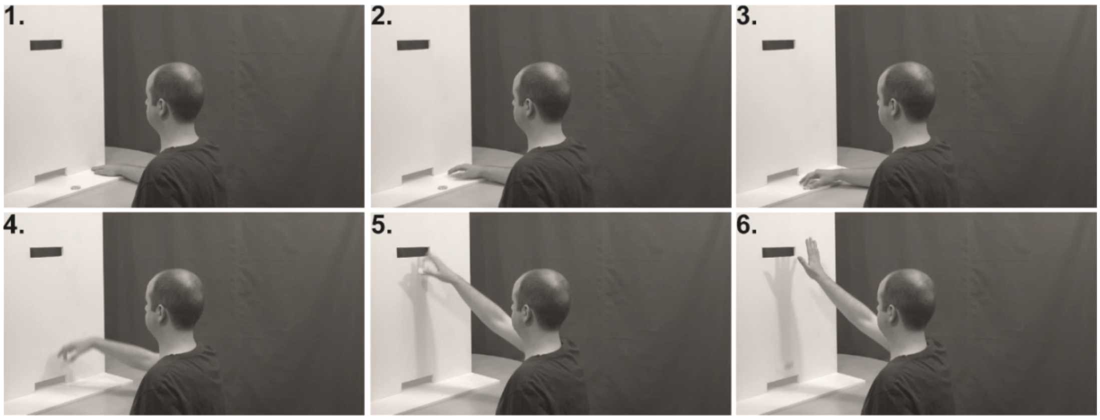

**Figure 3.2** Screenshots from one example video showing the frames that were displayed when the TMS pulses were applied at the 6 different time points. 1. When the hand was stationary, 2. After movement onset but before contact was made with the poker chip, 3. Initial movement of the poker chip, 4. During the movement of the chip before it was released, 5. Moment at which the chip was released, 6. End of the action, 20 frames after the chip was released.

## 3.4 Analysis

### *3.4.1 Behavioural data*

The mean proportion of correct responses were calculated for each task for every participant. Data screening identified that these data were not normally distributed and consequently, a log transformation was applied. Following the log transformation, the data still violated the assumption of normality so non-parametric analyses were conducted. A Friedman's ANOVA was used to examine potential differences in behavioural performance across tasks. The relationships between task performances and both levels of autistic traits and MNS activity were investigated using linear regression analyses.

### *3.4.2 MEP data*

For each participant, the mean and standard deviation of the root mean squared scores of the EMG background activity in the 50ms period prior to stimulus onset were calculated. As EMG background activity can modulate MEP magnitude (Hess, Mills, & Murray, 1987), MEPs were excluded from the analyses if EMG background activity exceeded more than 2.5 standard deviations (SD) from the mean EMG activity for that particular participant and muscle. MEP peak-to-peak amplitudes were measured, and in order to exclude outliers, those MEPs that differed more than 1.5 SD from the mean MEP size for that condition and muscle, were also excluded from subsequent analyses. From the remaining MEPs, median values were then calculated for each muscle and condition. Median MEP values were calculated rather than mean MEP values as Schmidt and colleagues showed that MEP values can be inflated on initial trials (Enticott et al., 2011; Schmidt et al., 2009) and this would have greater influence on mean rather than median values. In total, 12.07% of all MEPs were excluded from the analyses.

The median MEP values were averaged across both muscles (FDI and OP) and action type (pushing and grasping) as neither were factors of interest; the goal of the experiment being to investigate the effect of the interaction between TMS pulse timing and task on MEP sizes. This resulted in 18 median MEP values for each participant: for the 6 different TMS pulse timings, and for all three tasks (Mentalizing, Either, Action). Median MEP values violated the assumption of normality and therefore a log transformation was applied. Shapiro-Wilk tests established that median MEP sizes did not significantly differ from a normal distribution after the log transformation was applied. A repeated-measures ANOVA was conducted to identify the influence of task (Mentalizing, Either, Action) and pulse timing (1-6) on MEP sizes. Finally, linear regression analyses were conducted in order to investigate possible relationships between mentalizing-specific MEP sizes and autistic traits (as measured by AQ).

Pearson's correlation coefficients were calculated alongside all linear regressions and were additionally tested using Bayesian analyses (Dienes, 2008, 2011) to quantify the evidence in favor for or against each correlation. The resulting Bayes factor (B) quantifies how much more (or less) likely the data are under the alternative hypothesis than under the null hypothesis. For example, B=3.0 would indicate that the data are 3 times as likely to be correlated compared to the null hypothesis, whereas B=1/3 would indicate that the data are 3 times as likely to indicate the hull hypothesis over the alternative that the data are correlated. The suggested convention (Jeffreys, 1961) is that Bayes factors above 3 indicate substantial evidence for the alternative hypothesis (data are correlated), Bayes factors below 1/3 indicate substantial evidence for the null hypothesis (data are uncorrelated), whilst values in between 3 and 1/3 indicate neither support for the alternative nor the null hypothesis.

## 3.5 Results

### *3.5.1 Behavioural data*

Participants' abilities to make judgments of the actors' behaviours were not significantly different across tasks [Mentalizing (proportion correct M=.94, SD=.10), Either (M=.93, SD=.08), Action (M=.93, SD=.06); $\chi^2(2)=5.72$, p=.06]. In addition, linear regression analyses showed that the level of autistic traits that participants displayed did not significantly predict performance on the Mentalizing task [F(1,13)=.69, p=.42, $R^2$=.05, r=-.22, B=.69], the Either task [F(1,13)<.01, p=.93, $R^2$=<.01, r=-.03 B=.56] nor the Action task [F(1,13)=1.7, p=.22, $R^2$=.12, r=.34, B=1.02]. See Figure 3.3. The Bayes factors indicated that there was neither evidence for, nor against, correlations between the level of autistic traits and performance on any of the tests.

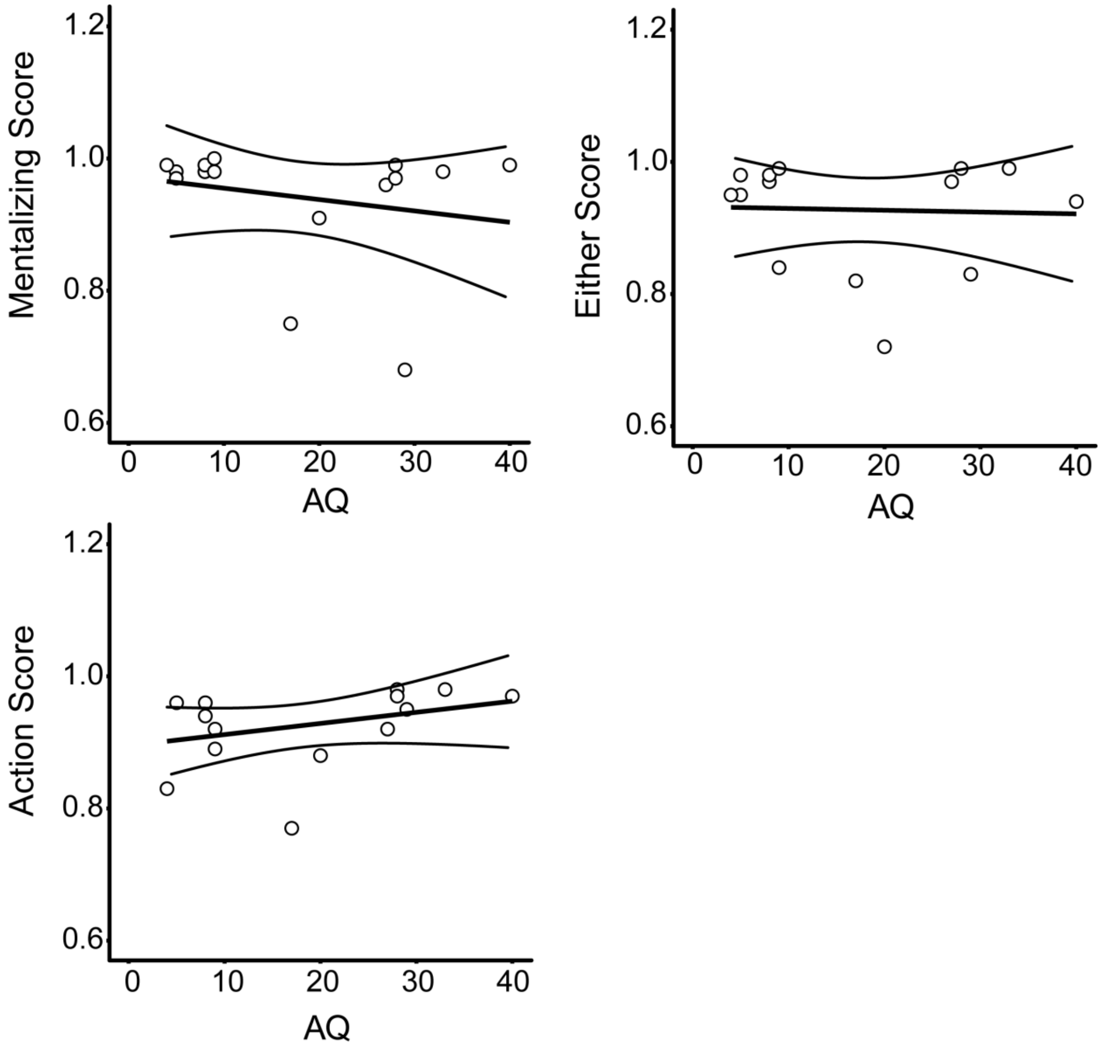

**Figure 3.3** The relationships between AQ score and behavioural performance (proportion correct) on each of the tasks. There were no significant relationships between AQ score and behavioural performance. Curved lines represent 95% confidence intervals.

*3.5.2 TMS data*

Median MEPs were larger during the Mentalizing task than other tasks when TMS was applied towards the end of the action (see Figure 3.4A). This was reflected in a significant interaction between task and the timing of the TMS pulse [F(4.71,65.87)=5.11, p=.001, $\eta_p^2$=.27; Greehouse-Geisser correction applied]. There were no main effects of task [F(2,28)=2.01, p=.15, $\eta_p^2$=.13] nor TMS pulse timing on MEP sizes [F(2.51,35.20)=1.34, p=.28, $\eta_p^2$=.09; Greehouse-Geisser correction applied].

The significant interaction between experimental task and TMS pulse timing was explored further by performing six separate one-way ANOVAs examining the effect of task on MEP sizes for each TMS pulse timing separately. There was only a significant effect of task at pulse timing 6 [F(2,28)=8.90, p=.001, $\eta_p^2$=.39, Bonferroni correction applied; see Figure 3.4B]. Subsequent paired samples t-tests showed that at time point 6, MEPs were significantly larger during the Mentalizing task (t(14)=-5.17, p<.001, *d*=1.34) and the Either task (t(14)=-2.62, p=.02, *d*=.68) compared to the Action task. However, once the significance level had been adjusted to correct for multiple comparisons using the Bonferroni correction, only the difference between the Mentalizing task and the Action task was significant. MEP sizes at time point 6 were not significantly different during the Mentalizing task compared to the Either task (t(14)=1.23, p=.24, *d*=.32.

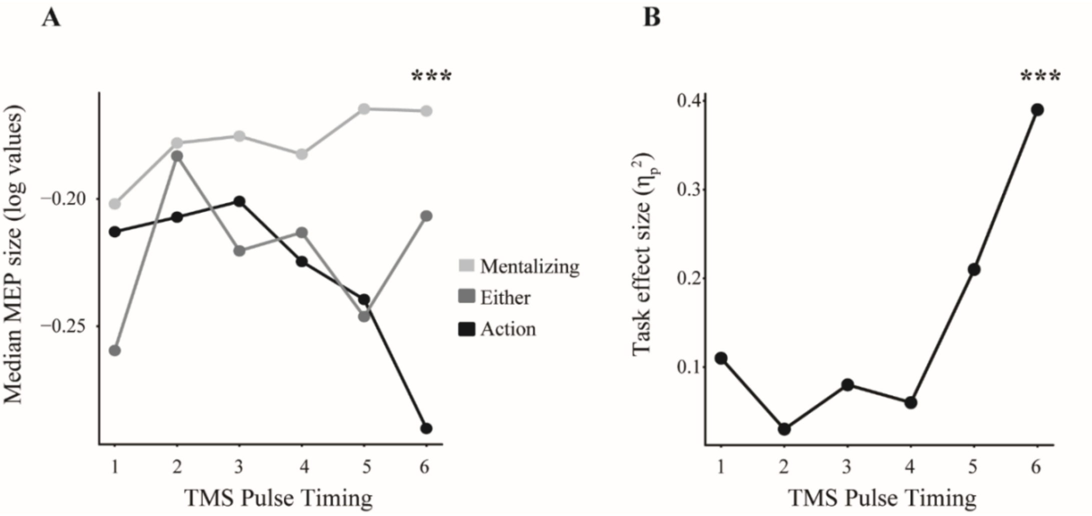

**Figure 3.4** The effect of behavioural task on levels of corticospinal excitability at different time points. The task that participants were engaged in, significantly influenced corticospinal excitability if TMS was applied at the end of the observed actions [pulse timing 6; F(2,28)=8.90, p=.001*, $\eta_p^2$=.39]. * Bonferroni-corrected threshold value for significance: .05/6= p<.008. Levels of corticospinal excitability

were larger during the Mentalizing task than the Action task when TMS was applied at the end of the actions ($t(14)=-5.17$, $p<.001$**). **Bonferroni-corrected threshold value for significance: $.05/3=p<.02$.

Finally, we examined the relationship between mentalizing-specific increases in MEP sizes at time-point 6 and the degree of autistic traits participants displayed. Linear regression analysis showed that participants' AQ scores did not significantly predict MEP sizes during the Mentalizing task at time point 6 [$F(1,13)=.13$, $p=.72$, $R^2=.01$, $r=-.10$, $B=.34$]. In addition, the degree of mentalizing-specific MNS activity didn't predict mentalizing behavioural performance [$F(1,13)=.93$, $p=.35$, $R^2=.07$, $r=-.26$, $B=.38$]. The Bayes factors indicated that there was neither evidence for, nor against, correlations between the level of autistic traits and MEP sizes, and between the degree of mentalizing-specific MNS activity and performance on the mentalizing task.

### 3.6 Discussion

This study measured corticospinal activity (as an indirect measure of mirror neuron system (MNS) activity) at different time points when inferring the intentions of others, and investigated the relationship between the degree of mentalizing-induced modulation of corticospinal activity and autistic traits. Our results show that, corticospinal excitability was higher when participants inferred actors' intentions compared to when they made judgments about actions that did not depict the actor's intention. Importantly, this mentalizing-induced modulation of corticospinal excitability was observed only at the end of the actions, when the actors' intentions had been revealed. No relationships were found between the degree to which corticospinal excitability was modulated and either the level of autistic traits that participants displayed or behavioural performance. These data helps us delineate between models of MNS involvement in mentalizing.

The larger MEP sizes (reflecting higher levels of MNS activity) found during the Mentalizing task compared to the Action task at the end of observed actions, suggest that the MNS plays a role in processing intentional information from hand actions (Giovanni Buccino et al., 2007; Ciaramidaro et al., 2014; de Lange et al., 2008; Iacoboni et al., 2005; Vingerhoets et al., 2010). These data oppose the mirroring-first hypothesis which argues that processing action kinematics in the MNS is a prerequisite for inferring others' intentions but the MNS does not process information about others' intentions directly (Hamilton & Marsh, 2013; Spunt, Satpute, & Lieberman, 2011). The mirroring-first model predicts that MNS activation at all time points would be equivalent irrespective of the task, and this was not observed here.

Second, the timing at which mentalizing was found to modulate MEP sizes provides evidence against the motor simulation theory which argues that MNS activity alone is sufficient to infer the intentions of others' from their actions (Rizzolatti & Sinigaglia, 2007). This theory predicts early mentalizing induced modulation of MNS activity given previous studies have shown others' intentions can be inferred during the early phases of movements of their actions (e.g. Manera, Becchio, Cavallo, Sartori, & Castiello, 2011; Sartori, Becchio, & Castiello, 2011). In contrast, we did not observe any early mentalizing induced modulation of MNS activity, rather the late mentalizing-induced modulation of MNS activation identified in our study suggests that early information about an individual's intentions is processed in an alternative system prior to MNS involvement. In addition, we did not see evidence for a relationship between the degree of mentalizing-induced modulation of MNS activity and ability to infer the intentions of others in our study, a Bayes factor of .38 indicated that there was more evidence for the null hypothesis:

that the mentalizing induced signal we observed in the MNS did not determine behavioural performance. A motor-simulation account would predict that MNS activity determines behavioural performance, and thus our data provide evidence against this theory of the role of the MNS in inferring intentions from observed actions.

The lack of a relationship between mentalizing performance and MNS activity supports the dual-process hypothesis which states that subconscious processing of intentional information available from action kinematics occurs in the MNS first before intentions are actively inferred in the mentalizing system (de Lange et al., 2008; Spunt & Lieberman, 2012b). This theory would not predict a direct relationship between MNS activity and mentalizing performance as it argues that the MNS is not involved in actively inferring others' intentions. However, similar to the motor simulation theory, the dual-process hypothesis would also predict early mentalizing-induced modulation of MNS activity, in contrary to our observed results. Instead, the timing of mentalizing-induced modulation of corticospinal activity identified in our study suggests that intentional information is initially processed by another cortical system before MNS involvement. It is possible that predictions about the intentions of unfolding actions, based on prior expectations and action context, are formed in the mentalizing system prior to intentional inferences made based on kinematic information in the MNS (Alexander & Brown, 2011a; Kilner et al., 2007).

The late timing of MNS involvement observed in our study implies that the interaction between the mentalizing system and the MNS, when inferring intentions from actions, might be best conceptualized within a predictive coding framework. The predictive coding theory in combination with the predicted outcome response model of mPFC functioning would suggest that intentions are initially inferred based on contextual information in the mPFC before MNS involvement in processing intentions information. This compliments data from neuroimaging studies which have shown that action context and prior expectations about an upcoming action modulate activity in the mPFC (Alexander & Brown, 2011; Becchio et al., 2012; Chambon et al., 2017b; Cooper, Kreps, Wiebe, Pirkl, & Knutson, 2010; Ferdinand & Opitz, 2014; Fogelson, Shah, Scabini, & Knight, 2009; Forster & Brown, 2011; Jahn, Nee, Alexander, & Brown, 2014; Leue, Cano Rodilla, & Beauducel, 2015; Schiffer, Krause, & Schubotz, 2014) and functional connectivity between the mPFC and the MNS increases when inferring intentions from actions (Ciaramidaro et al., 2014; Spunt & Lieberman, 2012a; 2012b). Action context has also been shown to modulate MNS activity (Amoruso, Finisguerra, & Urgesi, 2016; Amoruso & Urgesi, 2016; Iacoboni, Molnar-Szakacs, Gallese, Buccino, & Mazziotta, 2005), in the later stages of action processing, implying possible top-down connectivity from the mentalizing system to the MNS. If the interaction between the MNS and the mentalizing system is best conceptualised within a predictive coding framework, the higher activation we found in the MNS at the end of observed actions may reflect the signalling of a ‘prediction error’ once the actual intention of an action has been revealed by the kinematics at the end of observed actions. The predictive coding model suggests that prediction errors are formed in the MNS and used to inform future predictions about others’ intentions (cf. Brown & Brüne, 2012; Kilner, Friston, & Frith, 2007; Kilner & Frith, 2008).

Although our data suggest late modulation of the MNS when mentalizing, it is possible that MNS activation was maximal throughout the mentalizing task and a task-related difference was only found at the end of observed actions because this is when MNS activity decreased during the control tasks. This interpretation would mean that the MNS is involved in mentalizing throughout the task. MNS involvement in mentalizing throughout the task without a relationship between MNS activation and mentalizing performance would support the dual-process hypothesis of mentalizing. However, we have no evidence that MNS activation was maximal during the early stages of action processing in our control tasks and previous studies have shown that MNS activation during the early stages of action processing can be enhanced by other factors such as the weight of an object about to be lifted (Alaerts et al., 2012) or expertise (Candidi, Sacheli, Mega, & Aglioti, 2014). These data suggest that in fact, MNS activation was likely not maximal during the early stages of action processing and imply mentalizing only modulates MNS activation at the end of observed actions.

Although corticospinal activity was higher (MEP sizes were larger) during the Mentalizing task than the Action task, MEP sizes were not significantly larger during the Mentalizing task compared to the Either task. These data suggest that higher levels of MNS activation are, to a certain extent, automatically elicited when observing actions that reflect an individual’s intention, regardless of the task participants are engaged in. Both the Mentalizing and the Either tasks involved watching actions that portrayed the actor’s intentions but only the Mentalizing task required the intentions of the actors to be inferred in order to complete the task. The lack of a significant increase in MNS activity during the Mentalizing task compared to the Either task compliments previous studies which found higher levels of MNS activity during the observation of actions with social intent compared to non-social actions even in the absence of a mentalizing task (Bucchioni et al., 2013; Ciaramidaro et al., 2014; Enticott et al., 2013; Iacoboni et al., 2004). Additionally, a previous study found higher MNS activity when viewing actions with unusual intentions regardless of the task participants were performing and no differences in MNS activity associated changes in the mentalizing demand of the task (de Lange et al., 2008). However, MEP sizes in our study were also not significantly different between the Either task and the Action task. This implies that although higher MNS activity was elicited when viewing actions which portrayed the actors' intentions compared to viewing actions which did not portray intent, MNS activity was only significantly higher if participants were engaged in a mentalizing task. In summary, our data suggest that the MNS is involved in inferring others' intentions from their actions. However, MNS activation does not appear to be sufficient in order to do so; our data imply that intentional information is initially processed in another cortical system (possibly the mentalizing system) before the MNS signals the performer's intention once it has been revealed at the end of the observed action.

Although mentalizing-specific MNS activity and performance on the Mentalizing task were not correlated in our study, we should be cautious as performances were extremely high (mean proportion of correct responses: M=.94, SD=.10) across all participants. Potentially, behavioural performances were near ceiling, preventing a relationship between mentalizing performance and the degree of mentalizing-specific MNS activation to be found. If we had also recruited participants with a diagnosis of ASD, it is possible that these individuals would have displayed poorer performances and a wider range of behavioural performances may have allowed a relationship between mentalizing-induced modulation of MNS activity and behavioural performance to be identified. In addition, recruitment of adults with ASD may have allowed a relationship to be identified between autistic traits and mentalizing-induced modulation of corticospinal activity. MNS functioning may be atypical in adults with ASD when inferring others' intentions but reduced levels of MNS activity may only be seen in individuals with clinical levels of autistic traits. This suggestion is supported by a previous study by Holt and colleagues that found reduced activation in IFG in individuals with ASD when inferring others' mental states but this reduction in MNS activity was not found in siblings of those with ASD (Holt et al., 2014). However, the lack of evidence for a relationship between autistic traits and modulation of corticospinal activity in our study supports some previous studies that found adults with ASD exhibited typical levels of MNS activation during mentalizing tasks (e.g. Kana, Keller, Cherkassky, Minshew, & Just, 2009; Kirkovski, Enticott, Hughes, Rossell, & Fitzgerald, 2015). Therefore, it is possible that dysfunction in other brain systems (e.g. the mentalizing system) or dysfunctional connectivity between the MNS and the mentalizing system rather than atypical MNS activity may underlie the mentalizing deficits that individuals with ASD experience (Frith, 2001; Gallagher et al., 2000; Schurz, Radua, Aichhorn, Richlan, & Perner, 2014; Spunt & Lieberman, 2012b; Spunt et al., 2011).

Although the hand actions shown in the Mentalizing and Action tasks had very similar kinematics at the time in which the task-related difference in corticospinal excitability was found, they were not identical (see Figure 3.1). These small kinematic differences between the actions shown in different tasks in principal may have contributed to the task-related difference in MEP sizes. However, in both tasks the poker chip had been released at this time point, and the FDI and OP muscles in the actors' hands were no longer contracted. Previous studies have shown that increased MEP sizes during action observation are specific to the muscles involved in the observed actions (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Fadiga, Craighero, & Olivier, 2005; Urgesi, Candidi, Fabbro, Romani, & Aglioti, 2006). Therefore, if MEP sizes were solely dependent upon the kinematics of the observed actions, MEPs would be expected to be small in both tasks at the end of the observed actions (Fadiga et al., 2005; Fadiga et al., 1995; Urgesi et al., 2006). This was observed during the Action task, MEPs were maximal leading up to, and during interaction with the object and were smallest once the object had been released (see Figure 3.4A). However, during the Mentalizing task, MEP sizes were significantly larger at the end of the actions, even when there was no obvious muscle contraction indicating increased corticospinal excitability was instead a result of processing others' intentions.

The actions shown in both the Mentalizing and Either tasks were always unsuccessful and the actions shown in the Action task were always successful. Possibly action success could explain the differences in MNS activity between the Mentalizing and Action tasks. However, previous studies have shown greater levels of MNS activity during the observation of actions with higher likely reward in both monkeys (Caggiano et al., 2012; Roesch & Olson, 2003, 2004) and humans (Brown, Wiersema, Pourtois, & Brüne, 2013; Trilla Gros, Panasiti, & Chakrabarti, 2015). Given that the actions during our Mentalizing (and Either) tasks were unsuccessful, and thus reflected a lower likelihood of reward than the actions during the Action task, if MNS activity was commensurate with success/reward, we would have observed opposite effects to those that we show here.

In the current study, participants observed right-handed actions and MEPs were recorded from the muscles in their left hands. It has been previously shown that MEPs are smaller when they are measured from the hand which is contralateral to the observed hand executing the action compared to when recordings are made from the observer's hand which is ipsilateral to that of the acting hand (Aziz-Zadeh et al., 2002). In principal, we may have measured larger MEPs if we had applied TMS to the left hemisphere in our study. However, MNS activation is bilateral during the observation and imitation of right-handed actions (Caspers, Zilles, Laird, & Eickhoff, 2010). In addition, a previous fMRI study showed bilateral MNS activation during the observation of right-handed actions but a greater degree of activation in right MNS (Aziz-Zadeh, Iacoboni, Zaidel, Wilson, & Mazziotta, 2004). Higher levels of activity have also been shown in the right MNS when actions are shown in a context that reflect the goals of the actions compared to actions shown without context (Kaplan & Iacoboni, 2006). Higher levels of right IFG activity have also been found when inferring the goals of actions compared to processing action kinematics (Spunt & Lieberman, 2011). Finally, reduced activation in right IFG and bilateral IPL in adults with ASD has also previously been associated with poorer abilities to infer the intentions of others (Kana et al., 2014). Collectively, these data suggest that MNS activation is often bilateral when observing others' actions and suggest possible right-hemisphere dominance for MNS activity when inferring others' intentions.

In conclusion, our data add to the current knowledge of MNS involvement in mentalizing by identifying the timing in which MNS activity is modulated by mentalizing. These data allow us to start to delineate between the different models of MNS involvement in inferring others' intentions. The late timing in which MNS activity was found to be modulated by mentalizing suggests that the MNS plays a role in processing others' intentions but only once they have been clearly revealed. It appears that another cortical system is involved in making early inferences about the intentions of observed actions. MNS activity was also found to be independent of behavioural performance and the level of autistic traits displayed by the participants, suggesting that the MNS play a more automatic role in processing the intentions of others' actions, irrespective of mentalizing ability.

# Chapter 4: Investigating Mirror Neuron System (MNS) Activity in Adults with ASD when Inferring Others' Intentions Using Both TMS and EEG.

This chapter is adapted from: Cole, E.J., Barraclough N. E., Enticott. P.G. (in review). Investigating mirror neuron system (MNS) activity in adults with ASD when inferring others' intentions using both TMS and EEG. *Journal of Autism and Developmental Disorders*.[3]

## 4.1 Abstract

Atypical MNS activation may underlie mentalizing difficulties associated with ASD. We investigated MNS activity in adults with ASD when inferring others' intentions using both TMS and EEG. Eye-tracking data were collected to ensure MNS differences were not due to atypical fixation. Autistic traits were measured for all participants. Our EEG data show reduced right MNS activity in adults with high autistic traits when mentalizing and higher left MNS activity associated with superior mentalizing performances. The eye-tracking and TMS data show no differences associated with autistic traits. Our data suggest ASD is associated with reduced right MNS activity when mentalizing, TMS and EEG measure different aspects of MNS functioning and the MNS is directly involved in inferring others' intentions.

