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Integrated Functioning of the Mirror Neuron System and Its Role in Imitation Deficits in Autism

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INTEGRATED FUNCTIONING OF THE MIRROR NEURON SYSTEM AND ITS ROLE IN IMITATION DEFICITS IN AUTISM

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MEDICAL/CLINICAL PSYCHOLOGY

ABSTRACT

Deficits in imitation have been widely reported in children and adolescents with autism spectrum disorders (ASD). A proposed dysfunction of the mirror neuron system (MNS), the neural network underlying imitation, has been the subject of recent debate in ASD literature. However, while some research has found evidence of MNS abnormalities in ASD, recent studies have cast doubt onto the mirror neuron hypothesis of ASD as it is currently depicted. There is also behavioral evidence that the imitation deficit is not unitary, and therefore may not have one-to-ne correspondence with a malfunction of all aspects of MNS. Since functional imitation involves more than mirroring actions, a more complete understanding of the imitation deficit in ASD requires the analysis of the MNS in the context of the component skills comprising imitation and the wider neural networks involved in these skills. The current set of studies examined the component skills required for imitation and the role of the MNS functioning in autism at focal as well as network levels. The three functional MRI experiments that are part of this project investigated the functional integrity of the MNS using tasks involving visuospatial rotation, mental imitation, and motor imitation. For each study, behavioral, brain activation, functional connectivity, and brain-behavior relationships were analyzed. Results indicated that the ASD participants in this showed intact ability, reflected by their performance, in all tasks. However, the neural route with which tasks were accomplished differed between the TD and ASD groups. Specifically, aberrant activation and functional connectivity patterns were found for ASD participants in each of the three

studies. Neural responses emerging from these studies indicated alterations in the regulation of the MNS by other neural systems. Altered neural responses were also found to be correlated with autism symptomatology. The findings of this project provide important insights into the neural and cognitive mechanism underlying imitation in children and adolescents with autism. Implications of these findings for future research as well as clinical applications addressing imitation deficits in ASD are discussed.

Keywords: autism, imitation, mirror neuron, MNS, functional connectivity

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THE NEURAL BASIS OF MENTAL IMITATION IN AUTISM: IS IT ALL IN THE MIND?

IMITATION DEFICITS IN AUTISM: IS THE MIRROR NEURON SYSTEM TO BLAME?

LIST OF ABBREVIATIONS

AFNI	Analysis of Functional NeuroImages
ANT	Attention Networks Task
AQ	Autism-Spectrum Quotient
ART	Artifact Detection Tools
ASD	autism spectrum disorder
EQ	Empathy Quotient
IFG	inferior frontal gyrus
IPL	inferior parietal lobule
LCBELL	left cerebellum
LEBA	left extrastriate body area
LIFG	left inferior frontal gyrus
LINS	left insula
LIPL	left inferior parietal lobule
LMFG	left middle frontal gyrus
LMOG	left middle occipital gyrus
LMTG	left middle temporal gyrus
LPRCN	left precentral gyrus
LPSCN	left postcentral gyrus
LSMA	left supplementary motor area
LSTS	left superior temporal sulcus
MNI	Montreal Neurological Institute

MNS	mirror neuron system
PANESS	Physical and Neurological Examination for Soft Signs
PIQ	Performance IQ
PMv	ventral pre-motor cortex
psc	percent signal change
RBS-R	Repetitive Behavior Scale-Revised
RCBELL	right cerebellum
REBA	right extrastriate body area
RIFG	right inferior frontal gyrus
RIPL	right inferior parietal lobule
RMOG	right middle occipital gyrus
ROIs	regions of interest
RPRCN	right precentral gyrus
RPSCN	right postcentral gyrus
RSMA	right supplementary motor area
RSTS	right superior temporal sulcus
RT	reaction time
SCQ	Social Communication Questionnaire
SMP8	Statistical Parametrical Mapping, version 8 software
SQ	Systemizing Quotient
STS	superior temporal sulcus
TD	typically developing

INTRODUCTION

People imitate each other every day, to varying degrees, both consciously and subconsciously. Imitation is a fundamental component of human social behavior, and plays a crucial role in development (Piaget, 1945; Lovaas, 1979; Rogers & Pennington, 1991). It is through imitation that children learn about themselves, others, and establish an understanding of their relationship between themselves and their environment. Automatic imitation begins shortly following birth and assists in providing a sense of connectedness between an infant and its environment (Meltzoff & Moore, 1983). Early automatic imitation is believed to be less frequent around two months of age (Field et al., 1986; Fontaine, 1984), and more complex imitation reappears by the end of the first year of life (Meltzoff & Moore, 1992). These findings indicate that rudimentary imitation is present very early in development but that imitation skills develop and change significantly over time. By providing a child with information about the actions and intentions of the physical and social world, imitation assists with social learning (Rogers et al., 2003) and lays the foundation for future social development. Imitation is also important for the development of a wide range of social, communication, and motor skills (Tomasello, Kruger, & Ratner, 1993), and assists in comprehending the behavior of others (Goldenberg & Karnath, 2006). As children begin to speak, imitation of mouth movements is believed to teach them how to use their own articulators (Jordan & Rumelhart, 2002). Early in development, infants begin to engage in a range of coordinated facial motor and vocal activity routines including reciprocal vocalizations,

imitation of mouth openings, positive/negative facial expressions, and gaze. This early vocal-motor mirroring/synchrony reflects early interpersonal coordination of communication (Colonnesi et al., 2012) and may also be associated with later social-emotional and cognitive development (Feldman et al., 1996).

Imitation can be either purposeful or reflexive (mimicry). Children and adults often automatically match the postures, gestures, syntactic constructions, and prosody of others (Chartrand & Bargh, 1999; Niedenthal et al., 2005). This "chameleon effect" is not typically conscious but, instead, develops from long-term associative learning (Heyes, 2001,2011) and is subtle and flexible depending upon the specifics of the social situation (Chartrand & van Baaren, 2009; Brass, Ruby, & Spengler, 2009). This automatic imitation also carries significant social importance by helping to align the imitating individual with the individual being imitated. Being imitated helps to build rapport (Chartrand & Bargh, 1999), increases altruistic behavior (van Baaren et al., 2004), and also increases trust (Bailenson & Yee, 2005). Furthermore, imitation and mimicry help us feel what others feel, and respond compassionately to others' emotional states (Chartrand & Bargh, 1999; Eisenberg, 2000; Tangney, Stuewig, & Mashek, 2007; Iacoboni, 2009). Thus, imitation plays an important role in understanding other minds as well as in relating to others with appropriate affective response.

Imitation in ASD

While research continues to emphasize the importance of imitation to a wide range of developmental skills, a large body of literature has implicated imitation deficits as a relatively consistent and frequently replicated finding in autism spectrum disorders

(ASD) (e.g., Rogers et al., 2003; Williams, Whiten, & Singh, 2004; Charman et al., 2005; Rogers & Williams, 2006; Rogers et al., 2008; Stewart, McIntosh, & Williams, 2013). Individuals with ASD have been found to have difficulties with both symbolic and non-symbolic imitative behaviors, in imitating the use of objects, imitating facial gestures, and in vocal imitation (Rogers, 1999). Given the role of imitation in development, it has been suggested that reduced imitation may represent a core deficit in individuals with autism (Rogers & Pennington, 1991). It has been suggested that an imitation deficit in ASD could be related to a range of social impairments common in the disorder including deficits in face processing (Hadjikhani et al., 2007), theory-of-mind (Williams et al., 2006), and joint attention (Villalobos et al., 2005).

However, it should be noted that several studies, especially of late, have failed to replicate findings of imitation deficits in ASD (Bird et al. 2007; Dinstein et al. 2010; Gowen, Stanley, & Miall, 2008; Hamilton, Brindley, & Frith, 2007; Leighton et al. 2008; Press, Richardson, & Bird, 2010; Spengler, Bird, & Brass, 2010). Nevertheless, these studies still question the degree to which individuals with ASD would imitate automatically in real-world social settings and if they would do so for the same reasons as their typically developing (TD) peers (Loth & Gomez, 2006). The variability in previous findings may, in part, be attributed to uneven levels of difficulty that individuals with ASD display across different forms of imitation. More and more research suggests that not all types of imitation are equally impaired in autism. For instance, individuals with ASD often exhibit greater difficulty when imitating meaningless gestures rather compared with meaningful gestures or gestures that involve objects (Williams, Whiten, & Singh, 2004; Stone, Ousley, & Littleford, 1997). Similarly, Hobson and Hobson (2008)

found that children with autism had most difficulty when imitating the style of an action, especially when the style was not necessary for achieving the action's goal. These results may suggest that persons with autism are better at imitation when the to-be-imitated action is goal-oriented and there is a clear end state (as meaningless gestures and the "style" of a gesture are determined more by the process of the imitative act rather than the end state or goal of the gesture).

Recent research has also suggested that individuals with ASD may be able to imitate when explicitly instructed to do so (McDuffie et al., 2007; Hamilton, Brindley, & Frith, 2007; Whiten & Brown, 1998), in the absence of which they often fail to spontaneously imitate others. McIntosh and colleagues (2006) found that children with ASD imitated facial expressions successfully when explicitly instructed. However, in the absence of explicit instruction, they failed to display typical automatic mimicry of facial expressions. Based on these findings, it was suggested that deficits in automatic imitation may impair a variety of socioemotional skills that help in understanding the affective states of others. In addition to a failure of automatic imitation, the broader social context may have a significant influence on imitation in individuals with ASD, such that they are more likely to imitate actions when the model is familiar (Oberman, Ramachandran, & Pineda, 2008). In addition, individuals with ASD may have more difficulty imitating emotional stimuli compared with stimuli that do not have an emotional component (e.g., Dapretto et al., 2006; Beall et al., 2008). Thus, it is likely that many characteristics of the to-be-imitated action may impact the quality and performance of imitation in ASD.

There is also evidence that imitation likely follows a developmental progression in ASD. Despite being delayed, imitation appears to improve with age (Heimann & Ullstadius, 1999; Stone, Ousley, & Littleford, 1997) with the ability to imitate simple, single-step actions increasing with age in children with autism (Hepburn & Stone, 2006). Imitation deficits in older children and adults with ASD are thought to more likely reflect difficulty imitating the "attitude" or "style" of an action, rather than the basic motor components of the action (Hobson & Lee, 1999). The continued difficulty in imitating the "style" of an action has led to the suggestion that individuals with ASD often focus on the goal of an action and, therefore, miss the kinematic aspects of the action that may contain important social information (Gowen, 2012). Taken together, all these findings reflect the complexity and heterogeneity of imitation ability in ASD, and more importantly the need for better understanding the basic mechanisms of imitation in this disorder.

Is Imitation a Unitary Construct?

While a core deficit in imitation has been widely reported in autism, a predominant view of imitation as a unitary construct is a topic of debate. Almost all published studies on this topic examined imitation as a global construct with little emphasis on the component processes that constitute this skill. If imitation was a unitary construct, individuals with autism should exhibit equal impairment in all forms of imitation tasks. However, the previously mentioned findings of unequal levels of difficulty with different forms of imitation (e.g., Rogers et al., 1996; Vivanti et al., 2008; McIntosh et al., 2006; Colombi, Vivanti, & Rogers, 2011; Carmo et al., 2013) suggest

otherwise. Therefore, analysis of the component processes underlying imitation and their integration would likely provide vital insights in better characterizing imitation deficits in ASD (Want & Harris, 2002; Bennetto, 1999; Hamilton, 2008). A study by Bennetto (1999) classified imitation into the following five components: 1) basic motor functioning, 2) body schema, 3) dynamic spatiotemporal representation, 4) memory, and 5) motor execution of non-meaningful hand and arm gestures. Despite a global impairment in imitation, her data showed no significant differences between children and adolescents with autism and TD controls on spatiotemporal representation, body schema, and memory. However, impairments were found in motor functioning, and the planning and execution aspects of imitation. Other studies also suggest similar component processes underlying imitation (Decety, 2006) along with attentional flexibility (Klin et al., 2002) and motor deficits (Rogers et al., 2003; Mostofsky et al., 2006), all of which may impact imitation in autism (Williams & Waiter, 2006). For these component skills to work together to produce functional imitation, the integrated functioning of several neural networks is required.

Neural Basis of Imitation: The Mirror Neuron System

Insights into the neural mechanisms of imitation came through the discovery of a set of neurons in the F5 area of the non-human primate brain that fire not only when a monkey performs an action but also when a monkey observes an action being performed (Gallese et al., 1996; Rizzolatti et al., 1996). These “monkey see, monkey do” neurons, referred to as mirror neurons, are dedicated to the visual processing of actions of others and have provided clues as to how we perceive the actions of others and translate this

information to perform similar actions ourselves. The Mirror Neuron System (MNS) has been proposed as a network that mediates action understanding and action imitation (Iacoboni et al., 1999; Koski et al., 2003; Heiser et al., 2003). In nonhuman primates, mirror neurons were identified in area F5 and area PF (Rizzolatti & Craighero, 2004). While it is difficult to directly study the existence of mirror neurons in humans, a substantial number of fMRI and EEG studies have found evidence suggesting that a homologue of the monkey mirror neurons exists in humans (Iacoboni & Dapretto, 2006; for an opposing perspective see Turella et al., 2009). The monkey F5 is thought to correspond to the human inferior frontal gyrus (IFG)/ ventral premotor cortex (PMv), and the monkey PF is thought to correspond to the human inferior parietal lobule (IPL) (see Figure 1). In humans, these two core regions are thought to interact closely with the superior temporal sulcus (STS) to produce action understanding and mental action "mirroring" (Iacoboni, 2005; Iacoboni et al., 2001; Iacoboni & Dapretto, 2006). Research also suggests that different regions of the MNS each likely serve different functions. While the IFG/PMv has been found to be involved in action planning (Hamilton, 2008) and perception-action coupling (Newman-Norlund et al., 2010), the IPL is thought to mediate spatiotemporal, perceptual and goal coding aspects necessary for imitation (Rizzolatti & Matelli, 2003; Hamilton, 2008).

It is clear, however, that imitation is not limited to mirroring others (Southgate & Hamilton, 2008). Therefore, while the MNS is believed to be the primary neural network underlying imitation, successful imitation likely underlies the communication within this network as well as between this network and the rest of the brain (see Kana, Wadsworth, & Travers, 2011 for review). For example, previous research suggests that the MNS

works with both the dorsolateral prefrontal cortex (DLPFC) and areas associated with motor preparation when engaged in imitative learning (Buccino et al., 2004). Given that the MNS works to map the actions of another person onto one's own motor system, it is also intrinsically linked to parietal motor circuitry previously identified as important for praxis (Heilman & Valenstein, 2003; Wheaton & Hallett, 2007). Previous findings suggest that the MNS is both structurally and functionally connected to both the limbic system (Iacoboni, 2005; Dapretto et al., 2006; Carr et al., 2003; Wicker et al., 2003) and theory-of-mind networks (Schulte-Ruther et al., 2007), with integrated functioning of the systems being responsible for empathy and the production of self-other distinctions during face-to-face interactions.

Broken Mirror Theory of Autism

It has been hypothesized, by some researchers, that individuals with ASD who have deficits in imitation may also have a malfunctioning MNS (e.g., Oberman & Ramachandran, 2007; Williams et al., 2001). This "broken mirror" theory of autism has received substantial attention, with quite a few studies suggesting that atypicalities of the MNS may contribute to autism symptomatology (for recent reviews see Williams, 2008; Bernier & Dawson, 2009). This account also suggests that MNS dysfunction could help explain some of the primary symptoms of ASD including isolation, lack of empathy, and social skill difficulties (Ramachandran & Oberman, 2006). Evidence for MNS dysfunction in persons with autism comes from EEG studies (e.g., Bernier et al., 2007; Martineau et al., 2008; Oberman et al., 2005), MEG studies (e.g., Nishitani, Avikainen, & Hari, 2004), functional MRI studies (e.g., Dapretto et al., 2006; Williams et al., 2006),

and also from structural MRI studies (e.g., Hadjikhani et al., 2007). EEG studies point to mu wave attenuation in TD individuals when they both observe and perform an action; but in individuals with ASD only when they perform an action (Oberman et al., 2005; Bernier et al., 2007; Martineau et al., 2008). This abnormal pattern of mu wave attenuation was also found to be correlated with the degree of imitation deficit in autism (Bernier et al., 2007). fMRI studies have shown functional alterations in both anterior and posterior components of the core MNS (Dapretto et al., 2005; Williams et al., 2006). Structural MRI studies have shown anatomical abnormalities in the MNS in autism (Yamasaki et al., 2010; Hadjikani et al., 2006).

However, not all research points towards a dysfunction in the MNS in autism. There have been studies finding intact activation (e.g., Oberman, Ramachandran, & Pineda, 2008; Raymaekers, Wiersema, & Roeyers, 2009; Avikainen, Kulomaki, & Hari, 1999) as well as increased activation in the MNS in subjects with autism (Martineau et al., 2010; Baron-Cohen et al., 2006). For example, a study by Hamilton, Brindley, and Frith (2007) found performance of the subjects with autism was superior to controls on gesture recognition tasks that would likely recruit the MNS. Dinstein and colleagues (2010) found equivalent MNS activation in ASD and TD controls during the observation and execution of hand gestures. Research has also found abnormal activation patterns in regions outside of the MNS but no group differences in MNS activation (Schulte-Ruther et al., 2011; Marsh & Hamilton, 2011). Other studies do not point toward a fully functional MNS in autism, but instead critique the validity of MNS dysfunction as a core (primary) deficit in ASD. It is possible that the activation differences in the MNS could

represent differences in resting state activation levels since many fMRI studies compare activation at baseline to activation during the task (Dinstein et al., 2008).

Studies examining functional connectivity in the MNS have also resulted in mixed findings. While there are reports of task-related decrease in functional connectivity in the MNS in ASD (e.g., Hari & Nishitani, 2004; Villalobos et al., 2005; Schipul et al., 2012), examination of intrinsic connectivity in ASD has led to mixed results. For example, Lee and colleagues (2009) found reductions in functional connectivity of the right IFG in children and adolescents with ASD, which was negatively correlated with age. They suggested an atypical developmental trajectory rather than an overall reduction in functional connectivity in ASD. While some studies have implicated resting state functional connectivity abnormalities in MNS in ASD (e.g., Paakki et al., 2010), others have failed to replicate such findings (e.g., Shih et al., 2010). Complicating matters further, recent research examining children with ASD has resulted in findings of widespread hyperconnectivity including hyperconnectivity between the MNS and other neural regions (Supekar et al., 2013; Uddin et al., 2013; Di Martino et al., 2011). Based on these findings, it has been suggested that both over and under connectivity likely impacts the MNS in autism (Friedrich et al., 2014). Several additional hypotheses have also been proposed to explain the inconsistencies of previous findings including the need for taking developmental changes into account (Uddin, Supekar, & Menon, 2013).

Theories of MNS Function and Dysfunction in ASD

As noted above, individuals with ASD often have increased difficulty with automatic imitation or imitation of meaningless actions. However, they experience fewer deficits in the voluntary imitation of meaningful actions (Hamilton, Brindley, & Frith, 2007; Hobson & Hobson, 2008; Williams, Whiten, & Singh, 2004; McIntosh et al., 2006). This led to the proposal that mirroring may rely on two distinct pathways. The mimicry pathway (M), involving a direct communication between the STS and IFG, is involved in automatic mimicry. The emulation pathway (EP), however, involves a connection with the IPL and is involved in coding for goals and goal-directed actions. According to the EP-M model, individuals with ASD may experience a specific deficit in mimicry, but have intact connections within the emulation pathway (Hamilton, 2008). Difficulties with imitation of meaningless actions have also led to explanations based on the dual-route theory of imitation processing (Tessari et al., 2007), according to which, imitation of meaningful actions takes a semantic neural route, while imitation of meaningless actions relies on the kinematic aspects of an action. Individuals with ASD have difficulties using the kinematic pathway, and they rely on semantic representations for both forms of imitation (Hamilton, 2008; Wild et al., 2011).

Another factor that may underlie MNS functioning in ASD is the presence of social components in a task. A malfunctioning social filter may be responsible for the difficulties observed in ASD. More specifically, low social motivation in autism may lead to reduced "time on task" and, thus, impeded social development (Chevallier et al., 2012). A lack of familiarity with or interest in unfamiliar others may result in reduced activation of social brain networks (including the MNS) in ASD (McCleery et al., 2013)

and may explain findings that individuals with ASD experience less difficulty with imitation and other social tasks when the model is a familiar individual (Oberman, Ramachandran, & Pineda, 2008). This has led to the social top-down response modulation (STORM) theory of MNS functioning (Wang & Hamilton, 2012), which proposes that the MNS interacts with, and is controlled by a component of the mentalizing system, the medial pre-frontal cortex (mPFC). The mPFC exerts top-down control on the MNS, leading to enhancement or inhibition of imitation. According to the theory, it is this top-down control which goes awry in ASD, leading to aberrant activation in the MNS.

While these models all provide different possibilities for the inconsistent findings of imitation and MNS deficits in ASD, they also include several key components. Specifically, all theories suggest that it is likely the connections between nodes of the MNS, rather than the nodes themselves, that may be dysfunctional in ASD. These models also indicate that functioning of the MNS is best understood in the context of the larger neural environment, which may influence functioning of the MNS. The MNS does not function in isolation and, therefore, abnormalities in the functioning of this system may be caused by or result in aberrant functioning of other systems. Yet another theme cutting across all of these theories is the complexity of imitation. Imitation is often identified as a unitary skill. However, as these models emphasize, it requires the integrated functioning of many different neural systems and may be accomplished using different neural pathways depending on the type of imitation or the context in which it is performed.

Description of Current Set of Studies

The gaps emerging from previous research on neural bases of imitation in ASD are essentially two-fold: 1) most studies examined imitation as a unitary construct, with little information on the neural basis of spared and affected components of imitation in autism and how these components may map onto the functioning of the MNS; and 2) while recruitment, and lack thereof, of MNS regions have been reported, the connectivity of it has largely been ignored. The current set of studies approached imitation in children with ASD from the framework of connectivity account of autism and the MNS model of autism.

In order to examine the unique contribution of components of imitation, three event-related fMRI paradigms were developed. All three experiments were completed in a single MRI session. The first experiment focused on the visuospatial aspect of imitation thought to be associated primarily with the IPL aspect of the MNS. The second experiment retained the visuospatial aspect while adding an executive component using a mental imitation task. This is expected to target both the IPL and the frontal executive areas, such as IFG, and dorsolateral prefrontal cortex (DLPFC). The third experiment involved a motor imitation paradigm to address the coordinated functioning of IFG/PMv and IPL along with other motor regions in executing imitation. These three experiments together allowed for the examination of the functioning of each component of the MNS separately as well as how the components work together a unit and with other neural systems.

Seventeen high-functioning children and adolescents with ASD and 17 age and IQ-matched TD control participants took part in the current set of fMRI studies (age

range: 8 to 17 years; minimum Full Scale and Non-Verbal IQ: 75, measured using the Wechsler Abbreviated Scales of Intelligence). Although the same participants were involved in all studies, participants excluded due to excessive motion were unique to each study. Participants with ASD were recruited through the research subject database of the *Cognition, Brain, and Autism Laboratory* at the University of Alabama at Birmingham (UAB), the Alabama Autism Society, and flyers posted at local ASD-related treatment and evaluation centers. Typically developing (TD) participants were recruited using flyers and advertisements on the UAB campus, flyers posted in local community centers (e.g., libraries, YMCAs), and through the *Cognition, Brain, and Autism Laboratory's* research subject database.