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[3] The author, Eleanor Cole, designed the experiment, collected the data, analysed the results and wrote the manuscript under the joint supervision of A/Prof Peter Enticott and Dr Nick Barraclough.

## 4.2 Introduction

Experimental evidence and anecdotal reports suggest that individuals with autism spectrum disorder (ASD) diagnoses display difficulties inferring the thoughts, feeling and beliefs of others, collectively known as 'mentalizing' (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Castelli, Frith, Happé, & Frith, 2002; Jolliffe & Baron-Cohen, 1999; Kana, Libero, Hu, Deshpande, & Colburn, 2014; Senju, Southgate, White, & Frith, 2009). ASD is a term used by the most recent edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) to describe a number of neurodevelopmental disorders characterised by difficulties in social communication as well as restricted and repetitive behaviours (American Psychiatric Association, 2013). Due to the spectral nature of ASD, individuals with and without ASD diagnoses display varying degrees of autistic traits. Individuals without an ASD diagnosis but who display relatively high levels of autistic traits have also been shown to exhibit mentalizing deficits (Gökçen, Petrides, Hudry, Frederickson, & Smillie, 2014; Gökçen, Frederickson, & Petrides, 2016).

The 'broken mirror' theory of ASD suggests that dysfunction in brain areas known collectively as the mirror neuron system (MNS) underlie some of the social communication difficulties experienced by individuals with ASD (Iacoboni & Dapretto, 2006; Oberman & Ramachandran, 2007). The main components of the human MNS are considered to be the inferior frontal gyrus (IFG) and the inferior parietal lobe ( IPL; Rizzolatti & Craighero, 2004; Rizzolatti & Sinigaglia, 2010). Areas of the MNS are active during the performance of an action as well as the observation of similar actions (di Pellegrino et al., 1992; Rizzolatti et al., 1996). It is thought that by displaying similar activation patterns during the observation of actions as when performing actions, the MNS simulates observed actions in the observer's own motor system to facilitate action understanding (Rizzolatti & Craighero, 2004). This is known as the motor resonance theory (Agnew, Bhakoo, & Puri, 2007; Landmann et al., 2011; Leslie, Johnson-Frey, & Grafton, 2004; Rizzolatti, Fogassi, & Gallese, 2002). According to the broken mirror theory, atypical MNS activation in individuals with ASD results in reduced understanding of the actions of others, which in turn underlies some of the social communication difficulties these individuals experience (Iacoboni & Dapretto, 2006; Oberman & Ramachandran, 2007).

Although the broken mirror hypothesis is an attractive theory, the literature supporting the possibility of atypical MNS activation in individuals with ASD is limited, particularly in adults. A number of studies have shown that children with ASD display behavioural impairments and atypical MNS activity during tasks typically associated with MNS functioning such as imitation (Dapretto et al., 2005; Hobson & Hobson, 2008; Rogers et al., 2003; Williams, Whiten, & Singh, 2004), action planning (Cattaneo et al., 2007; Dowd et al., 2012; Fabbri-Destro, Cattaneo, Boria, & Rizzolatti, 2009) and gestural performance (Dewey et al., 2007). In contrast, some studies have shown that children with ASD display typical behavioural performances (Dapretto et al., 2006; Hamilton, Brindley, & Frith, 2007; Sowden, Koehne, Catmur, Dziobek, & Bird, 2016; Stoit, Van Schie, Slaats-Willemse, & Buitelaar, 2013) and MNS activation levels (Pascolo & Cattarinussi, 2012; Raymaekers, Wiersema, & Roeyers, 2009; Ruysschaert, Warreyn, Wiersema, Oostra, & Roeyers, 2014) when performing these tasks. Adults with ASD generally display typical behavioural performances on tasks traditionally associated with MNS functioning (Bird, Leighton, Press, & Heyes, 2007; Sari Avikainen, Wohlschläger, Liuhanen, Hänninen, & Hari, 2003) and the majority of neuroimaging studies have shown that adults with ASD display typical levels of MNS activity (Avikainen, Kulomäki, & Hari, 1999; Dinstein et al., 2010; Enticott et al., 2013, Marsh & Hamilton, 2011). Only a limited number of studies have provided evidence to suggest MNS activation is atypical during these tasks in adults with ASD (Bernier, Dawson, Webb, & Murias, 2007; Enticott et al., 2012; Honaga et al., 2010; Martineau, Andersson, Barthélémy, Cottier, & Destrieux, 2010) and adults with high levels of autistic traits (Cooper, Simpson, Till, Simmons, & Puzzo, 2013; Lepage, Tremblay, & Théoret, 2010; Puzzo, Cooper, Vetter, Russo, & Fitzgerald, 2009). Therefore, evidence to support general dysfunction of the MNS in ASD, particularly in adults, is limited (Hamilton, 2013).

Despite the limited evidence suggesting atypical MNS activity in adults with ASD during tasks traditionally associated with MNS functioning (e.g. imitation and action planning), adults with ASD have shown reduced MNS activation during mentalizing tasks compared to control participants (Baron-Cohen et al., 1999; Holt et al., 2014; Kana et al., 2014). A wide body of literature has provided evidence for MNS involvement in mentalizing in typically developing adults: higher levels of MNS activity have been shown during mentalizing tasks than non-mentalizing tasks (Adams et al., 2010; Brunet et al., 2000; Centelles et al., 2011; de Lange et al., 2008; Schurz et al., 2014) and higher MNS activation has also been elicited during the observation of actions with social context compared to non-social actions even in the absence of mentalizing tasks (Bucchioni, Cavallo, Ippolito, Marton, & Castiello, 2013; Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; Enticott et al., 2013; Iacoboni et al., 2004). Additionally, lesions to IFG, both in brain damaged patients (Besharati et al., 2016; Dal Monte et al., 2014) and when temporary functional lesions are induced via direct current stimulation in patients undergoing surgery to treat epilepsy (Herbet, Lafargue, Moritz-Gasser, Bonnetblanc, & Duffau, 2014), have been shown to impair mentalizing performances. Collectively, these data show that MNS has a role in mentalizing and that MNS functioning is atypical in adults with ASD when the mentalizing system is engaged. Therefore, it is possible that, reduced MNS activity during mentalizing tasks may contribute to the mentalizing difficulties these adults experience.

Despite numerous studies providing evidence for a role of the MNS in mentalizing, some mentalizing tasks have not elicited higher levels of MNS activity compared to non-mentalizing tasks in typically developing participants (Castelli, Happé, Frith, & Frith, 2000; Castelli et al., 2002; Gallagher et al., 2000; Spunt, Satpute, & Lieberman, 2011; White, Frith, Rellecke, Al-Noor, & Gilbert, 2014). Differences in the stimuli used are likely to have contributed to inconsistencies in the existing literature. Neuroimaging studies have shown that different brain areas are active during mentalizing tasks depending on the stimuli used (Gobbini et al., 2007; Schurz et al., 2014). Mentalizing tasks have been shown to elicit more MNS activation when dynamic stimuli are used rather than static stimuli and when stimuli depict bodies rather than faces (Schlochtermeier, Pehrs, Kappelhoff & Jacobs, 2015). The majority of mentalizing tasks that have not elicited MNS activation have used simplistic cartoons, still images or passages of text as stimuli (Castelli et al., 2000; Castelli et al., 2002; Gallagher et al., 2000; White et al., 2014). If MNS functioning is atypical in adults with ASD during mentalizing tasks then these individuals may display more prominent differences in brain activation and greater behavioural impairments on mentalizing tasks that typically elicit greater levels of MNS activity.

Transcranial magnetic stimulation (TMS) and electroencephalography (EEG) are two techniques that have often been used to non-invasively measure MNS activity, but it is unknown precisely how these two indices of MNS activity relate to each other. TMS involves administering brief magnetic pulses through a magnetic coil placed on the scalp in order to induce transient changes in activity in the underlying region of the cortex (Hallett, 2000). When single TMS pulses are applied to the primary motor cortex (M1), the resulting increases in corticospinal activity can be measured by recording increased activity in contralateral hand muscles via electromyography ( EMG; Aziz-Zadeh, Maeda, Zaidel, Mazziotta, & Iacoboni, 2002; Fadiga, Craighero, & Olivier, 2005). These increases in muscle activity induced by TMS (known as motor evoked potentials; MEPs) are larger when individuals view hand actions compared to when TMS is applied at rest and therefore these increases in MEP sizes during action observation are regarded as an index of MNS activity (Luciano Fadiga et al., 2005a; Patuzzo et al., 2003; Strafella & Paus, 2000). In contrast, *mu* rhythm; large amplitude oscillations in the alpha frequency band (8-12Hz) over sensorimotor cortex detected by EEG, is suppressed during action observation as well as the performance of actions and thereby provides another index of MNS activity (Fox et al., 2016; Frenkel-Toledo, Bentin, Perry, Liebermann, & Soroker, 2014; Oberman, Pineda, & Ramachandran, 2007). Two previous studies that have combined EEG and single-pulse TMS have shown that although measurements from both these techniques are sensitive to motor resonance mechanisms, they are not correlated with each other (Andrews, Enticott, Hoy, Thomson, & Fitzgerald, 2015; Lepage, Saint-Amour, & Théoret, 2008). Therefore, it is possible that these measurements reflect different aspects of MNS functioning. It is important to note that these indices of MNS activity also differ in their spatial and temporal properties; EEG measures the sum of post-synaptic neuronal activity over a large area, and an index of mu suppression is typically taken over a relatively long time period (i.e., >1s). By contrast, TMS measures brief induced increases in corticospinal activity from peripheral muscles (Andrews et al., 2015; Pineda, 2005; Rossini et al., 1994). Using both of these non-invasive measures of MNS activity simultaneously allows a more complete picture of MNS functioning to be collected.

This study aimed to investigate whether adults with diagnoses of ASD display atypical MNS activity when mentalizing and whether levels of MNS activation correspond to mentalizing performance. In this study, participants watched hand action videos, and after each video they had to either make decisions about the intention of the actor (mentalizing task) or the success of the action (non-mentalizing task). The video stimuli used showed different actors performing naturalistic hand actions to ensure the stimuli were sufficiently complex and optimally activated the MNS. TMS-induced MEPs and *mu* suppression were both used as indices of MNS activity. A preliminary TMS study, using the same stimuli, identified higher MNS activation during a mentalizing task compared to a non-mentalizing task once the actors' intentions had been revealed (Cole & Barraclough, in review). Therefore, in our study, single-pulse TMS was applied to the primary motor cortex (M1) at the end of each hand action when the outcome of the action or the intention of the actor had been revealed. Simultaneous EEG recordings were made throughout the experiment. It was predicted that larger TMS-induced MEP sizes and greater levels of *mu* suppression would be found during the mentalizing task compared to the non-mentalizing task in adults without ASD diagnoses, indicating higher levels of MNS activity. It was also predicted that ASD would be associated with reduced task-related changes in MNS activity and that lower levels of MNS activity would be related to poorer mentalizing performances.

## 4.3 Methods

### *4.3.1 Participants*

Forty-three adults were recruited for this study, of which 13 had a formal diagnosis of either Asperger's disorder (11) or Autism Spectrum Disorder (ASD). All of the participants with a diagnosis met the DSM-5 criteria for ASD and none of the participants had any existing learning difficulties or experienced delayed language development. Participants without an ASD diagnosis were recruited based on the level of autistic traits they displayed as measured by the Autistic Quotient (AQ; Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001). The average AQ score in the general population is 16.94 (Ruzich et al., 2015). Individuals were excluded from the study if they had AQ scores between 16-19. Participants with scores between 0-15 were assigned to the 'low AQ' group and participants with AQ scores higher than 20 were assigned to the 'high AQ' group. This resulted in three participant groups: low AQ (n=15), high AQ (n=15) and ASD (n=13). The participant groups did not significantly differ in age, verbal IQ, gender or years of formal education and all participants had verbal IQ scores within the normal range (>70; see Table 4.1).

Table 4.1

*Demographic information; group mean (SD) values*

| | Low AQ | High AQ | ASD | p | $\eta_p^2$ |
|---|---|---|---|---|---|
| N | 15 | 15 | 13 | | |
| Age | 23.40 (6.82) | 24.13(4.68) | 28.30 (9.40) | 0.16 | 0.09 |
| Gender (m:f) | 8:7 | 7:8 | 9:4 | 0.47 ($X^2$) | / |
| Years of formal Education | 15.60 (1.64) | 16.20 (1.66) | 15.38 (1.45) | 0.37 | 0.05 |
| Verbal IQ[1] | 109.67 (14.09) | 113.00 (9.22) | 11.62 (14.98) | 0.78 | 0.01 |

[1]The verbal IQ scores were measured using the test of pre-morbid functioning.

p values were obtained from one-way MANOVA unless otherwise stated

All participants were screened for symptoms of psychiatric disorders using the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). Individuals were not eligible to take part in this study if they were diagnosed with any psychiatric disorders or were identified by the MINI as displaying symptoms of any psychiatric disorders. An exception was made for mood disorders, anxiety and ADHD in the participants with ASD due to the high prevalence of these comorbidities (Matson, Rieske, & Williams, 2013; Matson & Williams, 2014). In the ASD group, six participants were taking psychotropic medication to treat ADHD, depression or anxiety (see Table 4.2). None of the participants without an ASD diagnosis were taking psychotropic medication.

Table 4.2

*Medication information for participants in the ASD group*

| Participant | Medication (daily dosage) |
|---|---|
| 1 | Dexamphetamine (20mg), Zoloft (150mg) |
| 2 | Dexamphetamine (20mg) |
| 3 | Ritalin (30 mg) |
| 4 | Valium (5 mg when needed) |
| 5 | Zoloft (50mg) |
| 6 | Zoloft (50mg) |

Participants were also screened for contraindications for TMS; history of seizures, serious head injuries, brain related conditions, severe headaches, implanted metal or medical devices, family history of epilepsy and current pregnancy (Rossi, Hallett, Rossini, & Pascual-Leone, 2009).

This research project was approved by the Human Research Ethics Committee at Deakin University and was performed in accordance with the ethical standards laid down in the 1990 Declaration of Helsinki. All participants provided signed informed consent.

### *4.3.2 Psychological Assessments*

All participants completed the Autism Quotient (AQ), Autism Diagnostic Observation Schedule (ADOS-2), The Awareness of Social Inference Test (TASIT), Social Responsiveness Scale (SRS-2) and Empathy Quotient (EQ). The AQ and ADOS-2 are designed to measure the level of autistic traits displayed, the SRS-2 and TASIT measure social functioning and the EQ provides a measure of empathy. The three groups significantly differed from each other on all these measures (see Table 4.1). These psychological tests have been shown to display good psychometric properties (Allison et al., 2011; Hurst et al., 2007; S McDonald et al., 2006; Oosterling et al., 2010). The AQ was administered in the form of an online questionnaire before the participants took part in the experiment. The other assessments were administered at the Cognitive Neuroscience Unit at Deakin University as a two-hour session prior to the TMS testing session. Psychological assessment sessions always took place on a separate date to the TMS session and both sessions were completed within a 2 week time frame.

### *4.3.3 Experimental set-up*

Participants sat 600mm away from an Eyelink 1000 plus eye-tracker (SR Research, Ontario) placed in front of a 24" LED computer monitor. Single EEG electrodes were placed at locations FCz, F3 and F4 according to the international 10-20 system of electrode placement. Typically, EEG recordings are taken from central electrodes (C3, C4, Cz) when investigating MNS activity. However, due to the placement of the TMS coil over the primary motor cortex (M1) EEG recordings were taken from frontal electrodes (F3, F4, FCz) to reduce TMS-induced artefacts, and to allow sufficient contact between the TMS coil and the scalp. It has previously been shown that *mu* suppression can be measured across the entire scalp when observing and imitating hand actions and ASD participants have been shown to display differences in *mu* power over frontal regions (Dumas, Soussignan, Hugueville, Martinerie, & Nadel, 2014). Reference electrodes were placed on the left and right mastoid bones and the ground electrode was placed on the forehead. Electrooculogram (EOG) electrodes were placed above and below the left eye to in order to identify EEG artefacts caused by blinking. EEG signals were recorded using Curry Neuroimaging Suite 7 (Compumetics Ltd, Australia). EEG signals were amplified using NeuroScan SynAmps RT (NeuroScan SynAmps, Compumedics Ltd.) and digitised at 1kHz. All electrode impedances were below 5KΩ. EEG analyses and bandpass filtering were conducted offline.

TMS was administered using a Magstim BiStim$^2$ stimulator (Magstim Company Ltd., Carmarthenshire, Wales, UK). Firstly, the location of the primary motor cortex (M1) was identified in each participant by measuring the position on the scalp five centimetres lateral and one centimetre anterior to Cz (according to the international 10-20 system of electrode placement). TMS pulses were then applied to this position on the scalp using a standard figure of eight 70mm coil held tangentially over the scalp at a 45 degree angle to the midline. An initial intensity of 40% stimulator output was used and then the intensity of TMS stimulation was increased in 5% increments until MEPs were produced. Stimulation was also applied around the estimated location of M1 in order to confirm that this was the optimal scalp location to produce motor evoked potentials (MEPs) in muscles of the right hand. MEPs were measured from the first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles using Ag/AgCl surface electrodes. EMG signals were amplified using PowerLab 4/35 (with dual BioAmp; AD Instruments, Colorado Springs, CO). Once the optimal location for stimulation had been identified, the intensity of the TMS stimulation was adjusted in order to find the participants' resting motor threshold (RMT). Participants' RMT was defined as the minimum stimulation intensity needed to induce MEPs with an average peak-to-peak magnitude of 1mV over 5 consecutive trials. Twenty MEPs induced by stimulation at RMT were used as a measure of baseline corticospinal excitability (CSE).

### *4.3.4 Experimental Task*

The experiment comprised of two tasks; a mentalizing task and a non-mentalizing task. In both tasks, participants watched short videos (4 seconds) of actors passing or attempting to pass a poker chip through slots in a wooden board to another person on the other side of the board (who was out of view; see Figure 4.1). Grasping and pushing actions were shown; grasping actions involved actors grasping a poker chip and placing it through a slot at head height, pushing actions involved pushing the poker chip with the index finger through a slot that was level with the table in front of them (see Figure 4.1). After each video, participants were asked to make a decision about the action and indicated their response by pressing buttons on the computer keyboard with their left hand. Videos were presented in mentalizing and non-mentalizing blocks. In the mentalizing block, participants watched videos that either showed an actor accidentally dropping a poker chip and therefore failing to pass the poker chip to the other player ('clumsy' action) or an actor deliberately not passing the poker chip ('spiteful' action). After each video, participants indicated whether they thought the action was 'clumsy' or 'spiteful'. In the non-mentalizing block, participants watched videos in which actors either successfully passed the poker chip to the other player (successful action) or accidentally dropped the poker chip (unsuccessful action). After each video, participants had to indicate whether the action was 'successful' or 'unsuccessful'. The unsuccessful actions shown in the non-mentalizing block were the same as the ‘clumsy’ actions shown in the mentalizing block. The videos shown in this experiment are a subset of the videos used in a previous study (Cole, Slocombe & Barraclough, 2017). Twenty actors (10 male) were shown performing each action (clumsy grasp, clumsy push, spiteful grasp, spiteful push, successful grasp, successful push) resulting in 80 action videos per block (clumsy actions were seen in both blocks). Two types of actions (grasping and pushing) were used in order to make the stimuli more varied and these particular actions were chosen as they both utilise the FDI muscle.

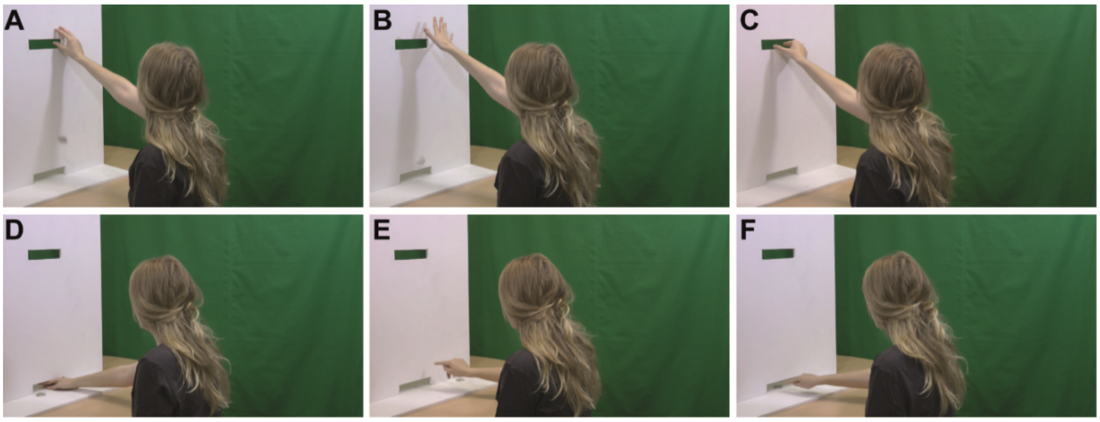

**Figure 4.1** Screenshots depicting the final frame of the video stimuli for one actor. The videos depict an actor: A. Accidently dropping a poker chip (clumsy grasp). B. Deliberately dropping a poker chip (spiteful grasp). C. Passing a poker chip through the higher slot in the board (successful grasp) D. Accidentally not passing a poker chip through the bottom slot (clumsy push) E. Deliberately not passing a poker chip through the bottom slot (spiteful push) F. Passing a poker chip through the bottom slot (successful push).

A single TMS pulse at 1mV RMT was delivered at the end of each video. A light sensor was used in order to time lock the TMS pulses to the timing of the videos. A black square was added to the top left corner of the videos and this black square was replaced with a white square for the last three frames in each video. The light sensor detected this change and sent a 5V TTL pulse to the TMS stimulator via a BNC cable which triggered a single TMS pulse to be fired. The TMS machine subsequently sent a trigger to a PowerLab 4/35 (ADInstruments Pty Ltd) to trigger EMG recording. EEG was continuously recorded throughout both the mentalizing and non-mentalizing tasks but triggers were sent to the EEG machine at the start of each trial to record the type of action being shown. The order in which mentalizing and non-mentalizing blocks were completed was counterbalanced across all participants and within each participant group. Once participants had completed both the mentalizing and the non-mentalizing task, twenty single TMS pulses were administered at RMT in order to compare baseline corticospinal excitability before and after the experiment.

### *4.3.5 Behavioural Analysis*

First, the ADOS-2, AQ, EQ, SRS-2 and TASIT scores were calculated and a one-way MANOVA was used to identify group differences in these scores. Then, the numbers of correct responses on the mentalizing and non-mentalizing tasks were calculated for each participant. Data screening identified that the behavioural data were not normally distributed and therefore a log transformation was applied. The log transformed data still violated the assumption of normality so non-parametric analyses were conducted. Potential group differences in behavioural performances were explored using a Kruskal-Wallis test and a possible task-related difference in performances across all participants was examined using a Wilcoxon signed rank test.

### *4.3.6 EMG analysis*

TMS was not performed on two participants in the high AQ group and four participants in the ASD group; two participants in the ASD group and one participant in the high AQ group found TMS too uncomfortable and the remaining three participants had motor thresholds deemed too high to continue (>75% stimulator output). Trials in which muscle activity was identified within a 200ms time window before the TMS pulse or trials in which FDI peak to peak MEP amplitudes were smaller than .2mV were removed from the analysis (4.02% of all MEPs were excluded). Two participants in the high AQ group were removed from the analyses for having only 50% or fewer valid FDI MEPs for either task. This resulted in 35 participants (15 low AQ, 11 high AQ and 9 ASD) being included in the EMG analysis.

Preliminary analyses were carried out on the EMG data in order to clarify that RMTs were not significantly different between groups, that the experiment did not alter participants' resting corticospinal activity and that the number of excluded MEPs did not significantly differ across tasks or participant groups. Group differences in RMTs were investigated using a one-way ANOVA. Changes in corticospinal activity as a result of the experiment were investigated by first calculating median MEP sizes (peak-to-peak amplitude [mV]) for both the 20 single TMS pulses given before the experiment and after the experiment for both muscles. Then, separate mixed-model ANOVAs were performed for each muscle investigating the influences of group (low AQ, high AQ, ASD) and time point (before or after the experiment) on MEP sizes. The data regarding the number of excluded MEPs violated the assumption of normality even after a log transformation was applied so non-parametric tests were used. An independent-samples Kruskal-Wallis test was used to investigate group differences and a related-samples Wilcoxon signed rank test was used to investigate differences in the number of MEPs excluded between tasks.

For the main TMS data analyses, median MEP values were calculated for both the FDI and the ADM muscles for each participant and each task (mentalizing/non-mentalizing). Median baseline MEP values were also calculated for both muscles for each participant by combining MEPs from both pre- and post-experiment baseline measures. The raw median MEP values for each task were then converted into motor resonance values by computing the relative MEP sizes in comparison to MEP sizes at baseline:

MR = [(median MEP during task-median MEP at baseline)/median MEP at baseline]*100

Data screening found that the motor resonance data for both FDI and ADM muscles violated the assumption of normality so a log transformation was used. This transformation cannot be performed on negative values so a constant of 100 was added to each motor resonance value prior to transformation to ensure that all values were positive. After the log transformation, the distribution of the FDI data did not significantly differ from a normal distribution but the ADM data still violated the assumption of normality. Therefore, parametric analyses were used for the log transformed FDI data, but non-parametric analyses were conducted on the log transformed ADM muscle data.

The FDI motor resonance data were analysed using a mixed-model ANOVA to investigate the influences of group (low AQ, high AQ, ASD) and task (mentalizing/non-mentalizing) on MEP sizes. Potential group differences in the ADM motor resonance data were investigated using a Kruskal-Wallis test and potential differences in motor resonances across experimental tasks were explored using a Wilcoxon signed rank test.

### *4.3.7 EEG Analysis*

Offline analyses of the EEG data were performed using Curry 7 Neuroimaging Suite software (Compumetics Ltd, Australia). Epochs of EEG data were created for videos shown in each task (mentalizing and non-mentalizing). Although, the action videos were 4000ms long, the last 350ms of each video was removed in order to eliminate the artefact created by the TMS pulse. Therefore, each video epoch was 3650ms long and 80 epochs of each type were created for every participant. EEG data collected when participants were viewing a fixation cross were used as a baseline measure. There were 160 fixation cross epochs (80 for each task), each 1500ms long. The first 500ms of each fixation cross epoch were removed from the analysis because the fixation cross was shown directly after participants were required to make a response and therefore removing the first 500ms reduced the possibility of increased *mu* power during fixation as a result of participants moving their left hand back to a resting position after they had made their responses. This resulted in 160 fixation epochs for each participant that were each 1000ms long.

EEG data were baseline corrected and band-pass filtered (1-30Hz). Data from the EOG electrodes were used to identify blink artefacts and these were removed using a threshold reduction method. Any epochs that still contained non-cerebral artefacts (>75 µV) were identified and removed from the analysis. Two participants were removed from the analysis (one participant from the high AQ group and one participant from the ASD group) because 62.5% or less of the epochs were valid for one or more of the individual conditions (mentalizing videos, non-mentalizing videos, mentalizing fixation crosses, non-mentalizing fixation crosses). Excluding these participants, only 5.6% of epochs were invalid across all participants. Preliminary analyses were carried out on the EEG data in order to clarify that the number of epochs that were excluded did not significantly differ between participant groups or experimental conditions. The numbers of excluded epochs were not normally distributed even after a log transformation was applied so non-parametric analyses were conducted. A Kruskal-Wallis test was used to investigate group differences in the number of epochs excluded and a Friedman's ANOVA was used to identify differences in the number of excluded epochs across experimental conditions.

A Fast Fourier Transform (FFT) was used to calculate *mu* power in both the low alpha frequency range (8-10Hz) and high alpha frequency range (10-12Hz) during all epochs. The majority of previous studies investigating MNS activity using EEG have used activity in the entire alpha frequency band (8-12/13Hz) as a measure of *mu* power (Andrews et al., 2015; Oberman et al., 2005; Oberman, Ramachandran, & Pineda, 2008; Perry, Stein, & Bentin, 2011; Ulloa & Pineda, 2007). However, there is accumulating evidence to suggest that lower (8-10Hz) and higher (10-12Hz) alpha bands reflect different processes and should therefore be analysed separately (Dumas et al., 2014; Frenkel-Toledo et al., 2014; Neuper, Scherer, Wriessnegger, & Pfurtscheller, 2009; Pfurtscheller, Neuper, & Krausz, 2000). Additionally, a previous study found reduced *mu* suppression in the 10-12Hz range over frontal regions in adults with ASD but not the 8-10Hz range when observing hand movements (Dumas et al., 2014). Consequently, lower and higher mu frequency bands were analysed separately in this study.