In addition to completion of the fMRI paradigms, demographic information, IQ, and other neuropsychological indices was also collected from participants. Cognitive functioning was measured using the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). Participant handedness was measured using the Edinburgh Handedness Inventory (Oldfield, 1971). Measures of ASD symptomatology were also collected from participants in both groups including the Empathizing Quotient and the Systemizing Quotient (EQ/SQ; Baron-Cohen et al., 2003), the "Reading the Mind in the Eyes Test" (RME) (Baron-Cohen et al., 2001), the Social Communication Questionnaire (SCQ) (Rutter, Bailey, & Lord, 2003), and the Repetitive Behavior Scale –Revised (RBS-R) (Bodfish et al., 2000; Lam & Aman, 2007). These measures were used to ensure that participants qualified for the studies, as well as to establish brain-behavior relationships. Outside of the scanner, participants also completed the Attention Networks Task (ANT; Fan et al., 2002) and completed the same motor imitations presented during imaging so

that these imitations could be coded for their style, accuracy, and precision based on the taxonomy suggested by Buxbaum and colleagues (2000) (please refer to Appendix 1 for additional information regarding each measure).

The current set of studies was designed to address the following overarching specific aims:

Specific Aim #1: Behavioral Performance

The first specific aim was to compare the behavioral performance of high-functioning children and adolescents with ASD and TD controls on tasks comprising various components of imitation.

Hypothesis 1a. It was predicted that participants with ASD would show intact or better performance than TD controls on a task assessing visuospatial rotation of body-parts.

Hypothesis 1b. Participants with ASD were predicted to exhibit greater difficulty (as expressed by lower accuracy rates and increased reaction time) than TD controls on a task involving mental imitation of actions.

Hypothesis 1c. Based on previous findings of intact ability (in older children and adolescents) for simple imitation, it was predicted that participants with ASD would demonstrate intact ability on a simple hand-based motor imitation task presented in the scanner.

Specific Aim #2: Functioning of the MNS

The second specific aim of the current set of studies was to delineate the specialized roles as well as integrative functions of the main cortical nodes (IFG, IPL) of

the mirror neuron system and the interaction of the MNS with other neural regions during action imitation in high-functioning children and adolescents with ASD.

Hypothesis 2a. While intact activation was predicted for the visuospatial rotation task, it was predicted that individuals with ASD would show reduced activation in the frontal component of the MNS (PMv/IFG) while engaged in both mental and motor imitation tasks.

Hypothesis 2b. It was predicted that there would be reduced functional connectivity between the posterior (IPL) and the anterior (IFG) components of the MNS when performing the imitation task. Participants with ASD were also expected to display intact or stronger connectivity in occipital-parietal areas.

Hypothesis 2c. Participants with ASD were expected to display aberrant activation and connectivity patterns between the MNS and other neural regions required to produce the various components of action imitation.

Specific Aim #3: Brain-behavior Relationships

The third specific aim was to establish the relationship between behavior and brain function by linking autism symptomatology and its effect on neural response to different aspects of imitation.

Hypothesis 3a. It was predicted that there would be a significant correlation between autism symptoms and activation in the MNS as well as the functional connectivity within the MNS and between the MNS and other neural systems.

Hypothesis 3b. Measures of attention, motor performance, and empathy were also predicted to be correlated with performance and neural activation/ connectivity patterns during performance of imitation-related tasks.

THE NEURAL MECHANISMS UNDERLYING MENTAL ROTATION OF BODY-RELATED STIMULI IN AUTISM SPECTRUM DISORDERS

by

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Abstract

Cognitive and behavioral studies over the last several years have identified visuospatial processing as a relatively unaffected area of functioning in individuals with autism spectrum disorders (ASD). Mental rotation is a visuospatial task where such advantage has been seen, although mental rotation of body-related stimuli in ASD has received little attention relative to rotation of objects and shapes. This fMRI study examined the neural mechanisms underlying mental rotation of hand stimuli in 13 high-functioning children and adolescents with ASD and 15 age-and-IQ-matched typically developing (TD) control participants. Results indicate that the ASD group was significantly more accurate, relative to TD group, in mental rotation, although this effect was related to their spatial skills, measured by performance IQ. Both ASD and TD groups showed activation in core visuospatial processing regions including inferior parietal, middle to superior occipital, and calcarine regions. Both groups also demonstrated activation in the left primary motor cortex extending to the postcentral gyrus. When activation during 45° rotations was contrasted with that of 90°rotations, the ASD group showed greater activation, relative to TD group, in the right fusiform gyrus, left and right middle occipital lobe, and right precuneus. The TD group, on the other hand, showed greater activation in LIFG, left MPFC, LSMA, and left anterior cingulate cortex. ASD participants also showed intact functional connectivity during this mental rotation task. Thus, the findings of this study suggest a visuospatial advantage in participants with autism, perhaps accompanied by an increased recruitment of relatively posterior visuospatial brain areas unlike the TD control participants who showed increased activity in motor areas.

Keywords: Mental rotation, fMRI, autism, visuospatial, functional connectivity

Word Count: 6,980

1. Introduction

While social communication impairment, and the presence of repetitive and restricted behavior and interests form the hallmark features of autism spectrum disorder (ASD), a key area of strength in ASD individuals is visuospatial processing (Falter et al., 2008; Hamilton et al., 2009; Caron et al., 2004; Mottron et al., 2003, 2006). Visuospatial skills, the ability to encode and mentally manipulate spatial information (Mathewson, 1999), are vital and form the foundation of problem solving and abstract reasoning (Smith, 1964; Cheng & Mix, 2014). Visuospatial rotation (sometimes referred to as spatial transformations) refers to the process through which a mental image is rotated around an axis in space (Zacks, 2008), often in order to determine if representations align with each other despite variations in position or orientation (Pearson et al., 2014). Such ability to imagine the rotation of an object in 2 or 3 dimensional space is termed mental rotation (Shepard, 1984; Corballis, 1997). Decades of behavioral research on mental rotation is inspired primarily by the classic 3-dimensional rotation task developed by Shepard and Metzler (Shepard and Metzler, 1971). Previous research examining the neural basis of object-oriented mental rotation in typically developing (TD) individuals has reported activation in a range of parietal and occipital-temporal areas including activation of the superior and inferior parietal lobule (SPL, IPL), the parietal-occipital border, secondary visual cortex, inferior temporal gyrus (ITG), supplementary motor area (SMA), thalamus, and basal ganglia (Dietrich et al., 2001; Cohen et al., 1996; Harris et al., 2000). Thus frontal, parietal, and motor cortex involvement is a relatively consistent pattern of activation in neuroimaging studies of mental rotation in healthy individuals.

Considering the visuospatial advantage seen in individuals with ASD, mental rotation tasks provide a unique platform to investigate the cognitive and neural mechanisms of such advantage. Evidence for a visuospatial advantage in ASD comes mainly from studies involving block design tasks (BDT), embedded figures tasks (EFT), and from studies examining the ability of ASD individuals to determine the shape of slanted circles in the absence of outside cues (Shah & Frith, 1983, 1993; Ring et al., 1999; Mottron et al., 2003; Mitchell & Ropar, 2004; Damarla et al., 2010; Kana et al., 2013). It has been suggested that intact or superior visuospatial processing in ASD may underlie the use of a "veridical" mapping process, which involves a feed-forward, bottom-up cognitive approach that relies less on the global top-down, higher cognitive processes and allows for a greater influence of low-level visual processing (Soulières et al., 2011). Neuroimaging studies of visuospatial processing have found intact or advanced behavioral skills with unique neural activation patterns, especially increased brain activity in relatively posterior areas, in individuals with ASD (Damarla et al., 2010; Samson et al., 2012; Keehn et al., 2013; Silk et al., 2006; McGrath et al., 2012; Kana et al., 2013). In addition, studies of functional connectivity reveal reduced frontal-posterior connectivity (along with intact or enhanced posterior area connectivity), indicating that typical higher-level cortical control was reduced in ASD providing additional support for reliance on enhanced lower-level visual regions (McGrath et al., 2012), and, subsequently, greater visuospatial ability.

While several studies have investigated visuospatial processing, far fewer studies have addressed mental rotation in ASD. Studies of mental rotation have consistently found that children and adults with autism demonstrate unimpaired or even superior

performance on these tasks (Falter et al., 2008; Hamilton et al., 2009; Silk et al., 2006; McGrath et al., 2012; Beacher et al., 2012; Nakano et al., 2012) despite showing altered patterns of neural activation. An overall reduction in activation in frontal, temporal, occipital, striatal, and cerebellar regions in ASD individuals has been reported in some studies (McGrath et al., 2012; Silk et al., 2006). Such alterations may emerge from increased reliance on detail-oriented information processing (Soulières et al., 2011). While mental rotation of objects appears to remain unimpaired in autism, studies have also suggested that this may not hold when the rotation involves other forms of stimuli. For example, while children with ASD showed unimpaired performance on a non-social mental rotation task, they exhibited impaired visual-perspective taking (a skill heavily influenced by mental rotation of socially presented stimuli) (Hamilton et al., 2009). This suggests potential differences in approach and performance of ASD individuals between different types of visuospatial transformation and rotation tasks. Given this distinction and previous findings of body-related processing deficits in autism (Minshew et al., 1997; Noterdaeme et al., 2002; Dawson et al., 2005; Hadjikhani et al., 2007), a better understanding of the cognitive and neural bases of body-related visuospatial transformations is particularly important in uncovering the dichotomy of visuospatial advantage and social deficit in ASD.

Mental rotations of body related stimuli such as hands have been found to evoke a unique cognitive strategy (Kosslyn et al., 1998) when compared with mental rotation of objects (Parsons, 1987, 1994; Sekiyama, 1983). In addition to visuospatial aspects, body-related mental rotation requires motor planning and biological motion processing (Zacks, 2008; Parsons et al., 1995; Kosslyn et al., 1998; Vingerhoets et al., 2002). This includes

regions such as the left primary motor area, SPL, IPL, and left frontal regions (Kosslyn et al., 1998). Many studies have also reported right hemisphere dominance for object-based mental rotation tasks (Ratcliff, 1979; Cohen et al., 1996; Ditunno & Mann, 1990; Harris et al., 2000) while hand-based mental rotation tasks results in left lateralized activation (Kosslyn et al., 1998). This additional activation is believed to represent a participant's tendency to imagine moving their own body part during mental rotation, which results in a qualitatively different approach to rotation of these stimuli as compared to mental rotation of characters, numbers, or abstract shapes (Kosslyn et al., 2001; Parsons, 1994). In other words, spatial rotations involving body-related stimuli such as hands, arms, and torso have been found to evoke a more "egocentric" (or self-based) cognitive strategy. Egocentric spatial transformation, which occurs when we place our body in a new alignment/ position in space (Zacks et al., 1999), is different from object-based mental rotation where individuals perform the task by imagining manipulation of the orientation of the object by external forces (Wraga et al., 2003). While the mental rotation of objects relies on analog spatial representations, hand stimuli additionally elicit representations in the sensorimotor system (Zacks, 2008). Previous studies have also indicated that mental rotation of hands is more likely to engage the mirror neuron system (MNS) (Kosslyn et al., 1998). For example, areas such as the IPL, appear to be uniquely activated in response to mental rotation of sensorimotor stimuli (Kosslyn et al., 1998) suggesting that these stimuli often result in an egocentric approach in which the individual either imagines themselves as in the position with the hand or imagines rotating their own hand.

Most studies examining mental rotation in ASD focus on rotations of abstract objects, such as the frequently utilized block stimuli developed by Shepard & Metzler

(1971). Considering the motor, mental imitation, and self-related difficulties seen in ASD (e.g., Rogers et al., 1996; Mostofsky et al., 2006; Dawson et al., 2005), body-related mental rotation may pose challenge to children with ASD. Although limited, there has been some behavioral research examining mental rotation of biological stimuli indicating an allocentric approach in ASD, rather than the egocentric approach seen in TD individuals (Pearson et al., 2014). When required to take an egocentric approach, individuals with ASD who had more symptoms (measured by the autism quotient, AQ) were slower to perform egocentric transformations than those with lower AQ scores (Brunye et al., 2012). Another study showed that adults with ASD had greater difficulty in egocentric spatial transformations (Pearson et al., 2014). However, several of these studies included the use of facial stimuli and full body diagrams (David et al., 2010; Brunye et al., 2012; Pearson et al., 2014), the transformation of which may be influenced by other skills unrelated to mental rotation ability. Preliminary studies examining mental rotation of drawings of hand stimuli rather than full body transformations in autism have found intact behavioral accuracy (Conson et al., 2013; Soulières et al., 2011) but differences in strategy suggesting that the hand stimuli were viewed as objects rather than body parts by the ASD group (Conson et al., 2013).

While previous research consistently indicates intact or superior visuospatial abilities in autism, body related and sensorimotor processing appears to be more impacted (Minshew et al., 1997; Noterdaeme et al., 2002; Dawson et al., 2005; Hadjikhani et al., 2007). There is also evidence of atypical motor planning in ASD individuals (Cattaneo et al., 2007; Gowen & Hamilton, 2012; Sacrey et al., 2014). The addition of these processing components for mental rotation of body parts, a skill

necessary for visual perspective taking (Michelon & Zacks, 2006; Pearson et al., 2014) and, as such, appropriate social interactions, highlights the importance of gathering a better understanding of this skill in autism. Previous studies examining hand-related mental rotation in ASD suggest the use of a unique cognitive approach but did not examine the neural mechanisms underlying completion of the task. Since aberrant activation patterns in regions associated with body-related processing were reported previously in ASD (e.g., Zilbovicius et al., 2006; Kleinhans et al., 2008; Di Martino et al., 2009), additional research is necessary to clarify the degree to which visuospatial strengths in autism are preserved when body-related stimuli are presented. Differences in the processing of these stimuli may provide important insight into the neural mechanisms underlying social difficulties in ASD individuals.

The current study used a unique fMRI mental rotation paradigm involving mental rotation of sensorimotor stimuli. Based on evidence of visuospatial advantage in ASD even using hand stimuli (Soulieres et al., 2011), we predict that the participants with ASD would perform as well, or better than TD control participants. At the neural level, such intact performance may be mediated by increased parietal and occipital activation in ASD as well as local overconnectivity in parietal areas associated with analog or object-based spatial representations. The TD group, however, may show activation in and around the precentral sulcus and in other neural regions specifically associated with spatial tasks that involve body-related or sensorimotor representations.

2. Materials & Method

2.1 Participants

Seventeen high-functioning children and adolescents with ASD and 17 age and IQ-matched TD control participants took part in the current fMRI study (age range: 8 to 17 years; minimum Full Scale and Non-Verbal IQ: 80, measured using the Wechsler Abbreviated Scales of Intelligence). Participants with ASD were recruited through the research subject database of the *Cognition, Brain, and Autism Laboratory* at the University of Alabama at Birmingham (UAB), UAB Civitan-Sparks Clinic, the Alabama Autism Society, and flyers posted at local ASD-related treatment and evaluation centers. All participants were diagnosed with an ASD. Current and past ASD symptoms were assessed using the Social Communication Questionnaire (SCQ) – Lifetime Version (Berument et al., 1999), the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001), and the Repetitive Behavior Scale – Revised (Lam & Aman, 2007) (see table 1 for detailed participant information). TD participants were recruited using flyers and advertisements on the UAB campus, flyers posted in local community centers (e.g., libraries, YMCAs), and through the *Cognition, Brain, and Autism Laboratory's* research subject database. Participants were not included in the study if they indicated having worked with metal or having metal implanted in their bodies (either surgically or accidentally) or if they had a history of psychiatric disorders. No participants indicated having a cognitive disorder, anxiety disorder, schizophrenia, or obsessive compulsive disorder. Out of the 34 participants scanned (17 ASD and 17 TD), six participants' data were not usable due to head motion artifacts resulting in a final sample of 13 ASD and 15 TD control participants.

Insert Table 1 about here

2.2 Overall Experimental Procedure

Participants took part in 2 separate sessions: 1) Assessment; and 2) MRI scanning. The first session lasted approximately two hours, during which the participants completed a series of neuropsychological tests as well as demographic information, measures of visuomotor skills, ASD-related symptomatology, attention shifting, medication history, and diagnosis. During the second session, participants completed their MRI scan. Before the scan, participants practiced the task on a laptop computer.

2.3 Stimuli and Experimental Paradigm

This study used a mental rotation task involving two different angles of rotation (45^0 and 90^0) and hence different levels of difficulty. Participants were shown high-resolution color images of two hands on left and right side of a cross on the computer screen, and were asked if the rotated hand on the right panel was the same hand as the hand shown on the left side. The angles of hand rotation were randomized between 45^0 and 90^0 (see Figure 1), which were chosen based on previous literature on mental rotation tasks in autism which used rotation angles up to 180^0 and indicated a positive linear relationship between the angle of rotation and task difficulty (Silk et al., 2006; Falter et al., 2008; Soulières et al., 2011). In an event-related design, each trial was presented for a period of 4000ms with an inter-stimulus interval (ISI) of 6000ms. There were a total of 24 trials, half of which were congruent (the hand on the right is the same as the hand on the left) and the other half were incongruent trials (the hand on the right is a different

hand than that on the left). The participants' task was to examine the two hands on the screen and press a button to indicate if the two hands were the same or different.

Insert Figure 1 about here

2.4 Image Acquisition

All fMRI scans were acquired using the Siemens 3.0 Tesla Allegra head-only scanner (*Siemens* Medical Inc., Erlangen, Germany) located at the UAB Civitan International Research Center (CIRC). For structural imaging, initial high resolution T1-weighted scans were acquired using a 160-slice 3D MPRAGE (Magnetization Prepared Rapid Gradient Echo) volume scan with TR = 200 ms, TE = 3.34 ms, flip angle = 12^0, FOV = 25.6 cm, 256 X 256 matrix size, and 1 mm slice thickness. A single-shot gradient-recalled echo-planar pulse sequence was used to acquire functional images (TR= 1000 ms, TE = 30ms, flip angle = 60 degrees, FOV = 24 cm, matrix =64 x 64). Seventeen adjacent oblique axial slices were acquired in an interleaved sequence with 5 mm slice thickness, 1 mm slice gap, a 24 X 24 cm field of view (FOV), and a 64 X 64 matrix, resulting in an in-plane resolution of 3.75 X 3.75 X 5 mm^3. The stimuli were rear-projected onto a translucent plastic screen and participants viewed the screen through a mirror attached to the head coil. Quality control checks were applied to the acquired data to examine the signal to noise ratio, temporal signal to noise ratio, ghosting, and head motion artifacts. Data that did not meet quality standards were not included in further analyses. In addition, the head motion for each participant, quantified in three translational (x, y, and z) and three rotational (pitch, roll, and yaw) dimensions were entered into the general linear model as regressors of no-interest.

2.5 Behavioral and Neuropsychological Data Analyses

Scores from neuropsychological measures for each participant were entered into SPSS 16.0 statistical software (*SPSS* Inc., Chicago, IL). Between-group t-tests were run in order to compare group means on each measure. Data on each participant's reaction time and accuracy was also entered into SPSS. Analyses were run examining group means on accuracy and reaction time for each condition (45^{o} rotation and 90^{o} rotation) separately as well as together (all rotation conditions combined). Since PIQ may have a role in mental rotation tasks, an ANCOVA was run using PIQ as a covariate in order to determine if significant group effects remained.

2.6 fMRI Data Analyses

Imaging analysis included brain activation, change in percent signal intensity (psc), and functional connectivity, along with correlation analyses involving these measures and assessment scores.

Brain activation. To examine brain activation, the data were pre-processed and statistical analyses were carried out using Statistical Parametrical Mapping, version 8 software (SPM8; Wellcome Department of Cognitive Neurology, London, UK). Images were corrected for slice acquisition timing, motion-corrected, normalized to the Montreal Neurological Institute (MNI) template, re-sampled to 8-mm^3 voxels, and smoothed with an 8 mm FWHM filter. The general linear model was utilized to perform statistical analyses on both individual and group data. Activated regions of interest (ROIs), or clusters with statistically significant activation were identified using a t-statistic on a voxel by voxel basis. In order to control for motion during scanning, artifact detection

was performed using the Artifact Detection Tool (ART) toolbox (http://www.nitrc.org/projects/artifact_detect/). This tool provided information regarding each subject's motion (in mm) as a function of time. These motion variables (in the x, y, and z planes as well as yaw, pitch, roll rotational dimensions) were then utilized as nuisance regressors for analysis of each subject's activation data. Since PIQ scores were marginally significant between the two groups, PIQ was added as a covariate for within-group and between-group activation analyses in order to determine areas of activation that remain after controlling for its effect. Differences in activation between groups were calculated by examining differences in the number of voxels activated in corresponding ROIs between the groups for each paradigm. Monte Carlo simulations were applied to the data based on the $8mm^3$ voxel size using AlphaSim in Analysis of Functional NeuroImage (AFNI) software (Ward et al., 2000) to determine the minimum number of voxels required in each cluster to be equivalent to the level of statistical significance at a family-wise error corrected threshold of $p<0.05$. According to Lieberman and Cunningham (2009), simulations can implicate cluster size thresholds that produce the best balance between Type I and Type II error. Based on the results of these simulations, the within-group analysis used a cluster size of 80 contiguous voxels at an uncorrected P-value of 0.001. Between-group analyses used a cluster threshold of 144 contigous voxels at an uncorrected P-value of 0.005.

Percent Signal Change (psc). In addition to the activation analyses run using the general linear model in SPM8, psc values were extracted for mental rotation task as a whole (45° + 90° rotations together) when contrasted with a fixation baseline. This contrast was used as it best represented the brain activation across different conditions.

Anatomical ROIs were defined using the WFU Pickatlas toolbox (Maldjian et al., 2003). These ROIs included 8 sets of bilateral regions: inferior parietal lobule (LIPL, RIPL), inferior frontal gyrus (LIFG, RIFG), superior temporal sulcus (LSTS, RSTS), middle occipital gyrus (LMOG, RMOG), supplementary motor area (LSMA, RSMA), precentral gyrus (LPRCN, RPRCN), postcentral gyrus (LPSCN, RPSCN), cerebellum (LCBELL, RCBELL); and left middle temporal gyrus (LMTG), left insula (LINS), left hippocampus, and left middle frontal gyrus (LMFG). Labels for the ROIs were assigned with reference to the parcellation of the Montreal Neurological Institute (MNI) single subject T1 weighted dataset carried out by Tzourio-Mazoyer and colleagues (Tzourio-Mazoyer et al., 2002). In addition to these ROIs, bilateral extrastriate body area (LEBA, REBA) was also defined spherically (centralized at: +/-46 -70 1; radius=8mm) considering its involvement in this task. The time course extracted for each participant over the activated voxels within each ROI originated from the normalized and smoothed images, which were high-pass filtered and had the linear trend removed. For each participant, the intensity of fMRI signal per voxel in all the ROIs was compared to that for the fixation baseline using a *t*-test with a statistical significance level of $p < 0.05$. Thus for each ROI, for each participant, the mean percent change in signal intensity reflected the amount of difference in the BOLD contrast-related changes between the experimental task and the fixation baseline.

Functional Connectivity. Functional connectivity (synchronization of activation across brain regions) was computed separately for each participant as a correlation between the average time course of all the activated voxels in each member of a pair of regions of interest (ROIs). This analysis utilized the same ROIs used for the psc analysis.

The time course extracted for each participant over the activated voxels within the ROI originated from the normalized and smoothed images, which were high-pass filtered and had the linear trend removed. The functional connectivity correlation was computed on the images from the mental rotation condition (45° + 90° rotations together Fisher's *r* to *z* transformation was then applied to the correlation coefficients for each participant prior to averaging and statistical comparison of the two groups. These ROIs were also separately clustered into networks based on function resulting in the following networks: MNS (LIFG, RIFG, LIPL, RIPL), motor regions (LPSCN, RPSCN, LSMA, RSMA), visual regions (LMOG, RMOG), insula (LINS), extrastriate body area (LEBA, REBA), and superior temporal sulcus (LSTS, RSTS). A network analysis was then run analyzing the correlation between activation time courses between networks. In order to explore the relationship between functional connectivity patterns and scores on neuropsychological measures, bivariate correlations were run in SPSS between scores on measures of ASD symptoms (AQ, SCQ), empathy, and PIQ with FCA network connectivity values for the overall group.

3. Results

Overview

This study examined the neural basis of mental rotation of sensorimotor stimuli in ASD. The main results are: 1) The ASD group was significantly more accurate, relative to TD group, in mental rotation, although this effect was related to PIQ; 2) Both groups showed activation in core visuospatial processing regions including IPL, middle to superior occipital lobe, and calcarine sulcus, also in regions associated with motor functioning; 3) Within-group activation analyses also indicated that when 45° rotation

was contrasted with the 90° rotation, the TD group showed additional activation in regions associated with self-processing; 4) For rotation as a whole, the ASD group, relative to TD, showed greater activation in right hemisphere regions including the RIFG, right mid insula, and RSTG. No areas of increased activation were found for the TD group; 5) The TD group also showed significantly greater psc in the LSTS, and activation in this region was positively correlated with EQ scores and negatively correlated with AQ scores for the overall group; and 6) The ASD group showed intact functional connectivity between all regions involved in the task compared with the TD group.

3.1 Behavioral Results

A two-way between-groups analysis of variance (ANOVA) was conducted to explore the effect of group (ASD or TD) and condition (45° rotation and 90° rotation) on accuracy and reaction time separately. Results of this analysis revealed a statistically significant main effect of group [$F(1,696) = 31.75$, $p<0.01$, partial eta squared = 0.04], with greater accuracy for the ASD group (ASD M=0.86; TD M=0.68). The main effect of condition did not reach statistical significance [$F(1,696) = 0.42$, $p>0.05$]; nor was there any significant interaction between group and condition [$F(2,696) = 2.40$, $p>0.05$]. Regarding reaction time, a statistically significant main effect of condition was found [$F(1,696) = 9.02$, $p<0.01$]. However, the effect size was relatively small (partial eta squared = 0.01). There was not a significant main effect of group [$F(1,696) = 0.09$, $p>0.05$] or a significant group by condition interaction [$F(2,696) = 0.01$, $p>0.05$]. Both groups showed significantly slower reaction times for the 90° rotation condition than for

the 45° rotation condition [45° rotation *M*=2228.52ms; 90° rotation *M*=2398.22ms]. (See Figure 2)

Insert Figure 2 about here

Given that the ASD group also had a marginally significant higher PIQ score, relative to TD controls, a one-way between-groups analysis of covariance was conducted to determine if the group difference in accuracy remained after controlling for the effects of PIQ. Analyses were run to ensure that the assumptions of an ANCOVA (assumptions of normality, linearity, homogeneity of variances, homogeneity of regression slopes, and reliable measurement of the covariate) were not violated. Results of the ANCOVA indicated that after adjusting for PIQ, there was no significant difference between the two groups on overall spatial accuracy (45° rotation + 90° rotation) [$F(2, 696)=0.51$, $p>0.05$].

As hypothesized, and suggested in previous literature (e.g., Baron-Cohen & Wheelwright, 2004), the ASD group received significantly lower scores on the EQ (ASD M=24) than the TD group (TD M=43) [$t(27)=-5.41$, $p<0.01$]. The ASD group also scored significantly higher on both measures of autism symptomatology, the AQ [$t(26)=4.58$, $p<0.01$] and the SCQ [$t(26)=5.28$, $p<0.01$]. Regarding motor skills, the ASD group displayed significantly greater difficulty on the PANESS [$t(27)=2.44$, $p=0.02$]. Results of testing indicated that the groups did not differ significantly on other measures including those assessing both IQ and theory-of-mind.

Brain Activation Results

3.2 Within-Group Activation

Within-group analyses of mental rotation (combining 45^0 and 90^0 rotations), indicated activation in regions associated with spatial processing (precentral gyrus, postcentral gyrus, IPL) and in calcarine sulcus in both TD and ASD groups ($p<0.001$; $k = 80$). Both groups also displayed activation in the left insula, thought to be involved in orienting of attention to spatial tasks (Yassa et al., 2008). The ASD group also showed activation in areas related to motor and spatial memory (i.e., the right cerebellum and left thalamus respectively) as well as the right IFG. The TD group, however, showed activation in additional areas such as the superior occipital lobe, fusiform gyrus, and the right SMA (See Table 2 & Figure 3).

Insert Table 2 and Figure 3 about here

Since the ASD group has slightly higher PIQ scores ($p=0.06$) than our TD group, PIQ was covaried in the activation analysis to determine the effects independent of the effects of PIQ. After the effects of PIQ were controlled for, both ASD and TD groups still showed activation in areas involved in spatial rotation tasks such as right IPL, calcarine sulcus, and insula ($p<0.001$; $k = 80$). The TD group still had activation in the SMA and superior occipital regions, and the ASD group in the right cerebellum and left pre- and post-central gyrus (See Table 3).

Insert Table 3 about here

When 90° rotation was directly contrasted with 45° rotation, the TD group showed significantly greater activation in the right middle and superior occipital areas ($p<0.001$, $k=80$). The ASD group did not show any areas of significantly increased

activation for this contrast. However, when 45° rotation was contrasted with 90° rotation, the ASD group showed greater activation in the left insula as well as the left precentral and postcentral gyri ($p<0.001$, $k=80$); whereas TD group showed greater activation in right supramarginal gyrus (BA 40) and LSMA (BA 24). The TD group also showed recruitment of additional regions in the prefrontal cortex including LIFG (BA 44) and LMFG (BA 10) (See Figure 4).

Insert Figure 4 about here

3.3 Group Differences in Brain Activation

Group differences in brain activation were examined using a two-sample t-test. When mental rotation (45^0 and 90^0 rotations together) was contrasted with fixation, the ASD group demonstrated greater activation in the right insula, RIFG, and RSTG. There were no areas of significantly greater activation for the TD group. When activation during 45° rotations was contrasted with that of 90°rotations, the ASD group showed greater activation in the right fusiform gyrus, left and right middle occipital lobe, and right precuneus. The TD group, on the other hand, showed greater activation in LIFG, left MPFC, LSMA, and left anterior cingulate cortex ($p<0.005$, $k=144$) (See Figure 5).

Insert Figure 5 about here

3.4 Percent Signal Change (psc)

PSC values were extracted for individual participants from activated voxels in several anatomical ROIs. The only statistically significant group difference found in this analysis was reduced levels of activation in LSTS in the mental rotation task for the ASD

group (ASD M=0.16), relative to TD controls (TD M=0.41) [$t(21)=2.29$, $p<0.05$]. To examine the relationship between activation in identified ROIs and measures of behavioral characteristics, bivariate correlations were run between psc values and scores on neuropsychological measures related to ASD symptoms, IQ, motor, and visual-motor skills. A significant positive correlation was found between psc in the LSTS and EQ scores ($r= 0.55$, $p<0.01$) for the overall group, indicating that higher levels of empathy were correlated with greater reliance on the LSTS for this task. Activation in the LSTS was also negatively correlated with AQ scores ($r=-0.53$, $p=0.01$). Greater motor difficulty (as indicated by higher PANESS scores) was positively correlated with psc in the RIPL ($r=0.44$, $p<0.05$) (See Figure 6).

Insert Figure 6 about here

3.5 Functional Connectivity Analysis

An analysis of functional connectivity between the time-course of activated regions of interest (ROIs) revealed significantly greater connectivity between the LIFG and both the left insula [$t(18)=2.74$,$p<0.01$] and left middle occipital lobe [$t(17)=2.13$, $p<0.05$] for the ASD group. However, it should be noted that these areas of increased connectivity for the ASD group did not survive correction for multiple comparisons. No areas of increased connectivity were found for the TD group, when compared with the ASD group. A subsequent connectivity network analysis, by grouping the ROIs into networks based on their primary role in different tasks, did not reveal any statistically different differences in connectivity between the two groups.

To examine the relationship between network functional connectivity and measures of behavioral characteristics, bivariate correlations were run between connectivity values obtained from the network analysis and scores on neuropsychological measures. A significant negative correlation was found between scores on the VMI and functional connectivity between the MNS and insula ($r=-0.57$, $p<0.01$). Functional connectivity between the MNS and motor networks showed a significant positive correlation with PANESS scores ($r=0.55$,$p<0.01$), indicating that individuals with greater motor difficulties showed significantly stronger connectivity between these regions (See Figure 7).

Insert Figure 7 about here

4. Discussion

While participants with autism performed the mental rotation task involving hand stimuli as well as TD controls, it was accompanied by differences at the neural level, in terms of altered activation and increased functional connectivity. The intact ASD performance on this task is consistent with previous findings of intact or superior visuospatial ability in ASD (Falter et al., 2008; Hamilton et al., 2009; Caron et al., 2004; Mottron et al., 2003; 2006; Mitchell & Ropar, 2004; Soulieres et al., 2011). However, it should also be noted that this advantage may be limited to hand rotation tasks, and may not extend to tasks involving full body transformations (David et al., 2010; Pearson et al., 2013; Pearson et al., 2014). The difficulties with full body transformations may likely underlie the visual-perspective taking deficits found in autism (Hamilton et al., 2009). It should be noted that improved performance in ASD participants is a product of their

performance IQ, rather than an effect of group. However, this advantage is still meaningful as visuospatial ability is a major component of tasks measuring PIQ. Therefore, the borderline significantly higher PIQ scores in the ASD group may be indicative of this group's advantage in spatial tasks including mental rotation. This is consistent with previous research indicating that there is a subset of ASD individuals who have a particular strength in block design (one of the subtests used to assess PIQ) and that individuals displaying this peak show increased speed and accuracy for a variety of visuospatial tasks when compared with peers matched on FSIQ (Caron et al., 2006).

4.1 Activation Specific to Mental Rotation of Biological Stimuli

In addition to visuospatial activation (superior parietal and occipital areas), both TD and ASD groups also showed activation in regions previously associated with mental rotation of body parts including activation in the motor network (precentral gyrus/primary motor cortex) and body-related processing regions (insula, inferior parietal lobule). While both groups displayed activation in core regions associated with mental rotation of body stimuli, within-group analyses indicated unique activation patterns for each group, with the TD group also showing activation in SMA and fusiform areas. This may be associated with self-referential sensorimotor processing (Kircher et al., 2000; Penfield & Jasper, 1954; Lim et al., 1994) and the processing of biological forms (Peelen & Downing, 2005). The ASD group showed activation in the thalamus and cerebellum, areas associated with processing/relaying sensory information and motor coordination. When the effect of PIQ was controlled, this activation pattern was mostly maintained, with individuals with ASD continuing to show activation in areas associated with motor

control including precentral and cerebellar regions. The TD group continued to display activation in areas associated with self-referential sensorimotor and processing of biological forms. Unlike traditional mental rotation tasks, the current study found activation in motor and biological motion processing areas in addition to visuospatial processing. This underscores the extra effort required (through the recruitment of additional neural resources) in order to accomplish mental rotation of hand stimuli.

4.2 Group Differences in Neural Approach to Task

While both groups activated networks associated with visuospatial, motor, and body-related processing, between group activation and psc differences were primarily centered around regions associated with processing of body-related or socially based stimuli. This finding of a difference in neural approach between groups is consistent with previous research indicating a tendency for individuals with ASD to adopt an allocentric rather than an egocentric approach to mental rotation (Kessler & Wang, 2012; Pearson et al., 2014) and within-group activation patterns indicating that the TD group activated regions related to self-processing and sense of agency (SMA, left MPFC) that have been found to be specifically associated with adapting an egocentric processing (Kosslyn et al., 1998). Between-group contrasts revealed greater activation in several right hemisphere regions for the ASD group including the right insula, RIFG, and RSTG. Activation of right hemisphere regions has been previously associated with adoption of an "other" or third-party based approach to spatial rotations (Ratcliff, 1979; Cohen et al., 1996; Ditunno & Mann, 1990; Harris et al., 2000). Also consistent with the hypothesis that the ASD group is using a third-party based approach to the task, the RSTG has been

previously implicated in the perception of biological motion (Pelphrey et al., 2005). Thus, this activation also suggests that ASD participants may be imagining viewing the rotation of the hands, rather than imagining their own hands. Insula activation also indicates that ASD individuals might be experiencing greater levels of difficulty switching between self and other processing (Menon & Uddin, 2010) while RIFG activation suggests the use of a more analytic or serial strategy for the task (Weiss et al., 2003; Thompson et al., 2000). PSC analyses also revealed significantly increased activation of the LSTS for the TD group during spatial conditions. The LSTS has previously been found to be activated during visual perspective taking, when a person takes a first person perspective to perform the task (David et al., 2006).

Participants with ASD also demonstrated increased activation in the insula. The insula is associated with body-related processing (Karnath et al., 2005; Farrer & Frith, 2002; Tsakiris et al., 2007) and has also been considered as a critical node of the brain's salience network (SN), which may be dysfunctional in autism (Uddin & Menon, 2009). However, increased activation in the insula may also suggest that the social nature of the images presented in our study may pose additional difficulty to participants with ASD. Given the SN's role in switching between the default mode and active processing (Menon & Uddin, 2010; Goulden et al., 2014), this increase in activation might also indicate that the switch between processes may not be as smooth or automatic in ASD individuals.

4.3 Functional Connectivity Differences

Based on previous findings of enhanced or unimpaired visuospatial skills in individuals with ASD, it was hypothesized that functional connectivity within the regions

associated with these skills would also remain intact. Consistent with prior research (e.g., Villalobos et al., 2005), results indicated that functional connectivity between relatively posterior neural regions remained unimpaired. The TD group did not show any areas of increased connectivity over the ASD group, even between frontal to posterior neural regions. Therefore, results of the current study indicate that functional connectivity across regions involved in visuospatial rotation of body-related stimuli remains intact in ASD.

4.4 Brain-Behavior Relations

Results of correlation analyses indicate that activation in the LSTS was positively correlated with EQ scores for all participants (ASD +TD). The correlation provides further evidence of the potential role of this region in both visuospatial processing and empathy (Decety & Lamm, 2007; Iacoboni & Dapretto, 2006). Prior research also indicates that this region is specifically activated during utilization of an egocentric visuospatial rotation strategy (Zacks et al., 2003). Trait empathy has also been found to be positively correlated with an egocentric approach to visuospatial transformations (Thakkar & Park, 2010; Gronholm et al., 2012). Thus, it has been suggested that the strategy used for body or socially related visuospatial transformations may moderate the relationship between empathy and visuospatial transformations, with individuals having the option of completing many of these tasks through an empathetic or non-empathetic route (Gronholm et al., 2012).

Activation in the LSTS was also negatively correlated with AQ scores. Based on the previously described role of this region in empathy, one potential reason for this finding is the negative correlation between AQ scores and levels of empathy

(Wheelwright et al., 2006). The LSTS has also been implicated in connecting the actions of others to one's own actions (Molenberghs et al., 2010) and in theory-of-mind (Schulte-Rüther et al., 2007; Otsuka et al., 2009). Thus, it follows that aberrant activation patterns involving this region may be related to deficits in the integration of visuospatial and social processing (Decety & Lamm, 2007) and, therefore, may also assist in explaining the dichotomy of visuospatial advantage and social impairment in ASD. These findings suggest on a larger scale a connection between the activation pattern or neural approach utilized and behavioral characteristics related to ASD including difficulties with social interaction. Given that this is a correlation, however, it is not possible to determine causality. It remains unclear as to if this pattern of neural activation causes or results from reduced amounts of empathy or other social skill deficits.

The cognitive approach utilized was also found to be related to PANESS score, used to measure motor ability in participants. More specifically, it was found that individuals whose scores on the PANESS indicated greater motor difficulties showed greater activation in the RIPL (a component of the human mirror neuron system) during the visual-spatial rotation task. While additional information is necessary to determine the exact cause of this correlation, it could indicate that increased activation of this region is indicative of a greater reliance on visuospatial aspects to perform the task. It may also indicate increased difficulty imagining self-based or other-based movement.

4.5 Limitations/Future Directions

One limitation of this study was that the experimental design relied on two dimensional hand images that exhibited a limited range of rotation angles. These angles

were selected in order to minimize the level of difficulty and, therefore, the need for reliance on executive functioning systems. However, the limited number of angles utilized, along with the unrealistic nature of the two dimensional images, reduces the generalizability of results to real-world social situations in which individuals encounter situations necessitating more complex mental rotations along with more interference from additional social stimuli. The exclusion of the hand images from other body-related aspects may minimize the degree to which these images are processed as a social stimulus. Further examination of this aspect would be of particular importance given that previous research suggests a visual-perspective taking deficit in ASD (Hamilton et al., 2009).

While previous research suggests that these neural patterns may indicate the use of a more third-person or allocentric approach by individuals with ASD (Kosslyn et al., 1998; Pearson et al., 2014), this cannot be confirmed. Future studies involving longitudinal designs could clarify questions regarding whether this allocentric neural approach may be causing social difficulties or if this relationship is switched with a history of social difficulties resulting in activation of this neural system in response to sensorimotor rotation stimuli. It would also provide additional information regarding how these findings may change over time. This may be of importance given the recent findings that neural networks utilized in children with ASD may differ significantly from those observed in adults with autism, resulting in some of the heterogeneity of results present in the autism literature (Dickstein et al., 2013).

5. Conclusions

Even when presented with body-related stimuli, the ASD group was able to perform the mental rotation task indicating this skill remains intact in high-functioning children and adolescents with autism. Additionally, no areas of significantly decreased functional connectivity were present in ASD participants. However, it should be noted that mental rotation of body stimuli such as hands recruit additional motor and body-related processing networks and that individuals with ASD show unique patterns of activation (compared with TD participants) in regions associated with body-related processing. The differences in neural activation patterns observed imply that the TD and ASD groups utilized different cognitive approaches to the task. This variation in approach may underlie difficulties observed in individuals with ASD when faced with more complex social stimuli requiring visual-perspective taking or full-body rotations. Results also suggest that differences in neural activation patterns are related to both the ability to empathize and levels of autism symptomology. While this indicates a relationship between these characteristics, it remains unclear if this difference in cognitive approach to the task underlies, or results from, the social difficulties observed in autism. More research is necessary to clarify the possible relationship between the alteration in neural approach observed and the with social deficits characteristic of ASD.

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Table 1. Table displaying scores on neuropsychological measures by group.

SPATIAL PROCESSING

	ASD			TD			
Measure	**n = 13**	**Range**	**S.D**	**n = 15**	**Range**	**S.D**	**p-value**
Age	13	10-17	2.66	11	8-15	1.86	0.11
EQ	24	9-42	7.61	43	23-54	9.64	<0.01
SQ	27	16-50	8.52	28	14-40	7.9	0.75
FSIQ	112	85-126	14.97	103	83-130	14.68	0.16
VIQ	109	75-128	16.74	105	84-134	19.41	0.62
PIQ	111	93-124	11.35	102	84-133	12.34	0.06
SCQ	16	1-31	8.74	2	0-10	3.52	<0.01
VMI	93	66-117	13.23	99	86-110	6.14	0.15
PANESS	55	39-75	10.8	46	36-71	9.19	0.02
AQ	80	32-135	28.7	31	5-67	21.62	<0.01

Table 2. Table displaying areas of activation for each group when all spatial conditions are contrasted with fixation.

ASD (Spatial Vs. Fixation)

Region	X	Y	Z	Hem	BA	Cluster	t
Precentral/Postcentral	-44	-18	56	L	4	2432	12.22
Cerebellum/Vermis	18	-62	-26	R	NA	954	8.95
Insula	-42	-8	6	L	NA	193	7.63
Thalamus	-16	-22	6	L	NA	204	6.49
Inferior Parietal	54	-30	56	R	2	329	6.25
Calcarine	10	-96	4	R	NA	1048	6.23
Inferior Frontal	48	0	16	R	NA	134	5.25

TD (Spatial Vs. Fixation)

Region	X	Y	Z	Hem	BA	Cluster	t
Supplementary Motor	2	18	50	R	8	452	6.9
Precentral/Postcentral	-40	-14	64	L	6	572	6.23
Insula	-28	24	-2	L	NA	103	5.93
Calcarine	0	-82	6	L	NA	343	5.71
Fusiform	24	-82	-16	R	NA	230	5.68
Superior Occipital	16	-96	16	R	18	338	5.67
Insula	42	22	-4	R	NA	141	5.44
Inferior Parietal	46	-46	54	R	40	181	4.86

Table 3. Table displaying areas of activation remaining after the effects of PIQ are controlled.

PIQ Covary (ASD) SPATIAL-FIXATION

Region	X	Y	Z	Hem	BA	Cluster	t
Precentral/Postcentral	-44	-16	56	L	4	2852	14.35
Cerebellum/Vermis	22	-60	-26	R	NA	457	8.22
Insula	46	-2	0	R	NA	177	7.26
Calcarine	8	-98	2	R	NA	103	5.84
Calcarine/Middle Occ.	-8	-94	-2	L	NA	124	5.74
Inferior Parietal	56	-28	56	R	2	164	5.6

PIQ Covary (TD) SPATIAL-FIXATION

Region	X	Y	Z	Hem	BA	Cluster	t
Supplementary Motor	2	18	50	R	8	366	6.63
Superior Frontal	-30	-10	70	L	6	568	5.15
Fusiform	24	-82	-16	R	NA	178	5.57
Calcarine	0	-82	6	L	NA	210	5.57
Superior Occipital	16	-96	16	R	18	262	5.48
Insula	42	22	-4	R	NA	104	5.23
Inferior Parietal	46	-46	54	R	40	125	4.74

Figure Captions

Figure 1. Figure displaying sample mental rotation stimuli. The participants were asked to identify if the rotated hand on the left was the same hand (left or right hand) as the upright hand presented on the left side of the screen. Hand rotations were randomized between 45 and 90 degree rotations. This figure displays: (A) A 45 degree RH rotation that is congruent; (B) A 45 degree LH rotation that is not congruent; (C) A 90 degree LH rotation that is congruent; and (D) A 90 degree RH rotation that is not congruent.