Average *mu* power in both frequency bands was then calculated for each epoch type (four epoch types: mentalizing videos, non-mentalizing videos, mentalizing fixation and non-mentalizing fixation) for every participant. The degree of *mu* suppression during each experimental condition was calculated by comparing average *mu* power during the video epochs compared to the fixation epochs in the same condition:[(fixation-video)/fixation]*100. Larger values indicated greater degrees of *mu* suppression. The *mu* suppression data for both frequency bands violated the assumption of normality so the data were log transformed. Log transformations cannot be carried out on negative values so a constant of 1300 was added to each data point to ensure that all values were positive before the log transformation. After the log transformation was applied, the data still violated the assumption of normality and therefore non-parametric analyses were conducted. Kruskal-Wallis tests were used to investigate group differences in *mu* suppression and related-samples Wilcoxon signed rank tests were carried out to investigate task-related changes in *mu* suppression. Analyses were carried out separately for both frequency bands.

### *4.3.8 Eye-tracking Analysis*

The eye-tracking data were analysed using EyeLink DataViewer software (SR Research Ltd., Ontario, Canada). Three dynamic rectangular regions of interest (ROIs) were created for each action video individually. These ROIs corresponded to the head of the actor, the actor’s hand and the poker chip (see Figure 4.2). These three interest areas were chosen based on eye-tracking data from a previous behavioural study using the same stimuli (Cole, Slocombe & Barraclough, 2017). The total number and total duration of fixations in each ROI during each task (mentalizing/non-mentalizing) were calculated for each participant. ROIs were analysed separately as these data are not independent (participants cannot fixate in more than one ROI at once). The eye-tracking data were not normally distributed even after a log transformation was applied and therefore non-parametric analyses were conducted. Independent-samples Kruskal-Wallis tests were used to investigate group differences and related-samples Wilcoxon signed rank tests examined differences between tasks for each ROI.

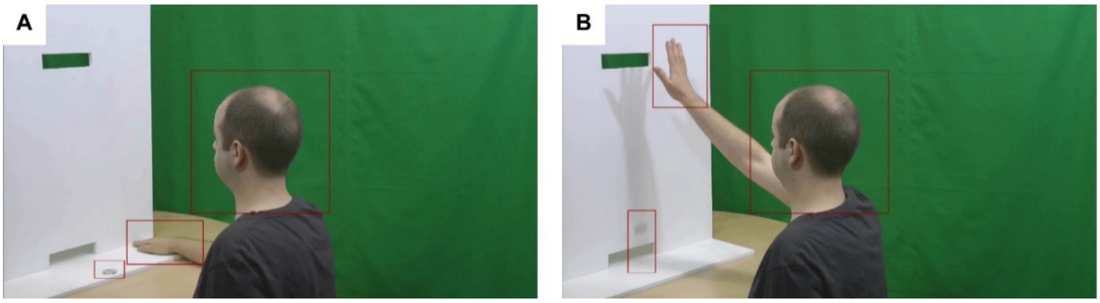

**Figure 4.2** The dynamic regions of interest (ROIs) used in the eye-tracking data analysis for one of the action videos are shown overlaid onto screenshots from (A) the start and (B) the end of that particular video. Three dynamic ROIs corresponding to 1. The poker chip, 2. The actor's head and 3. The actor's hand, were created for each of the 120 videos individually.

### *4.3.9 Additional analyses*

For all analyses (behavioural, EMG, EEG and eye-tracking), any significant task-related differences that were identified were investigated further by analysing the data collected during the presentation of clumsy actions across the two tasks. Identical clumsy actions were shown during both the mentalizing and non-mentalizing tasks. Analysing the data in this way eliminates the possibility that apparent effects of the task are due to differences in observed action kinematics.

Due to the spectral nature of ASD, any significant group differences that were found were also examined across the continuum of autistic traits. A principal components analysis (PCA) was performed on all the psychological test scores in order to obtain a single score for each participant that reflected the level of autistic traits that they displayed. Linear regression analyses were then used to examine whether the levels of autistic traits significantly predicted the outcome variable e.g. levels of *mu* suppression. These additional analyses were conducted to further support the relationships between the outcome variables and ASD.

## 4.4 Results

### *4.4.1 Psychological tests*

A one-way MANOVA identified that scores on all psychological tests (ADOS-2, AQ, EQ, TASIT and SRS-2) were significantly different between groups (see Table 4.3). Post-hoc pairwise comparisons identified that all groups were significantly different from each other on the ADOS-2, AQ and SRS-2 measures (Bonferroni correction applied; see Table 4.4). The high AQ group and the ASD group did not display significantly different TASIT scores and EQ scores did not significantly differ between low and high AQ groups. All other group comparisons were significant (see Table 4.4). In all cases, where significant group differences were found, the ASD group had scores that reflected the highest level of autistic traits and the low AQ group had scores reflected the lowest level of autistic traits.

Table 4.3

*Participants' psychological test scores; group mean (SD) values*

| | Low AQ | High AQ | ASD | p | $\eta_p^2$ |
|---|---|---|---|---|---|
| AQ | 8.80 (4.38) | 24.07 (4.27) | 32.00 (6.67) | <.001 | 0.79 |
| EQ | 53.07 (11.82) | 42.60 (13.61) | 27.23 (12.09) | <.001 | 0.43 |
| TASIT[1] | 58.73 (3.10) | 54.07 (4.13) | 52.38 (5.49) | 0.001 | 0.30 |
| ADOS-2 | 1.40 (.99) | 4.67 (1.40) | 8.54 (3.28) | <.001 | 0.78 |
| SRS-2[2] | 27.87 (15.70) | 58.60 (19.10) | 101.46 (26.00) | <.001 | 0.69 |

[1] The TASIT scores were obtained from part 3 (social inference test).

[2] The reported SRS-2 scores are the unstandardized, raw scores, where scores above 60 indicate some social impairment and scores above 75 reflect severe social impairment.

Table 4.4

*Bonferroni post-hoc pairwise comparisons for psychological test scores*

| | Low AQ-High AQ | Low AQ-ASD | High AQ-ASD |
|---|---|---|---|
| AQ | p<.001 | p<.001 | p=.001 |
| EQ | p=.08 | p=.001 | p<.01 |
| TASIT | p=.02 | p=.001 | p=.92 |
| ADOS | p<.001 | p<.001 | p<.001 |
| SRS | p=.001 | p<.001 | p<.001 |

Principal component analysis (PCA) was conducted using the psychological test scores in order to obtain a single value for each participant that represented the level of autistic traits that they displayed. The psychological test scores correlated with each other (all rs>.35) meaning that they were suitable for PCA. The Kasier-Meyer-Olkin measure of sampling accuracy was .84 (above .6), Barlett's test of sphericity was significant $\chi^2(10)=146.07$, p<.001 and the communalities were all above .7 which collectively supported the inclusion of all the psychological tests in the PCA. PCA with varimax rotation was used. The initial eigenvalues from the PCA analysis showed that one factor (with an eigenvalue of 3.57) explained 71.36% of the variance in psychological test scores. No other factors had eigenvalues higher than Kaiser's criteria of 1 and therefore only one factor was extracted. This factor was labelled 'autistic traits'.

### *4.4.2 EEG data*

#### *4.4.2.1 8-10Hz*

##### *4.4.2.1.1 Group differences*

There were significant differences in the levels of mu suppression in the 8-10 frequency band between groups during the mentalizing task at F4 (H(2)=6.21, p<.05;

see Table 4.5). Additionally, linear regression analysis demonstrated that the level of autistic traits that participants displayed significantly predicted the amount of *mu* suppression in 8-10Hz band at F4 during the mentalizing task [F(1,38)=.47, p=.04, $R^2$=.11; see Figure 4.3]. Pairwise-comparisons with adjusted p values demonstrated a borderline significant difference between the high AQ group and the ASD group (p=.05, r=.47; with lower levels of *mu* suppression in the ASD group). After applying a Bonferroni correction, the new significance threshold was p=.02 (.05/ 3) meaning that this borderline significant difference between the high AQ group and ASD group did not survive correction for multiple comparisons. There were also no significant differences between ASD and low AQ groups (p=.18, r=.37) or the between the low and high AQ groups (p=1.00, r=-.10).

Table 4.5

*Mu Suppression (8-10Hz): Median (IQR) values*

| | | Low AQ | High AQ | ASD |
|---|---|---|---|---|
| 8-10Hz | F3 Mentalizing | 66.40 (73.62) | 76.14 (159.40) | 64.07 (26.80) |
| | F3 Non-mentalizing | 80.25 (20.02) | 63.85 (34.49) | 80.94 (15.34) |
| | FCz Mentalizing | 74.42 (25.32) | 75.70 (80.67) | 58.88 (64.91) |
| | FCz Non-mentalizing | 74.61 (28.31) | 62.09 (38.42) | 78.19 (14.02) |
| | F4 Mentalizing | 74.59 (66.71) | 79.23 (40.92) | 46.19 (116.16) |
| | F4 Non-mentalizing | 71.91 (37.54) | 60.95 (49.27) | 75.91 (21.36) |

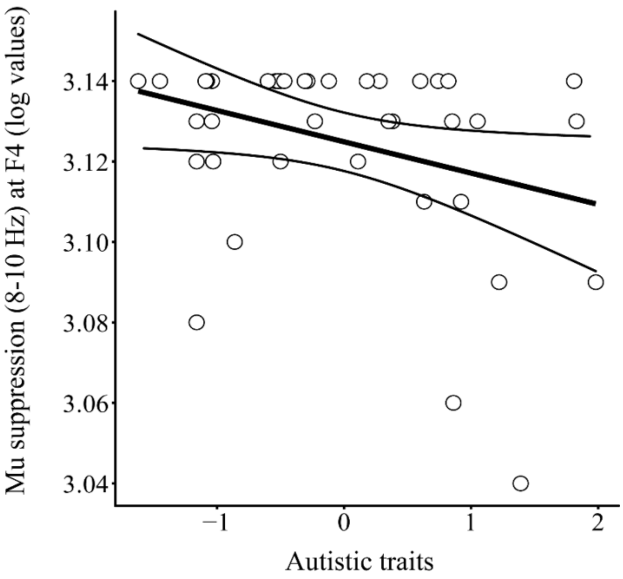

**Figure 4.3** The relationship between the level of autistic traits that participants displayed and the level of *mu* suppression in the 8-10Hz frequency range at F4. Levels of autistic traits significantly predicted the degree of *mu* suppression at F4; participants that exhibited higher levels of autistic traits showed lower levels of *mu* suppression (8-10Hz) at F4 [F(1,38)=.47, p=.04, $R^2$=.11]. The curved lines represent 95% confidence intervals.

*Mu* suppression in the 8-10Hz frequency band was significantly different between groups during the non-mentalizing task at F3 (H(2)=10.10, p=.006) and FCZ (H(2)=7.32, p=.03). Pairwise comparisons showed that the high AQ group displayed significantly lower levels of mu suppression than the low AQ group at F3 (p=.01, r=.54). No other group differences were significant once threshold significance values had been adjusted using the Bonferroni correction (p=.02; see supplementary material). Linear regression analysis demonstrated that the level of autistic traits that participants displayed was not a significant predictor of the amount of *mu* suppression in 8-10Hz band during the non-mentalizing task at F3 [F(1,38)=.02, p=.90, $R^2$<.001] or FCZ [F(1,38)=.03, p=.86, $R^2$<.01].

There were no other significant group differences in *mu* suppression in the 8-10Hz frequency band (see supplementary material).

#### *4.4.2.1.2 Task-related differences*

Initial analyses identified that *mu* suppression in the 8-10Hz band was significantly lower during the mentalizing task than the non-mentalizing task at F3 across all participants (T=581, p=.02, r=.38). However, when this apparent significant task-related difference in *mu* suppression was investigated using data from the clumsy actions alone (in order to control for differences in action kinematics), there was no significant difference in *mu* suppression in the 8-10Hz range between clumsy actions shown in the mentalizing task compared to the non-mentalizing task (T=518, p=.15, r=.23). There were also no task-related differences in *mu* suppression in the 8-10Hz band at FCZ (T=501, p=.21, r=.20) or F4 (T=495, p=.25, r=.18).

#### *4.4.2.2 10-12Hz*

There were no significant differences in *mu* suppression in the 10-12Hz frequency band between groups or across tasks at any of the cortical sites (see Table 4.6 and supplementary material).

Table 4.6

*Mu Suppression (10-12Hz): Median (IQR) values*

| | | Low AQ | High AQ | ASD |
|---|---|---|---|---|
| 10-12Hz | F3 Mentalizing | 82.10 (64.85) | 71.27 (51.51) | 62.40 (63.67) |
| | F3 Non-mentalizing | 73.34 (40.03) | 72.09 (43.26) | 70.91 (36.56) |
| | FCz Mentalizing | 73.52 (48.86) | 66.79 (36.94) | 71.03 (96.40) |
| | FCz Non-mentalizing | 72.58 (42.96) | 72.60 (48.57) | 78.43 (29.70) |
| | F4 Mentalizing | 83.38 (37.30) | 73.15 (27.42) | 69.86 (.40) |
| | F4 Non-mentalizing | 71.80 (23.32) | 68.72 (26.15) | 78.20 (23.88) |

### *4.4.3 TMS data*

Across all participants, there was no significant difference in motor resonance values in the FDI muscle between the mentalizing and non-mentalizing tasks (F(1,32)=.30, p=.59, $\eta_p^2$<.01), there was no significant interaction between participant group and the task (F(2,32)=.73, p=.49, $\eta_p^2$=.04) and there were no significant group differences in motor resonance values (F(2, 32)=.73, p=.49, $\eta_p^2$=.04; see Table 4.7 for motor resonance values).

There were also no significant results for the ADM muscle (see Table 4.8 and supplementary material).

Table 4.7

*Motor resonance log values FDI muscle: Mean (SD) values*

| | Mentalizing task | Non-mentalizing task |
|---|---|---|
| Low AQ | 2.15 (.24) | 2.17 (.23) |
| High AQ | 2.16 (.23) | 2.14 (.24) |
| ASD | 2.07 (.22) | 2.03 (.32) |

Table 4.8

*Motor resonance log values ADM muscle: Median (IQR) values*

| | Mentalizing task | Non-mentalizing task |
|---|---|---|
| Low AQ | 2.02 (.56) | 2.14 (.59) |
| High AQ | 2.00 (.28) | 2.00 (.25) |
| ASD | 1.94 (.41) | 1.96 (.52) |

### *4.4.4 Eye-tracking*

Across all participants, significantly more and longer fixations were made in the hand and head ROIs during the mentalizing task compared to the non-mentalizing task [hand ROI: number: (T=169, p<.001, r=-.52), duration: (T=288, p= .03, r=-.33); head ROI: number: (T=271, p=.02, r=-.34), duration: (T=344, p= .02, r=-.35)]. See Tables 4.8 and 4.9 for descriptive statistics. There was borderline significantly more fixations in the poker chip ROI during the mentalizing than the non-mentalizing task

(T=297, p=.05, r=.29). However, there was no significant task-related difference in the total duration of fixations within the poker chip ROI (T=431, p=.61, r=-.08).

Table 4.8

*Number of fixations: Median (IQR) values*

| | | Low AQ | High AQ | ASD |
|---|---|---|---|---|
| Mentalizing | Hand ROI | 132.00 (89.00) | 154.00 (129.00) | 206.00 (157.20) |
| | Head ROI | 183.00 (90.00) | 203.00 (126.00) | 161.00 (126.50) |
| | Poker chip ROI | 90.00 (80.00) | 93.00 (62.00) | 92.00 (78.50) |
| Non-mentalizing | Hand ROI | 103.00 (37.00) | 115.00 (69.00) | 139.00 (73.00) |
| | Head ROI | 158.00 (102.00) | 201.00 (1.03) | 169.00 (109.00) |
| | Poker chip ROI | 93.00 (65.00) | 56.00 (57.00) | 84.00 (63.00) |

Table 4.9

*Duration of fixations (ms): Median (IQR) values*

| | | Low AQ | High AQ | ASD |
|---|---|---|---|---|
| Mentalizing | Hand ROI | 29366 (32683) | 45431 (27323) | 53280 (56637) |
| | Head ROI | 54584 (59841) | 60864 (42150) | 44154 (58472) |
| | Poker chip ROI | 23850 (25791) | 27003 (25843) | 21126 (37765) |
| Non-mentalizing | Hand ROI | 29624 (25911) | 27452 (21443) | 48775 (33693) |
| | Head ROI | 42858 (41739) | 68264 (40723) | 44889 (51148) |
| | Poker chip ROI | 28313 (39537) | 14469 (13128) | 20584 (34201) |

When the eye-tracking data from the clumsy actions were analysed alone, all previously significant results (including the borderline significant difference) were still significant except for the duration of fixations within the head ROI (T=344, p=.12, r=-.24; see supplementary material for all results).There were no significant group differences in the eye-tracking data (see supplementary material).

### *4.4.5 Relationships between data from different techniques*

#### *4.4.5.1 EEG and behavioural performance*

Linear regression analysis found that *mu* suppression in the 8-10Hz frequency band at F3 during the mentalizing task significantly predicted performance on this task across all participants [F(1,38)=5.64, p=.02, $R^2$=.13; see Figure 4.4]. There were no other significant relationships between the EEG data and behavioural performance (see supplementary material). Although one ASD participant was identified as an outlier (Cook's distance>1) this point had a relatively low leverage value (.06, <n/4) and when this outlier was removed, the linear regression was still significant [F(1,38)=17.90, p<.001, $R^2$=.33].

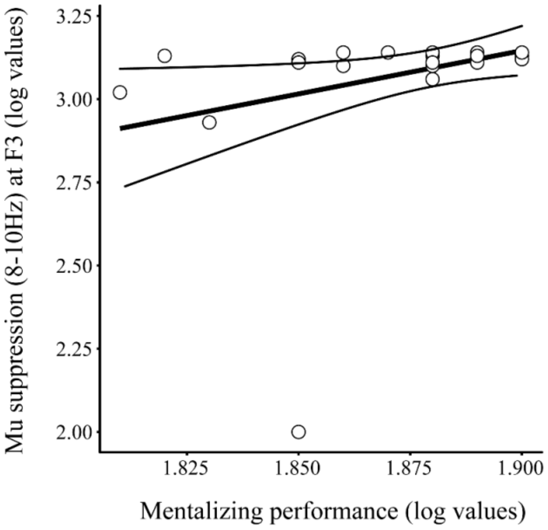

**Figure 4.4** The relationship between performance on the mentalizing task and the level of *mu* suppression in the 8-10Hz frequency band at F3. Mentalizing performance significantly predicted the degree of *mu* suppression at F3; participants with superior mentalizing performances also showed greater levels of *mu* suppression at F3 in the 8-10Hz frequency band [F(1,38)=5.64, p=.02, $R^2$=.13]. The curved lines represent 95% confidence intervals.

#### *4.4.5.2 Eye-tracking and behavioural performance*

The total duration of fixations within the poker chip ROI during the non-mentalizing task significantly predicted performance on the non-mentalizing task [F(1,41)=5.14,

$p=.03$, $R^2=.11$]. There were no other significant relationships between eye-tracking data and behavioural performance (see supplementary material).

#### *4.4.5.3 Eye-tracking and EEG*

Linear regression analyses found that the degree of *mu* suppression did not significantly predict fixation patterns for any of the cortical sites (see supplementary material).

#### *4.4.5.4 TMS and other measures*

Linear regression analyses found that motor resonance values did not significantly predict behavioural performances or levels of *mu* suppression in either task (see supplementary material).

## **4.5 Discussion**

This study aimed to investigate the possible association between ASD and atypical MNS activity when mentalizing, as well as the relationship between MNS activity and mentalizing performance. Both TMS-induced MEPs and *mu* suppression (measured by EEG) were used as indices of MNS activity. The EEG data show that higher levels of autistic traits (across clinical and non-clinical populations) were associated with lower levels of MNS activation in the right hemisphere when mentalizing. However, these lower levels of MNS activity in the right hemisphere were not associated with poorer mentalizing performances. In contrast, lower levels of MNS activity in the left hemisphere were associated with poorer mentalizing performance but not associated with the levels of autistic traits that participants displayed. The TMS data did not show differences in MNS activity between groups or a relationship between MNS activity and mentalizing performances. Consequently, the EEG data provide evidence for MNS involvement in mentalizing and reduced MNS activity in adults with high levels of autistic traits. However, as there were lateralisation differences between MNS activity associated with task performance and reduced MNS activity associated with high levels of autistic traits, our data do not provide evidence that atypical MNS functioning underlies mentalizing difficulties associated with ASD.

Across all participants, the level of autistic traits displayed significantly predicted levels of *mu* suppression in the 8-10Hz frequency band at F4 during the mentalizing task (see Figure 4.3). These data imply that high levels of autistic traits are associated with reduced MNS activity in the right hemisphere when mentalizing. Our results support previous studies which have found atypical MNS activation in adults with ASD (Baron-Cohen et al., 1999; Kana et al., 2014; Martineau et al., 2010; Oberman et al., 2005) and adults without a diagnosis but high levels of autistic traits (Cooper et al., 2013; Puzzo et al., 2009). The right lateralisation of the reduced MNS activity associated with high levels of autistic traits in this study compliments findings from a previous study which found reduced right IFG activity in adults with ASD when inferring the intentions of characters in a comic strip task (Kana et al., 2014). In contrast, reduced left IFG activity has been found in adults with ASD during the ‘reading the mind in the eyes’ (RME) task which involves viewing still images of people’s eyes and selecting the appropriate mental state from four written options (Baron-Cohen et al., 1999; Holt et al., 2014). Differences in the laterality of reduced MNS activity associated with ASD between studies may reflect differences in task demands; activity in the right IFG has been linked to inhibiting self-perspective (Samson, Apperly, Kathirgamanathan, & Humphreys, 2005; Samson, Houthuys, & Humphreys, 2015) and processing action context (Villarreal, Fridman, & Leiguarda, 2012) whereas left IFG has been associated with face (Feurra, Fuggetta, Rossi, & Walsh, 2010; Kesler-West et al., 2001) and language processing

(Cornelissen et al., 2009; Liakakis, Nickel, & Seitz, 2011; Purcell, Napoliello, & Eden, 2011; Tyler et al., 2011; Wright, Randall, Marslen-Wilson, & Tyler, 2010). Therefore, mentalizing tasks which rely more heavily on face or language processing are likely to recruit the left IFG to a greater degree than right IFG in neurotypical participants, meaning that larger group differences will be found in left IFG if the MNS is disengaged in adults with ASD. In our study, individuals with low levels of autistic traits may have used methods which preferentially recruited the right MNS in order to infer the intentions of others from their actions. This is supported by previous studies which have found right lateralised MNS activity when identifying deceitful actions (Grezes, 2004), when implicitly processing the intentions of actions (Iacoboni et al., 2005) and when viewing intended compared to non-intended hand actions (Liepelt, Cramon, et al., 2008).

Although our EEG data suggest that MNS activation in the right hemisphere is reduced in adults with high levels of autistic traits when mentalizing, no significant relationship was found between right-lateralised MNS activity and mentalizing performance. Consequently, our data do not provide evidence that atypical MNS activation in the right hemisphere underlies mentalizing difficulties associated with ASD. These data contradict the broken mirror theory, which states that atypical MNS activity underlies the social communication deficits that individuals with ASD experience (Iacoboni & Dapretto, 2006; Lindsay M Oberman & Ramachandran, 2007; Ramachandran & Oberman, 2006). The typical performances of adults with high levels of autistic traits on the mentalizing task despite the reduced levels of right MNS activity in the current study may reflect compensatory strategies that these individuals have adopted in order to successfully infer the intentions of others from action kinematics, despite atypical disengagement of the right MNS. All participants in this study were high-functioning adults with IQ scores within the typical range (>70). It is possible that if younger participants were recruited they may not have yet developed compensatory mechanisms to allow intentions to be successfully inferred from others' actions and a relationship between right IFG activation and mentalizing performance may have been found.

The lower levels of mu suppression in the right hemisphere associated with high levels of autistic traits in the mentalizing task were not observed in the non-mentalizing task. This suggests mentalizing induces atypical suppression of the right MNS in these adults. Both the mentalizing task and the non-mentalizing task involved watching hand action videos but the tasks differed in the inferences that the participants were required to make. Making judgements about others' internal states, such as their intentions, reliably induces activation in brain areas known as the 'mentalizing system' (Ciaramidaro et al., 2014; de Lange et al., 2008; Lombardo et al., 2010; Spunt et al., 2011; Van Overwalle & Baetens, 2009). The core areas of the mentalizing system are thought to be the medial prefrontal cortex (mPFC), temporoparietal junction (TPJ) and the temporal poles (Frith & Frith, 2006). As lower levels of MNS activation were only observed in adults with high levels of autistic traits during the task in which the mentalizing system was engaged, this suggests that activation of the mentalizing system causes atypical suppression of MNS functioning in adults with high levels of autistic traits. Atypical connectivity between the mentalizing system and the MNS has previously been reported in ASD (Damarla et al., 2010; Fishman et al., 2014; Just, Cherkassky, Keller, Kana, & Minshew, 2007; Just, Cherkassky, Keller, & Minshew, 2004; Kennedy & Courchesne, 2008; Noonan, Haist, & Müller, 2009; Shih et al., 2010). Additionally, neuroimaging studies have provided evidence to suggest that MNS activation is influenced by top-down processing; MNS activity is higher when actions are shown in context compared to actions shown without context (Iacoboni et al., 2005) and MNS activation is higher during socially relevant movements compared to non-social movements (Becchio et al., 2012; Centelles et al., 2011). Therefore, our data in conjunction with the previous literature suggest that top-down connectivity from the mentalizing system to the MNS may be atypical in adults with high levels of autistic traits, which results in reduced levels of right MNS activity in these individuals compared to adults with low levels of autistic traits when inferring the intentions of others.

In contrast to the right-lateralised MNS data, left-lateralised MNS activity was not related to levels of autistic traits,  but a significant positive relationship was found between left MNS activity and mentalizing performance. Participants who exhibited superior performances on the mentalizing task also displayed higher levels of *mu* suppression in the 8-10Hz frequency band at F3 during this task. These data support the motor resonance (or motor simulation) theory (Decety & Grèzes, 2006; Landmann et al., 2011; Uithol et al., 2011). This theory states that observed actions are internally simulated in the observer's own MNS in order to infer the internal states of the individuals performing the actions. In our study, right-handed actions were viewed and therefore internal simulation of these actions would be predicted to result in particularly increased activation in left hemisphere motor areas (Aziz-Zadeh et al., 2002). The relationship found between MNS activity and mentalizing performance implies that individuals who formed stronger internal simulations of the observed actions displayed superior abilities to infer the internal states of the actors. Our data therefore support the notion that internal simulation of observed hand actions by the contralateral MNS is an important process in order to successfully infer others intentions. This compliments previous studies that have shown bilateral but stronger left MNS activity when inferring the intentions of right-handed actions (de Lange et al., 2008), higher left MNS activation when viewing social right-handed actions compared to those without social context (Becchio et al., 2012; Bucchioni et al., 2013; Enticott et al., 2013) and poorer mentalizing performances in patients with MNS lesions (Besharati et al., 2016; Dal Monte et al., 2014).

Initial analyses of task-related differences in *mu* suppression suggested that left-lateralised MNS activity was lower during the mentalizing task than the non-mentalizing task. However, this task-related difference in MNS activity was eliminated when only identical ('clumsy') actions were analysed across tasks. This implies that the apparent task-related difference in MNS activity in the left hemisphere was likely to be the product of differences in action kinematics between the videos shown across the mentalizing and non-mentalizing tasks. During the non-mentalizing task, successful actions were shown which involved an actor passing a poker chip to another player through a slot in a board and returning to their hand to their side of the board at the end of the action. In comparison, during the mentalizing task, spiteful actions were shown which involved an actor deliberately releasing a poker chip before it could be passed through the slot (see Figure 4.1). Determining the success of the successful actions required participants to process that the actors' hands had returned to their side of the board without the poker chip. Therefore, it is likely that the successful actions were internally simulated for slightly longer periods of time, resulting in overall greater levels of MNS activation during the non-mentalizing task. This could explain the apparent significantly higher levels of MNS activity during the non-mentalizing task compared to the mentalizing task and the elimination of this apparent difference in MNS activity when only activity during the presentation of identical action videos ('clumsy' actions) was compared across tasks.

Our EEG data show differences in *mu* suppression in the 8-10Hz frequency band rather than the 10-12Hz frequency band were associated with autistic traits and mentalizing performance. No significant relationships were found between *mu* suppression in the 10-12Hz frequency band and any other measures. These data support previous studies which have found *mu* suppression only in the lower alpha frequency band (8-10Hz) and not the higher alpha frequency band during action observation (Cochin, 1999; Simon & Mukamel, 2016). These EEG data also support the functional segregation of *mu* rhythm into two discrete sub-bands, complimenting previous work that found distinct *mu* responses in low and high alpha bands (Dumas et al., 2014; Frenkel-Toledo et al., 2014; Neuper et al., 2009; Pfurtscheller et al., 2000). The majority of previous studies investigating mu rhythm in individuals with ASD have not split mu rhythm into sub-bands (Bernier, Aaronson, & McPartland, 2013; Dumas et al., 2014; Fan, Decety, Yang, Liu, & Cheng, 2010; Oberman et al., 2005, 2008; Raymaekers et al., 2009). A previous study that did investigate *mu* suppression in two discrete sub-bands in adults with ASD found reduced *mu* suppression in the 11-13Hz frequency band when observing hand actions and no atypicalities in the 8-10Hz frequency band (Dumas et al., 2014). This previous study investigated *mu* suppression during passive action observation, not during a mentalizing task. Similar to this previous study, our data show no atypicalities in *mu* suppression in the 8-10Hz range in adults with ASD during the non-mentalizing task. The reduced *mu* suppression in the upper sub-band during passive action observation in this previous study may be the result of the slightly higher frequency band used. This frequency band encroaches into the beta frequency range 12(/13)-30(/35)Hz

(Haenschel, Baldeweg, Croft, Whittington, & Gruzelier, 2000; Kilavik, Zaepffel, Brovelli, MacKay, & Riehle, 2013; Miller, 2007). Similar to *mu* suppression, oscillatory activity in the beta frequency range is suppressed when observing biological motion (Babiloni et al., 2002; Milston, Vanman, & Cunnington, 2013; Perry et al., 2010) and atypical oscillatory activity in the beta frequency range has previously been reported in adults with ASD (Cooper et al., 2013; Honaga et al., 2010). In summary, mu suppression in the 8-10Hz frequency sub-band (and not 10-12Hz) appears to reflect MNS activity in the left hemisphere that is related to mentalizing performance, and MNS activity in the right hemisphere is reduced in adults with high levels of autistic traits when mentalizing.

The TMS data show no relationship between motor resonance values and either mentalizing performance or autistic traits and no differences in motor resonance values across tasks. These data were unexpected as we predicted that a positive relationship would be found between motor resonance values and mentalizing performances. We predicted that motor resonance values would be larger during the mentalizing task (Enticott et al., 2013), and that task-related differences in motor resonance values would be reduced in adults with high levels of autistic traits (Enticott et al., 2012; Puzzo et al., 2009; Théoret et al., 2005). In this study, TMS stimulation was applied to the left hemisphere meaning that the TMS data reflects MNS activity in the left hemisphere. The lack of a task-related difference in MNS activity in the TMS data compliments our EEG data, which also suggest no task-related difference in MNS activity when differences in action kinematics are controlled for. Additionally, the lack of a relationship between MNS activity and autistic traits in the TMS data supports our EEG data, which also found no relationship between left MNS activity and autistic traits. However, the relationship between left MNS activation and mentalizing performance shown in the EEG data were not replicated in the TMS data.

A possible reason for the inconsistency between the TMS data and the EEG data is that these methods measure different aspects of MNS functioning. We found that, across all participants, motor resonance values did not significantly predict the degree of *mu* suppression (see supplementary material). These data support previous studies that found TMS-induced MEP sizes did not correspond with levels of *mu* suppression when observing actions (Andrews, Enticott, Hoy, Thomson, & Fitzgerald, 2015; Lepage, Saint-Amour, & T Théoret, 2008). Results from both MEG studies (Cheyne et al., 2003; Jones et al., 2009) and a combined MRI-EEG study (Arnstein, Cui, Keysers, Maurits, & Gazzola, 2011) suggest that *mu* rhythms correspond to activation in S1. Although S1 is not considered to be a 'core region' of the MNS, S1 has been reliably shown to display mirror properties (Confalonieri et al., 2012; Gazzola & Keysers, 2009; Molenberghs, Cunnington, & Mattingley, 2012; Porro et al., 1996). TMS on the other hand, is very unlikely to cause MEPs in muscles of the hand through means other than the stimulation of M1 (Lepage et al., 2008). TMS-induced MEPs measured during action observation are thought to measure increased excitability in M1 as the result of excitatory cortico-cortical connections from prefrontal MNS areas (IFG/vPMC; Fadiga, Craighero, & Olivier, 2005; Loporto, McAllister, Williams, Hardwick, & Holmes, 2011). Therefore, if *mu* suppression measured by EEG reflects MNS activity in S1 and TMS-induced MEPs provide an index of prefrontal MNS activity then this could explain the differences between the results from these two measures.

An alternative reason for the inconsistency between the EEG and TMS data could be due to the differences in the spatial and temporal properties of these two measurements of MNS activity. The EEG measurements in this study reflect the sum of post-synaptic neuronal activity over a large cortical area and a relatively long time period (throughout video or fixation cross display) whereas TMS measures brief induced increases in corticospinal activity from peripheral muscles, induced by stimulating a relatively small population of neurons at a discrete time point (Andrews et al., 2015; Pineda, 2005; Rossini et al., 1994). Therefore, even if EEG and TMS methods were measuring the same aspects of MNS functioning, the results may differ simply due to differences in the spatial and temporal properties of the measurements. Consequently, it is unsurprising that these two indices of MNS activity do not correlate with each other. Measuring MNS activity using both these measures allowed us to obtain a more complete measure of MNS activity.

The total duration of fixations in the poker chip ROI predicted non-mentalizing task performance but there were no significant relationships between any of the eye-tracking measures and mentalizing performance. This implies that the visual information within the poker chip ROI was vital for performance on the non-mentalizing task; this is to be expected as performances relied upon identifying whether the poker chip was successfully passed to another player or was dropped before being passed to another player. Therefore, visual information from the other ROIs was not needed, only the final position of the poker chip was important for task performance. In contrast, during the mentalizing task, the final location of the poker chip was always the same (all actions were unsuccessful) but participants were required to infer the intentions of the actors from the kinematics of their actions in order to complete the task. The lack of any significant relationships between the eye-tracking data and mentalizing performances suggests that participants did not have a rigid method in which they did this. This is supported by the fact that overall, participants displayed a greater number of fixations during the mentalizing task than the non-mentalizing task suggesting a greater degree of re-diverting attention during this task perhaps reflecting an increased level of uncertainty regarding where to direct their visual attention.

There were no significant differences in the eye-tracking data associated with high levels of autistic traits during the mentalizing task. This means that the lower levels of MNS activity during the mentalizing task exhibited by adults with high levels of autistic traits were not due to reduced fixation on the observed action kinematics in these individuals.

There are a number of limitations associated with this study including the small sample size, particularly for the TMS data, which may have resulted in limited power to detect differences in MNS functioning associated with ASD. The particularly small sample size for the TMS data was due to a number of participants (n=6) not being able to complete the TMS element of this study either due to not tolerating stimulation or having particularly high motor thresholds. This particularly small sample size may have contributed to the lack of differences in motor resonance values found both across tasks and between groups. The lack of significant mentalizing deficits in adults with high levels of autistic traits in our study may have also limited our ability to detect a relationship between motor resonance values and autistic traits; it is possible that if ASD participants with higher levels of autistic traits and significant mentalizing deficits were recruited then a significant relationship may have been observed. However, adults with higher levels of autistic traits did show lower levels of mu suppression which suggests that MNS atypicalities were detectable in these high-functioning adults despite typical behavioural performances. Additionally, our TMS data support other studies that have previously reported typical motor resonance in adults with ASD (Enticott et al., 2013; Kirkovski et al., 2016). It is possible that the interesting laterality difference in the relationship between mu suppression (8-10Hz) and behavioural performance compared to the relationship between mu suppression (8-10Hz) and autistic traits may have been the result of TMS stimulation to the left hemisphere in our study. Therefore, replication of the study with stimulation applied to the right hemisphere is required in order to confirm this laterality difference. The inclusion of medicated participants in this study may have also influenced the TMS data; six participants in the ASD group were taking psychotropic medications which have been shown to increase corticospinal excitability (Gilbert et al., 2006; Minelli et al., 2010). Due to the high comorbidity of ADHD, depression and anxiety in ASD, the inclusion of adults taking psychotropic medication is common in TMS studies with ASD participants (Enticott, Rinehart, Tonge, Bradshaw, & Fitzgerald, 2010; Enticott et al., 2013; Oberman, Pascual-Leone, & Rotenberg, 2014). Despite the possible influence of psychotropic medication on corticospinal excitability, our data show no group differences in resting motor thresholds and there was no significant difference in resting motor threshold values between medicated and non-medicated participants (see supplementary material).

Despite these limitations, our EEG data add to the existing literature by identifying lower levels of right MNS activity in adults with high levels of autistic traits when inferring the intentions of others from their actions and higher levels of left MNS activity associated with superior mentalizing performances. These EEG data suggest that the MNS has a role in inferring the intentions of others from their actions, providing support for the motor resonance theory of social cognition (Agnew et al., 2007; Landmann et al., 2011; Leslie et al., 2004; Rizzolatti et al., 2002). Additionally, adults with high levels of autistic traits appear to display atypical suppression of the MNS in the right hemisphere when inferring the intentions of others. Therefore, this study provides evidence for reduced MNS activity in adults with high levels of autistic traits when mentalizing and a potential role of the MNS in inferring the intentions of others.

# Chapter 5: Reduced Connectivity between Mentalizing and Mirror Neuron Systems May Underlie Difficulties Inferring Social Intentions in Autism Spectrum Disorder

This chapter is adapted from: Cole, E.J., Barraclough N. E., Andrews T.J. (in preparation). Reduced connectivity between mentalizing and mirror neuron systems may underlie difficulties inferring social intentions in autism spectrum disorder.[4]

## 5.1 Abstract

Autism spectrum disorder (ASD) is associated with difficulties inferring the social intentions of others. Two neural systems known as the mentalizing system and the mirror neuron system (MNS) are thought to play an important role in making inferences about the actions of others. The aim of this fMRI study was to determine whether differences in the connectivity between these networks can explain behavioural difficulties associated with ASD. We recruited 40 adult participants (20 with ASD and 20 typically-developing). Brain activity was monitored while participants watched videos in which actors performed hand actions. The videos were divided into mentalizing and non-mentalizing blocks. During mentalizing blocks, participants were asked to indicate whether hand actions were clumsy or spiteful. During non-mentalizing blocks, participants indicated whether the actions were successful or unsuccessful. Behavioural performance on the mentalizing task was negatively correlated with levels of autistic traits. To explore the neural

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[4] The author, Eleanor Cole, designed the experiment, collected the data, analysed the results and wrote the manuscript under the joint supervision of Prof Timothy Andrews and Dr Nick Barraclough.

correlates of this behavioural deficit in adults with high levels of autistic traits, the neural response was compared during the mentalizing and non-mentalizing blocks. Higher activity was elicited during the mentalizing blocks in regions associated with the mentalizing system, such as the dorsal medial prefrontal cortex (dmPFC) and the temporo-parietal junction (TPJ), as well as in regions associated with the MNS, such as the inferior frontal gyrus (IFG) and the inferior parietal lobe (IPL). Next, connectivity between regions was measured as a function of task. Increased functional connectivity between the dmPFC and both the IFG and supplementary motor area (SMA) was evident during mentalizing compared to non-mentalizing blocks. Interestingly, connectivity between the dmPFC and IFG was negatively correlated with autistic traits. Together, these data emphasise the importance of functional connectivity between the MS and MNS in inferring social intentions and show that reduced connectivity between these systems could explain some of the social difficulties experienced by adults with ASD.

## 5.2 Introduction

Inferring the internal states of others from their movements is important for successful social interactions. Two neural systems; the mirror neuron system (MNS) and the mentalizing system, have been shown to be active when making inferences about the internal state of others based on their actions (Becchio et al., 2012; Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; de Lange, Spronk, Willems, Toni, & Bekkering, 2008; Hooker, Verosky, Germine, Knight, & D'Esposito, 2010; Spunt & Lieberman, 2012a). Inferring aspects of someone's internal state such as their emotions, intentions or beliefs is known as 'mentalizing' (Chung, Barch, & Strube, 2014; Denny, Kober, Wager, & Ochsner, 2012; Frith & Frith, 2006; Kampe, Frith, & Frith, 2003).

The main components of the mentalizing system are considered to be the medial prefrontal cortex (mPFC) and the temporoparietal junction (TPJ; Frith & Frith, 2006; Schurz, Radua, Aichhorn, Richlan, & Perner, 2014). The mPFC can be subdivided into distinct subregions and meta-analyses have identified different roles for each subregion (Amodio & Frith, 2006; Bzdok et al., 2013; Jérôme Sallet et al., 2013). The most dorsal region of the mPFC (dmPFC) appears to be the subregion that is particularly involved in inferring internal states of others from their actions (Amodio & Frith, 2006; Frith & Frith, 2006; Sallet et al., 2013).

Regions in the MNS show similar activation patterns during both the performance and observation of actions (di Pellegrino et al., 1992; Rizzolatti et al., 1996). It is thought that this pattern of activation reflects the internal simulation of observed actions on the observer's own motor system (Rizzolatti & Craighero, 2004). Some researchers propose that internally simulating the actions of others aids the understanding of the internal states motivating their actions (de Lange et al., 2008; Gallese & Goldman, 1998; Lindsay M Oberman & Ramachandran, 2007; Trilla Gros et al., 2015; Uddin et al., 2007). The core components of the MNS are considered to be the inferior frontal gyrus (IFG) and the inferior parietal lobe (IPL; Rizzolatti & Craighero, 2004; Rizzolatti & Sinigaglia, 2010). Despite the activation of both the MNS and the mentalizing system during mentalizing tasks, it is not clear how these two systems interact and the role of the MNS is debated (Catmur, 2015; de Lange et al., 2008; Hamilton & Marsh, 2013).

Identifying the brain activation and functional connectivity patterns elicited when mentalizing may be important for understanding the difficulties experienced by individuals with autism spectrum disorder (ASD). A number of studies have shown that individuals with ASD have difficulties mentalizing (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Castelli, Frith, Happé, & Frith, 2002; Jolliffe & Baron-Cohen, 1999; Kana, Libero, Hu, Deshpande, & Colburn, 2014; Senju, Southgate, White, & Frith, 2009). Difficulty inferring others' social intentions (i.e. their intended impact on others such as the intent to help or to harm) is of particular importance as this deficit can result in individuals with ASD making inappropriate social decisions and consequently being vulnerable to mistreatment (Fisher, Moskowitz, & Hodapp, 2013; The National Autistic Society, 2014). To date, brain activation and functional connectivity patterns when inferring social intentions have not been investigated in adults with ASD.

The aim of this fMRI study was (1) to determine whether connectivity between areas associated with the MNS and mentalizing system is important when inferring social intentions and (2) to explore whether differences in connectivity between these systems can explain the difficulties experienced by individuals with ASD in inferring social intentions of others. To address this issue, we recruited adults with and without a diagnosis of ASD. Participants viewed videos in which actors performed actions. During the mentalizing videos, participants had to report whether the actor was being clumsy or spiteful and during the non-mentalizing videos, participants had to report whether the action was successful or unsuccessful. To localize regions involved in mentalizing, the response during mentalizing blocks was compared to non-mentalizing blocks. The prediction was that this contrast should define regions associated with the MNS and mentalizing system. Next, we examined whether connectivity between these regions differed between the mentalizing blocks compared to non-mentalizing blocks. The prediction was that functional connectivity between these systems would be higher during mentalizing blocks. Finally, we explored whether differences in connectivity across individuals could be explained by the level of autistic traits displayed.

## 5.3 Methods

### *5.3.1 Participants*

Twenty adults with a diagnosis of autism spectrum disorder (ASD; 8 female) and twenty typically developing (TD) adults were recruited for this study. Each participant with ASD was individually matched on both gender and age to a TD participant and the groups did not significantly differ in years of formal education (demographic information is shown in Table 5.1). One participant pair were left-handed. All participants had normal or corrected-to-normal vision. This research project was approved by the Ethics Committee at York Neuroimaging Centre and was performed in accordance with the ethical standards laid down in the 1990 Declaration of Helsinki. Six participants in the ASD group were taking psychotropic medication to treat depression or anxiety and one of these participants was additionally taking a very low dose of an antipsychotic (see Table 5.2). None of the participants in the control group were taking psychotropic medication for any psychiatric disorders.

Table 5.1

*Participant demographic information; group mean (SD) values*

| | ASD | TD | p | $\eta_p^2$ |
|---|---|---|---|---|
| N | 20 | 20 | | |
| Age | 29.8(12.87) | 29.6 (13.50) | 0.96 | 0.00 |
| Gender (m:f) | 12:8 | 12:8 | 1.00 ($X^2$) | / |
| Years of formal Education | 16.20 (1.42) | 17.20 (1.88) | 0.07 | 0.09 |

p values were obtained from a one-way MANOVA unless otherwise stated

Table 5.2

*Medication information for participants in the ASD group*

| Participant | Medication (daily dosage) |
|---|---|
| 1 | Fluoxetine (20mg) |
| 2 | Mirtazapine (30mg) |
| 3 | Sertraline (200mg) |
| 4 | Sertraline (200mg) |
| 5 | Sertraline (200mg) |
| 6 | Aripiprazole (2.5mg), Citalopram (30mg) |

### *5.3.2 Stimuli*

The stimuli were action videos used in a previous behavioural study (Cole, Slocombe & Barraclough, 2017). Videos were filmed at a resolution of 1080 x 1920 pixels and 50 frames per second. The videos showed actors either successfully or unsuccessfully passing a poker chip through slots in a white wooden board to another player on the other side that was out of view (see Figure 5.1). The actors in the unsuccessful videos either did not pass the poker chip to the other player deliberately (‘spiteful’ action) or accidentally (‘clumsy’ action). All action types (successful, spiteful and clumsy) were carried out by both pushing the poker chip (with the index finger) through a lower slot or by grasping the poker chip (with the index finger and thumb) and passing it through a higher slot (see Figure 5.1). Eighteen different actors (9 female) were shown performing these actions and each video was four seconds long.

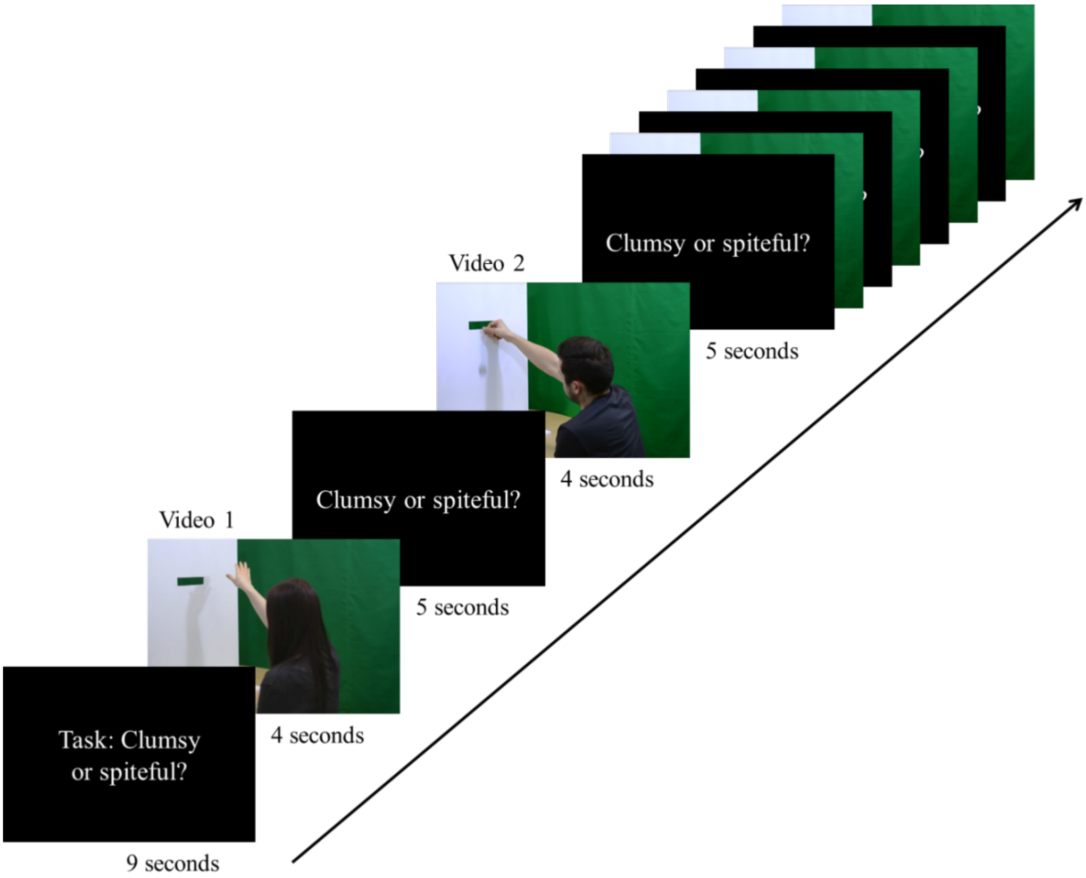

**Figure 5.1.** Schematic representation of the mentalizing task. At the beginning of each block, an instruction screen was shown for 9 seconds to indicate whether participants would be completing a mentalizing or non-mentalizing task. Subsequently, six videos were shown sequentially. After each video a response screen was shown and participants indicated whether the action shown was 'clumsy' (accidental) or' spiteful' (deliberate). In this example, video 1 portrays a spiteful action and video 2 depicts a clumsy action.

### *5.3.3 Experimental design*

Participants completed a behavioural task whilst inside the scanner which had a block-design with two conditions: mentalizing and non-mentalizing. In mentalizing blocks, participants were shown unsuccessful (clumsy or spiteful) actions and after each video a response screen was shown, prompting participants to indicate whether the action was clumsy or spiteful. In the non-mentalizing blocks, participants were shown successful or unsuccessful (clumsy) actions and were asked to indicate whether the action was successful or unsuccessful. In both tasks, participants indicated their answers by pressing buttons on a response box placed in their right hand. Each block contained six videos (three of each type). Videos were counterbalanced, so that gender and identity of the actors were not predictive of the actions they were performing.

At the beginning of each block, an instruction screen was shown for nine seconds to make participants aware of the task they would be completing during the upcoming block. After each video the response screen was shown for five seconds and afterwards a central fixation cross was shown for one second before the next video in the block was shown. Participants completed twelve blocks (six mentalizing and six non-mentalizing) lasting a total of 12 minutes 36 seconds. The videos were projected onto a rear projection screen in the bore of the scanner using an Epson EB-G5900 projector with a long throw lens. Participants viewed the videos using a mirror attached to the head coil. Videos were shown full screen (40 x 23cm and degrees of visual angle, 1920 x 1080 resolution).

Prior to scanning, participants completed practice trials (one mentalizing and one non-mentalizing block) on a laptop outside of the scanner in order to familiarise them with the structure of the task. The actors shown in the practice blocks were not shown in the main experiment to avoid previous experience with the actors influencing decisions in the main experiment.

### *5.3.4 Data acquisition*

A 3T GE HD Excite MRI scanner with an 8-channel phase array head coil tuned to 127.4 MHz was used to acquire fMRI data. A gradient-echo EPI sequence was used to collect data from 38 interleaved axial slices (TR=3000ms, TE=35ms, voxel size=2.25 x 2.25, flip angle =90º, FOV=288mm, matrix size= 128 x 128, slice thickness= 3mm). Localiser scans were carried out in order to align EPI images. High-resolution T1-weighted anatomical images (TR=7.96ms, TE=3.05ms, voxel size= 1.13 x 1.13 mm, FOV=290mm, matrix size= 256 x 256, slice thickness=1mm) were also obtained. T1-weighted fluid-attenuated inversion recovery sequence (T1-FLAIR) images with the same spatial prescription as the EPI data were collected to aid co-registration to structural images (voxel size=.56 x .56mm, matrix size=512 x 512). The first three frames of each scan were discarded to allow time for magnetisation to stabilise.

### *5.3.5 Psychological Assessments*

We used standardised psychological assessments to measure the level of autistic traits displayed by all participants (both those with and without a diagnosis of ASD). This was done so that behavioural and neural measures could be investigated in relation to the level of autistic traits displayed. This method was used rather than simply examining group differences based on diagnosis because of the high variability in levels of autistic traits known to exist within clinical and non-clinical populations (American Psychiatric Association, 2013; Robinson et al., 2011; van Boxtel & Lu, 2013; Von Dem Hagen et al., 2011). Individuals without an ASD diagnosis but relatively high levels of autistic traits, exhibit subtler versions of the behavioural and neurological characteristics associated with ASD (Best, Arora, Porter, & Doherty, 2015; Di Martino et al., 2009; Lindell, Notice, & Withers, 2009; Ridley, Homewood, & Walters, 2011; van Boxtel & Lu, 2013), including mentalizing deficits (Gökçen, Petrides, Hudry, Frederickson, & Smillie, 2014; Gökçen, Frederickson, & Petrides, 2016). Therefore, high variability in autistic traits within groups would mean high variability in behavioural and neural characteristics

(Gökçen, Petrides, Hudry, Frederickson, & Smillie, 2014; Gökçen et al., 2016; van Boxtel & Lu, 2013) and may prevent group differences being identified.

Participants completed three psychological assessments after the scanning session; two self-report questionnaires and one interactive task. The questionnaires completed were the Autism Quotient (AQ) scale and the Social Responsiveness Scale (SRS-2). The task used was the Awareness of Social Inference Test (TASIT) which involves watching video clips of social interactions and subsequently answering questions about the thoughts and feelings of characters. These psychological tests have been shown to display good psychometric properties (Hurst et al., 2007; Skye McDonald et al., 2006; Wigham et al., 2012). The AQ, SRS-2 and TASIT scores were calculated and a one-way MANOVA was used to identify group differences in these scores. The scores on all psychological tests significantly differed between groups (see Table 5.3). In all cases, scores in the ASD group indicated higher levels of autistic traits/greater degree of social impairment.

Table 5.3

*Participants' psychological test scores; group mean (SD) values*

| | ASD | TD | p | $\eta_p^2$ |
|---|---|---|---|---|
| AQ | 37.4 (8.04) | 12.90 (6.23) | <.001*** | 0.75 |
| TASIT[1] | 46.35 (10.92) | 58.20 (4.26) | <.001*** | 0.35 |
| SRS[2] | 114.75 (26.37) | 35.85 (20.90) | <.001*** | 0.74 |

[1] The TASIT scores were obtained from part 3 (social inference test), scores are out of 64.

[2] The reported SRS scores are the unstandardized, raw scores, where scores above 75 reflect severe social impairment.

p values were obtained from a one-way MANOVA

A principal components analysis (PCA) was then performed on all the psychological test scores in order to obtain a single score for each participant that reflected the level of autistic traits that they displayed. The psychological test scores correlated with each other (all rs>.6) meaning that they were suitable for PCA. The Kasier-Meyer-Olkin measure of sampling accuracy was above .6, Barlett's test of sphericity was significant $\chi^2(3)=100.03$, $p<.001$ and the communalities were all .7 or above; this collectively supported the inclusion of all the psychological tests in the PCA. PCA with varimax rotation was used. Only one factor had an eigenvalue above Kaiser's criteria of 1 (2.51) and this factor explained 83.56% of the variance in psychological test scores. Consequently, only one factor was extracted and this factor was labelled 'autistic traits'.