Figure 2. Figure displaying the behavioral results (accuracy and reaction time) for each condition and each group. This graph shows the significantly greater accuracy of the ASD group for all spatial conditions but no significant differences in reaction time for any of the spatial conditions.

Figure 3. The activation patterns for each condition (contrasted with a fixation baseline) are shown for each group. For this contrast, analyses were run on $p<0.001$ uncorrected with a voxel threshold of 80 (as determined by Monte Carlo simulations).

Figure 4. Areas of increased activation for the 45° rotation condition when contrasted with the 90° rotation condition for both groups. They exhibited greater activation in the left middle temporal gyrus, left angular gyrus, and left caudate. For this analysis, results were examined at a $p<0.001$ with a voxel threshold of 80.

Figure 5. Group differences in activation for the 45° rotation condition when contrasted with the 90° rotation condition. The ASD group shows greater levels of activation in posterior brain regions, while the TD group shows a greater reliance on frontal regions.

Figure 6. Scatterplots displaying significant correlations between PSC values and scores on neuropsychological measures. These plots display the positive correlation between EQ scores and PSC in the LSTS, the negative correlation between EQ scores and PSC in the LSTS, and the positive correlation between PANESS scores and PSC in the RIPL.

Figure 7. Scatterplots displaying significant correlations between network-based functional connectivity and scores on neuropsychological measures. These plots display the negative correlation between Insula:MNS connectivity and VMI scores as well as the positive correlation between MNS:Motor system connectivity and PANESS scores.

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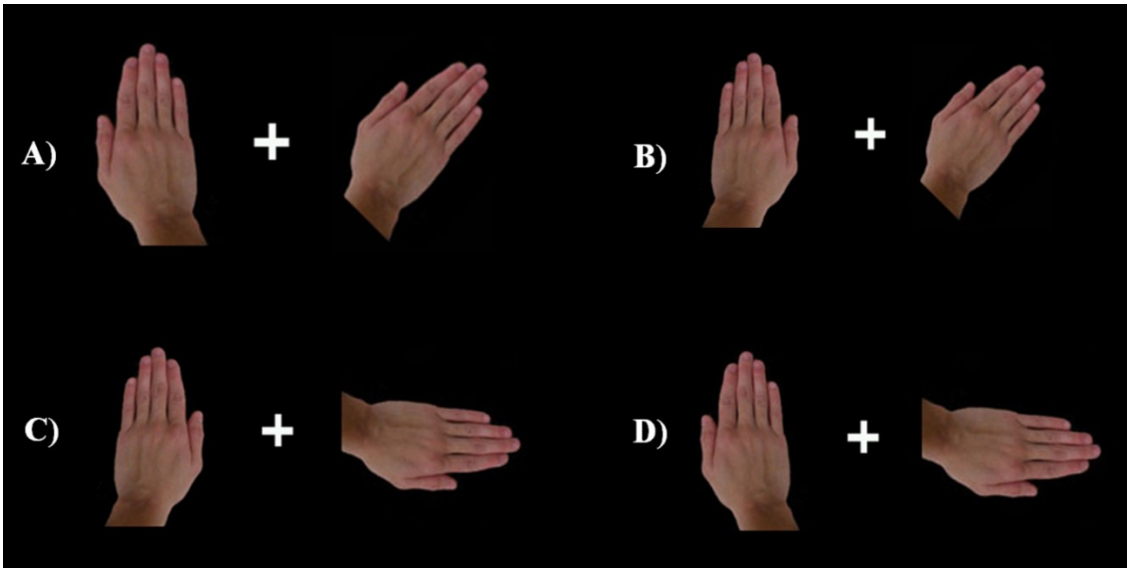

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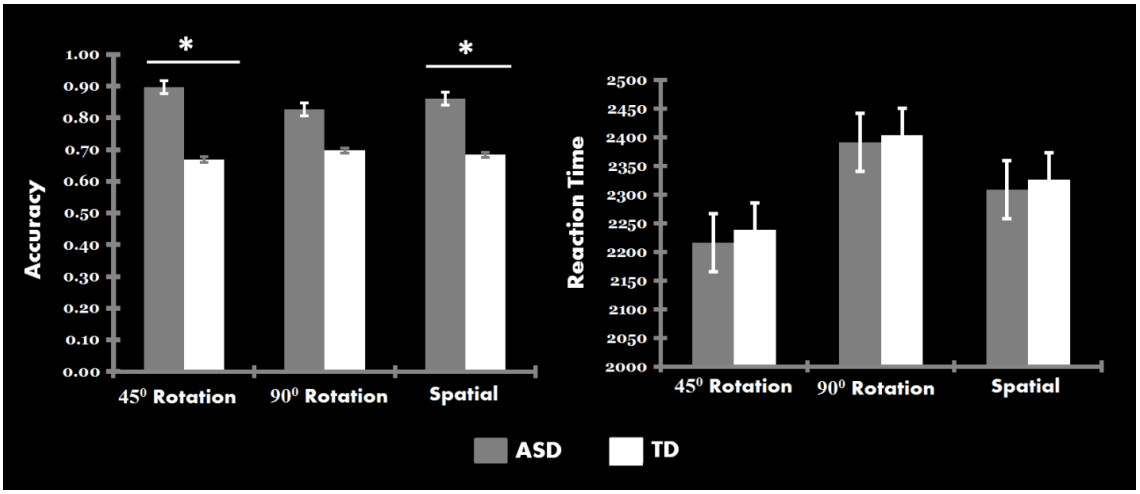

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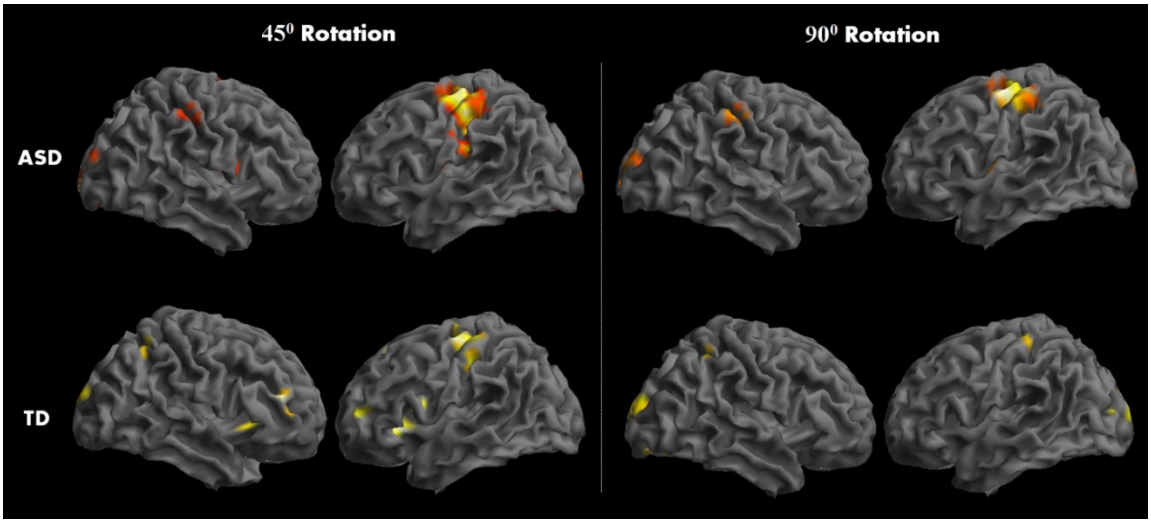

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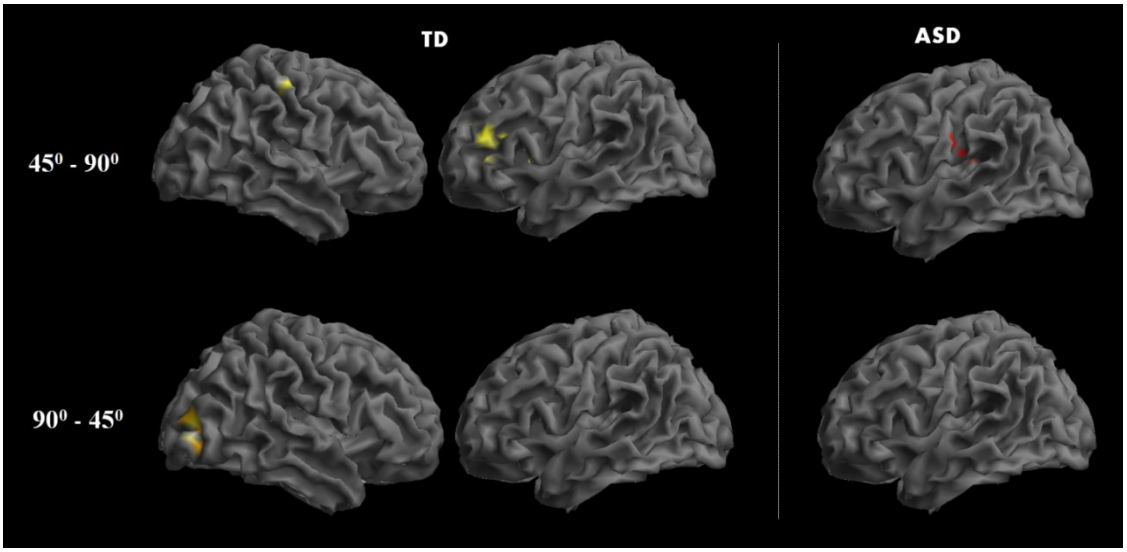

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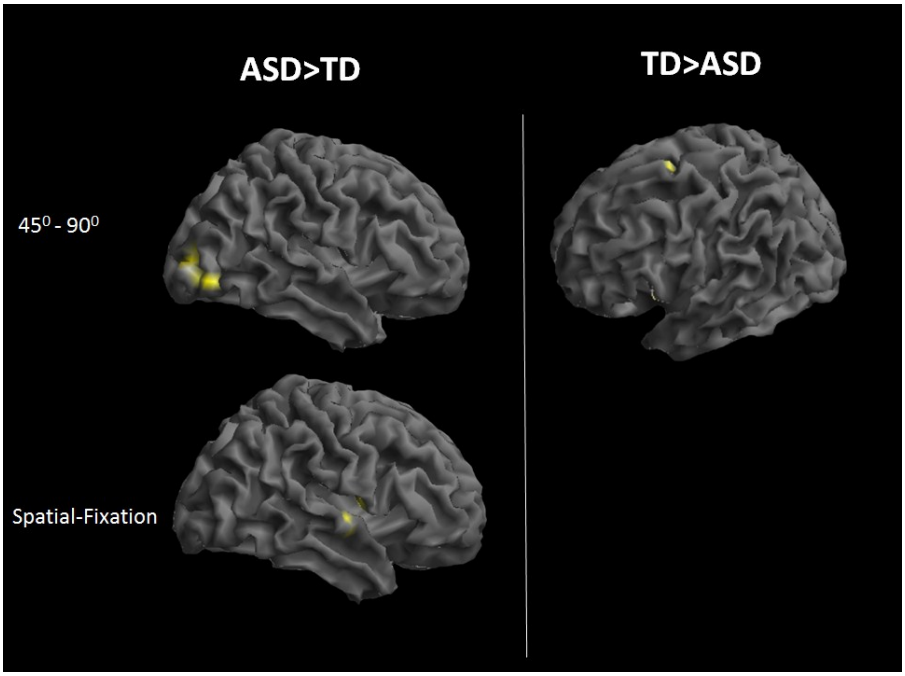

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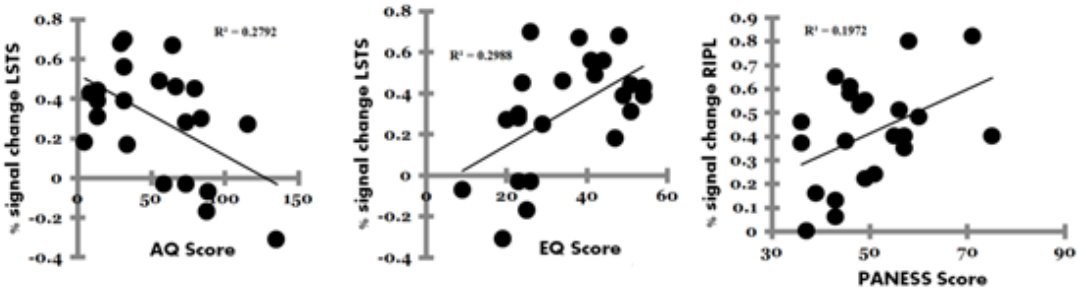

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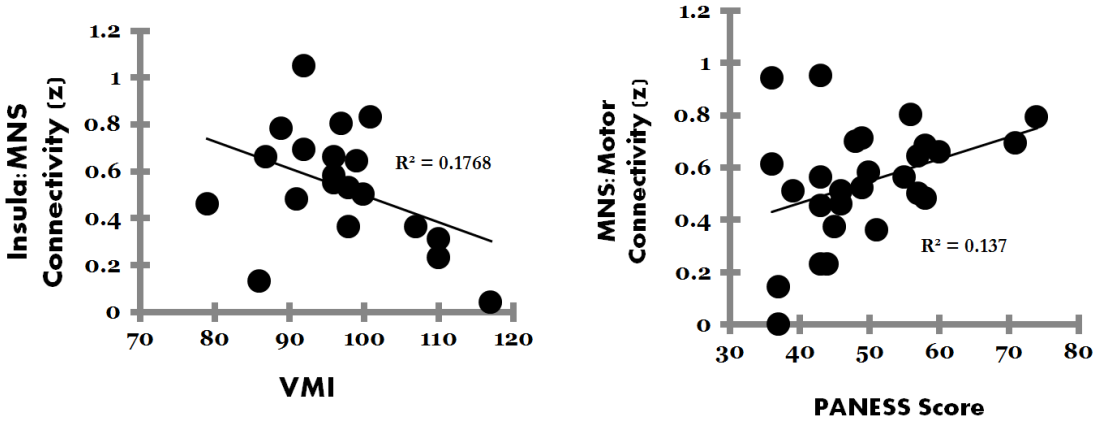

THE ROLE OF MIRROR NEURON SYSTEM IN ACTION SIMULATION IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

by

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Abstract

Although a deficit in the ability to imitate is widely reported in children with autism spectrum disorders (ASD), the exact nature of it is still a topic of debate. It is possible that impairments in motor production as well as action simulation in ASD may impact their ability to successfully imitate actions. This fMRI study examined the role of mirror neuron system (MNS) in mediating action simulation (mental imitation of an action) in children and adolescents with ASD. Seventeen high-functioning children and adolescents with autism and 17 age and IQ-matched typically developing (TD) control participants took part in the study. Participants were shown cartoon pictures of people performing everyday actions (e.g., ironing clothes) but with the hand portion missing. They were asked to identify which hand (of three answer choices) would best fit the gap. There were no significant group differences on performance accuracy. While both ASD and TD groups showed robust activity in most parts of the MNS during action simulation, participants with ASD showed reduced activity, relative to TD, in right angular gyrus of the IPL during simulation of transitive actions and increased activity in right middle temporal cortex during intransitive actions. The ASD group also showed increased activity (measured by percent signal change) in cerebellum, precentral and postcentral gyrus during action simulation. Overall, the present study did not find any abnormalities, behaviorally and neurally, in participants with ASD in engaging successfully in action simulation.

Keywords: autism, fMRI, mirror neuron, motor, functional connectivity

Word Count: 6,909

1. Introduction

Imitation, a fundamental aspect of human social behavior, plays a crucial role in development. Imitation acts as a precursor to symbolic functioning necessary for the development of both play and language skills (Piaget, 1962), and is important for the development of social, communication, and motor skills (Tomasello et al., 1993). By providing the child with information about the actions and intentions of the physical and the social world, imitation assists in social learning (Rogers et al., 2003) and forms the foundation for future social development. While research continues to emphasize the importance of imitation in a range of developmental areas, children with developmental disorders, such as autism spectrum disorders (ASD) exhibit significant difficulties in imitating actions appropriately and successfully (Williams et al., 2001). The finding of an imitation deficit in ASD has been frequently replicated (e.g., Williams et al., 2001; Rogers et al., 2003; Williams et al., 2004; Rogers & Williams, 2006; Rogers et al., 2008; Stewart et al., 2013).

However, the exact nature of this deficit is not clear. Several recent studies have found intact imitation skills in autism (Bird et al. 2007; Dinstein et al. 2010; Gowen et al., 2008; Hamilton et al., 2007; Leighton et al. 2008; Press et al., 2010; Spengler et al., 2010). In addition, not all types of imitation are equally impaired in ASD as individuals with autism often exhibit the greatest difficulty while imitating meaningless gestures than while imitating meaningful gestures or gestures that involve objects (Williams et al., 2004; Stone et al., 1997) or when imitating the style of an action (Hobson & Hobson, 2008). Imitation in ASD also appears to follow a clear developmental trajectory. Studies indicate that, although delayed, children with ASD show improvement in imitation skills over time (Heimann & Ullstadius, 1999; Stone et al., 1997), with the

ability to imitate simple, single-step actions increases with age in children with autism (Hepburn & Stone, 2006). These findings point to the need for understanding the subtle processes and components of imitation.

The discovery of mirror neurons in nonhuman primates has provided a neural base for imitation. These neurons were found to not only fire when a monkey performs an action but also when a monkey watches an action being performed (Gallese et al., 1996; Rizzolatti et al., 1996). Although still debated, research indicates the presence of mirror neurons in humans (Iacoboni & Dapretto, 2006) with the core regions of the human mirror neuron system (MNS) located in the caudal inferior frontal gyrus (IFG)/ ventral pre-motor cortex (PMv) and the rostral inferior parietal lobe (IPL). These core regions interact closely with the superior temporal sulcus (STS) to produce action understanding and mental action mirroring (Iacoboni, 2005; Iacoboni et al., 2001; Iacoboni & Dapretto, 2006). While the IFG/PMv has been found to be involved in action planning (Hamilton, 2008) and perception-action coupling (Newman-Norlund et al., 2010), the IPL is thought to mediate spatiotemporal, perceptual and goal coding aspects necessary for imitation (Rizzolatti & Matelli, 2003; Hamilton, 2008). Successful imitation likely relies not only on these regions but also on their communication with other neural networks (see Kana et al., 2011 for review) including interactions with limbic regions (Carr et al., 2003; Wicker et al., 2003) and theory-of-mind (ToM) networks (Schulte-Ruther et al., 2007).

It has been hypothesized that individuals with autism who have deficits in imitation (Williams et al., 2004) may also have a malfunctioning MNS (Oberman & Ramachandran, 2007; Williams et al., 2001). However, not all research points towards a dysfunction in the MNS in children with ASD. There have also been studies finding

intact activation (e.g., Oberman et al,, 2008; Raymaekers et al., 2009; Avikainen et al., 1999) as well as studies suggesting increased activation in the MNS for subjects with autism (Martineau et al., 2010; Baron-Cohen et al., 2006). While previous research has suggested reduced functional connectivity in the MNS in autism, recent studies have called this finding into question, with studies reporting both hyper-connectivity and hypo-connectivity in autism (Fishman et al., 2014).

Recent research has also indicated abnormal connectivity patterns between regions of the MNS and complimentary neural regions, particularly increased connectivity with components of the limbic system (Murphy et al., 2012; Shih et al., 2010). These findings lead to the hypothesis that the MNS may be under increased subcortical control in ASD (Williams et al., 2006). Current evidence also supports atypical functional connectivity (or cross-communication) between the MNS and mentalizing system in ASD (Fishman et al., 2014), indicating reduced separation between two neural networks key for appropriate social understanding. Together with inconsistent findings of behavioral studies examining imitation in autism, current literature regarding the neural substrate of imitation suggests that imitation is likely comprised of multiple skills. Isolating the potential effects of specific component skills is important for developing a more comprehensive understanding of the imitation deficits observed in autism.

One area of particular importance to previous research examining imitation in autism is the possible effect of motor difficulties. Research has suggested that motor abnormalities can be seen in individuals with ASD starting in infancy (Brian et al., 2008; Provost et al., 2007; see Ozonoff et al., 2008 for differing opinion). Rogers (1999)

proposed that mimicry deficits in ASD may result from cerebellar abnormalities leading to motor difficulties. A review of the current literature regarding motor skills in autism suggested that there were two main motor deficits present in ASD, difficulties integrating information necessary for motor planning and an increase in the variability between sensory inputs and motor outputs (Gowen & Hamilton, 2012). Research has also suggested difficulties related to action chaining (Cattaneo et al., 2007) and in control of reaching movements (Glazebrook et al., 2009). Given the joint presentation of motor and imitation difficulties in autism, it has been proposed that the imitation deficits observed may result from dyspraxia (DeMyer et al., 1981). Several researchers have suggested that dyspraxia may be responsible for imitation difficulties in autism as well as underlie a range of other symptoms related to the disorder (Bennetto, 1999; Rogers et al., 1996; Minshew et al., 1997).

However, recent research has also questioned this hypothesis. For example, Rogers and colleagues (2003) found no evidence of autism-specific motor difficulties on fine motor, gross motor, and a non-imitative praxis task for a group of toddlers with ASD as compared to children without ASD matched for developmental level. The toddlers with ASD did, however, still show difficulty on an imitation task providing evidence against a global dyspraxia hypothesis. Recent research has also evaluated the effect of basic motor skill functioning on the dyspraxia observed in autism. A study performed by Mostofsky and colleagues (2006) found that dyspraxia in ASD cannot be attributed solely to deficits in basic motor functioning. Smith and Bryson (1998) attempted to examine the contribution of effects of motor skill deficits on imitation by covarying scores of participants on a standard task of manual dexterity. They found that, while motor deficits

appeared important for imitation (accounting for 37% of the variance observed), the deficit in imitation remained for the ASD group even after controlling for motor deficits. Given the ongoing debate regarding the exact nature of motor difficulties in autism and their potential effect on autism, this remains an essential aspect for developing a clearer understanding of the behavioral and neural nature of imitation deficits in ASD.

The current study attempted to fill gaps in the previous literature in several ways. Most studies have examined imitation as a single skill which makes determining the impact of motor deficits difficult. To remove the motor production component, the current study examined a "mental imitation" paradigm. Mental imitation has been defined as being comprised of visual perspective taking and motor imagery (Jeannerod, 1994; Goldman, 2005). The importance of examining mental imitation is emphasized in the simulation theory, which proposes that we gain insight into the mental workings (plans, beliefs, desires) of others by covertly or mentally simulating the actions ourselves but without actually performing the action. This suggests that mental imitation may be the most important aspect of imitation influencing deficits in social understanding in ASD, such as deficits with theory of mind.

Since motor production is not a component of the experimental design, this allows for both behavioral and neuroimaging data to be collected during a task requiring more complex imitative strategies. This allows the experiment to examine abilities related to dyspraxia, a deficit that would have clear implications on imitation ability, without the confounding variable of producing a motor act. Neuroimaging data can thus be collected on activation within the MNS without impact from motor production. By examining a complex imitative task, it can also evaluate not only the connectivity within the MNS but

also between the MNS in other regions, such as those involved in mentalizing. This study aimed to determine the activation patterns and functional connectivity observed in individuals with ASD when presented with a mental imitation task involving both transitive actions (actions involving an object) and intransitive actions (actions not involving an object).