### *5.3.6 Behavioural analysis*

The numbers of correct responses on the mentalizing and non-mentalizing tasks were calculated for each participant. Linear regression analyses were used to investigate relationships between autistic traits and performance on both tasks.

### *5.3.7 MRI analysis*

fMRI analyses were conducted using FEAT v5.98 (http://www.fmrib.ox.ac.uk/fsl). Firstly, slice-timing correction, spatial smoothing (6 mm FWHM), motion correction (MCFLIRT, FSL) and temporal high-pass filtering were applied. Brain extraction was completed using the FSL brain extraction tool (BET). The individual data were then analysed using a general linear model (GLM) with two regressors: 'mentalizing' and 'non-mentalizing'. Parameter estimates were created for each condition by regressing the hemodynamic response against a box-car regressor showing the onset and offset of each video convolved with a gamma response function.

In order to identify areas that displayed higher levels of activation during the mentalizing blocks compared to the non-mentalizing blocks, statistical images were created for the mentalizing>non-mentalizing contrast. Functional data for each participant were firstly registered to the participant's high-resolution T1-image (using the T1-FLAIR image as an intermediate step to aid co-localisation) and then data were registered onto the standard MNI brain (ICBM152). Individual participant data were entered into a higher-level analysis using a mixed-effects design (FLAME; http://www.fmrib.ox.ac.uk/fs), cluster thresholded at $z<3.1$, $p<.001$. Group-level analyses were conducted with 1) autistic traits and 2) mentalizing performance as covariates in order to examine differences in brain activity which corresponded with autistic traits or performance.

### *Functional connectivity analysis*

Regions of interest (ROIs) for regions associated with the MNS and mentalizing system were defined using the areas of peak activation for the mentalizing>non-mentalizing contrast when thresholded at $z > 3.1$, $p< 0.001$. ROI masks were drawn using FSLView software (http://www.fmrib.ox.ac.uk/fsl) and fslmaths commands were used to restrain the masks to voxels which were active ($z > 3.1$) for the mentalizing>non-mentalizing contrast. The ROIs were: bilateral inferior frontal gyrus (IFG), bilateral inferior parietal lobe (IPL), bilateral temporoparietal junction (TPJ) and dorsal medial prefrontal cortex (dmPFC). ROIs were labelled based on comparisons to regions identified in existing fMRI studies (Becchio et al., 2012; Liew, Han, & Aziz-Zadeh, 2011), meta-analyses (Schurz et al., 2014; Van Overwalle & Baetens, 2009) and using the Juelich histological atlas from the FSL anatomy toolboxes as a reference (Eickhoff et al., 2005). The time series of activation was extracted from each ROI for both mentalizing and non-mentalizing blocks.

Time series correlations were calculated for ROIs within the MNS, within the mentalizing system and between MNS and mentalizing system ROIs for each participant.

Prior to statistical analysis, correlations were then transformed using a Fisher's z transform to ensure a normal distribution (Fisher, 1921). In order to explore task and regional differences in connectivity both within each system individually and between the MNS and the mentalizing system, we performed repeated measures ANOVAs for within system connectivity (task x connection) and a repeated measures ANOVA for between system connectivity (task x MNS region x mentalizing system region). The relationships between the degree of mentalizing-induced functional connectivity changes and autistic traits were then examined using linear regression analyses.

## 5.4 Results

### *5.4.1 Behavioural performance*

The performance of participants on the mentalizing and non-mentalizing tasks was measured during the fMRI scan. Scores on these tasks (mentalizing: M=32.45, SD= 4.0); non-mentalizing: M=34.53, SD=2.81) were then correlated with autistic traits. Figure 5.2 shows that the level of autistic traits that participants displayed predicted their performance on the mentalizing task (F(1,38)=5.50, p=.02, $R^2$=.13, 95% CI [-2.65, -.20]). A similar relationship was not evident for the non-mentalizing task (F(1,38)=.01, p=.94, $R^2$<.001, 95% CI [-.96, .89]).

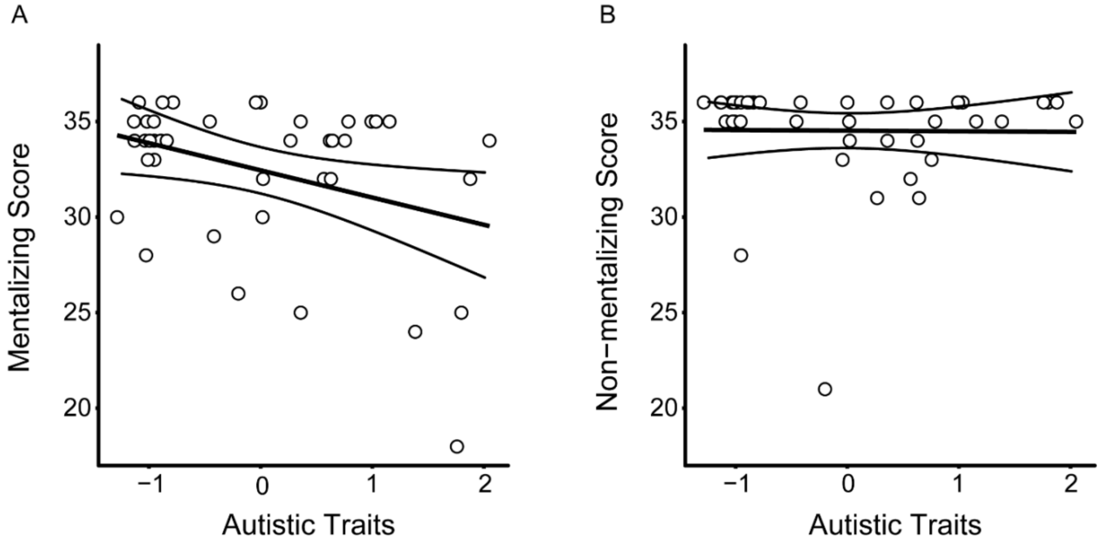

**Figure 5.2** Relationships between autistic traits and behavioural performance. Linear regression analyses showed that autistic traits significantly predicted performance on the mentalizing task [F(1,38)=5.50, p=.02, $R^2$=.13] but not the non-mentalizing task [F(1,38)=.01, p=.94, R2<.001]. The curved lines represent 95% confidence intervals.

### *5.4.2 Brain response during mentalizing and non-mentalizing blocks.*

A group analysis across all participants was performed on the response to the mentalizing and non-mentalizing blocks relative to baseline. Figure 5.3 shows that similar patterns of activation were elicited during both the mentalizing and non-mentalizing blocks, indicating that our stimuli were well matched across conditions. During both mentalizing and non-mentalizing blocks, activity was evident in regions of visual (posterior occipital lobe, lateral occipital lobe, fusiform gyrus) and motor cortex (pre-central gyrus, supplementary motor area), reflecting the visual and motor components of the tasks. There was also activity in the inferior frontal gyrus (IFG), inferior parietal lobe (IPL), temporoparietal junction (TPJ), superior parietal lobe (SPL), and posterior cingulate cortex (PCC) that reflects regions more often associated with the mentalizing and MNS. The coordinates of the peak activation are shown in Table 5.4).

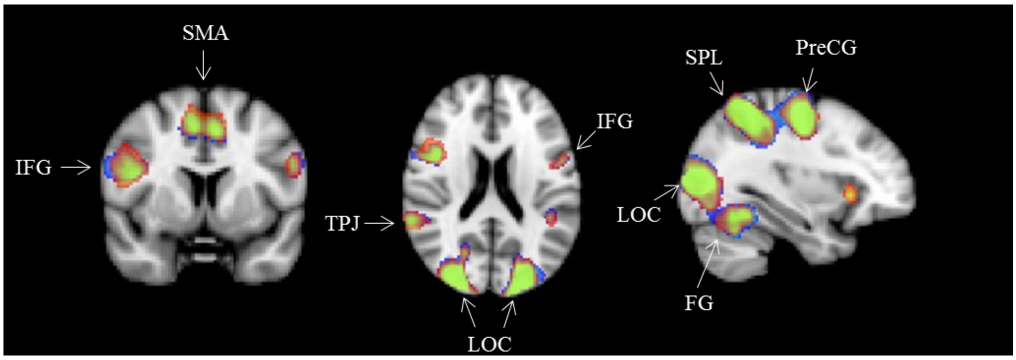

**Figure 5.3** Whole-brain group analysis showing regions that were active during the mentalizing (red-yellow) and non-mentalizing (blue) tasks compared to baseline. Both tasks elicited bilateral activation in inferior frontal gyrus (IFG), supplementary motor area (SMA), temporoparietal junction (TPJ), fusiform gyrus (FG), lateral occipital cortex (LOC), precentral gyrus (preCG) and the superior parietal lobe (SPL). Images thresholded at z > 3.1, p<0.001. MNI co-ordinates (x, y, z): -32, 6, 20.

Table 5.4

*Areas of peak activation during the mentalizing task compared to baseline (p < 0.001 uncorrected for multiple comparisons)*

| | | | Peak MNI co-ordinates | | | |
|---|---|---|---|---|---|---|
| Contrast | Anatomical Region | BA | x | y | z | t-value |
| Mentalizing task>baseline | EVC | 18 | -6 | -82 | -6 | 8.50 |
| | Left LOC | 18 | -22 | -92 | 12 | 8.33 |
| | Left M1 | 6 | -26 | -10 | 52 | 8.13 |
| | Right LOC | 37 | 48 | -70 | -2 | 8.13 |
| | Left ventral stream | 18 | -14 | -76 | -14 | 7.64 |
| | Left SPL | 7 | -34 | -48 | 54 | 7.53 |
| | Right ventral stream | 37 | 30 | -52 | -14 | 6.9 |
| | Right SPL | 7 | 16 | -66 | 58 | 6.71 |
| | Right M1 | 6 | 24 | -8 | 54 | 6.62 |
| | Left SMA | 6 | -6 | 0 | 52 | 6.35 |
| | Left PCC | 31 | -14 | -26 | 40 | 6.08 |
| | Left IPL | 40 | -54 | -28 | 36 | 5.92 |
| | Right SMA | 6 | 8 | 6 | 52 | 5.38 |
| | Right TPJ | 39 | 62 | -44 | 20 | 5.08 |
| | Left IFG | 44/6 | -54 | 4 | 28 | 5.03 |
| | Right IFG | 44/6 | -48 | 0 | 28 | 4.9 |
| | Right IPL | 40 | 54 | -26 | 36 | 4.26 |
| | Left TPL | 22 | -44 | -40 | 18 | 4.25 |
| | Right PCC | 31 | 14 | -28 | 42 | 4.03 |

Abbreviations: EVC; early visual cortex, LOC; lateral occipital cortex, M1; primary motor cortex, SPL; superior parietal lobe, SMA; supplementary motor area, PCC; posterior cingulate cortex, IPL; inferior parietal lobe, TPJ; temporoparietal junction, IFG; inferior frontal gyrus.

Next, we asked whether there were differences in the response to the mentalizing and non-mentalizing conditions. Figure 5.4 shows that significantly higher activation was evident in the dorsal medial prefrontal cortex (dmPFC), bilateral IFG, orbitofrontal cortex (OFC), bilateral TPJ, bilateral IPL and bilateral SMA during mentalizing blocks. The SMA has been shown to display mirror properties (Confalonieri et al., 2012; Gazzola & Keysers, 2009; Molenberghs et al., 2012; Porro et al., 1996) and neuroimaging (Mainieri et al., 2013) and lesion studies (Stone, Baron-Cohen, & Knight, 1998; Stuss, 2001) have shown OFC involvement in mentalizing tasks. Therefore, although these regions are not ‘core’ regions of either system, they are considered to be regions of the extended MNS and mentalizing system respectively. In order to support that our IFG and IPL ROIs are core areas of the MNS, the locations of these ROIs were compared with areas displaying activity for successful>unsuccessful contrast. Figure 5.5 shows the overlap between our ROIs and the statistical map for successful>unsuccessful contrast, therefore providing support that these areas are part of the MNS. The non-mentalizing>mentalizing contrast identified activation in the left primary somatosensory cortex (S1) and the cingulate gyrus. The co-ordinates of peak activation for the mentalizing>non-mentalizing and non-mentalizing>mentalizing contrasts are shown in Table 5.5.

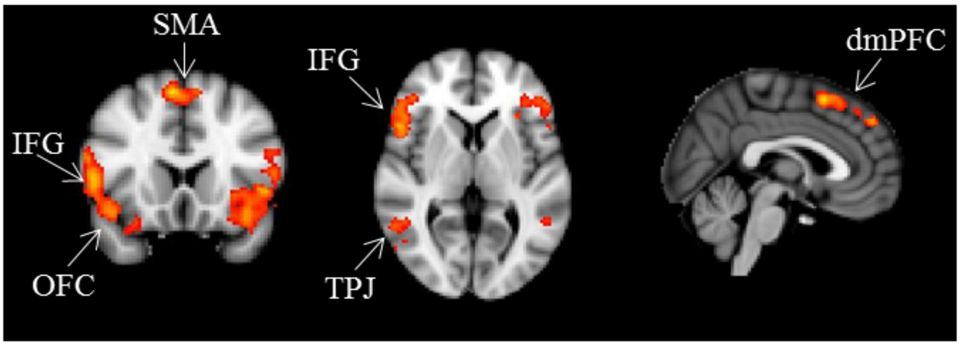

**Figure 5.4** Whole-brain group analysis of regions showing higher activity during the mentalizing task compared to the non-mentalizing task. Higher activation was found in the dorsal medial prefrontal cortex (dmPFC), bilateral inferior frontal gyrus (IFG), bilateral temporoparietal junction (TPJ), orbitofrontal cortex (OFC), bilateral supplementary motor area (SMA) and bilateral inferior parietal lobe (IPL). Images are thresholded at $z > 3.1$, $p < 0.001$. MNI co-ordinates: 2, 18, 4.

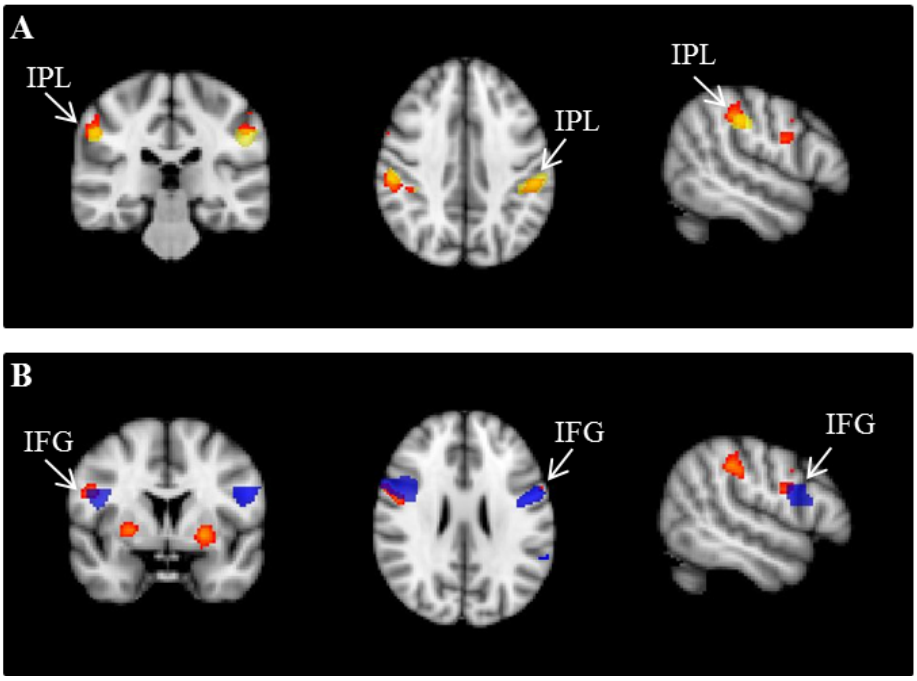

**Figure 5.5** Whole-brain group analysis of regions showing higher activity during the presentation of successful actions compared to unsuccessful actions in the non-mentalizing task with A) IPL ROIs (yellow) and B) IFG ROIs (blue) overlaid onto the statistical map. Our IFG and IPL ROIs defined using the mentalizing>non-mentalizing contrast overlap with areas identified by the successful>unsuccessful contrast, supporting the labelling of our ROIs as MNS regions. Images are thresholded at $z > 3.1$, $p < 0.001$. MNI co-ordinates: A) 54, -28, 38 B) 54, 2, 26.

Table 5.5

*Areas of peak activation for the mentalizing>non-mentalizing contrast ($p < 0.001$ uncorrected for multiple comparisons)*

| | | | Peak MNI co-ordinates | | | |
|---|---|---|---|---|---|---|
| Contrast | Anatomical Region | BA | x | y | z | t-value |
| Mentalizing>non-mentalizing | | | | | | |
| | Right OFC | 47 | 52 | 32 | -8 | 5.72 |
| | Left OFC | 47 | -50 | 24 | -4 | 5.60 |
| | Right SMA | 6 | 6 | 18 | 56 | 4.71 |
| | Right IFG | 44 | 52 | 20 | 12 | 4.36 |
| | Right TPJ | 37 | 56 | -52 | 10 | 4.19 |
| | dmPFC | 9 | 4 | 48 | 38 | 4.11 |
| | Left IFG | 44 | -52 | 14 | 20 | 3.79 |
| | Left IPL | 39 | -62 | -46 | 28 | 3.54 |
| | Right IPL | 40 | 62 | -42 | 24 | 3.53 |
| | Left SMA | 6 | -6 | 12 | 60 | 3.51 |
| | Left TPJ | 21 | -52 | -50 | 4 | 3.47 |
| Non-mentalizing>mentalizing | CG | 23 | 0 | -42 | 36 | 4.65 |
| | Left S1 | 1 | -52 | -22 | 46 | 3.93 |
| Associated with mentalizing score (Positively correlated) | Right dmPFC | 9 | 10 | 42 | 28 | 3.52 |
| | Right SMA | 6 | 20 | -8 | 60 | 3.40 |
| | Right SPL | 7 | 18 | -56 | 52 | 3.39 |
| | Left SPL | 7 | -16 | -52 | 50 | 3.36 |
| Associated with mentalizing score (Negatively correlated) | No areas. | | | | | |

Abbreviations: OFC; orbitofrontal cortex, SMA; supplementary motor area, IFG; inferior frontal gyrus, TPJ; temporoparietal cortex, dmPFC; dorsomedial prefrontal cortex, IPL; inferior parietal lobe, CG; cingulate gyrus, S1; primary somatosensory cortex.

Superior performances on the mentalizing task correlated with higher levels of activation in dmPFC, SMA and bilateral SPL (see Table 5.5). No brain areas showed activation which was negatively correlated with autistic traits.

### *5.4.3 Differences in functional connectivity during mentalizing and non-mentalizing tasks.*

We compared functional connectivity between regions that showed more activity during the mentalizing compared to the non-mentalizing blocks. These regions can be divided into associated with the MNS (IFG, IPL, SMA; Rizzolatti & Craighero, 2004) and the mentalizing system (TPJ, dmPFC, OFC; Frith & Frith, 2006).

Table 5.6

*Sizes of ROIs used in the functional connectivity analysis*

| | Voxels | Volume (mm³) |
|---|---|---|
| dmPFC | 1031 | 8248 |
| Left TPJ | 43 | 344 |
| Right TPJ | 177 | 1416 |
| Left OFC | 1551 | 12408 |
| Right OFC | 1520 | 12160 |
| Left IFG | 191 | 1528 |
| Right IFG | 144 | 1152 |
| Left IPL | 19 | 152 |
| Right IPL | 22 | 176 |

First, we asked whether there was a difference in functional connectivity *within* each system during the mentalizing and non-mentalizing blocks. The time-course of response in each region associated with the MNS or the mentalizing system was correlated with the time-course of each of the other regions associated with the MNS or mentalizing system. This analysis was performed separately for the mentalizing and non-mentalizing blocks. Figure 5.6 shows the connectivity between regions associated with the MNS and mentalizing system during mentalizing and non-mentalizing blocks. We asked whether connectivity during the mentalizing blocks was higher than during the non-mentalizing blocks. Connectivity between areas associated with the MNS significantly differed between different regions [F(2,78)=25.26, p<.001, $\eta_p^2$=.39], but connectivity was not significantly higher during mentalizing blocks compared to non-mentalizing blocks [F(1,39)=1.27, p=.27, $\eta_p^2$=.03] and the comparative strength of different connections did not change across mentalizing and non-mentalizing blocks [F(2,78)=.15, p=.89, $\eta_p^2$=.004]. Similarly, the strength of connectivity between areas of the mentalizing system were significantly different between different regions [F(2,78)=49.36, p<.001, $\eta_p^2$=.56], but connectivity did not differ across tasks [F(1,39)=2.17, p=.15, $\eta_p^2$=.05] and the relative strength of connectivity between these regions did not alter as a function of the task [F(2,78)=.81, p=.45, $\eta_p^2$=.02].

Next, we asked whether there was a difference in functional connectivity *between* the MNS and mentalizing system. Figure 5.7 shows the functional connectivity between the mentalizing and MNS regions. A 3-way ANOVA revealed a significant interaction between task and mentalizing system region [F(2,78)=3.37, p=.04, $\eta_p^2$=.08], a significant interaction between MNS and mentalizing system regions [F(4,156)=2.65, p<.001, $\eta_p^2$=.44] as well as main effects of MNS region [F(2,78)=88.15, p<.001, $\eta_p^2$=.69] and mentalizing system region [F(2,78)=38.40, p<.001, $\eta_p^2$=.50].

Individual ANOVAs for each mentalizing system region were then conducted in order to examine which areas of the mentalizing system showed differences in functional connectivity across tasks. The dmPFC showed differences in connectivity across tasks (F(1,39)=5.81, p=.02, $\eta_p^2$=.13] and between MNS regions [$F(2,78)=100.30$, $p<.001$, $\eta_p^2=.72$] but the relative strength of connectivity with different MNS regions did not change as a result of experimental task [$F(2,78)=1.21$, $p=.30$, $\eta_p^2=.03$]. Paired t-tests revealed that connectivity was significantly higher between dmPFC and IFG [$t(39)=2.01$, $p=.05$] and between dmPFC and SMA [$t(39)=2.33$, $p=.03$] during the mentalizing task. Connectivity between dmPFC and IPL was not significantly different across tasks [$t(39)=1.28$, $p=.21$].

The OFC showed significantly different levels of functional connectivity with different MNS regions [$F(2,78)=23.04$, $p<.001$, $\eta_p^2=.37$] but connectivity did not significantly differ across tasks [$F(1,39)=.10$, $p=.76$, $\eta_p^2=.002$] and the relative strength of connectivity between OFC and different MNS regions did not differ across tasks [$F(2,78)=.36$, $p=.68$, $\eta_p^2=.009$].

The TPJ did show significantly different levels of functional connectivity with areas associated with the MNS across tasks [$F(1,39)=1.14$, $p=.29$, $\eta_p^2=..03$], between MNS regions [$F(2,78)=.92$, $p=.41$, $\eta_p^2=.02$] and there was no significant interaction between MNS area and task [$F(2,78)=.60$, $p=.55$, $\eta_p^2=.02$].

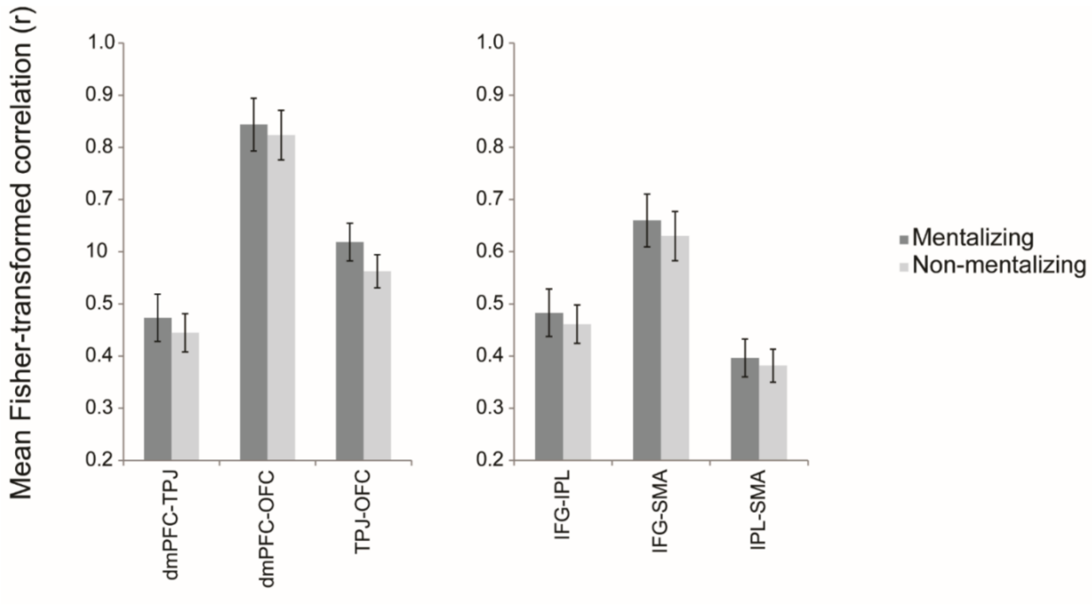

**Figure 5.6** Mentalizing-induced changes in functional connectivity within the mentalizing system and the MNS. Functional connectivity between regions within the MNS and within the mentalizing system did not significantly differ across tasks.

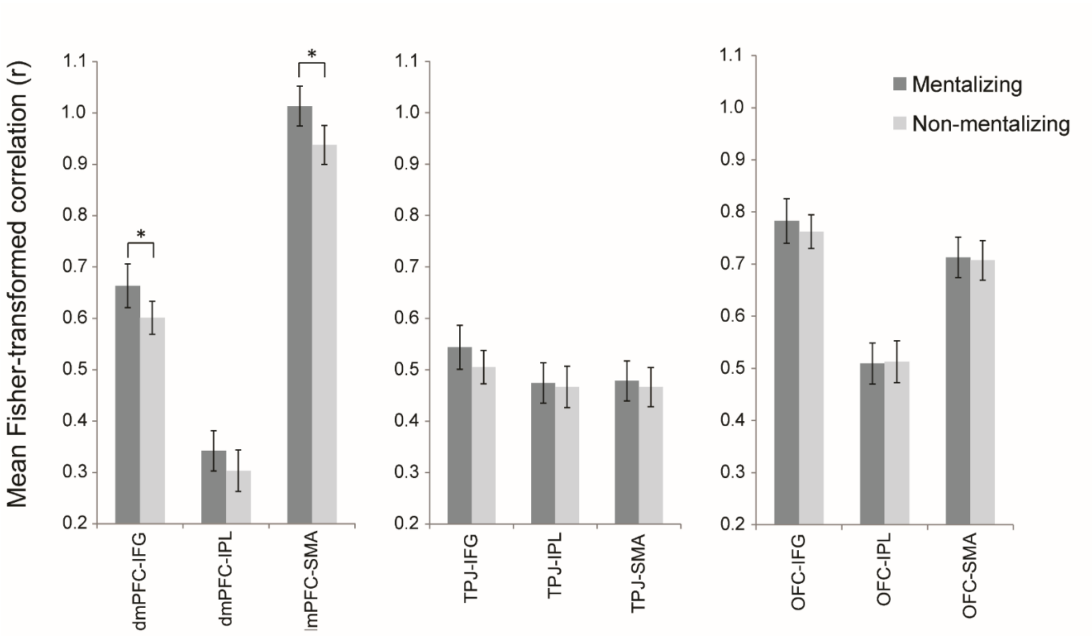

**Figure 5.7** Mentalizing-induced changes in functional connectivity between areas of the MNS and the mentalizing system. Higher connectivity was found between the dmPFC and the IFG [t(39)=2.01, p=.05] and between dmPFC and SMA [t(39)=2.33, p=.03] during mentalizing blocks.

These results suggest that connectivity between the dmPFC and IFG and between dmPFC and SMA play an important role in mentalizing. In the final analysis, we asked whether the connectivity between these regions is predicted by the level of autistic traits. To do this, we performed linear regression analyses. Autistic traits were shown to significantly predict the degree to which connectivity between dmPFC and IFG was increased during mentalizing blocks. Adults with high levels of autistic traits showed reduced mentalizing-induced changes in functional connectivity between IFG and dmPFC [F(1,38)=5.61, p=.02, $R^2$=.13; See Figure 5.8]. Autistic traits did not significantly predict mentalizing-induced changes in functional connectivity between SMA and dmPFC [F(1,38)=.03, p=.87, $R^2$=.001].

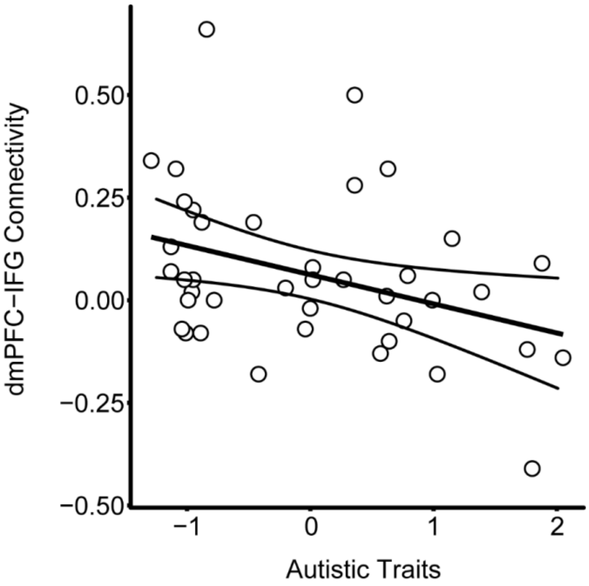

**Figure 5.8** The significant negative relationship between autistic traits and the degree of mentalizing-induced functional connectivity between dmPFC and IFG. Linear regression analysis found that autistic traits significantly predicted the mentalizing-induced change in functional connectivity between dmPFC and IFG: [F(1,38)=5.61, p=.02, $R^2$=.13]. The curved lines represent 95% confidence intervals.

Due to reports of increased right IFG connectivity with dmPFC when inferring the internal states of others from their actions (Rudie et al., 2012; Spunt & Lieberman, 2012a; Spunt & Lieberman, 2012b; Tettamanti et al., 2017), the possibility of lateralisation in the increased connectivity found between IFG and dmPFC was explored by comparing functional connectivity between IFG and dmPFC across tasks for each hemisphere separately. Functional connectivity was increased between right IFG and dmPFC during the mentalizing task [t(39)=2.05, p=<.05] but not between left IFG and dmPFC [t(39)=.57, p=.57]. The degree of mentalizing-induced increase in connectivity between right IFG and dmPFC was significantly predicted by the level of autistic traits that participants displayed [F(1,38)=5.34, p=.02, $R^2$=.12].

### 5.5 Discussion

This study aimed to identify whether functional connectivity between areas associated with the MNS and the mentalizing system is higher when inferring the social intentions of others from their actions and whether differences in functional connectivity between these systems may underlie the mentalizing difficulties associated with ASD. Inferring others' social intentions (mentalizing task) elicited higher levels functional connectivity between the dorsal medial prefrontal cortex (dmPFC; mentalizing system) and regions associated with the MNS (inferior frontal gyrus - IFG and supplementary motor area - SMA). Moreover, adults with high levels of autistic traits were impaired on the mentalizing task and this corresponded with reduced functional connectivity between IFG and dmPFC. Together, these results suggest that reduced connectivity between areas associated with the MNS and the mentalizing system may underlie the difficulties in inferring social intentions experienced by adults with ASD.

Our findings highlight the importance of both areas associated with the MNS and the mentalizing system in inferring social intentions from actions. Higher levels of activation were found in regions associated with the MNS and the mentalizing system during mentalizing blocks compared to non-mentalizing blocks. These regions included the dmPFC, temporoparietal junction (TPJ), IFG and inferior parietal lobe (IPL). Behavioural performance was also correlated with increased activity in the dmPFC and the SMA during the mentalizing task. These findings are consistent with previous studies, which have identified higher levels of activity in areas associated with the MNS and mentalizing system when inferring social intentions from hand actions (Becchio et al., 2012; Chambon, Domenech, et al., 2017).

A number of theories have been proposed to explain the role of the MNS when mentalizing. Our data are not consistent the mirroring-first theory (Hamilton & Marsh, 2013; Spunt, Satpute, & Lieberman, 2011). This proposes that, although action processing in the MNS is a necessary prerequisite for mentalizing, the MNS does not process internal state information. According to this theory equivalent levels of MNS activation would be expected during the mentalizing and non-mentalizing blocks, because both tasks involved watching hand action videos. In contrast to the mirroring first theory, the motor simulation theory proposes that the MNS internally simulates observed actions and this is sufficient to infer the internal state of others (Rizzolatti & Sinigaglia, 2007). This theory would predict higher MNS activation during the mentalizing block than the non-mentalizing block and a strong correlation between MNS activity and mentalizing performance. Our findings show higher responses in areas associated with the MNS during the mentalizing task, consistent with the motor simulation theory. However, although levels of response in the SMA correlated with performance on the mentalizing task, we did not find any correlation between activity in the core regions associated with the MNS (IFG, IPL) and mentalizing performance. We also found that activity in the mPFC correlated with mentalizing performance and connectivity between areas associated with the

MNS and MS is higher when mentalizing. These data are more consistent with the dual-process theory of mentalizing which proposes that both the MNS and the mentalizing system have roles in inferring the internal states of others from their actions (de Lange et al., 2008; Spunt & Lieberman, 2012b). This theory suggests that the MNS converts kinematic information into internal state information which can then be actively interpreted in the mentalizing system. This theory would predict higher MNS activation during the mentalizing task compared to the non-mentalizing task and that activation in the mentalizing system would correlate with mentalizing performance but there would not be a strong correlation between MNS activation and behavioural performance.

As well as providing evidence to support a role areas associated with the MNS in processing social intentions, our data provide support for the importance of connectivity between the MNS and the mentalizing system when inferring social intentions. Our data show increased connectivity between dmPFC and IFG as well as increased connectivity between dmPFC and SMA when inferring the social intentions of others from their actions. Previous studies have also shown increased functional connectivity between areas of the MNS and the mentalizing system when inferring internal states from the actions of others (Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; Lombardo et al., 2010; Marsh et al., 2010; Sperduti, Guionnet, Fossati, & Nadel, 2014; Spunt & Lieberman, 2012a; 2012b; Trapp et al., 2014; Xu, Gannon, Emmorey, Smith, & Braun, 2009). The most commonly reported connection between areas of the MNS and the mentalizing system when inferring others' internal states from their actions has been between dmPFC and right IFG (Rudie et al., 2012; Spunt & Lieberman, 2012; Spunt & Lieberman, 2012; Tettamanti et al., 2017). These studies have shown increased connectivity between right IFG and dmPFC when inferring motive underlying observed actions (Spunt & Lieberman, 2012b), when inferring the reasons for displays of emotion in movie clips (Spunt & Lieberman, 2012a) and when viewing emotional facial expressions (Rudie et al., 2012). Follow-up analyses on our data suggest that the increase in IFG connectivity with dmPFC in our study was also right-lateralised.

In contrast, previous studies which have used mentalizing tasks which did not involve viewing others' actions e.g. imaging social actions (Trapp et al., 2014) or when making mental state inferences from passages of text (Lombardo et al., 2010; Tettamanti et al., 2017) have reported connectivity between left IFG/ventral premotor cortex (vPMC) and the mPFC. Collectively, these data suggest that inferring internal states from observed actions may elicit functional connectivity between right IFG and mPFC but inferring internal states in the absence of visual action information (e.g. from passages of text) may elicit connectivity between the left IFG/vPMC and mPFC. This suggestion of lateralisation of MNS connectivity with the mPFC is supported by a recent study which showed increased functional connectivity between the right IFG and dmPFC when inferring communicative intentions from gestures but between left IFG and dmPFC when inferring communicative intentions from passages of text (Tettamanti et al., 2017).

Knowledge of brain areas which show increased functional connectivity when inferring social intentions is important for understanding the neural basis of deficits associated with ASD. Our behavioural data show that adults with high levels of autistic traits were impaired at inferring the social intentions of others from their actions, but there was no significant relationship between autistic traits and performance on the non-mentalizing task. These data imply the existence of a mentalizing-specific deficit in individuals with high levels of autistic traits rather than a more generalised deficit in the ability to perform experimental tasks. These data compliment findings from previous studies that have shown mentalizing-specific deficits in adults with ASD (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Castelli, Frith, Happé, & Frith, 2002; Jolliffe & Baron-Cohen, 1999; Kana, Libero, Hu, Deshpande, & Colburn, 2014) and individuals with high levels of autistic traits (Gökçen, Petrides, Hudry, Frederickson, & Smillie, 2014; Gökçen, Frederickson, & Petrides, 2016), including difficulties inferring the social intentions of others (Cole, Slocombe & Barraclough, 2017; Happé, 1994; Moran et al., 2011).

In addition to the behavioural deficits we found, participants with high levels of autistic traits also exhibited reduced functional connectivity between IFG and dmPFC during mentalizing blocks. These data compliment findings of reduced functional connectivity between the MNS and mentalizing system in individuals with ASD when inferring intentions from passages of text (Mason et al., 2008) or from comic strips (Kana et al., 2014). However, this is the first study to investigate differences in functional connectivity in adults with ASD when inferring the intentions of others from their actions. Identifying neural differences in adults with ASD when inferring others' intentions from their actions is important for understanding the neural basis of the mentalizing deficits these individuals experience because adults with ASD show consistent difficulties inferring aspects of someone's internal state from their actions but not in the absence of human action (Kana et al., 2009; Kirkovski, Enticott, et al., 2016; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001; Rosenblau et al., 2015; Spek et al., 2010). Neuroimaging studies have shown different patterns of functional connectivity between areas of the MNS and the mentalizing system in typically developing individuals when mentalizing in the presence of human action compared to in the absence of action information (Lombardo et al., 2010; Spunt & Lieberman, 2012b; 2012c; Tettamanti et al., 2017). The reduced functional connectivity found between IFG and dmPFC in adults with high levels of autistic traits could underlie difficulties individuals with ASD have in inferring others' social intentions from their actions.

It has been proposed that inferring the social intentions of others from their actions requires the integration of contextual information and prior experience with action kinematics (Chambon, Domenech, et al., 2017b; de Lange et al., 2008; Jacob & Jeannerod, 2005; Liew et al., 2011; Mainieri et al., 2013). Existing fMRI studies have provided evidence that action context and prior expectations about an upcoming action are processed in the mPFC (Alexander & Brown, 2011b; Becchio et al., 2012; Chambon, Domenech, et al., 2017b; Cooper et al., 2010; Fogelson et al., 2009; Forster & Brown, 2011) and IFG activation has been associated with inferring intentions based on kinematic cues (Becchio et al., 2012; Buccino et al., 2007; Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; Herbet et al., 2014). Therefore, reduced connectivity between the mPFC and IFG in adults with high levels of autistic traits may result in reduced integration of kinematic and contextual information, leading to difficulties inferring social intentions (de Lange et al., 2008; Keysers & Gazzola, 2007; Spunt & Lieberman, 2012c; Uddin et al., 2007). The theory of reduced integration of action context and kinematic information in ASD is supported by the results of a meta-analysis which concluded that adults with ASD are significantly impaired on mentalizing tasks that involve integrating information regarding others' internal states with surrounding context (Baez et al., 2012). Additionally, previous behavioural studies have found atypical utilisation of prior experience relative to incoming sensory information in adults with ASD when inferring social intentions from actions (Chambon, Farrer, et al., 2017) and children with ASD show impaired abilities to infer intentions when this requires processing of both contextual information and kinematic information (Boria et al., 2009). These data imply that integration of prior expectations and incoming sensory information is atypical in ASD during action observation. This reduced integration may result from reduced functional connectivity between IFG and dmPFC and consequently give rise to poorer abilities to infer others' social intentions from their actions.

This study focussed on investigating relationships between autistic traits and both behavioural and neural differences rather than potential group differences in behavioural and neural measures. We conducted the analyses in this way because ASD is a spectrum condition and high levels of variability in autistic traits exist within clinical and non-clinical populations (American Psychiatric Association, 2013; Robinson et al., 2011; van Boxtel & Lu, 2013; Von Dem Hagen et al., 2011). Individuals with relatively high but not clinically significant levels of autistic traits have been shown to display subtler versions of the behavioural and neurological characteristics associated with ASD (Best, Arora, Porter, & Doherty, 2015; Di Martino et al., 2009; Lindell, Notice, & Withers, 2009; Ridley, Homewood, & Walters, 2011; van Boxtel & Lu, 2013) including mentalizing deficits (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Chung, Barch, & Strube, 2014; Happé, 1994; Kana et al., 2014; Moran et al., 2011). Therefore, high variability in autistic traits within ASD and TD groups could reduce the chances of observing group differences in behavioural and neural characteristics associated with ASD. Our data show that the significant relationships between autistic traits and both abilities to infer social intentions and the degree of mentalizing-induced changes in functional connectivity would not be significant if participants were analysed as two distinct groups (see supplementary material). The participants in this study displayed a range of autistic traits rather than the existence of two discrete groups (see Figures 5.2 and 5.6) meaning that variability within ASD and TD groups likely reduced the ability to observe group differences. Our data suggest that, in comparison to investigating potential group differences, examining the relationship between autistic traits and neural and behavioural measures is a more sensitive method, which can identify characteristics associated with high levels of autistic traits which are not identified when simply investigating group differences.

In conclusion, activation in areas associated with the MNS and the mentalizing system as well as increased functional connectivity between these two systems was found when inferring social intentions from their actions of others. Adults with high levels of autistic traits displayed reduced abilities to infer social intentions and exhibited reduced connectivity between these systems. Our data highlight the possibility that reduced connectivity between areas associated with the MNS and the mentalizing system could underlie the difficulties adults with ASD experience in inferring social intentions.

# Chapter 6: General Discussion

The studies presented in this thesis aimed to identify the brain regions involved in inferring the social intentions of others from their actions, the nature of difficulties in inferring social intentions associated with ASD and the neural basis of these deficits. First, a behavioural study found adults with ASD were impaired in explicitly but not implicitly inferring the social intentions of others from their actions. Second, the results of a TMS study suggested that the mirror neuron system (MNS) is involved in inferring others' intentions from their actions but only at the end of observed actions. Third, a combined TMS-EEG experiment found reduced right MNS activity in individuals with high levels of autistic traits when inferring social intentions but left MNS activity correlated with behavioural performance. Finally, an fMRI study found increased functional connectivity between areas of the MNS and the mentalizing system when inferring social intentions which was reduced in adults with high levels of autistic traits. Collectively, the results of these studies provide evidence to support that the MNS has a role in inferring others' intentions and reduced functional connectivity between the MNS and the mentalizing system in adults with high levels of autistic traits may underlie difficulties in explicitly inferring the social intentions of others from their actions.

## 6.1 MNS involvement in mentalizing

The data presented in this thesis suggest that the MNS has a role in processing the intentions of others from their actions but not actively interpreting intentional information. Higher levels of MNS activation were found in the first TMS experiment (Chapter 3) and the fMRI experiment (Chapter 5) when inferring others' social intentions compared to when viewing similar actions but performing a non-mentalizing task which suggest that the MNS is involved in processing intentional information. These data provide evidence against the mirroring-first theory which states that although motor simulation is a necessary prerequisite for mentalizing, the MNS does not process internal state information (Hamilton & Marsh, 2013). The mirroring-first theory was constructed based on fMRI studies showing increased MNS activation when actions were viewed which had with unusual kinematic profiles but increased mentalizing system, not MNS, activation when actions with unusual intentions were viewed (Brass et al., 2007; de Lange et al., 2008). However, despite the evidence provided for higher MNS activation during mentalizing tasks from the experiments presented in Chapters 3 and 5, this task-related difference in MNS activity was not found in the TMS-EEG experiment presented in Chapter 4. There are a couple of factors that may explain why a mentalizing-induced increase in MNS activity was not found in this experiment. First, TMS was applied to the left hemisphere in this experiment. In our first TMS experiment (Chapter 3), the right hemisphere was stimulated and a mentalizing-induced increase in MEP sizes (reflecting increased MNS activity) was found at the end of observed actions. The time point in which larger MEP sizes were found in this experiment was then used as the time point for stimulation in the TMS-EEG study but a mentalizing-induced increase in MEP sizes was not found. Therefore, the mentalizing-induced increase in MNS activity found at the end of observed actions in the first TMS experiment may restricted to the right-hemisphere. A second reason why a mentalizing-induced increase in MNS activity might not have been found in the TMS-EEG experiment is that the index of MNS activity used for our EEG data (*mu* suppression) may reflect activity in the extended MNS rather than core areas associated with the MNS (Arnstein et al., 2011; Cheyne et al., 2003; Jones et al., 2009). Previous MEG studies

(Cheyne et al., 2003; Jones et al., 2009) and a combined MRI-EEG study (Arnstein et al., 2011) suggest that *mu* suppression corresponds to activation in the primary somatosensory cortex (S1). S1 has been shown to display mirror properties (Confalonieri et al., 2012; Gazzola & Keysers, 2009; Molenberghs et al., 2012; Porro et al., 1996) but is not considered a 'core' region associated with the MNS (Rizzolatti & Craighero, 2004; Rizzolatti & Sinigaglia, 2010). In comparison, TMS-induced motor-evoked potentials (MEPs) measure increased excitability in M1 which is considered to be the result of excitatory cortico-cortical connections from prefrontal MNS areas (IFG/ventral premotor cortex (vPMC); Fadiga, Craighero, & Olivier, 2005; Loporto, McAllister, Williams, Hardwick, & Holmes, 2011). Therefore, the larger TMS-evoked MEPs during the mentalizing task in Chapter 3 as well as the fMRI (Chapter 5) suggest that higher activation was elicited in core frontal regions associated with the MNS during the mentalizing task. If *mu* suppression reflects S1 activation then the EEG data do not contradict these findings. Data from all the studies presented in this thesis therefore suggest that activity in core regions associated with the MNS is higher when inferring the social intentions of others from their actions but not S1, part of the extended MNS, and the temporally-specific increase in activation in core MNS areas identified at the end of observed actions may be right-lateralised.

Despite the higher levels of activation found in core areas associated with the MNS when mentalizing than when performing a non-mentalizing task, mentalizing performance was not significantly related to the level of MNS activity in our TMS experiment (Chapter 3), the TMS data from the TMS-EEG study (Chapter 4) or activity in core regions associated with the MNS in the fMRI experiment (Chapter 5). These data oppose the motor simulation theory of mentalizing which states that

MNS activation alone is sufficient in order to infer others' internal states (Rizzolatti & Sinigaglia, 2007). The motor simulation is based on empirical evidence showing actions with different intentions exhibit different kinematic profiles and these different kinematic profiles can be used to successfully infer the intentions of others (Manera, Becchio, Cavallo, Sartori, & Castiello, 2011; Sartori, Becchio, & Castiello, 2011). The motor simulation theory would predict that activity in core regions associated with the MNS would be directly related to behavioural performance when inferring other individuals' intentions. In contrast, the EEG data from the TMS-EEG experiment do show a relationship between mentalizing performance and left-lateralised *mu* suppression. Differences in the methods used to measure MNS activity may account for these different results. Given that *mu* suppression likely reflects activity in S1 (Arnstein et al., 2011; Cheyne et al., 2003; Jones et al., 2009) then the EEG data do not contradict the finding that activity in core areas associated with the MNS are not associated with superior abilities to infer the social intentions of others from their actions.

The lack of a significant relationship between activation in the core MNS areas and mentalizing performance but higher levels of MNS activation when inferring social intentions compared to completing a non-mentalizing task, suggest the MNS has a role in processing the internal states of others but not actively interpreting this information. This is potentially consistent with a proposed dual-process hypothesis of mentalizing in the presence of human action which states that the MNS is involved in pre-reflective processing of internal state information embedded within action kinematics whereas the mentalizing system actively infers the internal states motivating observed actions (de Lange, Spronk, Willems, Toni, & Bekkering, 2008; Spunt & Lieberman, 2012b). Spunt and Lieberman generated the dual-process hypothesis based on results from their fMRI studies in combination with classic social psychology theory of social casual attribution (Spunt & Lieberman, 2012a). Data from their fMRI studies found both MNS and mentalizing system activation during mentalizing tasks, increased connectivity between these two systems when mentalizing, particularly when action information was available, but only mentalizing system activation increased in response to increased mentalizing demand of experimental tasks (Spunt & Lieberman, 2011; Spunt & Lieberman, 2012a; 2012b). Spunt and Lieberman argued that these data reflected the importance of both the MNS and the mentalizing system in inferring internal states of others but that these systems have different roles. Social casual attribution theory states that when inferring internal states from actions, incoming sensory information must be translated into internal state information (Gilbert, 1998). Additionally, previous fMRI studies provided evidence that IFG encodes contextual information which indicates the actors' intentions (Iacoboni et al., 2005; Hamilton and Grafton, 2008). Collectively, these data and social causal attribution theory led them to hypothesise that the MNS translates kinematic information into internal state information at the subconscious level and passes internal state information to the mentalizing system for this to be actively interpreted.

## 6.2 Connectivity between the MNS and the mentalizing system

As well as providing evidence to support a role of the MNS in inferring the social intentions of others from their actions, the data presented in this thesis indicate that connectivity between the MNS and the mentalizing system is important for this task. Increased functional connectivity between the dmPFC (region of the mentalizing system) and both IFG and SMA (regions of the MNS) was found when inferring the social intentions of others in the fMRI experiment (Chapter 5). The increased connectivity found between areas of the MNS and the mentalizing system compliments findings of previous fMRI studies which have shown increased functional connectivity between these systems when inferring others' internal states from their actions (Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; Lombardo et al., 2010; Marsh et al., 2010; Sperduti, Guionnet, Fossati, & Nadel, 2014; Spunt & Lieberman, 2012b; Trapp et al., 2014; Xu, Gannon, Emmorey, Smith, & Braun, 2009).

The most commonly reported connection between MNS and mentalizing system areas in the previous literature when inferring internal states from actions has been between dmPFC and right IFG (Rudie et al., 2012; Spunt & Lieberman, 2012a; 2012b; Tettamanti et al., 2017). Further analyses carried out on our fMRI data imply that the increased connectivity between IFG and dmPFC found when inferring social intentions is also lateralised to right IFG. These data highlight the possibility of a right-hemisphere lateralisation of functional connectivity between the IFG and dmPFC when inferring internal states of others from their actions. In contrast, previous studies that have used mentalizing tasks which do not involve viewing others' actions e.g. imagining social actions (Trapp et al., 2014) or inferring the mental states of characters from passages of text (Lombardo et al., 2010) have elicited higher functional connectivity between left IFG or left vPMC and the mPFC. The IFG and vPMC occupy similar regions of the cortex and some previous studies have combined these areas and referred to one core frontal region of the MNS (IFG/vPMC;Bastiaansen, Thioux, & Keysers, 2009; Mehta, Thirthalli, Basavaraju, Gangadhar, & Pascual-Leone, 2013; Spunt & Lieberman, 2012b). Collectively, these data suggest that inferring others' internal states from observed actions may elicit greater functional connectivity between IFG/cPMC in the right hemisphere and mPFC but inferring internal states in the absence of visual action information may elicit greater connectivity between left IFG/vPMC and the mPFC. This suggestion of lateralisation of MNS connectivity with the mPFC is supported by the results of a recent study which investigated patterns of functional connectivity when inferring communicative intentions from both gestures and passages of text (Tettamanti et al., 2017). This study found increased connectivity between right IFG and mPFC when inferring communicative intentions from gestures but increased connectivity between left IFG and mPFC when inferring communicative gestures from passages of text.

### 6.3 Nature of connectivity between the MNS and the mentalizing system

The timing of MNS activity identified in Chapter 3 suggests that connectivity between the MNS and the mentalizing system when inferring others' intentions, may be best conceptualised within a predictive coding framework. The late timing of MNS activity when inferring the social intentions of others from their actions, identified in Chapter 3, provides evidence against the stages of intentional information processing proposed by the dual-process and mirroring-first models. Both of these models suggest that internal state information is only derived in the mentalizing system after MNS involvement. However, the data presented in this thesis in combination with the existing literature suggest that information about an individual's internal state is processed in the mentalizing system first before MNS involvement. The results from our first TMS experiment (Chapter 3) suggest that others' social intentions are only signalled by the MNS once they have been fully revealed at the end of observed actions. Previous behavioural studies have shown that others' intentions can be inferred from the early kinematics of the action before the outcome is evident at the end (Manera, Becchio, Cavallo, Sartori, & Castiello, 2011; Sartori, Becchio, & Castiello, 2011). The data presented in this thesis therefore suggest that intentional information is processed elsewhere before the MNS, and this may fit within a predictive coding framework.

The predictive coding theory of MNS functioning states that action context is signalled to the MNS prior to motor simulation, in order for predictions to be made about the outcome of upcoming actions (Kilner et al., 2007). The predictive coding model is based on empirical evidence showing that when observing actions, EEG signals reflecting motor preparation are generated (Kilner et al., 2004), suggesting the intended outcome of observed actions are predicted in order to prepare the appropriate response. Additionally, increased muscle activation in observer's mouths has been found when observing grasp-to-eat actions but not grasp-to –place actions before the outcome of the actions are shown (Cattaneo et al., 2007). This suggests that predicted intentions of observed actions are encoded in the observer's MNS. The predicted outcome response model of mPFC function was constructed by Alexander and Brown based on neuroimaging studies providing evidence that the mPFC encodes both predicted outcomes of observed actions and prediction errors (Alexander & Brown, 2011). The predicted outcome response model suggests that the mPFC forms predictions about upcoming actions based on surrounding context and prior experience (Alexander & Brown, 2011). The predictive coding theory of MNS functioning in combination with the predicted outcome response model of mPFC function therefore imply that inferences regarding the internal state underlying an action are formed in the mPFC first, based on previous experience and contextual information, before MNS involvement. Neuroimaging studies have provided evidence that prior expectations about upcoming actions and action context are processed in the mPFC (Alexander & Brown, 2011b; Becchio et al., 2012; Chambon, Domenech, et al., 2017b; Cooper et al., 2010; Fogelson et al., 2009;

Forster & Brown, 2011) and event-related potential (ERP) studies have provided evidence to show mPFC activation as early as 200ms after stimulus onset during mentalizing tasks (Van der Cruyssen, Van Duynslaeger, Cortoos, & Van Overwalle, 2009; Van Duynslaeger, Van Overwalle, & Verstraeten, 2007; Van Overwalle, Van den Eede, Baetens, & Vandekerckhove, 2009). These data and the late timing of MNS involvement in processing intentional information found in our TMS data (Chapter 3) is consistent with the predictive coding framework.

The increased MNS activation we identified at the end of observed actions could reflect signalling of prediction error once the actual intentions of the observed actions have been revealed by the kinematics of the action. The predictive coding theory of MNS functioning proposed by Kilner suggests that signalling of prediction error is confined within the MNS but existing neuroimaging data and the predicted outcome response model of mPFC functioning imply that the mPFC also signals prediction errors (Alexander & Brown, 2014; Jahn et al., 2014; Malekshahi et al., 2016; Zarr & Brown, 2016). This raises the possibility that prediction errors formed in the MNS may be signalled to the mPFC.

## 6.4 Mentalizing impairments in ASD

The behavioural data presented in this thesis suggest that ASD is associated with difficulties explicitly inferring social intentions of others from their actions. The behavioural experiments presented in Chapter 2 assessed both implicit and explicit abilities of adults with ASD to infer the social intentions of others using the same naturalistic stimuli and behavioural outcome measures for both tasks. The results of these experiments suggest that adults with ASD are significantly impaired at explicitly but not implicitly inferring the social intentions of others from their actions. The behavioural data from our fMRI experiment presented in Chapter 5 also show that adults with high levels of autistic traits are significantly impaired at explicitly inferring the social intentions of others from their actions, supporting the association between ASD and this explicit mentalizing deficit. In contrast, the behavioural data from the TMS-EEG experiment presented in Chapter 4 show no relationship between autistic traits and abilities to explicitly infer the social intentions of others from their actions. This conflicting result may have been due to the comparatively lower levels of autistic traits displayed by the participants in the TMS-EEG experiment (see Table 6.1) and therefore there may not have been a wide enough range of autistic traits in this participant sample to detect a relationship between autistic traits and behavioural performance. When the behavioural data from all experiments are combined, higher levels of autistic traits significantly predict poorer explicit mentalizing performance [$F(1,99)=7.09$, $p<.01$, $R^2=.07$]. Adults with an ASD diagnosis ($n=41$) also display significantly poorer explicit mentalizing abilities compared to adults without an ASD diagnosis [$n=61$; $t(43.58)=-2.46$, $p=.02$]. The combined data from all studies therefore support the association between ASD and deficits in explicitly inferring the social intentions of others from their actions.