It was hypothesized that children with ASD would make more errors relative to TD children on this task. This is based on previous findings of impaired action planning, a skill mediated by the frontal component of the MNS – the ventral premotor cortex (Davare et al., 2006), in children with ASD (Fabbri-Destro et al., 2009). At the neural level, it was predicted that participants with autism would show aberrant or decreased levels of activation in the frontal component of the MNS but similar levels of activation in the parietal region of the MNS (e.g., IPL). It was also hypothesized that participants with ASD would show aberrant functional connectivity patterns within the core components of the MNS as well as between the MNS and other neural systems.

2. Materials & Method

2.1 Participants

Seventeen high-functioning children and adolescents with autism and 17 age and IQ-matched typically developing participants took part in the current fMRI study (age range: 8 to 17 years; minimum Full Scale and Non-Verbal IQ: 80, measured using the Wechsler Abbreviated Scales of Intelligence). Participants with autism were recruited through the research subject database of the Cognition, Brain, and Autism Laboratory at the University of Alabama at Birmingham (UAB), the Alabama Autism Society, and flyers posted at local ASD-related treatment and evaluation centers. All participants had

previous diagnoses of an ASD. Current and past ASD symptoms were assessed using the Social Communication Questionnaire (SCQ) – Lifetime Version (Berument et al., 1999), the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001), and the Repetitive Behavior Scale – Revised (Lam & Aman, 2007). The average SCQ scores for the participants with ASD was 18 (*SD* = 7.25) while the average SCQ scores for typically developing participants was 3 (*SD*= 3.56 $p<0.01$). The average AQ for ASD participants was 78 (*SD*= 30.31) while the average AQ for the typically developing group was 31 (*SD*=21.62; $p<0.01$). Typically developing participants were recruited using flyers and advertisements on the UAB campus, flyers posted in local community centers (e.g., libraries, YMCAs), and through the Cognition, Brain, and Autism Laboratory's research subject database. Participants were not included in the study if they indicated having worked with metal or having metal implanted in their bodies (either surgically or accidentally) or if they had a history of psychiatric disorders. No participants indicated having a cognitive disorder, anxiety disorder, schizophrenia, or obsessive compulsive disorder. Out of the thirty-four participants scanned, we obtained 15 usable fMRI datasets for the TD group and 14 usable data sets for the ASD group after taking into account subject attrition due to head motion in the scanner and/or poor data quality (see table 1 for detailed participant information).

2.2 Overall Experimental Procedure

Participants took part in 2 separate sessions: 1) Assessment; and 2) MRI scanning. The first session lasted approximately two hours, during which time the participants completed a series of neuropsychological tests to ensure that they qualified for the study and to gather additional information, such as demographics, cognitive level, visual-motor

skills, ASD-related symptomology, ability to maintain and shift attention, medication history, and diagnosis. During the second session, which also lasted approximately 2 hours, participants were prepared for the MRI scan, performed a computer practice task outside the scanner to ensure that they understood the directions for the task and then performed the tasks in the MRI scanner. They then performed a hand-based imitation task outside of the scanner in order to determine if the groups differed on their ability to imitate simple hand motions and were debriefed regarding their overall experience with the study.

Prior to participants' arrival for the first session, participants' families received an email including the informed consent forms, a social story about his/her MRI day, and a schedule for the first and second sessions. Participants were reimbursed $25 for the first session and, if they qualified for the second session, received a total of $50 upon completion of the MRI scan.

2.3 Stimuli and Experimental Paradigm

The fMRI component of the project consisted of a mental imitation experiment designed in an event-related format. This experiment focused on the aspects of imitation that can be separated from motor ability and was expected to target both the anterior component of the system (the IFG) as well as the posterior component of the system (the IPL). In order to control for potential practice effects, the order of presentation of stimuli within the experiment was randomized across participants.

This experiment was aimed at measuring mental imitation ability, requiring subjects to perform all the necessary components of imitation except for the motor execution aspect. In other words, this task involved imagining the imitative act, which is usually a

precursor to the motor action. This part also comprised planning the imitative act, a step which may prove critical in determining the ultimate outcome. The stimuli for this experiment were based on a paradigm developed by Heilman and colleagues (Mozaz et al., 2002). During this experiment, participants were shown cartoon pictures of people performing everyday actions (e.g., ironing clothes) but with the hand missing. Ten of these stimuli showed transitive acts (which require an object and are generally less social in nature) and ten stimuli showed intransitive acts (which do not require an object and are generally social in nature). For each item, there were 3 options of hand grasps presented as high-quality images beneath the cartoon picture. The participant was asked to identify which hand (a, b, or c) would best fill in the gap for a series of 2 practice and 20 test stimuli (see Figure 1). Each picture was presented for a period of 6000 ms followed by an inter-stimulus interval of 4000 ms. This experiment not only targeted visuospatial ability (by requiring an individual to mentally rotate hands to fill in the gap correctly), but also action planning (by requiring the individual to plan and simulate the action in their mind).

Insert Figure 1 about here

2.4 Image Acquisition

All fMRI scans were acquired using the Siemens 3.0 Tesla Allegra head-only scanner (*Siemens* Medical Inc., Erlangen, Germany) located at the UAB Civitan International Research Center (CIRC). For structural imaging, initial high resolution T1-weighted scans were acquired using a 160-slice 3D MPRAGE (Magnetization Prepared Rapid Gradient Echo) volume scan with TR = 200 ms, TE = 3.34 ms, flip angle = 12^0, FOV = 25.6 cm, 256 X 256 matrix size, and 1 mm slice thickness. A single-shot gradient-

recalled echo-planar pulse sequence was used to acquire functional images (TR= 1000 ms, TE = 30ms, flip angle = 60 degrees, FOV = 24 cm, matrix =64 x 64). Seventeen adjacent oblique axial slices were acquired in an interleaved sequence with 5 mm slice thickness, 1 mm slice gap, a 24 X 24 cm field of view (FOV), and a 64 X 64 matrix, resulting in an in-plane resolution of 3.75 X 3.75 X 5 mm. The stimuli were rear-projected onto a translucent plastic screen and participants viewed the screen through a mirror attached to the head coil. Quality control checks were applied to the acquired data to examine the signal to noise ratio, temporal signal to noise ratio, ghosting, and motion artifacts. Data that did not meet quality standards were not included in further analyses.

2.5 Behavioral and Neuropsychological Data Analyses

Scores from neuropsychological measures for each participant were entered into SPSS 16.0 statistical software (*SPSS* Inc., Chicago, IL). Between-group t-tests were run in order to compare group means on each measure. Data on each participant's reaction time and accuracy was also entered into SPSS. Analyses were run examining group means on accuracy and reaction time for each condition (transitive actions and intransitive actions) separately as well as together (all rotation conditions combined).

2.6 fMRI Data Analyses

Imaging analysis included several components, such as activation patterns, change in percent signal intensity, and functional connectivity. In addition, bivariate correlation analyses were used to examine the correlation between percent signal change values and functional connectivity in specified ROIs and scores on neuropsychological measures related to ASD symptoms, IQ, motor, and visual-motor skills.

Brain Activation. To examine activation, the data was pre-processed and statistical analyses were carried out using Statistical Parametrical Mapping, version 8 software (SPM8; Wellcome Department of Cognitive Neurology, London, UK). Images were corrected for slice acquisition timing, motion-corrected, normalized to the Montreal Neurological Institute (MNI) template, re-sampled to 8-mm^3 voxels, and smoothed with an 8 mm FWHM filter. The general linear model was utilized to perform statistical analyses on both individual and group data. Activated regions of interest (ROIs), or clusters with statistically significant activation were identified using a t-statistic on a voxel by voxel basis. In order to control for motion during scanning, artifact detection was performed using the Artifact Detection Tools (ART) toolbox (http://www.nitrc.org/projects/artifact_detect/). This tool provided information regarding each subject's motion (in mm) as a function of time. These motion variables (in the x, y, and z planes as well as yaw, pitch, roll) were then utilized as a covariate for analysis of each subject's activation data. Differences in activation between groups were calculated by examining differences in the number of voxels activated in corresponding ROIs between the groups for each paradigm. In order to correct for false positives and false negatives, Monte Carlo simulations were applied to the data based on the $8mm^3$ voxel size using AlphaSim in Analysis of Functional NeuroImage (AFNI) software (Ward et al., 2000) to determine the minimum number of voxels required in each cluster to be equivalent to the level of statistical significance at a family-wise error corrected threshold of $p<0.05$. This analysis resulted in an extent threshold of 80 contiguous voxels at a $p<0.001$ level for all contrasts with fixation and direct contrasts between conditions (transitive actions and intransitive actions) within groups.

Percent Signal Change. In addition to the activation analyses run using the general linear model in SPM8, percent signal change (PSC) values were extracted for all mental imitation conditions (transitive + intransitive) when contrasted with a fixation baseline. This contrast was utilized due to the fact that it included the largest amount of activation and activation that encompasses that seen in the separate contrasts. As such, utilization of this contrast offered the least chance of missing areas of activation present in any other contrast. Anatomical ROIs were defined using the WFU Pickatlas toolbox (Maldjian et al., 2003). These ROIs included bilateral regions associated with the Mirror Neuron System (IPL, IFG), motor functioning (cerebellum, SMA, precentral and postcentral gyri), and visual processing (extrastriate body area (EBA), MOG) as well as left insula, left hippocampus, STS, and LMFG. Bilateral EBA ROIs were defined spherically (centralized at: +/-46 -70 0.65; r=8mm). The time course extracted for each participant over the activated voxels within each ROI originated from the normalized and smoothed images, which were high-pass filtered and had the linear trend removed. For each participant, the intensity of fMRI signal per voxel in all the previously defined anatomical ROIs for the experimental task was compared to that for the fixation baseline using a *t*-test with a statistical significance level of $p < 0.05$. The average percentage change in signal intensity was then calculated for each ROI and the statistically significant difference was tested between the mental rotation task and fixation baseline. Thus for each ROI, for each participant, the mean percent change in signal intensity reflected the amount of difference in the BOLD contrast-related changes between all experimental tasks and the fixation baseline. In order to explore the relationship between neural activation and scores on neuropsychological measures, bivariate correlations were

run in SPSS between scores on measures of ASD symptoms (AQ, SCQ), empathy, and PIQ with PSC values for the overall group.

Functional Connectivity. Functional connectivity (correlation in time between activation of brain regions) was computed separately for each participant as a correlation between the average time course of all the activated voxels in each member of a pair of regions of interest (ROIs). This analysis utilized the same ROIs used for the PSC analysis which were based on areas main clusters of activation in the group activation map for each experimental condition contrasted to the fixation baseline as well as ROIs based on apriori hypotheses. Labels for the ROIs were assigned with reference to the parcellation of the Montreal Neurological Institute (MNI) single subject T1 weighted dataset carried out by Tzourio-Mazoyer and colleagues (2002). The activation time course extracted for each participant over the activated voxels within the ROI originated from the normalized and smoothed images, which were high-pass filtered and had the linear trend removed. The functional connectivity correlation was computed on the images from the experimental conditions (transitive + intransitive). As such, it reflects the synchronization between the activation in two areas while the participant is performing the task and not during the fixation condition. Fisher's *r* to *z* transformation was then applied to the correlation coefficients for each participant prior to averaging and statistical comparison of the two groups. These ROIs were also separately clustered into networks based on function (MNS, motor regions, visual regions, insula, thalamus) and a network analysis was run analyzing the correlation between activation time courses between networks. In order to explore the relationship between functional connectivity patterns and scores on neuropsychological measures, bivariate correlations were run in

SPSS between scores on measures of ASD symptoms (AQ, SCQ), empathy, and PIQ with FCA network connectivity values for the overall group.

3. Results

Overview

This study examined the role of MNS in mediating mental simulations of actions without actual motor movement. The main results are: 1) There were no statistically significant group differences in performance accuracy across simulating transitive and intransitive actions. However, the participants with ASD were significantly slower, relative to TD, in response time in both conditions; 2) Processing mental imitation when contrasted with fixation yielded significant activity in core MNS regions in both ASD and TD groups. The ASD group also demonstrated additional activation in the right fusiform and left hippocampus; 3) Neither group demonstrated greater levels of activation for transitive actions when directly contrasted with intransitive actions. However, the ASD group showed several areas of increased activation while processing intransitive actions relative to transitive actions; 4) Group differences in activation involved ASD group, relative to TD, showing decreased right angular gyrus activation for transitive actions; and increased activation in ASD, relative to TD, in right middle temporal cortex for intransitive actions; 5) The ASD group showed significantly increased percent change in signal intensity in several areas including LIFG, and this activity was correlated with motor ability; and 6) Greater functional connectivity was seen between the insula and several brain regions in the ASD group compared to TD group.

3.1 Neuropsychological Testing Results

While the participants in both ASD and TD groups were matched on age and IQ, they differed significantly on several assessment measures. Consistent with the previous literature and our hypothesis, the ASD group had significantly lower scores on the EQ (Mean=24) than the TD group (Mean=43) [$t(27)= -5.84$, $p<0.01$]. The ASD group also scored significantly higher on both measures of autism symptomatology, the AQ [$t(27)=4.25$, $p<0.01$] and the SCQ [$t(26)=6.89$, $p<0.01$] suggesting more autistic traits in ASD participants. In terms of motor skills, the ASD group displayed significantly greater difficulty on the PANESS compared to the TD group [$t(26)=4.25$, $p<0.01$]. There were no significant group differences on other neuropsychological measures including those assessing IQ and theory-of-mind (see Table 1).

Insert Table 1 about here

3.2 Behavioral Results

A two-way between-groups analysis of variance (ANOVA) was conducted to explore the effect of group (ASD or TD) and condition (transitive or intransitive) on accuracy and reaction time separately. Results of this analysis revealed a statistically significant main effect of condition [$F(1,516) = 4.29$, $p<0.05$], with greater accuracy for processing transitive actions (Transitive M=0.45; Intransitive M=0.36). However, the effect size was relatively small (partial eta squared = 0.01). The main effect of group did not reach statistical significance [$F(1,516) = 1.41$, $p>0.05$]; nor was there any significant interaction between group and condition [$F(2,516) = 0.06$, $p>0.05$]. Regarding reaction time, a statistically significant main effect of group was found [$F(1,516) = 34.75$, $p<0.01$,

partial eta squared = 0.06]. However, there was not a significant main effect of condition [$F(1,516) = 1.57$, $p<0.05$] or a significant group by condition interaction [$F(2,516) = 3.65$, $p<0.05$]. For all stimuli (transitive and intransitive), the ASD group showed significantly slower reaction times than the TD group [ASD M=3304.28ms; TD M=2715.96ms]. Since IQ can be a factor influencing participants' performance, a regression analysis was conducted to determine the amount of variance in accuracy that could be accounted for by IQ (VIQ and PIQ). Results of this analysis indicated no significant relationship between IQ and overall accuracy for either the ASD group [$F(12,2)=0.97$, $p>0.05$] or the TD group [$F(13,2)=0.94$, $p<0.05$].

Brain Activation Results

3.3 Within-Group Activation

When mental imitation was contrasted with fixation, significantly increased activation was seen in core Mirror Neuron System (MNS) regions (bilateral IFG and IPL) in both TD and ASD groups ($p<0.001$; k = 80 contiguous $2mm^3$ voxels determined by Monte Carlo simulation). Both groups also displayed activation in the right anterior insula and right inferior to right middle temporal gyrus. Both the TD and ASD group also displayed thalamus activation, with the ASD group in the right hemisphere and TD in the left. Additionally, the TD group demonstrated activation in the calcarine sulcus and the right supplementary motor area (SMA), regions primarily associated with visual processing and motor control respectively. The ASD group, however, showed greater activation in the fusiform gyrus and hippocampus, suggesting a possible reliance on working memory to perform the task (see Figure 2 & Table 2).

Insert Figure 2 and Table 2 about here

Processing transitive actions, when compared with processing intransitive actions, did not elicit increased activation in ASD or TD group. However, the opposite contrast (intransitive > transitive), had the ASD group showing greater levels of activation in a few different regions, such as the left lingual gyrus, left middle occipital gyrus (LMOG), left calcarine sulcus (LCALC) as well as the right precuneus. The TD group did not have any areas of increased activation for the intransitive action condition, perhaps indicating a similar neural approach to both tasks (see Figure 3).

Insert Figure 3 about here

3.4 Group Differences in Brain Activation

Group differences in brain activation were examined using a two-sample t-test. When mental imitation was contrasted with fixation, there were no statistically significant differences in group activation patterns ($p<0.005$, cluster size = $80mm^3$). However, when processing transitive actions was compared with fixation, the ASD participants, relative to TD, showed significantly reduced activation in the right angular gyrus. The ASD group did not demonstrate any areas of increased activation over the TD group in this contrast. Processing intransitive actions (relative to fixation), on the other hand, elicited significantly greater activity in the ASD group, relative to TD, in right middle temporal gyrus (RMTG). ASD group also showed increased activity (intransitive vs. transitive) in right thalamus and right precentral gyrus, regions found to be involved in motor control and integration of sensory information (see Figure 4).

Insert Figure 4 about here

3.5 Percent Signal Change (PSC)

Percent Signal Change values were extracted for individual participants for activated voxels from anatomically defined ROIs. These ROIs were defined based on the group level activation for all participants (ASD + TD) for the mental imitation vs. fixation contrast so that it best represented the activation in response to the task. Significantly increased psc values were found in ASD participants, relative to TD, in areas related to motor control/execution and integration of sensory input including the right precentral gyrus [$t(21)=2.05$, $p=0.05$], the right postcentral gyrus [$t(21)=2.13$, $=0.05$], and the right cerebellum [$t(21)=4.13$, $p<0.01$]. The ASD group also displayed increased psc in the LIFG [$t(21)=2.68$, $p=0.01$]. The TD group did not show any regions of increased psc as compared to the ASD group (see Figure 5).

Insert Figure 5 about here

3.6 Functional Connectivity

Functional connectivity was examined comparing individual ROIs and their connectivity with all other ROIs. Since this resulted in a large number of statistical comparisons, none of the results of this analysis survived multiple comparison for statistical significance in group difference. Thus, a follow-up network connectivity analysis was conducted to examine group differences in functional connectivity between the MNS and related brain networks (including the motor system and visual system as well as regions like insula, EBA and STS). Results of this analysis indicated significantly greater connectivity (ASD > TD) between the insula and motor system in ASD participants [$t(21)=2.04$, $p=0.05$]. A second level network analysis involving networks

created based on lobes and hemispheres (where different ROIs belonged to) revealed greater functional connectivity (ASD > TD) of the insula with left parietal [$t(22)=2.15$, $p<0.05$] and right temporal lobe [$t(21)=2.08$, $p=0.05$] networks (see Figure 6). These findings are consistent with recent research findings of aberrant, rather than reduced, connectivity in ASD and, in particular, findings of increased functional connectivity within many circuits involving early developing areas such as the insula (Di Martino et al., 2011).

Insert Figure 6 about here

3.7 Brain-Behavior Relationships

To examine the relationship between brain activation and measures of neuropsychological characteristics, bivariate correlations were conducted between psc values and scores on neuropsychological measures related to ASD symptoms, IQ, motor, and visual-motor skills. A significant negative correlation was found between psc in the LIFG and VMI scores [$r= -0.53$, $p<0.05$] for the overall group of participants. VMI scores were also negatively correlated with psc in the left precentral gyrus [$r=-0.51$, $p<0.05$]. These results demonstrate that higher levels of visual-motor ability were correlated with less reliance on activation of these regions (LIFG and left precentral gyrus) for this task (see Figure 7). Functional connectivity was also found to be correlated with behavioral measures. For example, greater connectivity between the MNS and Motor System was significantly positively correlated with overall accuracy in all participants [$r=0.47$, $p<0.05$]. In addition, the MNS-Insula connectivity was also positively correlated with accuracy [$r=0.55$, $p<0.05$] (see Figure 7).

Insert Figure 7 about here

4. Discussion

4.1 Intact Ability for Mental Imitation in ASD?

Results indicated that the groups performed equally well on mental imitation tasks. Both groups also demonstrated increased difficulty with stimuli depicting intransitive actions. While these findings contradict the hypothesis of decreased accuracy on the mental imitation task for the ASD group, the ASD group did take significantly longer to perform the tasks. In conjunction with the neuroimaging results, this suggests the ASD group is relying on a different neural approach to the task. By reducing the ease and immediacy, use of a different cognitive approach could affect the ability to use these strategies in a real world social environment.

There are several possible explanations for the finding of intact accuracy for the ASD group on a task requiring complex mental imitation. Given that imitation skills increase with age (Williams et al., 2004) and with cognitive functioning (Turner, Pazdol, & Stone, 2002), the current findings of intact accuracy in ASD may reflect characteristics of the study participants (all pre-adolescents or older with average to above average cognitive functioning). The explicit nature of the task may have also resulted in increased accuracy, while individuals with ASD may have more difficulty utilizing these skills naturally in a social setting (Raymaekers et al., 2009). The intact ability to perform this task may also suggest that the core deficit in imitation in autism is motor execution difficulties.

4.2 Findings of Intact (or Even Increased) MNS Functioning in ASD

Analysis of within-group activation patterns revealed that both groups show activation in core MNS regions (IFG and IPL) during mental imitation tasks. Additionally, an analysis of between-group activation differences reveals no areas of reduced activation in the MNS for the ASD group. These results are inconsistent with the hypothesis of reduced IFG activation in the ASD group but in line with recent studies failing to find reduced activation within the MNS in autism (Dinstein et al., 2010; Press et al., 2010; Fan et al., 2010; Raymaekers et al., 2009). PSC results indicated, instead, that the ASD group showed greater activation in the LIFG than TD controls (Tesink et al., 2009; Moss et al., 2005). In conjunction with increased reaction times for the ASD group, greater activation in the LIFG may indicate that they are working harder to produce the same results. Martineau and colleagues (2010) suggested that hyperactivation of the pars opercularis (a component of the IFG) in subjects with autism provides support for the hypothesis of atypical (but not reduced) activity within the MNS. The majority of group activation and functional connectivity differences for the current study, however, fell within regions outside of the MNS. This finding suggests that the abnormal pattern of reliance on additional neural regions may underlie the noted deficits in imitation.

4.3 Increased Reliance on Motor Planning and Theory-of-Mind in the TD Group

Despite both activating core regions of the MNS, the ASD and TD groups displayed several differences in activation patterns. The TD group showed increased activation in the right SMA and the right angular gyrus. The SMA is believed to be

involved in planning and performing complex movements requiring sequences of actions (Decety et al., 1997; Grezes et al., 1998; Nachev et al., 2008) and has been demonstrated to show reduced activation in autism (Marsh & Hamilton, 2011). It has been suggested that SMA dysfunction in ASD may help explain difficulties understanding as well as performing chained action sequences in individuals with ASD (Fabbri-Destro et al., 2009; Cattaneo et al., 2007; Marsh & Hamilton, 2011).