Table 6.1

*Psychological test scores across all studies with ASD participant; mean (SD) values*

| | ADOS | AQ | SRS | TASIT* |
|---|---|---|---|---|
| Chapter 2: Behavioural experiment | 5.3(3.34) | 25.3 (11.9) | 75.8 (39.96) | 54.3 (6.93) |
| Chapter 4: TMS-EEG experiment | 4.70 (3.28) | 21.24(10.91) | 60.83 (36.03) | 55.19 (4.99) |
| Chapter 5: fMRI experiment | N/A | 25.15 (14.29) | 75.3 (46.34) | 52.28 (10.15) |

*higher values indicate lower levels of autistic traits

A trend was found between higher levels of autistic traits and poorer abilities to implicitly infer the social intentions of others from their actions in Chapter 2. It is possible that with a larger sample size, a significant relationship between autistic traits and implicit mentalizing performance would be found. However, the much larger effect size for the explicit task (r=.39) compared to the implicit task (r=.11) further supports the existence of a significant explicit mentalizing deficit but no clear implicit mentalizing deficit in these adults with ASD. Additionally, the TMS experiment presented in Chapter 3 did not find a significant relationship between autistic traits and implicit mentalizing performance in individuals without ASD diagnoses.

The finding of impaired explicit mentalizing but no clear deficit in implicit mentalizing performance contradicts data from the existing literature which suggest that adults with ASD display consistent implicit but not explicit mentalizing difficulties (Kana et al., 2009; Kirkovski et al., 2015; Rosenblau et al., 2015;

Schuwerk et al., 2014; Senju et al., 2009). However, the comparison between implicit and explicit mentalizing abilities in the previous literature is confounded by differences in the methods used to measure mentalizing abilities, the aspects of someone's internal state being inferred and the stimuli used. The use of simplistic stimuli in explicit tasks may have allowed some individuals with ASD to explicitly mentalize, perhaps with the help of learned strategies, which are less useful in more complex, naturalistic settings. The measurement of implicit mentalizing abilities using eye-tracking data alone rather than measurable behavioural outcomes may have also contributed to the existence of a consistent implicit mentalizing deficit (Schuwerk et al., 2014; Senju et al., 2009); atypical fixation patterns are often reported for adults with ASD when processing social stimuli, even in the absence of impaired behavioural performances (Pelphrey et al., 2002; Rutherford & Towns, 2008; Spezio et al., 2007). Our results suggest that differences in the stimuli and the methods used to measure mentalizing abilities may have contributed to the apparent difference in explicit and implicit mentalizing abilities in adults with ASD in the existing literature. However, our behavioural study is the first to measure implicit abilities of adults with ASD to infer the intentions of others; previous studies measuring implicit mentalizing abilities in adults with ASD have assessed abilities to infer mental states or false beliefs (Rosenblau et al., 2015; Schuwerk et al., 2014; Senju et al., 2009). It is possible that adults with ASD display greater impairments in inferring certain aspects of someone's internal state compared to others. Inferring different aspects of someone's internal state may involve different processes (Call & Tomasello, 2008; Pineda & Hecht, 2009) and different brain regions have been shown to be active depending on the mentalizing task being performed (Gobbini et al., 2007; Pineda & Hecht, 2009). Therefore, adults with ASD may exhibit neural atypicalities which impair their abilities to perform certain internal state inferences more than others.

## 6.5 Evidence against the theory of reduced mentalizing system activity in ASD

The fMRI experiment presented in Chapter 4 provides evidence suggests mentalizing system activation is not reduced in adults with ASD when inferring others' intentions from their actions. These data contradict previous findings of reduced mentalizing system activation during mentalizing tasks in adults with ASD (Baron-Cohen et al., 1999; Happé et al., 1996; Holt et al., 2014; Kana, Keller, Cherkassky, Minshew, & Just, 2009). However, some previous fMRI studies have also found no differences in mentalizing system activation (Ciaramidaro et al., 2015; Kana et al., 2014; Kirkovski et al., 2015; Vander Wyk, Hoffman, & Pelphrey, 2014) or higher mentalizing system activation (Marsh & Hamilton, 2011; Mason, Williams, Kana, Minshew, & Just, 2008) in adults with ASD during mentalizing tasks. Our data provide evidence against the hypothesis that reduced mentalizing system activation is the neurobiological basis for the social impairments associated with ASD.

The absence of reduced mentalizing system activation is supported by behavioural data which show that adults with ASD do not show consistent impairments on mentalizing tasks which do not involve human action (Kana et al., 2009; Kirkovski et al., 2015; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001; Spek et al., 2010); these tasks do not consistently activate the MNS and consequently, mentalizing system activation alone without MNS activation is considered to be sufficient in order to complete these tasks (Castelli et al., 2000; Castelli et al., 2002; Gallagher et al., 2000; White et al., 2014). Adults with ASD show more consistent deficits on mentalizing tasks that involve inferring internal states from others' actions (Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001; Rosenblau et al., 2015) which suggest greater impairments on mentalizing tasks which elicit MNS activity as well as mentalizing system activation. However, it could also be argued that tasks involving inferring internal states from human actions are more complex than inferring internal states of characters in passages of text or from still images of people's eyes and that increased task difficulty resulted in poorer performances rather than specific action-related deficits. In dispute of this argument, in the studies which found action-specific mentalizing deficits, ASD participants were IQ matched with control participants, meaning increased task difficulty should have resulted in equal reductions in performance across both groups if a specific deficit was not present.

### 6.6 Mixed results regarding reduced MNS activation associated with ASD

The results of the studies presented in this thesis are inconsistent regarding whether activity in the MNS is reduced in adults with high levels of autistic traits when mentalizing. The broken mirror neuron theory would predict reduced MNS activation during mentalizing tasks in adults with high levels of autistic traits and this would underlie the poorer mentalizing performances observed. A number of previous studies have found reduced MNS activation in adults with ASD when mentalizing (Baron-Cohen et al., 1999; Hadjikhani et al., 2009; Holt et al., 2014; Kana et al., 2014; Wicker et al., 2008). However, the existing literature is also inconsistent, with some studies reporting typical levels of MNS activation (Kana et al., 2009; Kirkovski et al., 2015; Marsh & Hamilton, 2011; Vander Wyk et al., 2014) and others reporting increased MNS activation (Libero et al., 2014; Mason et al., 2008) in adults with ASD during mentalizing tasks. The results of the TMS experiment presented in Chapter 3 suggest that the level of MNS activation when inferring the social intentions of others is not related to the level of autistic traits displayed in typically developing individuals. However, this experiment did not include participants with a diagnosis of ASD and therefore we may not have had a wide enough range of both autistic traits and levels of MNS activation to find a relationship between these two variables. The fMRI experiment presented in Chapter 5 and the TMS data from the TMS-EEG experiment in Chapter 4 also show no relationship between autistic traits and MNS activation; these studies had participant populations which included both individuals with and without ASD diagnoses. In contrast, the EEG data from the TMS-EEG experiment did show a significant negative relationship between autistic traits and right-lateralised MNS activation. The relationship between right-lateralised *mu* suppression and autistic traits may reflect a right-lateralised decrease in the extended MNS, possibly S1, when mentalizing. However, this evidence is limited because the fMRI data (Chapter 5) did not show reduced activation in these areas in individuals with high levels of autistic traits when mentalizing and therefore this result was not replicated. Overall, the data from the studies presented in this thesis provide little evidence to support the existence of reduced MNS in adults with high levels of autistic traits when inferring the social intentions of others from their actions, opposing the broken mirror neuron theory.

The lack of a consistent finding of reduced MNS activation in the presence of explicit mentalizing deficits could provide support for the visual inference model which suggests that internal states underlying others' actions are inferred in the mentalizing system without MNS involvement (Hamilton & Marsh, 2013). In support of this theory, some previous mentalizing tasks, not involving human action processing, have not elicited MNS activation (Castelli, Happé, Frith, & Frith, 2000; Castelli et al., 2002; Gallagher et al., 2000; Spunt, Satpute, & Lieberman, 2011; Van Overwalle et al., 2009; White, Frith, Rellecke, Al-Noor, & Gilbert, 2014), suggesting mentalizing does not require MNS activation and that MNS activation may simply reflect action processing. This theory would suggest that dysfunction in other brain areas, other than the MNS, underlie mentalizing deficits associated with ASD. This would be consistent with the theory that mentalizing system dysfunction, rather than MNS dysfunction underlies mentalizing deficits in ASD (Frith, 2001; Hamilton, 2009) or that the mentalizing deficits associated with ASD are attributable to more generalised deficits such as executive functioning (Hill, 2004; White 2013). However, the higher levels of MNS activation found during mentalizing compared to non-mentalizing tasks in Chapters 3 and 5 as well as the relationship between MNS activation and mentalizing performance in Chapter 4 suggest that the MNS does have a role in inferring the intentions of others from their actions.

## 6.7 Atypical connectivity between the MNS and the mentalizing system in ASD

The data from the studies presented in this thesis suggest that perhaps connectivity between the MNS and the mentalizing system is dysfunctional rather than activation in either system individually. The lack of evidence to support reduced mentalizing system and MNS activation in adults with high levels of autistic traits in our studies was unexpected; due to the mentalizing difficulties associated with ASD (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Chung, Barch, & Strube, 2014; Frith, 2001; Holt et al., 2014; Jolliffe & Baron-Cohen, 1999) and activation in both systems reliably found when inferring internal states from actions (Becchio et al., 2012; Ciaramidaro, Becchio, Colle, Bara, & Walter, 2014; de Lange, Spronk, Willems, Toni, & Bekkering, 2008; Hooker, Verosky, Germine, Knight, &

D'Esposito, 2010; Spunt & Lieberman, 2012a; 2012b), reduced activation in both systems was predicted. However, the theory of reduced connectivity between these two systems rather than reduced activation in either system individually compliments the existing behavioural data which suggest impairments are more pronounced when reliance on connectivity between these systems is higher. The finding of reduced connectivity between the dmPFC and IFG in adults with high levels of autistic traits, in Chapter 5, supports the results of previous studies which have shown reduced connectivity between these two systems in individuals with ASD when mentalizing (Ciaramidaro et al., 2015; Kana et al., 2014; Mason et al., 2008; Rudie et al., 2012b). Reduced connectivity between IFG and the mPFC specifically, has been reported in adults with ASD when inferring intentions from comic strips (Deshpande, Libero, Sreenivasan, Deshpande, & Kana, 2013), when inferring intentions and emotions from passages of text (Mason et al., 2008) and during rest (Itahashi et al., 2014; Kennedy & Courchesne, 2008). However, our fMRI study was the first to investigate patterns of functional connectivity when inferring others' social intentions from their actions in adults with ASD. Adults with ASD show consistent deficits on mentalizing tasks involving human action but not mentalizing tasks which do not involve action processing (Kana et al., 2009; Kirkovski, Enticott, et al., 2016; Ponnet, Roeyers, Buysse, De Clercq, & Van Der Heyden, 2004; Roeyers et al., 2001; Rosenblau et al., 2015; Spek et al., 2010) and a previous neuroimaging study showed higher connectivity between the MNS and the mentalizing system when inferring internal states from actions rather than text (Spunt & Lieberman, 2012b). Similarly, adults with ASD have shown consistent impairments when inferring social intentions but not inferring immediate motor goals (known as 'motor intentions'; Aldridge et al., 2000; Baron-Cohen et al., 1986;

Broekhof et al., 2015; Carpenter et al., 2001; Cole, Slocombe & Barraclough, 2017; Fisher et al., 2013). It has been proposed that because motor intentions are directly related to observed actions, kinematic processing without the integration of contextual information may be sufficient to infer these intentions (Catmur, 2015; Jacob & Jeannerod, 2005). Therefore connectivity between the MNS and the mentalizing system may not be required when inferring motor intentions, unlike when inferring social intentions (Liew et al., 2011; Mainieri et al., 2013; Spunt & Leiberman, 2012c; de Lange et al., 2008). These data suggest that mentalizing impairments in ASD are more pronounced when the reliance on connectivity between the MNS and the mentalizing system is higher and therefore support our finding of reduced functional connectivity between these systems in adults with ASD.

Reduced functional connectivity between the MNS and the mentalizing system but typical activation in the MNS could also explain the findings of an explicit mentalizing deficit but absence of a clear implicit mentalizing deficit in Chapter 2. If internal state information embedded in action kinematics is subconsciously processed in the MNS before being actively interpreted in the mentalizing system, as has been previously proposed (de Lange, Spronk, Willems, Toni, & Bekkering, 2008; Spunt & Lieberman, 2012b). Typical MNS activation in adults with ASD could allow these individuals to select the preferable action between a choice of two actions (required for our implicit mentalizing task) but not explicitly infer aspects of someone’s internal state, which is proposed to require kinematic information to be signalled from the MNS to the mentalizing system (de Lange, Spronk, Willems, Toni, & Bekkering, 2008; Spunt & Lieberman, 2012c). Therefore, according to the dual-process theory, intact implicit mentalizing abilities in the presence of explicit mentalizing deficits may reflect intact MNS functioning but dysfunctional connectivity between the two systems.

### 6.8 Mentalizing deficits explained in terms of the predictive coding framework

The possibility that reduced functional connectivity between the MNS and the mentalizing system may underlie difficulties inferring others' social intentions, in adults with high levels of autistic traits, could potentially be explained within a predictive coding framework. The predictive coding model in combination with the predicted outcome response model would suggest that top-down connectivity from the mPFC to the MNS is important for integrating prior expectations about an action outcome with incoming sensory information. The neuroimaging data and the predicted outcome model also suggest that the mPFC signals prediction errors as well as the MNS, implying that prediction errors, improving future predictions, may be signalled from the MNS to the mPFC (Alexander & Brown, 2011a; Jahn et al., 2014; Malekshahi et al., 2016; Zarr & Brown, 2016). If this combined model correctly characterises connectivity between the MNS and the mentalizing system and connectivity between these two systems is dysfunctional in individuals with ASD then these individuals would be expected to display impairments in integrating expectations with incoming kinematic information (impaired top-down connectivity) and impaired abilities to update associations between kinematics and internal states (dysfunctional bottom-up connectivity).

The theory of impaired integration of prior expectations and incoming kinematic information in ASD compliments the findings of a recent behavioural study which found that adults with ASD display dysfunctional integration of incoming sensory information and prior expectations and this correlated with the degree of social impairment (Chambon et al., 2017). Another previous behavioural study showed children with ASD were impaired at inferring intentions when this involved integrating contextual information with kinematic information but not when intentions could be inferred based on contextual information alone (Boria et al., 2009). Finally, a meta-analysis which investigated mentalizing deficits in adults with Asperger's concluded that these individuals are significantly impaired on mentalizing tasks that involve integrating information regarding others' internal states with surrounding context (Baez, et al., 2012). Previous behavioural studies have also provided evidence to support the suggestion that abilities to update future predictions of upcoming actions may be impaired in ASD; children and adolescents with ASD have shown reduced abilities to predict the outcome of others' actions and displayed no benefit when shown familiar actions, unlike typically developing individuals (Zalla, Labruyère, Clément, & Georgieff, 2010). Another experiment showed that after observing a communicative action, typically developing individuals displayed improved visual discrimination of a complimentary responsive gesture which was masked compared to when viewing this masked gesture after a non-communicative action was shown (von der Lune et al., 2016). Adults with ASD did not exhibit improved recognition of the masked responsive gesture after being shown the communicative gesture (von der Lune et al., 2016). Data from both studies suggest that prior expectations about upcoming actions are not efficiently updated as a result of previous experience. These behavioural data support the proposal that integration of prior expectations and incoming information is reduced in ASD as well as impaired abilities to update predictions about upcoming actions based on previous experience. These difficulties, which may result from reduced connectivity between the MNS and the mentalizing system, may underlie difficulties adults with high levels of autistic traits experience in inferring others' social intentions.

## 6.9 Particular importance of the right MNS underlying deficits associated with ASD

It is possible that reduced connectivity of right IFG, in particular, underlies the mentalizing difficulties experienced by adults with ASD. The data from our fMRI study suggest that increased connectivity between dmPFC and IFG when inferring social intentions is lateralised to right IFG and this mentalizing-induced increase in connectivity is reduced in adults with high levels of autistic traits. Previous fMRI studies have shown increased functional connectivity between dmPFC and right IFG when inferring internal states of others from their actions (Rudie et al., 2012; Spunt & Lieberman, 2012a; 2012b; Tettamanti et al., 2017) and adults with ASD show consistent impairments in inferring internal states of others from their actions (Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Rosenblau et al., 2015; Schuwerk et al., 2014; Senju et al., 2009). In contrast, increased functional connectivity has been reported between dmPFC and left MNS regions when inferring others' internal states in the absence of human action (Lombardo et al., 2010; Tettamanti et al., 2017; Trapp et al., 2014) and adults with ASD have not shown consistent impairments in inferring internal states in the absence of human action (Kana et al., 2009; Kirkovski et al., 2015; Ponnet, Roeyers, Buysse, De Clercq, & Van der Heyden, 2004; Roeyers et al., 2001; Spek et al., 2010). The theory of reduced right-lateralised IFG connectivity in adults with ASD when mentalizing, compliments the findings of a previous study found reduced connectivity of the right IFG in adults with ASD when inferring intentions from a comic strip (Deshpande et al., 2013). Data from resting-state fMRI (rsfMRI) studies have also shown atypical connectivity between right IFG and areas of the mentalizing system in individuals with ASD (Fishman et al., 2014; Itahashi et al., 2014; Kennedy & Courchesne, 2008; Rudie et al., 2012a; Shih et al., 2010), providing evidence for right-lateralised dysfunction in these individuals irrespective of task. The reduced *mu* suppression found in adults with high levels of autistic traits in our TMS-EEG experiment (Chapter 4) was also right-lateralised which further supports the concept of right-lateralised MNS dysfunction. Therefore, our data in conjunction with the existing rsfMRI, fMRI and behavioural data suggest that dysfunctional connectivity between right IFG and the mentalizing system may be of particular importance in underlying the mentalizing deficits associated with ASD.

## 6.10 Potential gender differences

The cumulative data from all studies presented in this thesis suggest that impairments in explicitly inferring the intentions of others from their actions associated with ASD may only be experienced by males and not females. Across all studies in this thesis, males with higher levels of autistic traits displayed poorer explicit mentalizing performances [F(1,56)=17.37, p<.001, $R^2$=.24] and males with ASD (n=25) exhibited significantly poorer performances than typically developing males [n=33; t(30.20)=-2.39, p=.02]. However, these differences were not seen in females with high levels of autistic traits [F(1,42)=.41, p=.52, $R^2$=.01] or females with ASD [n=16, TD= 28; t(17.96)=-.77, p=.45]. Research into gender differences in ASD is extremely limited due to small sample sizes of females with ASD as four times as many males have a diagnosis of ASD than females (Centers for Disease Control and Prevention, 2014). One previous study found that males but not females with ASD were impaired at inferring others' mental states from still images of their eyes (Holt et al., 2014) and another study found atypical neural activation in males but not females with ASD whilst inferring mental states of animated triangles (Kirkovski et al., 2015). However, no previous studies have investigated gender differences in abilities to infer others' internal states from their actions in adults with ASD or gender differences in brain activation patterns during these tasks.

Every experiment presented in this thesis had a relatively large proportion of female ASD participants compared to previous studies but each study is still individually underpowered in their ability to accurately report gender differences. However, the data in both the behavioural experiment presented in Chapter 2 and the fMRI experiment in Chapter 5 show the same gender difference in explicit mentalizing abilities as seen in the overall data set. Initial analysis of potential gender differences in the relationship between autistic traits and the degree of mentalizing-induced increased functional connectivity between dmPFC and right IFG in the fMRI data suggest that there were no differences between male and female participants [males: $F(1,22)=3.17$, $p=.09$, $R^2=.13$; females: $F(1,14)=2.06$, $p=.17$, $R^2=.13$]. The relationship between autistic traits and right-lateralised *mu* suppression in the EEG data from the TMS-EEG experiment (Chapter 4) was significant in males [$F(1,21)=5.24$, $p=.03$, $R^2=.20$] but not females [$F(1,15)=.40$, $p=.54$, $R^2=.03$]. Males with high levels of autistic traits showed reduced right-lateralised *mu* suppression (indicating reduced MNS activity) during the mentalizing task. Due to the small samples of females in each study, these results are preliminary. However, potential gender differences in brain activation and functional connectivity when inferring others' internal states from their actions is an area worth exploring with larger sample sizes; especially as the cumulative behavioural data (n=102) suggest a gender difference in explicit mentalizing abilities in adults with ASD.

## 6.11 Using the continuous measure of autistic traits

Examining the relationship between the continuous measurement 'autistic traits' and behavioural and neural characteristics associated with ASD, may be a more sensitive method than investigating group differences in behavioural and neural measures. The studies presented in this thesis investigated the relationship between the continuous variable of autistic traits and both neural and behavioural measures because ASD is a spectrum condition and therefore high variability in levels of autistic traits within both clinical and non-clinical populations may have prevented differences associated with high levels of autistic traits being found across groups (American Psychiatric Association, 2013; Robinson et al., 2011; van Boxtel & Lu, 2013; Von Dem Hagen et al., 2011). Data from both our TMS-EEG study (Chapter 4) and our fMRI study (Chapter 5) suggest that investigating differences associated with autistic traits is a potentially more sensitive method than investigating group differences; the significant findings of reduced right-lateralised MNS activity (Chapter 4) and reduced connectivity between dmPFC and right IFG (Chapter 5) would not have been significant if participants were only analysed in terms of participant groups. Measuring levels of autistic traits displayed by all participants may therefore be an effective method of assessing neural differences associated with ASD that would not be identified in group comparisons due to within group variability.

## 6.12 Limitations

There are a number of limitations associated with the studies presented in this thesis. First, although the sample sizes of all studies presented in this thesis are comparable to similar existing studies, the sample sizes were all relatively small. The small sample sizes may have contributed to the limited evidence we found to support a relationship between MNS activity and mentalizing performance as well as the, perhaps inconsistent, findings regarding the relationship between autistic traits and levels of MNS activation. The smaller sample size in the TMS data, compared to the EEG data, in the TMS-EEG experiment may have contributed to the lack of significant relationships in the TMS data between MNS activation and both autistic traits and behavioural performance whilst significant relationships were found in the EEG data. However, the small effect sizes found for the relationships between MNS activity and both behavioural performance and autistic traits in all studies, suggest that significant relationships would not have been found even with larger sample sizes. The Bayes factors calculated in the TMS experiment (Chapter 3) also suggest that significant relationships between MNS activity and both autistic traits and mentalizing performance were unlikely to be found regardless of sample size.

Another possible reason why significant relationships may not have been found between MNS activity and behavioural measures is that only high-functioning adults with ASD were included in all studies; these individuals may have developed compensatory strategies which improve behavioural performance and therefore alter the relationship between neural measures and performance. If different cognitive methods are used by these individuals in order to infer social intentions, these may also utilise different brain regions and therefore compensatory strategies could influence patterns of neural activity. Therefore, it remains to be seen whether the results of the studies presented in this thesis can be generalised to younger or lower-functioning populations who may not have developed compensatory strategies. However, the eye-tracking data in all of our studies suggest no differences in fixation patterns and our fMRI data (Chapter 5) show identical brain regions were activated in both individuals with high and low levels of autistic traits, with the exception of reduced functional connectivity during the mentalizing task. These data suggest that the participants in our study were likely using equivalent methods to infer social intentions; adults with high levels of autistic traits were just less effective at utilising these techniques.

Despite the inclusion of only high-functioning adults with ASD, a number of ASD participants both the TMS-EEG experiment and the fMRI were taking psychotropic medication. Due to the high comorbidity of depression, ADHD and anxiety in ASD, the inclusion of individuals taking these medications is common in the ASD literature (Enticott, Rinehart, Tonge, Bradshaw, & Fitzgerald, 2010; Enticott et al., 2013; Kirkovski et al., 2016; Oberman, Pascual-Leone, & Rotenberg, 2014). Psychotropic medications have been shown to increase baseline measures of corticospinal excitability in TMS experiments (Gilbert et al., 2006; Minelli et al., 2010) and increase functional cortico-cortical connectivity in fMRI data sets (Linke, Olson, Gao, Fishman, & Müller, 2017; Narayanan et al., 2010). However, there were no group differences in baseline measures of corticospinal excitability in our TMS data and participants with high levels of autistic traits displayed reduced rather than increased functional connectivity in our fMRI experiment. It is possible that if only unmedicated participants had been included in our fMRI experiment then we would have observed a greater difference in functional connectivity between the MNS and the mentalizing system in adults with high levels of autistic traits.

The exclusive inclusion of high-functioning adults meant that performances on the non-mentalizing tasks were very high in all experiments meaning that the mentalizing-specific behavioural deficit we found in individuals with high levels of autistic traits may have been due to non-mentalizing task performances being at ceiling and thus preventing a relationship between autistic traits and non-mentalizing task performance being identified. However, the relationships between neural measures and autistic traits support the existence of a mentalizing-specific deficit associated with ASD; reduced *mu* suppression (Chapter 4) and reduced connectivity between IFG and dmPFC (Chapter 5), in adults with high levels of autistic traits, were found during the mentalizing task but not the non-mentalizing task. The mentalizing-specific deficit we found in adults with high levels of autistic traits also compliments findings from previous studies which have shown mentalizing-specific behavioural impairments in adults with ASD (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Castelli, Frith, Happé, & Frith, 2002; Jolliffe & Baron-Cohen, 1999; Kana, Libero, Hu, Deshpande, & Colburn, 2014) and individuals with high levels of autistic traits (Gökçen, Petrides, Hudry, Frederickson, & Smillie, 2014; Gökçen, Frederickson, & Petrides, 2016). As well as high performances on the non-mentalizing tasks in our studies, performances on the mentalizing task were very high in the first TMS study (Chapter 3; mean proportion of correct responses= .94, SD=.10) perhaps because this study did not include individuals with a diagnosis of ASD. The high performances on the mentalizing task in this study may therefore have contributed to the absence of relationships between mentalizing score and both autistic traits and MNS activity in this experiment.

Although the hand actions shown in the mentalizing and non-mentalizing tasks in our studies had very similar kinematics, they were not identical. These small kinematic differences between the actions shown in different tasks, in principal, may have contributed to higher levels of MNS activation found in the mentalizing task compared to the non-mentalizing task in Chapters 3 and 5. However, the larger TMS-induced MEP sizes reported in Chapter 3 during the mentalizing task were found at the end of observed actions when the muscles recording from were not contracted in the observed action. Therefore, if MEP sizes were only dependent on the kinematics of the observed action, smaller MEP sizes would be predicted at this time point (as was found in the non-mentalizing task at this time point). Larger MEP sizes at this time point therefore imply a role of the MNS in inferring social intentions beyond basic kinematic processing. The fMRI experiment, presented in Chapter 5, showed clumsy actions in both the mentalizing and the non-mentalizing tasks. If the comparison of brain activation across tasks is restricted to activity recorded during the observation of clumsy actions, higher activation is found in right IFG and right SMA when these actions were shown during the mentalizing task compared to the non-mentalizing task (see Figure 6.1). These data provide further support for the finding of higher MNS activation when mentalizing.

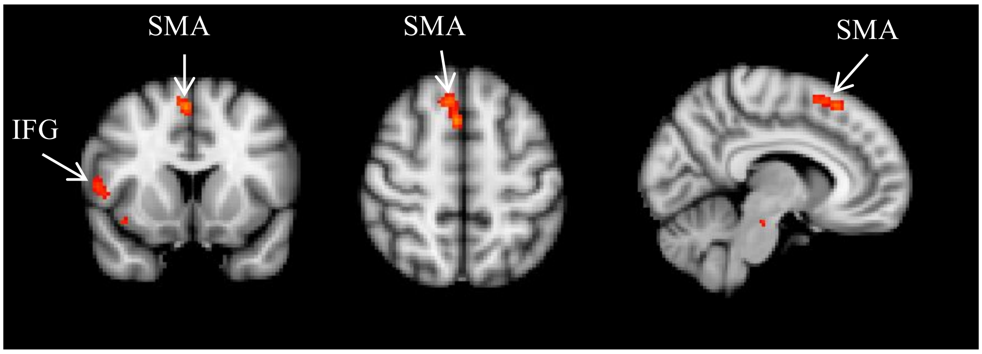

**Figure 6.1** Whole-brain group analysis of regions showing higher activity during the presentation of clumsy actions during the mentalizing task compared to the non-mentalizing task. Higher activation was found in the right inferior frontal gyrus (IFG) and right supplementary motor area (SMA).

It is possible that the timing of MNS activation differs depending on the explicitness of task instructions. Chapter 3 investigated MNS activation during a more implicit task and therefore the timing of MNS activation identified may not apply to mentalizing tasks with directly explicit instructions. The differences in the nature of the tasks in Chapter 3 and 4, may have contributed to the inconsistent task-related differences in MNS activity in the TMS data. However, previous studies which have investigated mentalizing system activation during mentalizing tasks have shown identical timing of mentalizing system activation across implicit and explicit tasks (Van der Cruyssen et al., 2009; Van Duynslaeger et al., 2007) suggesting that the timing of neural activation may generalise across mentalizing tasks irrespective of task instructions.

Finally, the temporal and spatial resolution of the imaging techniques used did not allow us to visualise areas of brain activity at different time points during the task so although our data provide evidence to suggest social intention processing within the MNS and the mentalizing system is best conceptualised within a predictive coding framework, we cannot draw firm conclusions regarding the precise timing of interactions between the MNS and the mentalizing system. However, separately, the high temporal resolution of TMS allowed us to identify the timing of right MNS activation, and the high spatial resolution of fMRI allowed us to identify the areas of the MNS and the mentalizing system that display greater functional connectivity during this task. This information can help us to start to delineate between different models of connectivity between the MNS and the mentalizing system.

### 6.13 Ideas for future research

In attempt to fully characterise the interplay between areas of the MNS and the mentalizing system when inferring the social intentions of others from their actions, a magnetoencephalography (MEG) study could be conducted. The very high temporal resolution and the reasonable spatial resolution of this technique, allows the timing of activation in different brain areas to be identified. If the predictive coding framework is an accurate characterisation of information processing whilst inferring the social intentions from actions then mPFC activation would be predicted before MNS activation. It would also be beneficial to examine potential differences in the timing of activation across both implicit and explicit tasks to investigate whether task instructions alter interactions between the MNS and the mentalizing system. A previous MEG study which investigated the timing of activation in areas of the MNS and the mentalizing system when explicitly inferring intentions from cartoon comic strips (Vistoli, Brunet-Gouet, Lemoalle, Hardy-Baylé, & Passerieux, 2011) found simultaneous activation in both systems during the early stages of intentional processing. The authors conclude these data provide evidence against the traditional view that intentional information processing is exclusively 'bottom-up' from the MNS to the mentalizing system (Vistoli et al., 2011). However, a similar study has not been conducted when naturalistic, video stimuli are used.

It would also be useful to identify whether adults with ASD exhibit greater impairments at inferring certain aspects of someone's internal state than others or whether the results of previous studies are confounded by differences in the stimuli used and the explicitness of the task instructions. The abilities of adults with ASD to explicitly infer different aspects of someone's internal state such as their mental states, beliefs and emotions could be assessed using naturalistic, complex video stimuli. Furthermore, differences in patterns of brain activation and functional connectivity when making these different internal inferences should be explored. Inferring different aspects of someone's internal state appear to activate different brain regions (Gobbini et al., 2007; Pineda & Hecht, 2009). However, neuroimaging studies have not investigated differences across tasks when using the same stimuli. The stimuli used in mentalizing tasks, not only affect the difficulty of the task but appear to alter the lateralisation of functional connectivity between areas of the MNS and the mentalizing system (Tettamanti et al., 2017). Identifying the brain areas involved in making different internal state inferences as well as identifying how changing the stimuli alters the neural signatures of these internal state inferences will help uncover the different cognitive methods used to perform these different mentalizing tasks. Additionally, by pinpointing the aspects of mentalizing in which adults with ASD are impaired, we can start to uncover the particular areas of neural dysfunction underlying these impairments.

The cumulative behavioural data from all studies presented in this thesis suggest that gender differences are an interesting avenue to explore in future studies. Males with high levels of autistic traits, unlike females, appear to exhibit difficulties inferring the social intentions of others from their actions and may therefore show more prominent neural differences e.g. reduction in functional connectivity between the MNS and the mentalizing system. Females with ASD may show less pronounced neural differences when inferring others' social intentions from their actions or display compensatory activity in other cortical areas.

## 6.14 Conclusions

The data from the studies presented in this thesis suggest that the MNS is involved in processing information regarding the internal states of others. However, MNS activity does not appear to correlate with abilities to explicitly infer the social intentions of others from their actions, suggesting that the MNS does not have a role in actively interpreting internal state information. Functional connectivity between the MNS and the mentalizing system appears to increase when inferring the social intentions of others from their actions and our data imply that connectivity between these systems may be best conceptualised within a predictive coding framework. We found that ASD is associated with deficits in explicitly inferring the social intentions of others from their actions and that connectivity between mPFC (mentalizing system) and IFG (MNS) is dysfunctional in adults with high levels of autistic traits. According to the predictive coding model, reduced connectivity between the mPFC and IFG may result in reduced integration of prior expectations with incoming kinematic information and, or reduced abilities to use previous experience to update future predictions about others' actions. Therefore, reduced connectivity between the MNS and the mentalizing system may underlie some of the social difficulties that adults with ASD experience.

# Appendices

## A.1 Supplementary Data from Chapter 4

### A.1.1 Behavioural data

There were no significant group differences in behavioural performance in either the mentalizing ($H(2)=1.92$, $p=.38$) or the non-mentalizing task ($H(2)=4.70$, $p=.10$). However, across all participants, performance was significantly poorer on the mentalizing task than the non-mentalizing task ($T=537$, $p<.001$, $r=.70$). There were no significant relationships between autistic traits and behavioural performance; see supplementary Figure A.1.

### A.1.2 TMS data

*A.1.2.1 Preliminary analysis of the TMS data*

There were no group differences in motor threshold values, no significant differences in baseline levels of corticospinal excitability before and after the experiment and no differences in the number of excluded MEPs both between groups and tasks (see below).

*A.1.2.2 Motor thresholds*

A one-way ANOVA showed that the 1mv motor thresholds were not significantly different between low AQ ($M=49.47$, $SD=4.9$), high AQ ($M=51.29$, $SD=10.36$) and ASD ($M=45.89$, $SD=7.40$) groups [$F(2, 35)=1.29$, $p=.29$, $\eta_p^2=.07$].

An independent samples t-test identified that within the ASD group, motor threshold values were not significantly different between medicated ($M=44.00$, $SD=4.90$) and non-medicated ($M=47.50$, $SD=10.66$) participants [$t(6)=.60$, $p=.57$].

### *A.1.2.3 Differences in Baseline Corticospinal Excitability*

Three participants (one from each group) were not included in this section of the analysis because their post-experiment baseline corticospinal excitability measures were invalid (>50% MEPs in the FDI muscle <.2mV). For two of the participants this was due to the 'hot spot' for the FDI being particularly difficult to maintain and the post-experiment baseline was missing for one participant due to an error when acquiring the data.

A (3 x 2) repeated measures ANOVA found that baseline MEP sizes in the FDI muscle were not significantly different before and after the experiment across all participants (F(1, 30)=3.62, p=.07, $\eta_p^2$=.11), there were no significant differences in baseline MEP sizes between groups (F(2, 30)=.56, p=.58, $\eta_p^2$=.04) and there was no significant interaction between participant group and the time point in which baseline measures were taken (F(2, 30)=.18, p=.84, $\eta_p^2$=.01).

There were no significant group differences in pre-experiment (H(2)=2.23, p=.33) and post-experiment (H(2)=.53, p=.77) baseline MEP sizes in the ADM muscle. Additionally, baseline MEPs in the ADM muscle were not significantly different before and after the experiment (T=218, p=.26, r=-.19) across all participants.

### *A.1.2.4 Excluded MEPs*

There were no significant group differences in the number of excluded MEPs in the mentalizing task[H(2)=1.60, p=.45] or the non-mentalizing task [H(2)=3.59, p=.17].

There was also no significant difference in the number of MEPs that were excluded between the two tasks across all participants [T=78, p=.49, r=-.12].

#### *A.1.2.5 ADM data*

Motor resonance in the ADM muscle was not significantly different between the mentalizing and non-mentalizing tasks [T=374, p=.33, r=.16]. There were also no group differences in ADM motor resonance values in the mentalizing task [H(2)=.96, p=.62] or the non-mentalizing task [H(2)=1.67, p=.43].

### A.1.3 EEG data

#### *A.1.3.1 Excluded Epochs*

There was a significant group difference in the number of video epochs excluded from the non-mentalizing task [H(2)=8.79, p=.01]. Pairwise comparisons with adjusted p values identified significantly more non-mentalizing video epochs were excluded in the low AQ group than the ASD group (p=.019) but there were no other significant group differences (High AQ & ASD groups: p=1.00; low & high AQ groups: p=.06).

There were no significant group differences in the number of epochs excluded for any of the other epoch types [mentalizing videos: H(2)=3.35, p=.19, fixation cross in the mentalizing task: H(2)=1.93, p=.38, fixation cross in the non-mentalizing task: H(2)=2.66, p=.26].

Across all participants, there were significant differences in the number of epochs excluded between epoch types [$\chi^2(3)=16.01$, p<.01]. Subsequent Wilcoxon tests found that a larger number of non-mentalizing video epochs were excluded compared to the mentalizing video epochs (T=51.01, p<.01, r=.50), the fixation cross epochs in the mentalizing task (T=54.50, p<.001, r=-.58) or fixation epochs in the non-mentalizing task (T=119, p<.01, r=-.48). There were no other significant differences in the number of epochs excluded between different epoch types (mentalizing videos vs mentalizing fixation: T=198.50, p=.14, r=-.23; mentalizing fixation vs non-mentalizing fixation: T=309.50, p=.39, r=.13; mentalizing videos vs non-mentalizing fixation: T=260, p=.52, r=-.10).

### *A.1.3.2 8-10Hz Data*

Pairwise comparisons with adjusted p-values showed that *mu* suppression in the 8-10Hz range at F3 during the non-mentalizing task was significantly lower in the high AQ group than the ASD group (p=.03, r=-.51) or the low AQ group (p=.01, r=.54). Only the difference between the high AQ and low AQ groups was significant once the significance threshold had been adjusted using the Bonferroni correction for multiple comparisons (p=.02).There was no significant group difference between the low AQ group and the ASD group (p=1.00, r=.02).

*Mu* suppression in the 8-10Hz range during the non-mentalizing task at FCz was significantly lower in the high AQ group than the ASD group (p=.04, r=-.49) but this did not survive the Bonferroni correction. Levels of mu suppression were not significantly different between the low & high AQ groups (p=.10, r=.40) or the low AQ group and the ASD group (p=1.00, r=-.09) at FCz during the non-mentalizing task.

There were no significant group differences in mu suppression during the mentalizing task at F3: H(2)=.34, p=.84, FCZ: H(2)=5.65, p=.06 or during the non-mentalizing task at F4: H(2)=3.38, p=.19].

### *A.1.3.3 10-12Hz Data*

There were no significant group differences in *mu* suppression in the 10-12Hz frequency band during either task at any of the cortical sites [mentalizing task: F3:

H(2)=.92, p=.63; FCZ: H(2)=.10, p=.95; F4: H(2)=.94, p=.62; non-mentalizing task: F3: H(2)=.42, p=.81; FCZ: H(2)=.58, p=.75; F4: H(2)=.82, p=.67]. There were also no significant differences in *mu* suppression in the 10-12Hz band between tasks at any of the cortical sites across all participants (F3: T=526, p=.12, r=.24; FCZ: T=511, p=.18, r=.21; F4: T=404, p=.94, r=-.13).

### A.1.4 Eye-tracking data

#### *A.1.4.1 Hand ROI*

When task-related differences were investigated using data from the clumsy actions alone, the number (T=229, p<.01, -.45) and duration (T=264, p=.01, -.38) of fixations made during the mentalizing task were still significantly higher than the non-mentalizing task.

There were no significant group differences in the number or duration of fixations within the hand ROI in the mentalizing task[number of fixations: H(2)=1.89, p=.39; duration: H(2)=3.59, p=.17] or the non-mentalizing task[number of fixations: H(2)=2.25, p=.32; duration: H(2)=4.21, p=.12].

#### *A.1.4.2 Head ROI*

When task-related differences were investigated using data from the clumsy actions alone, significantly more fixations were made within the head ROI during the mentalizing task (T=284, p=.02, r=-.35) but the total duration of fixations in the head ROI was not significantly different between tasks (T=344, p=.12, r=-.24).

There were no significant group differences in the number or duration of fixations within the head ROI in the mentalizing task [number of fixations:

H(2)=.82, p=.66; duration: H(2)=1.46, p=.48] or the non-mentalizing task [number of fixations: H(2)=3.10, p=.21; duration: H(2)=3.49, p=.17].

### *A.1.4.3 Poker Chip ROI*

When the borderline significant difference in the number of fixations in the poker chip ROI was investigated using the clumsy actions alone this difference was significant (T=278, p=.03, r=-.33; significantly more fixations were made during the mentalizing task than the non-mentalizing task).

There were no significant group differences in the number or duration of fixations within the poker chip ROI in the mentalizing task [number of fixations: H(2)=1.45, p=.48; duration: H(2)=.65, p=.72] or the non-mentalizing task[number of fixations: H(2)=1.79, p=.41; duration: H(2)=1.81, p=.41].

## A.1.5 Relationships between data from different techniques

### *A.1.5.1 TMS and behavioural performance*

Motor resonance values for the FDI muscle during the mentalizing task[F(1,33)=.11, p=.75, $R^2$<.01] and the non-mentalizing task[F(1,33)=.91, p=.35, $R^2$=.03] did not significantly predict performances on these tasks.

### *A.1.5.2 EEG and behavioural performance*

The degree of *mu* suppression in the 8-10Hz frequency band at the other cortical sites and the 10-12Hz frequency band at all cortical sites during the mentalizing task did not significantly predict mentalizing performance [8-10Hz at FCZ: F(1,38)=2.77, p=.10, $R^2$=.07; F4: F(1,38)=3.61, p=.07, $R^2$=.09; 10-12Hz: F3: F(1,38)=.34, p=.57, $R^2$<.01; FCZ: F(1,38)=.40, p=.53, $R^2$=.01; F4: F(1,38)=.44, p=.51, $R^2$=.01].

Performances on the non-mentalizing task were not significantly predicted by the degree of *mu* suppression at any cortical site in either frequency band [8-10Hz: F3: F(1,38)=3.26, p=.08, $R^2$=.08; FCZ: F(1,38)=1.98, p=.17, $R^2$=.05; F4: F(1,38)=.76, p=.39, $R^2$=.02; 10-12Hz: F3: F(1,38)=.03, p=.87, $R^2$<.01; FCZ: F(1,38)=.05, p=.83, $R^2$<.01; F4: F(1,38)=.16, p=.69, $R^2$<.01].

### *A.1.5.3 EEG and TMS*

FDI motor resonances during the mentalizing task did not predict levels of *mu* suppression at any of the cortical sites in either frequency band: 8-10Hz: F3:[F(1,30)=.17, p=.68, $R^2$<.01] FCZ: [F(1,30)=.48, p=.50, $R^2$=.02] F4: [F(1,30)<.01, p=.96, $R^2$<.01]; 10-12Hz: F3:[F(1,30)=.22, p=.65, $R^2$<.01] FCZ: [F(1,30)=.17, p=.69, $R^2$<.01] F4: [F(1,30)=.21, p=.65, $R^2$<.01].

FDI motor resonances during the non-mentalizing task also did not predict levels of *mu* suppression at any of the cortical sites in either frequency band: 8-10Hz: F3: [F(1,30)=.25, p=.62, $R^2$<.01] FCZ: [F(1,30)=.42, p=.52, $R^2$=.01] F4: [F(1,30)<.001, p=.99, $R^2$<.001]; 10-12Hz: F3:[F(1,30)=.05, p=.82, $R^2$<.01] FCZ: [F(1,30)=.02, p=.90, $R^2$<.01] F4: [F(1,30)=.06, p=.80, $R^2$<.01].

### *A.1.5.4 Eye-tracking and behavioural*

Mentalizing performances were not predicted by the number of fixations or total duration of fixations within any of the ROIs during the mentalizing task [number of fixations: hand [F(1,41)=1.67, p=.20, $R^2$=.04]; head [F(1,41)=1.43, p=.24, $R^2$=.03]; poker chip [F(1,41)=.06, p=.82, $R^2$<.01]; duration of fixations: hand [F(1,41)=1.90, p=.18, $R^2$=.04]; head [F(1,41)=1.04, p=.31, $R^2$=.03]; poker chip The total duration of fixations within the ROIs other than the poker chip ROI and the number of fixations within all the ROIs during the non-mentalizing task did not significantly predict performance on the non-mentalizing task [duration: hand F(1,41)=.54, p=.47, $R^2$=.01; head F(1,41)=.20, p=.65, $R^2$<.01; number of fixations: hand F(1,41)=.81, p=.38, $R^2$=.02; head [F(1,41)=.01, p=.91, $R^2$<.001 and poker chip F(1,41)=2.08, p=.16, $R^2$=.05.

[F(1,41)=.14, p=.72, $R^2$<.01].

### *A.1.5.5 Eye-tracking & EEG*

### *A.1.5.6 8-10Hz Data*

*Mu* suppression (8-10Hz) at F3 during the mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)<.01, p=.97, $R^2$<.001]; head: [F(1,38)<.01, p=.97, $R^2$<.001]; poker chip: [F(1,38)=.16, p=.67, $R^2$<.01]; total duration of fixations: hand: [F(1,38)<.01, p=.96, $R^2$<.001]; head: [F(1,38)<.01, p=.94, $R^2$<.001]; poker chip: [F(1,38)=.97, p=.33, $R^2$=.03].

*Mu* suppression (8-10Hz) at F3 during the non-mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)=.28, p=.60, $R^2$<.01]; head: [F(1,38)=.25, p=.62, $R^2$<.01]; poker chip: [F(1,38)<.01, p=.98, $R^2$<.01]; total duration of fixations: hand: [F(1,38)=.16, p=.69, $R^2$<.01]; head: [F(1,38)=.50, p=.49, $R^2$=.01]; poker chip: [F(1,38)=.08, p=.78, $R^2$<.01].

*Mu* suppression (8-10Hz) at F4 during the mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)=.04, p=.84, $R^2$<.01]; head: [F(1,38)=1.63, p=.21, $R^2$=.04]; poker chip: [F(1,38)=.43, p=.52, $R^2$=.01]; total duration: hand:

[F(1,38)<.001, p=.97, $R^2$<.001]; head: [F(1,38)=.63, p=.43, $R^2$=.02]; poker chip: [F(1,38)<.01, p=.95, $R^2$<.001].

*Mu* suppression (8-10Hz) at F4 during the non-mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)=.08, p=.79, $R^2$<.01]; head: [F(1,38)=.31, p=.58, $R^2$<.01]; poker chip: [F(1,38)=.07, p=.80, $R^2$<.01]; total duration of fixations: hand: [F(1,38)=.03, p=.86, $R^2$<.01]; head: [F(1,38)=.45, p=.51, $R^2$=.01]; poker chip: [F(1,38)=.08, p=.79, $R^2$<.01].

*Mu* suppression (8-10Hz) at FCZ during the mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)=.44, p=.51, $R^2$=.01]; head: [F(1,38)=1.06, p=.31, $R^2$=.03]; poker chip: [F(1,38)=2.92, p=.10, $R^2$=.07]; total duration of fixations: [F(1,38)=.04, p=.85, $R^2$<.01]; head: [F(1,38)=.47, p=.50, $R^2$=.01]; poker chip: [F(1,38)=2.04, p=.16, $R^2$=.05].

*Mu* suppression (8-10Hz) at FCZ during the non-mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)=.28, p=.60, $R^2$<.01]; head: [F(1,38)=.04, p=.84, $R^2$<.01]; poker chip; [F(1,38)<.01, p=.95, $R^2$<.01]; total duration of fixations: hand: [F(1,38)=.14, p=.71, $R^2$<.01]; head: [F(1,38)=.18, p=.67, $R^2$<.01]; poker chip: [F(1,38)=.05, p=.83, $R^2$<.01].

#### *A.1.5.7 10-12Hz Data*

*Mu* suppression (10-12Hz) at F3 during the mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)=.04, p=.85, $R^2$<.01]; head: [F(1,38)=.13, p=.72, $R^2$<.01]; poker chip:

[F(1,38)=.49, p=.49, $R^2$=.01]; total duration of fixations: hand: [F(1,38)=.11, p=.74, $R^2$<.01]; head: [F(1,38)<.01, p=.98, $R^2$<.001]; poker chip: [F(1,38)=.62, p=.44, $R^2$=.02].

*Mu* suppression (10-12Hz) at F3 during the non-mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)=.67, p=.42, $R^2$=.02]; head: [number of fixations: hand: [F(1,38)=1.82, p=.19, $R^2$=.05]; poker chip: [F(1,38)=.19, p=.67, $R^2$<.01]; total duration of fixations: hand: [F(1,38)=.73, p=.40, $R^2$=.02]; head: [F(1,38)=1.64, p=.21, $R^2$=.04]; poker chip: [F(1,38)=.06, p=.81, $R^2$<.01].

*Mu* suppression (10-12Hz) at FCZ during the mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)=.27, p=.60, $R^2$<.01]; head: [F(1,38)=.57, p=.45, $R^2$=.02]; poker chip: [F(1,38)=3.49, p=.07, $R^2$=.08]; total duration of fixations: hand: [F(1,38)=.16, p=.69, $R^2$<.01]; head: [F(1,38)=.02, p=.89, $R^2$<.01]; poker chip: [F(1,38)=3.94, p=.05, $R^2$=.09].

*Mu* suppression (10-12Hz) at FCZ during the non-mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)=.54, p=.47, $R^2$=.01]; head: [F(1,38)=.04, p=.84, $R^2$<.01]; poker chip: [F(1,38)=.03, p=.86, $R^2$<.01]; total duration of fixations: hand: [F(1,38)=.6`, p=.44, $R^2$=.02]; head: [F(1,38)=.10, p=.76, $R^2$<.01]; poker chip: [F(1,38)=.21, p=.65, $R^2$<.01].

*Mu* suppression (10-12Hz) at F4 during the mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [F(1,38)<.01, p=.93, $R^2$<.001]; head: [F(1,38)=.68, p=.41,

$R^2=.02$]; poker chip: [$F(1,38)=1.31$, $p=.26$, $R^2=.03$]; total duration of fixations: hand: [$F(1,38)<.01$, $p=.98$, $R^2<.001$]; head: [$F(1,38)=.09$, $p=.76$, $R^2<.01$]; poker chip: [$F(1,38)=1.42$, $p=.24$, $R^2=.04$].

*Mu* suppression (10-12Hz) at F4 during the non-mentalizing task did not significantly predict the number or duration of fixations in any of the ROIs [number of fixations: hand: [$F(1,38)=.62$, $p=.44$, $R^2=.02$]; head: [$F(1,38)=3.38$, $p=.07$, $R^2=.08$]; poker chip: [$F(1,38)=.45$, $p=.51$, $R^2=.01$]; total duration of fixations: hand: [$F(1,38)=.85$, $p=.36$, $R^2=.02$]; head: [$F(1,38)=2.47$, $p=.12$, $R^2=.06$]; poker chip: [$F(1,38)=.76$, $p=.39$, $R^2=.02$].

## A.2 Supplementary Data from Chapter 5: Group Comparisons

### A.2.1 Behavioural data

Data screening identified that the behavioural data were not normally distributed even after a log transformation had been applied so Mann-Whitney U tests were conducted in order to investigate potential group differences. There were no significant group differences in behavioural performance in either the mentalizing (U=146.00, p=.15, r=-.24) or the non-mentalizing task (U=149.00, p=.17, r=-.24; see supplementary Table S1 for descriptive statistics).

### A.2.2 Brain activation

No brain areas showed significantly higher levels of activation in TD participants compared to ASD participants at the p<.001 significance level (uncorrected for multiple comparisons).

### A.2.3 Functional connectivity

The reduced dmPFC-IFG connectivity identified associated with high levels of autistic traits was not significant when participants were analysed in terms of ASD and TD groups t(38)=-1.34, p=.77. Additionally, no group difference was found in right IFG-dmPFC connectivity in particular: t(38)=-1.34, p=.22

## A.3 Supplementary Tables

Table S.1

*Behavioural performances for Chapter 4: Median (IQR) values*

| | Mentalizing task | Non-mentalizing task |
|---|---|---|
| Low AQ | 77.00 (5.00) | 80.00 (1.00) |
| High AQ | 76.00 (7.00) | 79.00 (1.00) |
| ASD | 78.00 (3.50) | 79.00 (2.00) |

## A.4 Supplementary Figures

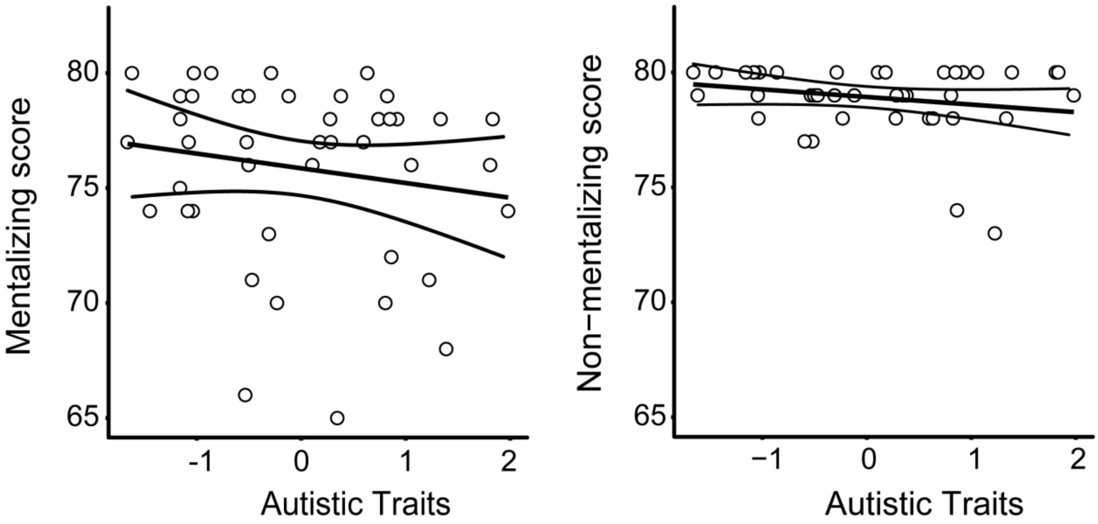

**Figure A.1** Relationship between autistic traits and behavioural performance for Chapter 4. There was no significant relationship between autistic traits and performance on the mentalizing task nor the non-mentalizing task.

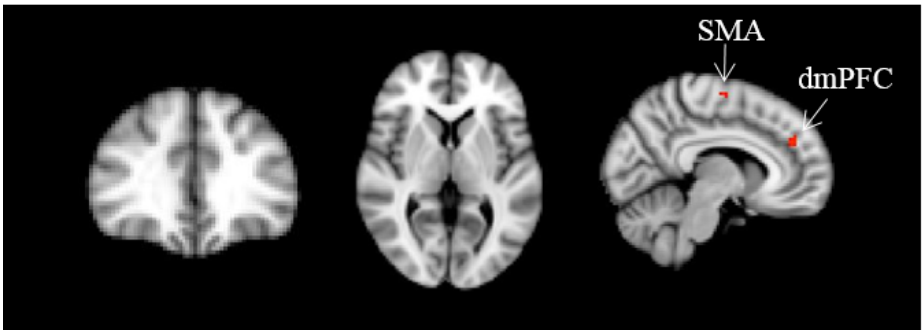

**Figure A.2** Whole-brain group analysis of brain areas in which activity correlated with mentalizing score. Activation in dorsal medial prefrontal cortex (dmPFC) and right supplementary motor area (SMA) correlated with mentalizing score.

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