Situated at the temporal-parietal junction (TPJ), the right angular gyrus is a key neural region for theory-of-mind (Happe et al., 1996; Frith & Frith, 1999; Saxe & Kanwisher, 2003; Decety & Lamm, 2007). Consistent with the current results, past studies have found reduced activation in this region for ASD (Castelli et al., 2002; Pelphrey et al., 2005; Murdaugh et al., 2014). In TD individuals, this region is not only a part of the mentalizing network, working with the MNS in order to facilitate self-other connections necessary for imitation. Activation in this region specific to the TD group may provide additional evidence that the ASD group deployed a separate – possibly compensatory – strategy to perform the task.

4.4 Increased ASD Reliance on Limbic System and Memory

The ASD group demonstrated an increased reliance on subcortical/ limbic structures to perform mental imitation tasks. When activation for all mental imitation stimuli were contrasted with fixation, the ASD group displayed unique activation in the right fusiform gyrus and the left hippocampus. Previous studies have frequently found the fusiform to be less activated in ASD individuals (Di Martino et al., 2009). However, activation in this region is modulated by selective attention (Hoffman & Haxby, 2000;

Hooker et al., 2003). Therefore, the explicit nature of the current task might explain the current findings. As part of the limbic system, the hippocampus is an early developing neural region. It is involved in recall of action sequences (Muller et al., 2002) and part of a bottom-up approach to imitation learning (Gaussier et al., 1998). Thus, activation in this region suggests that the ASD group is remembering previous events in which they performed or watched another person perform the action depicted.

Examination of areas of increased activation for intransitive (over transitive) actions, revealed increased activation in the right thalamus for the ASD group. The thalamus is believed to also play a crucial role in visuospatial memory (Van der Werf et al., 2003). Increases in activation in both the hippocampus and thalamus suggest that the ASD group is relying on memory to complete the task. However, the thalamus is also involved in focusing attention and screening out distracting stimuli (Van der Werf et al., 2003). Therefore, the increased activation for the intransitive condition could indicate difficulty determining salient features and filtering out extra stimuli. Consistent with this hypothesis, the ASD group also demonstrated increased activation for intransitive stimuli in the RMTG, a region involved in both verbal and nonverbal semantic functioning, including representation of motion and actions (Devlin et al., 2002; Martin, 2007; Tranel et al., 2005). Therefore, RMTG activation also suggests that the ASD group is utilizing extra neural resources attempting to search for and interpret meaningful nonverbal cues.

Regardless of the specific role played these regions, current findings are consistent with previous findings of increased connectivity with and reliance on limbic system structures in autism (Di Martino et al., 2011; Iidaka et al., 2012; Kleinhans et al., 2008; Murphy et al., 2012; Williams et al., 2006) and compliment a recent proposal that

the MNS in autism is more heavily connected with subcortical regions (Williams & Waiter, 2006). It has been suggested that this difference might explain the seemingly reduced social function of the MNS in autism (Lewis, 2004; Williams & Waiter, 2006).

4.5 Different Approach to Transitive vs. Intransitive Stimuli – Only for ASD?

Results of the current study indicate that the TD group utilized a similar neural approach to both tasks. However, while ASD participants did not show any increase in activation for the transitive condition (over the intransitive condition), they did rely on additional neural resources to complete stimuli involving intransitive actions. More specifically, they demonstrated increased activation in the LMOG, LCALC, and left lingual gyrus. Consistent with previously described findings of an increased reliance on memory in the ASD group, the left lingual gyrus has been implicated not only in visual processing but also the encoding and recollection of visual memories (Machielsen et al., 2000; Cho et al., 2012). The ASD group also showed increased activation in regions associated with motor imagery (Porro et al., 1996) and with identifying and inhibiting distracting stimuli (Van der Werf et al., 2003; Fischer & Whitney, 2012), potentially representing an increased reliance on compensatory mechanisms for intransitive stimuli.

4.6 Limitations and Future Directions

Contrary to hypotheses, the ASD group showed an intact ability to perform mental imitation tasks and activation in the core components of the MNS, with abnormal patterns of activation in regions external to the MNS. However, there are several aspects of the current study which may affect the generalizability of results. Given the

previously established link between age (Williams et al., 2004) and cognitive functioning (Turner et al., 2002) with imitation skills in autism, the current results may be significantly influenced by these subject characteristics. Additionally, the structured environment of the fMRI task may mask difficulties associated with imitation in real-world social settings including lack of attention and the presence of more motivating stimuli (Trevarthen & Aitken, 2001; Smith et al., 2006). While neural activation patterns suggest that the ASD group utilized a unique cognitive approach to the task, no qualitative data was gathered from subjects regarding the specific approach used.

Future research should focus on assessing the generalizability of current results. Longitudinal studies will be particularly important given recent findings that a critical developmental shift occurring around puberty may account for inconsistent findings in ASD (Peper et al., 2011; Uddin et al., 2013). It will also be important to continue to gather additional information regarding the impact of other participant characteristics including cognitive level, functional level, and gender. The results of the current study also suggest that research should examine both integrated functioning within the MNS and also between the MNS and related neural regions. Current findings indicate that imitation deficits may result from abnormalities in the integrated functioning of the MNS with limbic, visual, and mentalizing systems.

5. Conclusions

Overall, results of the current study indicate that the participants with ASD were able to perform the mental imitation task as well as TD participants and showed no areas of reduced activation in the core MNS regions compared to the TD group. However,

results of activation and functional connectivity analyses indicate that the ASD group utilized a different neural approach than subjects in the TD group. The main group differences were found in the activation and connectivity with regions outside the MNS. More specifically, the ASD group showed a stronger reliance on integration of the MNS with limbic regions and greater activation in areas contributing to nonverbal semantic processing. The TD group, however, relied more on activation in the right TPJ, an area associated with theory-of-mind. These results indicate that the ASD group may be utilizing a unique strategy to perform the task. Further research is needed to elucidate the degree to which subject and/or study characteristics could be influencing these findings and the extent to which the unique cognitive strategy used by the ASD group may influence the effective implementation of imitation skills in a real-world social environment.

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Table 1. Table displaying scores on neuropsychological measures by group.

MENTAL IMITATION

	ASD			TD			
Measure	**n = 14**	**Range**	**S.D**	**n = 15**	**Range**	**S.D**	**p-value**
Age	13	8-17	2.8	11	8-15	1.77	0.08
EQ	24	9-42	7.11	43	23-54	9.63	<0.01
SQ	26	15-50	8.77	29	18-40	7.01	0.42
FSIQ	111	85-126	14.9	103	83-130	15.17	0.21
VIQ	107	75-128	16.59	104	84-134	19.24	0.64
PIQ	111	93-124	11.37	102	84-130	12.04	0.08
SCQ	18	6-31	7.25	3	0-10	3.56	<0.01
VMI	92	66-117	13.04	97	71-110	9.41	0.26
VMI Motor	91	51-110	13.99	91	75-105	10.36	0.99
PANESS	57	39-78	12.31	46	36-71	9.19	0.01
AQ	78	28-135	30.31	31	5-67	21.62	<0.01
RME	19	15-24	2.27	20	17-25	2.32	0.23
Imitation	127	108-130	6.22	129	127-130	1.29	0.31

Table 2. Table displaying areas of activation for each group when all mental imitation conditions are contrasted with fixation.

Mental Imitation vs. Fix (TD)

Region	x	y	z	Hem	BA	Cluster	t
Middle Temporal/Calcarine	38	-50	4	R	NA	15974	13.76
Inferior Frontal	-48	6	28	L	44	2019	12.77
Insula/Inferior Frontal	34	24	-2	R	NA	474	8.59
Middle/Inferior Frontal	34	4	40	R	9	1477	7.04
Supplementary Motor	2	24	48	R	8	463	6.92
Thalamus	26	-30	2	R	NA	219	6.25

Mental Imitation vs. Fix (ASD)

Region	x	y	z	Hem	BA	Cluster	t
Inferior Temporal/Fusiform	46	-56	-8	R	NA	20893	16.03
Hippocampus/Thalamus	-30	-30	-6	L	NA	537	8.41
Inferior/Middle Frontal	-40	26	8	L	45	2892	8.11
Inferior Frontal	52	12	28	R	44	1572	7.43
Insula	38	22	-6	R	NA	113	5.81

Figure Captions

Figure 1. Figure showing example mental imitation stimuli. Participants were provided with a cartoon image of an individual with their hand missing. They were asked to choose from three answer choices the hand image that would most appropriately complete the picture. Top: Example transitive stimuli of person ironing (Correct response: C). Bottom: Example intransitive stimuli of people at a meeting clapping (Correct response: B).

Figure 2. The activation pattern for processing mental imitation contrasted with fixation for each group ($p < 0.001$; cluster = 80 mm^3).

Figure 3. The activation pattern for processing transitive and intransitive actions contrasted separately with fixation for each group ($p < 0.001$; cluster = 80 mm^3).

Figure 4. Group differences in activation for transitive and intransitive conditions when contrasted with a fixation. The TD group shows greater levels of activation in the right angular gyrus while the ASD group shows greater levels of activation in the right middle temporal area.

Figure 5. Graph displaying areas of increased psc values for the ASD group. The ASD group showed significantly greater psc in the LIFG, right cerebellum, right precentral gyrus, and right postcentral gyrus.

Figure 6. Graph displaying networks of increased functional connectivity in the ASD group(ASD >TD): increased connectivity of the insula with the left parietal and the right temporal networks.

Figure 7. Scatterplots displaying significant correlations between psc values and neuropsychological test scores: A) Negative correlation between VMI scores and psc in the LIFG and left precentral gyrus for all participants; (B) Significant positive correlations of *MNS:Motor* and *MNS:Insula* connectivity with performance accuracy.

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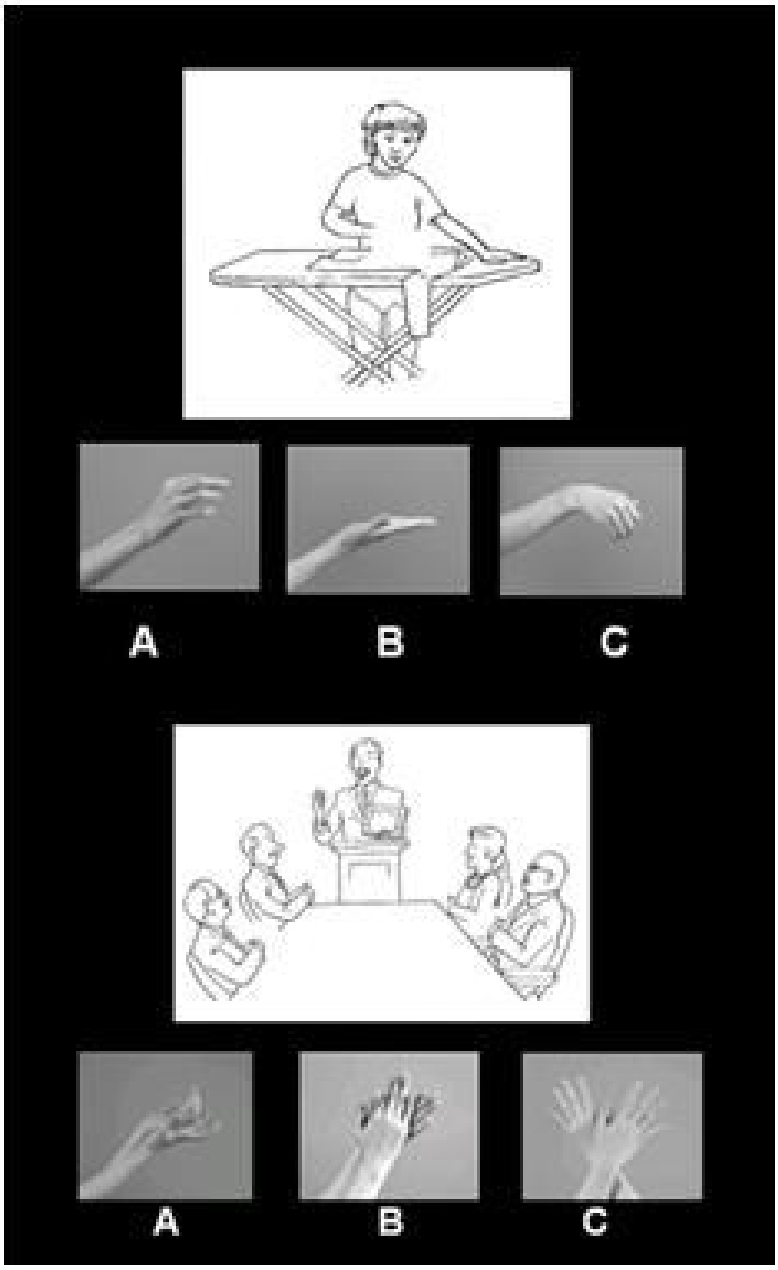

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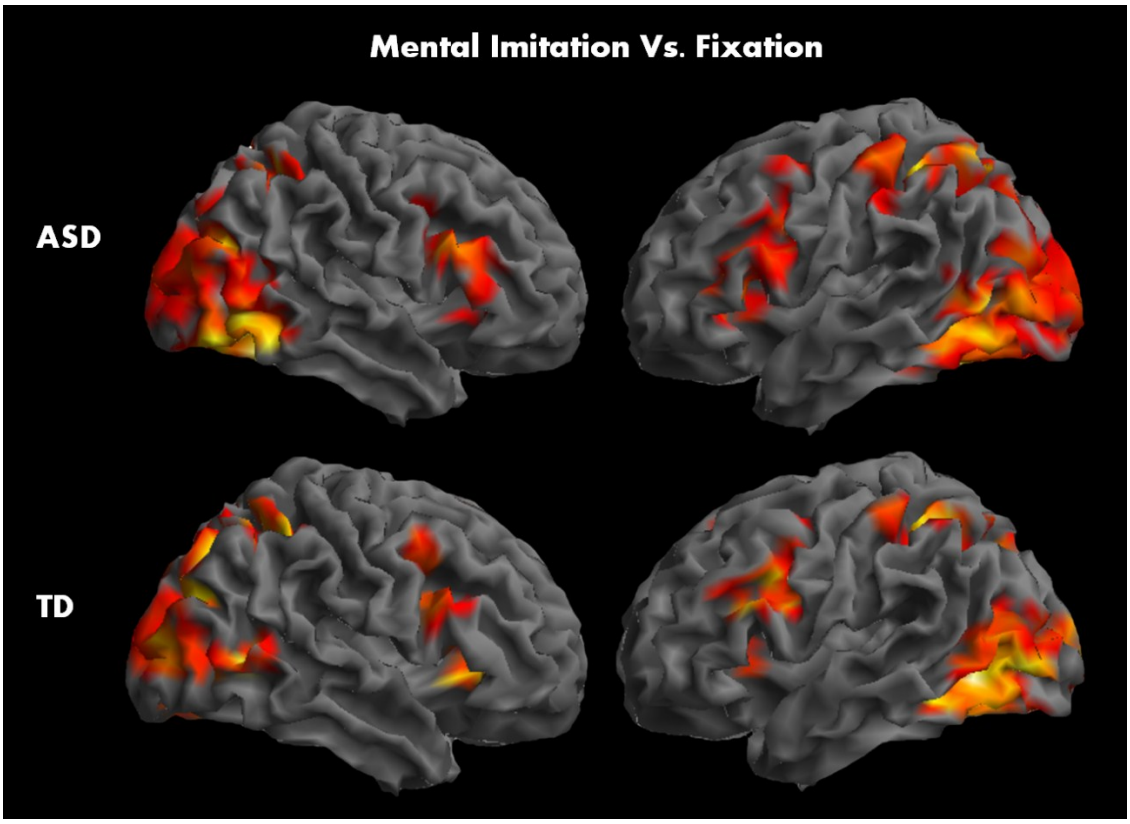

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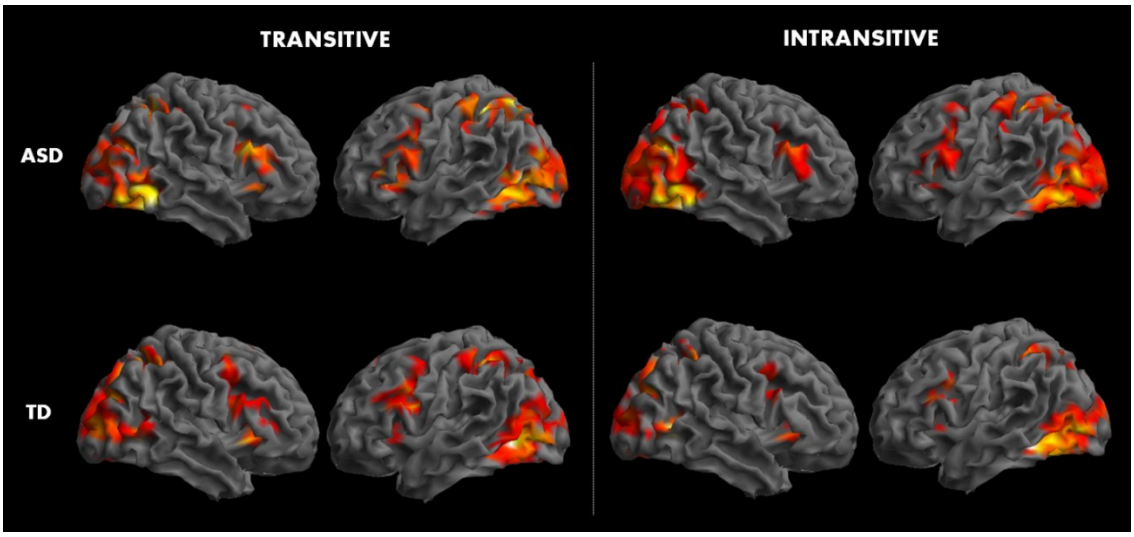

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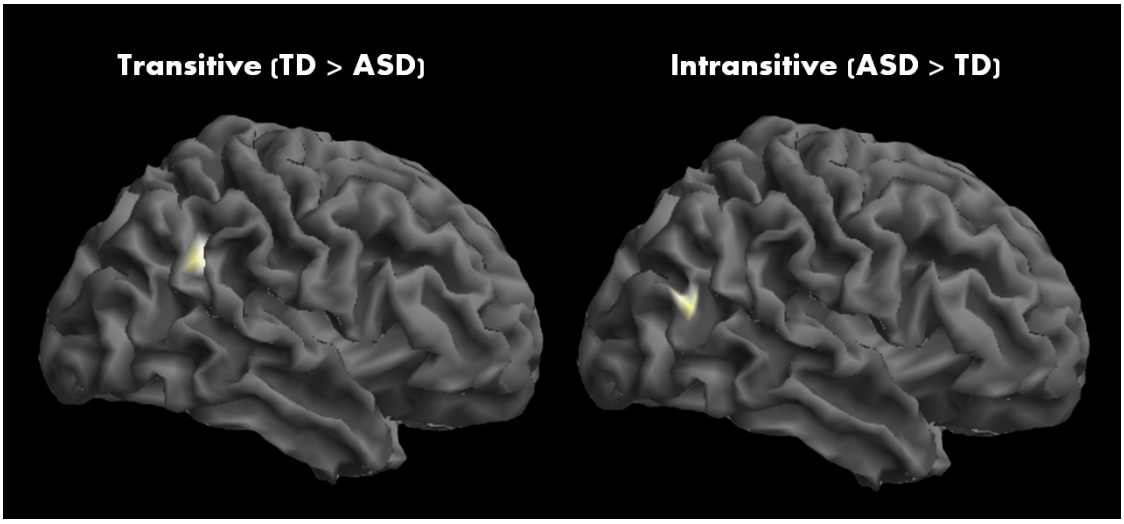

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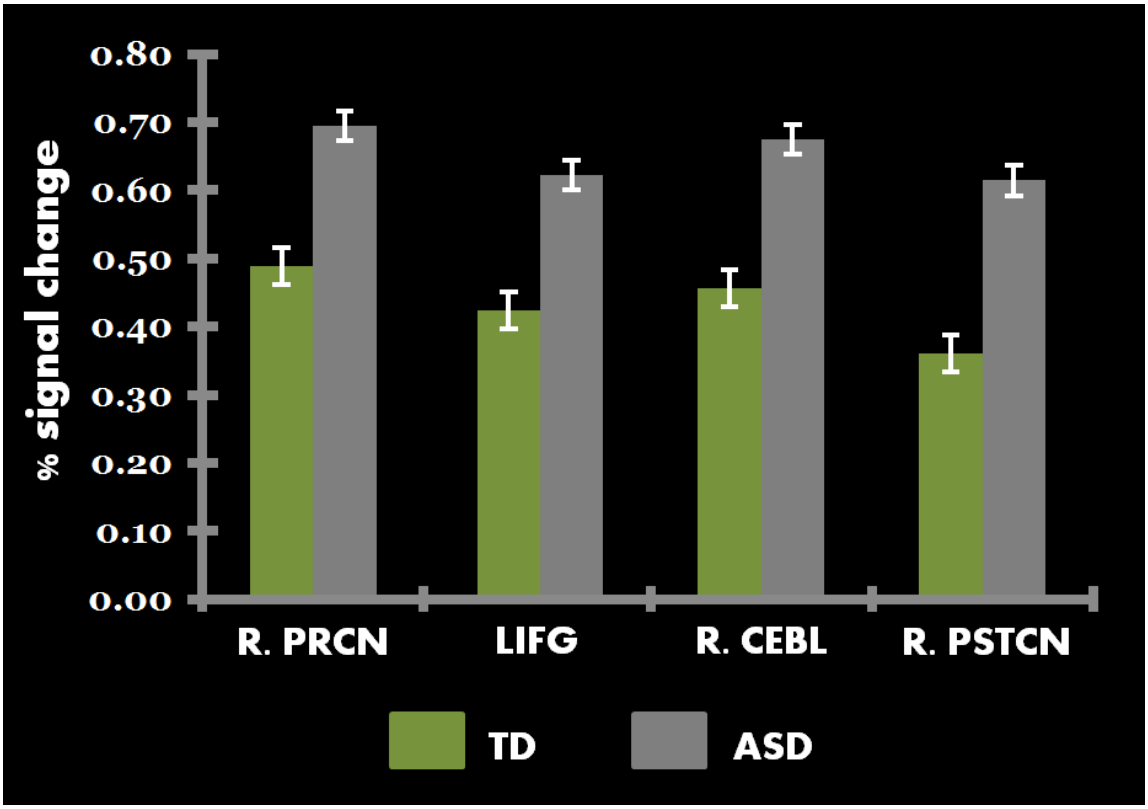

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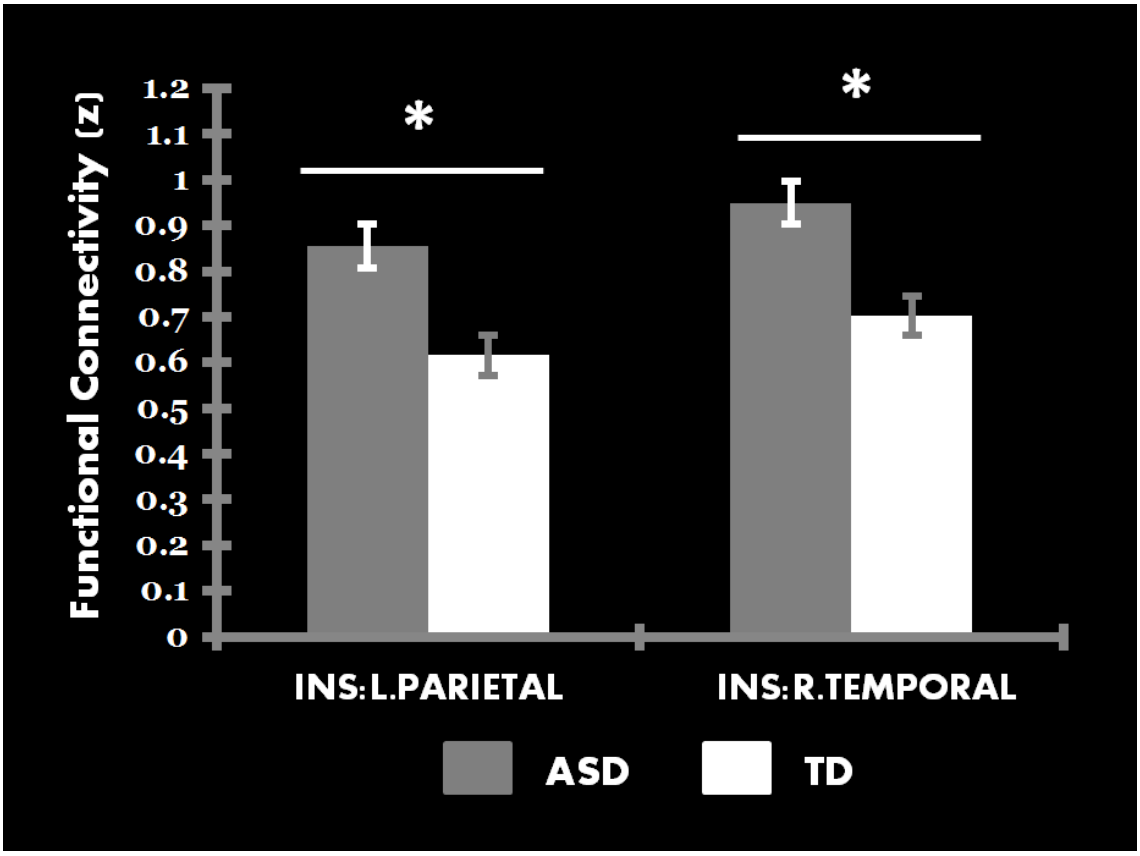

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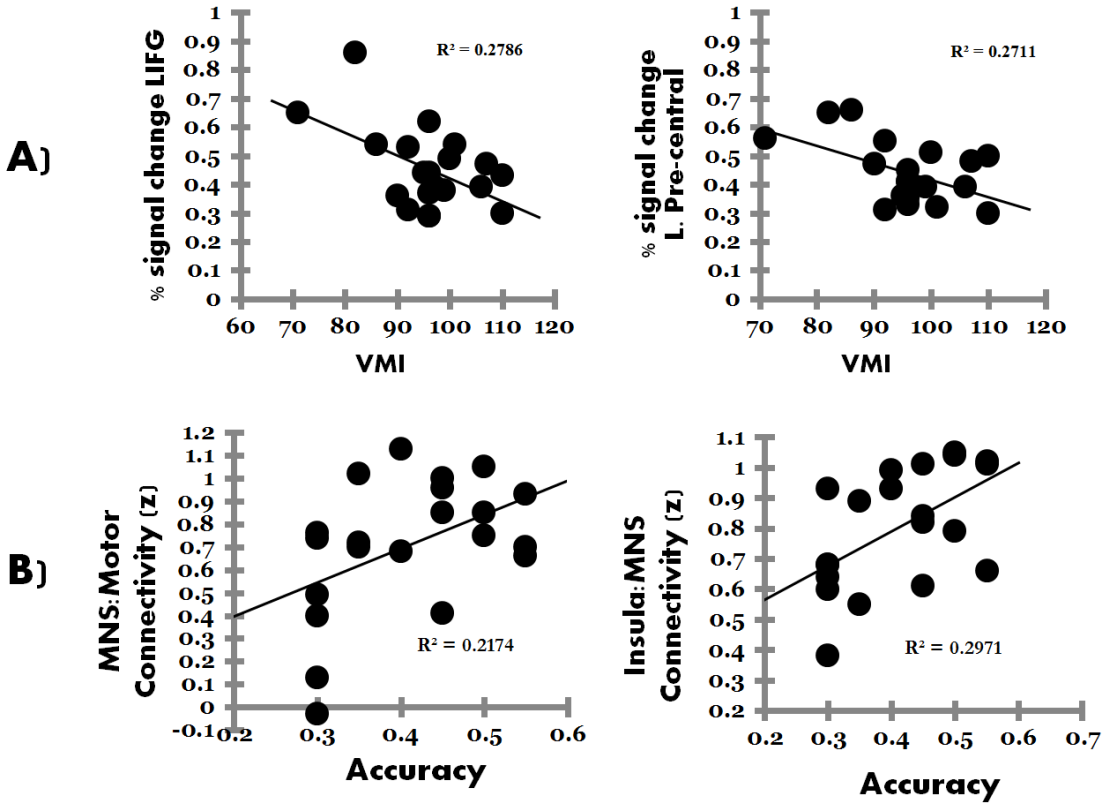

THE MIRROR NEURON NETWORK AND MOTOR IMITATION IN CHILDREN AND ADOLESCENTS WITH AUTISM

by

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Abstract

While imitation has consistently been reported to be an area of particular deficit in autism spectrum disorder (ASD), the exact nature of this deficit remains unclear. Although a dysfunction in the brain's mirror neuron system (MNS) has been proposed to explain this, the reliability of this account is under debate. The current study used fMRI to examine the integrated functioning of the regions that are part of the MNS, and the communication between this network and other brain areas in children and adolescents with ASD during a motor imitation task. Fifteen ASD and 13 age-and-IQ-matched typically developing (TD) children were asked to imitate, in the MRI scanner, a series of hand gestures. Intact performance on imitation in both ASD and TD groups was accompanied by strong activation in areas considered part of the MNS, such as the ventral premotor cortex (PMv) and the inferior parietal lobule (IPL). Group difference analyses revealed significantly increased activity in ASD participants, relative to TD, in mirror and motor areas including PMv, supplementary motor area (SMA), inferior frontal gyrus (IFG), and extrastriate body area (EBA). The ASD participants also showed significantly increased functional connectivity, than TD participants, of the visual network with insula and superior temporal sulcus (STS). Overall, our study found robust MNS activity accompanied by typical imitation performance in ASD children. These findings provide new evidence for a lack of MNS dysfunction in ASD at least in the context of a simple motor imitation task.

Keywords: mirror neuron, imitation, autism, fMRI, functional connectivity

Word Count: 7, 339

1. Introduction

Imitation is a fundamental aspect of human social behavior and plays a critical role in the acquisition of knowledge (Bandura, 1977; Hurley & Chater, 2005) as well as in the development of social, communication, and motor skills (Tomasello, Kruger, & Ratner, 1993). By providing children with information about the actions and intentions of the physical and the social world, imitation assists in social learning (Rogers et al., 2003) and forms the foundation for future social development. Early imitative skills are correlated with social engagement (Masur, 2006; Young et al., 2011) and predict nonverbal communication skills (Heimann et al., 2006), language development (McEwen et al., 2007; Rose, Feldman, & Jankowski, 2009), social understanding (Meltzoff, 1995), and cognitive skills (Strid et al., 2006). Additionally, being imitated helps build rapport (Chartrand & Bargh, 1999), increases altruistic behavior (van Baaren et al., 2004), and increases trust (Bailenson & Yee, 2005). Thus, the ability to imitate has benefits that span multiple levels of social and cognitive functioning.

Imitation plays an important role in characterizing the social and communicative impairments seen in individuals with autism spectrum disorders (ASD). Evidence from behavioral research has led to the proposal that imitation is a core deficit in autism (Rogers & Pennington, 1991; Williams, Whiten, & Singh, 2004; Rogers et al., 2003; Charman et al., 2005; Turan & Ökçün Akçamuş, 2013). Given the importance of imitation in social and cognitive development, it has been suggested that imitation deficits in ASD may result in a range of difficulties including deficits in face-processing (Hadjikhani et al., 2007), theory-of-mind (Williams et al., 2006), empathy (Oberman & Ramachandran, 2007), and joint attention (Villalobos et al., 2005). However, not all

forms of imitation appear equally impaired in autism. For instance, ASD individuals demonstrate specific deficits in spontaneous imitation, but perform better on tasks that elicit imitation explicitly (McDuffie et al., 2007; Ingersoll, 2008). They also seem to have greater difficulty imitating meaningless gestures over those involving meaning or those with objects (Williams, Whiten, & Singh, 2004). Studies indicate that children with ASD show improvement in imitation skills over time (Heimann & Ullstadius, 1999; Stone, Ousley, & Littleford, 1997), with the ability to imitate simple, single-step actions improving with age (Hepburn & Stone, 2006). Imitation deficits in older children and adults with ASD are more likely to reflect difficulty imitating more complex actions (Rogers, 1999; Rogers et al., 1996) or in getting the "attitude" or "style" of an action correct (Hobson & Lee, 1999).

Although, as studies mentioned above suggest, imitation deficit has been reported widely in autism, there are several recent studies that found intact imitation skills in autism (Bird et al., 2007; Dinstein et al., 2010; Hamilton, Brindley, & Frith, 2007; Press, Richardson, & Bird, 2010; Spengler, Bird, & Brass, 2010). Nevertheless, some of these studies question if ASD individuals imitate in the same way as those without autism and if they would automatically do so in a real-world situation (e.g., Carpenter, Pennington, & Rogers, 2001; Charman & Baron-Cohen, 1994; Cook & Bird, 2011). Previous findings suggest that typically developing (TD) children will imitate "socially", copying all of the actions of an adult, while those with ASD will imitate "efficiently", copying only the necessary steps (Marsh et al., 2013). If an action has a clear goal, most people emulate the goal of the action. However, if there is no clear goal, they imitate the kinematic aspects of the action (Rumiati & Tessari, 2002). ASD individuals rely on a

goal-directed strategy, even in the absence of visual goals (Wild et al., 2011). This is thought to result from difficulties identifying key kinematic clues (Gowen, 2012), an important component of understanding other people's actions and identifying when to imitate for appropriate social interaction (Hamilton, Brindley, & Frith, 2007). Thus, individuals with ASD may be less flexible in their approach to imitation resulting in successful imitation in some cases and failure in many others.

Imitation involves copying of both the goal and style of an action voluntarily (Whiten et al., 2004) and likely requires the integrated functioning of several component skills (Want & Harris, 2002; Bennetto, 1999; Hamilton, 2008), such as perception-action coupling, visual attention, short-term memory, body schema, mental state attribution, and agency (Decety, 2006). Imitation also likely involves attentional flexibility, deficits in which influence the foci of attention during social interaction tasks (Klin et al., 2002) and, as such, may impact imitation in autism (Williams & Waiter, 2006). Given the need for motor production for imitation, motor deficits may also affect imitation skills (Rogers et al., 2003; Mostofsky et al., 2006). It has been hypothesized that ASD individuals may also lack internal motivation, in that their decreased interest in others may lead them to look at other people less frequently and make them less motivated to copy their actions (Dawson et al., 2002; Trevarthen & Aitken, 2001). Thus, the social, cognitive, and motor aspects of imitation may be mediated by the coordinated functioning of several brain areas, especially the mirror neuron system (MNS) (Rizzolatti, Fogassi, & Gallese, 2001). It is thought that these neurons provide the basis for action understanding (Lestou, Pollick, & Kourtzi, 2008) and, as such, are directly involved in imitation (Iacoboni et al., 1999; Koski et al., 2002, 2003; Heiser et al., 2003). While debated, research suggests that

the core regions of the human MNS are the caudal inferior frontal gyrus (IFG)/ventral pre-motor cortex (PMv) and the rostral inferior parietal lobe (IPL) (Iacoboni, 2005; Iacoboni et al., 2001; Iacoboni & Dapretto, 2006). These individual regions likely serve separate functions, with the IFG/PMv involved in action planning (Hamilton, 2008) and perception-action coupling (Newman-Norlund et al., 2010), and the IPL in mediating the spatiotemporal, perceptual, and goal coding aspects necessary for imitation (Rizzolatti & Matelli, 2003; Hamilton, 2008). The superior temporal sulcus (STS) is believed to work with the MNS by coding visual input and sending those signals to the core regions of the MNS (Hamilton, 2008).

Previous findings of limited or lack of MNS response in autism have led to the "broken mirror hypothesis" (Williams et al., 2001; Oberman & Ramachandran, 2007; Ramachandran & Oberman, 2006), an account receiving substantial attention in recent years. Several studies have provided evidence that individuals with ASD improperly engage the MNS during imitation (for a review see Williams, 2008; Bernier & Dawson, 2009; Kana, Wadsworth, & Travers, 2011). However, several recent studies have failed to find a decreased MNS response in autism in a variety of imitation tasks (Press, Richardson, & Bird, 2010; Fan et al., 2010; Raymaekers, Wiersema, & Roeyers, 2009), providing evidence against a global dysfunction of this system in autism (Hamilton, 2013). Given that imitation is more than simply mirroring actions (Southgate & Hamilton, 2008), a more complete understanding of the imitation deficit in autism is likely to emerge from an analysis of the MNS in the context of the wider neural networks involved. As the MNS works closely with regions comprising the limbic system (Iacoboni, 2005; Dapretto et al., 2006), aberrant activation in limbic regions in ASD

(Dapretto et al., 2006; Uddin et al., 2009; Dickstein et al., 2013) may interfere with MNS functioning. The MNS is also strongly influenced by top-down control mechanisms (Spunt, Satpute, & Lieberman, 2011; Naeem et al., 2012), where ASD individuals experience considerable difficulties (e.g., Lopez et al., 2005; Hill, 2004; Kenworthy et al., 2010; Hamilton, 2008; Gomot & Wicker, 2012). In addition to aberrant executive control mechanisms, research also indicates that individuals with ASD show increased activation in regions associated with processing memory (Shih et al., 2010), which is important for imitation of meaningful actions. ASD individuals show greater reliance on these regions for imitation even in the absence of visual goals (Wild et al., 2011). Imitation difficulties may also arise from problems related to motor planning or execution. Since the MNS acts to map the actions of others onto one's own motor system, deficits in imitation may result from poor motor performance or ineffective connectivity between the MNS and motor systems. Motor difficulties seen in individuals with ASD (e.g., Fournier et al., 2010) have been implicated in the imitation deficits observed in the disorder (Mostofsky et al., 2006; Enticott et al., 2012; Théoret et al., 2005). Therefore, mirroring mechanism, mediated by motor as well as other networks, may respond differently in individuals with autism, especially in the context of imitation.

The goal of the current study was to examine the role of MNS in mediating motor imitation in high-functioning children and adolescents with autism using a hand gesture task. Since attention can have an impact on imitation performance, sustained attention and attentional flexibility were also assessed outside the scanner. Given the simple nature of the task, it was predicted that both groups would be able to imitate the gestures well. Based on previous research (Dapretto et al., 2006; Kana et al., 2009; Shih et al., 2010),

we also expected that the ASD group would show decreased activation and connectivity within the frontal component of the MNS (PMv/IFG) and demonstrate aberrant patterns of activation and connectivity between the MNS and other neural regions involved in the production of imitation.

2. Materials & Method

2.1 Participants

Seventeen high-functioning children and adolescents with ASD and 17 age and IQ-matched TD control participants took part in this fMRI study (age range: 8 to 17 years; minimum Full Scale and Non-Verbal IQ: 75, measured using the Wechsler Abbreviated Scales of Intelligence [WASI]). Participants with ASD were recruited through the research subject database of the *Cognition, Brain, and Autism Laboratory* at the University of Alabama at Birmingham (UAB), the Alabama Autism Society, and flyers posted at local ASD-related treatment and evaluation centers. All participants were diagnosed with an ASD. Current and past ASD symptoms were assessed using the Social Communication Questionnaire (SCQ) – Lifetime Version (Berument et al., 1999) (ASD mean/SD = 17/8.29; TD mean/SD = 3/3.66), the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001) (mean ASD/SD = 78/28.67; TD mean/SD = 32/23.18), and the Repetitive Behavior Scale – Revised (Lam & Aman, 2007) (ASD mean/SD = 29.71/26.70). TD participants were recruited using flyers and advertisements on the UAB campus, flyers posted in local community centers (e.g., libraries, YMCAs), and through the *Cognition, Brain, and Autism Laboratory's* research subject database. Participants were not included in the study if they indicated having worked with metal or having metal implanted in their bodies (either surgically or accidentally) or if they had a

history of psychiatric disorders. No participants indicated having a cognitive disorder, anxiety disorder, schizophrenia, or obsessive compulsive disorder. Out of the 34 participants scanned (17 ASD and 17 TD), six participants' data were not usable due to head motion artifacts resulting in a final sample of 15 ASD and 13 TD control participants (see table 1 for detailed participant information).

Insert Table 1 about here

2.2 Overall Experimental Procedure

Participants took part in 2 separate sessions: 1) Assessment; and 2) MRI scanning. The first session lasted approximately two hours, during which the participants completed a series of screening measures and neuropsychological tests including demographics, visual-motor skills, ASD-related symptomology, medication history, and diagnosis. In order to analyze the relationship between attention and imitation skills, attention was measured using the computerized children's Attention Networks Task (ANT; Fan et al., 2002). Motor skills were also analyzed using the Physical and Neurological Examination for Soft Signs (PANESS; Denckla, 1985). During the second session, which also lasted approximately 2 hours, participants were prepared for the MRI scan, performed a computer practice task outside the scanner to ensure that they understood the directions for the task, and performed the tasks in the MRI scanner.

2.3 Stimuli and Experimental Paradigm

Participants were shown 24 high-resolution hand images, presented one at a time and centered on the screen, showing a range of meaningless hand gestures (see Figure 1).

These stimuli were adapted from answer choice images included in a praxis paradigm developed by Heilman and colleagues (Mozaz et al., 2002). Images were selected for inclusion based on ability to perform while lying in the scanner as well as the representation of a meaningless gesture. Images selected for inclusion were modeled and re-photographed using a high-resolution color camera in order to increase the resolution of images presented. Each stimulus was displayed on the screen for a period of 4000ms with an inter-stimulus interval of 6000 ms in an event-related design. Participants were instructed to use their right hand to imitate the same hand action they saw in the image. In order to control for potential practice effects, the order of presentation of stimuli within the experiment was randomized across participants. Participants were also presented with a fixation condition after every four imitation stimuli for a total of 6 fixation periods. During fixation, participants were shown a white cross centered on a black background and instructed to relax and wait for the next image to appear. Each fixation was displayed on the screen for a period of 24000ms. In a post-scanning computer session, participants were asked to imitate hand motions of the same stimuli they saw in the scanner. Their imitative acts were video recorded and coded for their style, accuracy, and precision based on the taxonomy suggested by Buxbaum and colleagues (2000). Two independent researchers also coded the tapes and inter-rater reliability was established.

Insert Figure 1 about here

2.4 Image Acquisition

All fMRI scans were acquired using the Siemens 3.0 Tesla Allegra head-only scanner (*Siemens* Medical Inc., Erlangen, Germany) located at the UAB Civitan

International Research Center (CIRC). For structural imaging, initial high resolution T1-weighted scans were acquired using a 160-slice 3D MPRAGE (Magnetization Prepared Rapid Gradient Echo) volume scan with repetition time (TR) = 200 ms, echo tile (TE) = 3.34 ms, flip angle = 12^0, field of view (FOV) = 25.6 cm, 256 X 256 matrix size, and 1 mm slice thickness. A single-shot gradient-recalled echo-planar pulse sequence was used to acquire functional images (TR= 1000 ms, TE = 30ms, flip angle = 60 degrees, FOV = 24 cm, matrix =64 x 64). Seventeen adjacent oblique axial slices were acquired in an interleaved sequence with 5 mm slice thickness, 1 mm slice gap, a 24 X 24 cm field of view (FOV), and a 64 X 64 matrix, resulting in an in-plane resolution of 3.75 X 3.75 X 5 mm. The stimuli were rear-projected onto a translucent plastic screen and participants viewed the screen through a mirror attached to the head coil. Quality control checks were applied to the acquired data to examine the signal to noise ratio, temporal signal to noise ratio, ghosting, and head motion artifacts. Data that did not meet quality standards were not included in further analyses. In addition, the head motion for each participant, quantified in three translational (x, y, and z) and three rotational (pitch, roll, and yaw) dimensions were entered into the general linear model as regressors of no-interest.

2.5 Behavioral and Neuropsychological Data Analyses

Scores from neuropsychological measures for each participant were entered into SPSS 16.0 statistical software (*SPSS* Inc., Chicago, IL). Between-group t-tests were run in order to compare group means on each measure. Data on each participant's accuracy during the out of scanner imitation task was also entered into SPSS. A two-way between groups analysis of variance (ANOVA) was also performed to examine the effects of

group and condition on both reaction time and accuracy of the ANT. The relationship between behavioral performance on the ANT and neural activation in mirror neuron regions (LIFG, RIFG, LIPL, RIPL) was examined using multiple regression analyses.

2.6 fMRI Data Analyses

Imaging analysis included brain activation, change in percent signal intensity, and functional connectivity. In addition, bivariate correlation analyses were used to examine the correlation between percent signal change values and functional connectivity in specified ROIs and scores on neuropsychological measures assessing PIQ, empathy, and motor skills.

Brain activation. To examine brain activation, the data were pre-processed and statistical analyses were carried out using Statistical Parametrical Mapping, version 8 software (SPM8; Wellcome Department of Cognitive Neurology, London, UK). Images were corrected for slice acquisition timing, motion-corrected, normalized to the Montreal Neurological Institute (MNI) template, re-sampled to 8-mm^3 voxels, and smoothed with an 8 mm FWHM filter. The general linear model was utilized to perform statistical analyses on both individual and group data. Activated regions of interest (ROIs), or clusters with statistically significant activation were identified using a t-statistic on a voxel by voxel basis. In order to control for motion during scanning, artifact detection was performed using the Artifact Detection Tools (ART) toolbox (http://www.nitrc.org/projects/artifact_detect/). This tool provided information regarding each subject's motion (in mm) as a function of time. These motion variables (in the x, y, and z planes as well as yaw, pitch, roll rotational dimensions) were then utilized as

nuisance regressors for analysis of each subject's activation data. Differences in activation between groups were calculated by examining differences in the number of voxels activated in corresponding ROIs between the groups for each paradigm. In order to correct for false positives and false negatives, Monte Carlo simulations were applied to the data based on the $8mm^3$ voxel size using AlphaSim in Analysis of Functional NeuroImage (AFNI) software (Ward, 2000) to determine the minimum number of voxels required in each cluster to be equivalent to the level of statistical significance at a family-wise error corrected threshold of $p<0.05$. This analysis resulted in an extent threshold of 136 contiguous voxels at a $p<0.001$ level for all contrasts within groups.

Percent Signal Change. In addition to the activation analyses run using the general linear model in SPM8, percent signal change (PSC) values were extracted for all imitation stimuli when contrasted with a fixation baseline. Anatomical ROIs utilized were defined using the WFU Pickatlas toolbox (Maldjian et al., 2003). These ROIs included 8 sets of bilateral regions: inferior parietal lobule (LIPL, RIPL), inferior frontal gyrus (LIFG, RIFG), superior temporal sulcus (LSTS, RSTS), middle occipital gyrus (LMOG, RMOG), supplementary motor area (LSMA, RSMA), precentral gyrus (LPRCN, RPRCN), postcentral gyrus (LPSCN, RPSCN), cerebellum (LCBELL, RCBELL); and left middle temporal gyrus (LMTG), left insula (LINS), left hippocampus, and left middle frontal gyrus (LMFG). In addition to these anatomically defined ROIs, bilateral extrastriate body area (LEBA, REBA) was also defined spherically (centralized at: +/-46 -70 1; radius=8mm). The time course extracted for each participant over the activated voxels within each ROI originated from the normalized and smoothed images, which were high-pass filtered and had the linear trend removed. For

each participant, the intensity of fMRI signal per voxel in all the previously defined anatomical ROIs for the experimental task was compared to that for the fixation baseline using a *t*-test with a statistical significance level of $p < 0.05$. The average percentage change in signal intensity was then calculated for each ROI and the statistically significant difference was tested between the imitation task and fixation baseline. Thus for each ROI, for each participant, the mean percent change in signal intensity reflected the amount of difference in the BOLD contrast-related changes between the experimental (imitation) task and the fixation baseline.

Functional Connectivity. Functional connectivity (correlation in time between activation of brain regions) was computed separately for each participant as a correlation between the average time course of all the activated voxels in each member of a pair of regions of interest (ROIs). This analysis utilized the same ROIs used for the PSC analysis which were based on areas main clusters of activation in the group activation map for imitation conditions contrasted to the fixation baseline as well as ROIs based on apriori hypotheses. Labels for the ROIs were assigned with reference to the parcellation of the Montreal Neurological Institute (MNI) single subject T1 weighted dataset carried out by Tzourio-Mazoyer and colleagues (Tzourio-Mazoyer et al., 2002). The activation time course extracted for each participant over the activated voxels within the ROI originated from the normalized and smoothed images, which were high-pass filtered and had the linear trend removed. The functional connectivity correlation was computed on the images from all imitation stimuli. As such, it reflects the synchronization between the activation in two areas while the participant is performing the task and not during the fixation condition. Fisher's *r* to *z* transformation was then applied to the correlation

coefficients for each participant prior to averaging and statistical comparison of the two groups. These ROIs were also separately clustered into networks based on function (MNS, motor regions, visual regions, insula, thalamus) and a network analysis was run analyzing the correlation between activation time courses between networks. In order to explore the relationship between functional connectivity patterns and scores on neuropsychological measures, bivariate correlations were run in SPSS between scores on measures of motor skills (PANESS), empathy, and PIQ with FCA network connectivity values for the overall group.

3. Results

3.1 Overview

The main results of this study are summarized as follows: 1) Both ASD and TD groups showed strong activation in ventral premotor cortex along with ventral temporal and occipital areas while imitating actions; 2) The ASD group showed significantly increased activation (ASD >TD) in lingual gyrus and middle temporal cortex during imitation; 3) Analysis of percent change in signal showed significantly increased activity in ASD participants (ASD > TD) in several regions, such as RIFG, LEBA, right cerebellum, and RSMA; 4) Functional connectivity measures indicated greater posterior area connectivity in ASD participants (ASD > TD), especially connectivity of the visual network with STS and insula; and 5) There were no statistically significant group differences in imitation ability and attention.

3.2 Neuropsychological Testing

Participants in both groups differed significantly on several measures of neuropsychological assessment. For empathizing ability, the ASD group had significantly lower scores on the EQ (ASD M=24) than the TD group (TD M=42) [$t(26)= -5.34$, $p<0.01$]. The ASD group also scored significantly higher on both measures of autism symptomatology, the AQ [$t(25)=3.97$, $p<0.01$] and the SCQ [$t(25)=5.30$, $p<0.01$]. In terms of motor skills, the ASD group displayed significantly greater difficulty as indicated by the PANESS scores [$t(26)=2.36$, $p<0.05$]. While not significant, the ASD group also showed borderline significantly lower scores on the Reading the Mind in the Eyes Task [$t(27)=-1.99$, $p=0.06$]. The groups did not differ significantly on other neuropsychological measures including IQ and visual-motor skills (see Table 1).

Insert Table 1 about here

3.3 Behavioral Results

Measures of imitation ability were recorded and coded for accuracy outside the MRI scanner following each participant's scan. This was done in order to allow for better measurement of imitation ability in real-world environments where participants are able to see their hands/arms and are allowed a free range of movement, in contrast to the restricted space in the MRI scanner. A two-tailed between-group t-test performed to examine group differences in imitation ability revealed no statistically significant differences [ASD M=127; TD M=129; $t(25)=-1.30$, $p=0.21$]. In addition, qualitative analysis showed that all subjects within both groups were generally able to perform the task, with scores ranging from 108-130 for the ASD group and from 127-130 for the TD

group. A two-way between-groups analysis of variance (ANOVA) was conducted to explore the effect of group (ASD or TD) and attention networks task (ANT) task condition (congruent, incongruent, or neutral) on ANT accuracy and reaction time separately. Results of this analysis revealed a significant main effect of group [$F(1,69) = 6.26$, $p<0.05$] and a main effect of condition [$F(2,69) = 3.50$, $p<0.05$] but no group x condition interaction [$F(2,69)=0.11$, $p>0.05$] for reaction time. Follow up analyses revealed, however, that there were no significant differences in reaction time between groups for any of the conditions analyzed separately [Congruent: $t(26)=-1.40$, $p>0.05$; Incongruent: $t(26)=-1.55$, $p>0.05$; Neutral: $t(26)= -1.39$, $p>0.05$]. Both groups were significantly slower for incongruent trials compared with congruent trials [Incongruent $M= 841.95$, Congruent $M=723.88$; $t(54)= -2.43$, $p>0.05$]. There was also no main effect of group [$F(1,69) = 0.56$, $p>0.05$] or condition [$F(2,69) = 2.11$, $p>0.05$] for accuracy; nor was there any group x condition interaction [$F(2.69)=0.47$, $p>0.05$].

A multiple regression was conducted to determine the role of attention on imitation performance with psc values from core MNS ROIs as dependent variable and ANT accuracy and RT as independent variables. We found a marginally significant relationship between psc in RIFG and overall RT on the ANT for all participants [$F(3,25)= 2.60$, $p=0.08$]. Follow-up correlation analyses revealed a significant relationship between RIFG psc and RT for all conditions separately [Congruent: $r=0.40$, $p=0.05$; Incongruent: $r=0.51$, $p=0.01$; Neutral: $r=0.51$, $p=0.01$]. RIFG psc values were not, however, significantly related to accuracy [$F(3,25)= 0.60$, $p>0.05$]. Regression analyses also revealed that, within the ASD group, there was a significant relationship between LIFG psc values and RT for incongruent trials ($r=0.61$, $p<0.05$). A significant

relationship between psc in the RIPL and overall RT on the ANT was also found for TD participants [$F(3,10) = 4.01$, $p<0.05$]. Follow-up correlation analyses revealed a significant relationship between RIPL psc and RT for incongruent stimuli for the TD group [Incongruent: $r= -0.40$, $p<0.05$]. However, there was no relationship between psc in the RIPL and RT on the ANT for the ASD group [$F(3,12] = 1.17$, $p>0.05$]. Also, no relationship was found between ANT overall RT and psc in the LIPL for either the TD [$F(3,10) = 1.90$, $p>0.05$] or the ASD [$F(3,12) = 0.93$, $p>0.05$] group.

3.4 Within-Group and Between-Group Activation Patterns

The ASD and TD participants showed strong activation in core MNS regions (Ventral premotor cortex including IFG and precentral gyrus along with IPL) as well as in occipital and ventral temporal areas while imitating actions ($p<0.001$; $k = 136$ contiguous $2mm^3$ voxels determined by Monte Carlo simulation). In addition to these areas of common activation, the ASD group alone also displayed activation in the right anterior insula, thalamus, and right middle frontal gyrus (RMFG). The TD group, on the other hand, showed increased activation for imitation in the right cerebellum, right fusiform gyrus, right inferior temporal gyrus (RITG), LSTS, and right lingual gyrus (see Figure 3 & Table 2). A two-sample t-test in SPM8 to examine group differences revealed significant increase in activation during imitation in ASD, relative to TD, participants in the left middle temporal gyrus, the left lingual gyrus, and the left parahippocampal gyrus, areas involved in visual processing (Epstein & Kanwisher, 1998; Bogousslavsky et al., 1987; Mangun et al., 1998), memory retrieval (Decety et al., 1997; Visser et al., 2012; Cho et al., 2012; Menon et al., 2000), and encoding the location of items in space

(Sommer et al., 2005; Maguire et al., 1998). No significant differences in activation within core MNS regions were found. The TD group did not show any areas of increased activation over the ASD group ($p<0.005$, with an extent threshold of 144 contiguous $2mm^3$ voxels) (see Figure 2).

Insert Figure 2 and Table 2 about here

3.5 Percent Signal Change (psc)

PSC values were extracted for individual participants from activated voxels from a set of functionally determined, but anatomically defined ROIs. These ROIs were chosen based on the pattern of group level activation (for ASD + TD) in the imitation condition contrasted with a fixation baseline to best represent the activation for all participants during imitation. The ASD group, relative to TD, showed significantly increased psc in areas related to motor control/execution including the right cerebellum [$t(24)=2.30$, $p<0.05$] and the right SMA [$t(25)=2.52$, $p=<0.05$]. The ASD group also displayed increased psc in the left EBA [$t(22)=2.30$, $p<0.05$], a region involved specifically in the processing of visual stimuli related to the body (Downing et al., 2001). While there were no significant group differences in activation of the MNS areas in our SPM-based analysis, the ASD group displayed significantly greater psc for the anterior component of the MNS, the right IFG, when compared with the TD group [$t(23)=2.05$, $p<0.05$] (see Figure 4). A significant negative correlation between psc in the left EBA and PANESS scores was found for the overall group ($r= -0.48$, $p<0.05$). When the two groups were analyzed separately, both the ASD group and the TD group showed a significant negative correlation between PANESS scores and psc in the left EBA (ASD:

r= -0.62, p<0.05; TD: r= -0.78, p<0.05), suggesting greater motor difficulty was associated with less change in activation in this region.

Insert Figure 3 about here

A psc network analysis was also performed examining overall psc values during imitation in motor (LPSCN, RPSCN, LSMA, RSMA, REBA, LEBA, LCEBLL, RCEBLL), mirror (LIPL, RIPL, LIFG, RIFG), and visual (LMOG, RMOG) networks for each group. Results of the network analysis indicated increased motor related psc in the ASD group [t(171)=3.76, p<0.01]during the imitation task. Results also showed increased psc in both mirror [t(96)=3.31, p<0.01]and visual [t(49)=2.28, p<0.05] for the ASD group as compared with TD controls. Thus, results from psc analyses indicate not only an increased reliance on individual regions involved in these networks but in the overall networks for participants with ASD.

3.6 Functional Connectivity Analysis

Functional connectivity network analysis was conducted to examine group differences in connectivity between the MNS and related brain networks and regions (including the motor system and visual system as well as insula, EBA, and STS). There were no areas or networks of increased functional connectivity for the TD group. However, the ASD participants showed overconnectivity (ASD > TD) of the visual system with both the insula [t(22)=2.30, p<0.05] and STS [t(25)=2.57, p<0.05]. An additional lobe-based functional connectivity network analysis revealed significantly increased connectivity in ASD (ASD>TD) between the left and right occipital lobes [t(27)=2.71, p<0.05], between the left and right parietal lobes [t(24)=2.57, p<0.05], between left temporal and left occipital lobes [t(26)=2.69, p<0.05], and between the left

temporal and right occipital lobes [$t(26)=2.32$, $p<0.05$] (see Figure 5). Thus, there were significant overconnectivity in ASD participants in relatively posterior areas of the brain, especially involving occipital, temporal, and parietal networks.

Insert Figure 4 about here

To examine the relationship between functional connectivity in identified hypothesis-based networks and measures of behavioral characteristics, bivariate correlations were run between connectivity values obtained from the network analysis and scores on neuropsychological measures. Results of this analysis indicated that PIQ scores were positively correlated with functional connectivity between the MNS and visual cortex ($r=0.48$, $p<0.05$). In addition PANESS scores for the whole group were also positively correlated with functional connectivity between the MNS and motor systems ($r=0.44$, $p<0.05$) (see Figure 6).

Insert Figure 5 about here

4. Discussion

This fMRI study of motor imitation in children and adolescents revealed intact imitation performance in ASD participants accompanied by robust MNS activity and increased functional connectivity. This finding is consistent with recent studies failing to find decreased activation of the MNS in ASD (e.g., Oberman, Ramachandran, & Pineda, 2008; Raymaekers, Wiersema, & Roeyers, 2009; Avikainen, Kulomaki, & Hari, 1999), especially an fMRI study involving similar paradigm using repetitive suppression (Dinstein et al., 2010). These results are also congruent with recent findings suggesting that individuals with ASD, although delayed, develop imitation skills and that imitation deficits present in older children and adults with autism may be limited to more complex

imitative tasks (Rogers et al., 1996; Hobson & Lee, 1999; Avikainen et al., 2003). Studies previously finding decreased activation in the MNS in autism often included additional components that were not present in the current study including dynamic stimuli (e.g., Oberman et al., 2005; Martineau et al., 2008), emotional stimuli (e.g., McIntosh et al., 2006; Dapretto et al., 2006), or complex action planning components (e.g., Rogers et al., 1996). Such tasks are more demanding and may elicit deficits in activation and coordination in MNS in individuals with autism. Although no areas of decreased functional connectivity with frontal regions were found, these results are consistent with previous research indicating greater posterior and local functional connectivity in ASD (Courchesne et al., 2005; Sahyoun et al., 2010; Samson et al., 2012). Moreover, these findings are also consistent with recent neuroimaging studies which suggest that connectivity patterns in children with autism may be different from that of adults with children usually showing overconnectivity (Uddin, Supekar, & Menon, 2013; Supekar et al., 2013).

4.1 Increased Reliance on Visual and Memory Processing Regions in ASD

While the ASD group displayed differences in psc/functional connectivity patterns involving components of the MNS, between-group differences in neural recruitment were not limited to mirror regions. Consistent with previous research indicating an increased use of visual processing regions in ASD (e.g., Mottron et al., 2006; Soulières et al., 2011; Samson et al., 2012), the ASD group showed increased activation in several neural regions related to visual processing of information during the current imitation task. This included greater psc (compared with the TD group) in the

EBA, a region involved in visual processing of body-related images (Downing et al., 2001) and a key component of the action observation network (AON) (Caspers et al., 2010; Koski et al., 2002). This activation within the AON may indicate that individuals with ASD are using "visual thinking" in order to imagine the action of the other or to produce a mental image of their own movement to assist in performing imitation. Of potential importance to the current task, the EBA has also been hypothesized to play a role in sorting self and other body images and connecting them (Myers & Sowden, 2008). As such, it is thought that this region may play a vital role in visual processing of self-other discrimination (Vocks et al., 2010). Results also indicated additional activation for the ASD group in the left lingual gyrus during imitation. This is consistent with previous findings of increased grey matter volume (Cauda et al., 2011) and activation (Noonan, Haist, & Muller, 2009) in this region for autism. The lingual gyrus has been shown to play a role in visual processing and daydreaming (Ganis, Thompson, & Kosslyn, 2004). Along with activation in the AON, this increased activation provides additional evidence for the use of mental imagery to perform the task and suggests a more visual approach to the imitation task.

Functional connectivity analyses also revealed evidence of increased reliance on visual system regions for the ASD group. Compared with TD participants, ASD participants demonstrated increased connectivity of the visual system with both the insula and STS. The ASD group also showed increased functional connectivity between the left and right occipital lobes, and between the left temporal lobe with both the left and right occipital lobes. Together this pattern of both increased activation and functional

connectivity in ASD suggests greater reliance on low-level visual processing to perform these imitations.

Group differences in activation also reveal an increased reliance on memory processing for the ASD group. More specifically, greater activation in the parahippocampal gyrus and middle temporal gyrus may both be associated with increased reliance on neural processing related to action memories. The parahippocampal gyrus is involved in processing related to memory of actions (Decety et al., 1997) and in familiarity based recognition processes (Yonelinas et al., 2002; Holdstock et al., 2005). Increased activation in the LMTG also suggests that the ASD group is potentially relying on semantic memory processing to imitate these relatively simple hand postures (Martin & Chao, 2001; Menon et al., 2002; Wei et al., 2012).

In addition to the region's previously mentioned role in visual processing, the lingual gyrus is also involved in memory processing (Bogousslavsky et al., 1987; Cho et al., 2012; Menon et al., 2000). As such, increased activation in this region for the ASD group may indicate that participants are attempting to identify the gesture or symbol created by the hand image displayed (Price et al., 1996; Farah, 2004). Each of these neural regions are components of the ventral visual stream, which has been implicated specifically in semantic processing necessary for imitation of meaningful (rather than meaningless) gestures (Rumiati et al., 2005). However, all of the stimuli used for this study displayed meaningless hand postures. Thus, activation of these regions suggests that individuals with ASD may be processing these stimuli through the same neural pathways as those generally reserved for processing of meaningful actions, resulting in increased reliance on memory mechanisms to determine the semantic role. Given the

intact ability of the ASD group to perform the imitations, this activation pattern may indicate the use of a compensatory mechanism. However, further examination would be necessary in order to determine how this neural pattern would hold if presented to participants unable to perform the imitations.

4.2 Increased Reliance on Motor-Related Regions in ASD

The ASD group also showed increased psc over the TD group in key motor regions including the cerebellum and SMA. The cerebellum has been implicated in visuomotor tasks requiring a combination of visual and kinesthetic information (Liu et al., 1999; Imamizu et al., 2000), an integration that is vital to imitation (Hagura et al., 2009). The SMA is also involved in motor planning and sequencing (see Nachev, Kennard, & Husain, 2008 for review). Previous research has found atypical activation in both of these regions in autism when individuals are executing motor tasks (Mostofsky et al., 2009). In addition to increased activation in these key motor regions, the ASD group also displayed greater activation in other neural regions previously implicated in motor planning and control including the RIFG and EBA. Along with its role in the MNS, the RIFG has also been implicated in control of fine motor movements (Liakakis, Nickel, & Seitz, 2011) and in motor response inhibition (Picazio et al., 2013; Menon et al., 2001; Rubia et al., 2003; Aron, Robbins, & Poldrack, 2004; Kana et al., 2007). Given the role of the RIFG in motor inhibition, this increased activation in the ASD group could indicate a need for additional top-down control of motor functioning in order to accomplish the simple imitation task. The finding of lower activation for the TD group might result as an artifact of the ease of task for this group (Wang et al., 2006; Hampshire

et al., 2010; Hughes et al., 2013). Additional support for this hypothesis comes from regression results. These results indicated that psc in the RIFG was positively correlated with RT for all ANT attention tasks (congruent, incongruent, and neutral) for the overall group, suggesting that greater activation of this region is related to increased need for inhibition or control of attentional resources. Previous research suggests that reduced ASD symptoms are associated with increased grey matter volume in the RIFG (Parks et al., 2009), indicating that the additional activation in this region may serve a compensatory function.

While the EBA is primarily a visual region, it has also been found to activate not only during perception of other people's body parts, but also during goal-directed movements of the observer's body parts (Astafiev et al., 2004). As such, the EBA has been suggested to contribute to motor planning of goal-directed actions by providing a representation of the goal posture of an action plan (Zimmermann, Meulenbroek, & de Lange, 2012). Regression results indicated that PANESS scores were negatively correlated with psc in the left EBA for the overall group, suggesting that poorer motor skills were correlated with less reliance on the EBA during imitations. Thus, given the region's role in motor planning, increased activation in this region for ASD may also represent a compensatory neural approach to assist with motor functioning necessary for the task. Overall, increased activation in motor processing regions indicates that individuals with ASD required additional motor-related processing resources to perform the relatively simple imitations. Given a more complex imitation task, these motor difficulties may contribute to impaired or delayed performance in ASD (Mostofsky et al., 2006).

4.3 Overall Greater Reliance on Motor, Mirror, & Visual Regions in ASD

Overall results indicated that, despite intact ability to perform the simple hand imitations, the ASD group utilized a unique cognitive approach to accomplish the task. This approach was characterized by an increased reliance on mirror, visual, and motor processing regions for the ASD group compared to TD participants. In addition to activation and functional connectivity results indicating an increased reliance on individual regions comprising each of these networks, a psc analysis combining ROIs involved in each network (mirror, visual, and motor) further demonstrated significantly increased reliance on all three systems for this imitation task in the ASD group. There are two possible reasons for this finding. First, it could represent an innate difference in neural approach to imitation in individuals with ASD. However, it is also possible that the low activation in these regions for TD participants was due to the relatively simple nature of the imitation tasks included in the study. Therefore, the activation difference in the ASD group may not represent a unique neural approach to the task but, instead, result from increased difficulty of the task for these participants. Analyses examining brain-behavior relationships provide additional evidence that increased functional connectivity may be related to increased difficulty. More specifically, these results indicate that increased motor difficulty (as measured by the PANESS) is correlated with increased functional connectivity between mirror and motor system regions. Thus, a greater need for integrated use of the MNS and motor systems may represent an effect of motor difficulty. However, further research is necessary to determine the exact nature of group differences in neural activation and functional connectivity during imitation.

4.4 Study Limitations and Future Directions

Although the current study provided additional evidence of intact imitation ability despite the presence of aberrant activation and functional connectivity patterns in ASD, limitations of the current study should be considered when evaluating these findings. One limitation of the study was the use of a fairly homogeneous group of both TD and ASD participants. While research involving neuroimaging often requires specific subject characteristics (older, average cognitive functioning), these limitations also reduce the generalizability of results. Additional research is therefore necessary to determine the degree to which the unique patterns of activation in connectivity displayed in the ASD group are applicable to individuals of other ages or cognitive levels. Longitudinal or cross-sectional studies could further elucidate the developmental course of the aberrant processing pattern and imitation skills in ASD. Use of this study design and a range of imitation tasks of different complexities could also assist in determining if the aberrant processing pattern is related to the imitation difficulties observed in ASD and, if so, whether this processing pattern results in imitation or social processing deficits or develops later as a compensatory strategy.

Additionally, results from the current study suggest potential involvement of increased reliance on top-down motor control for the ASD group. Given that the controlled fMRI study environment may minimize reliance on these regions and, thus, mask the potential interfering effects of increased need for these regions, future research should evaluate neural processing patterns during imitation produced in a more realistic setting. The finding of increased MNS activation and intact behavioral performance for simple imitation in the ASD group provided additional evidence that the type and

complexity of imitation tasks used in previous research may underlie inconsistent of findings within the literature. Development of scales to determine the complexity of imitation tasks presented and the comparison of imitation tasks involving a range of complexity would assist in further elucidating the potential effects of the abnormal neural processing observed and at which point this results in decreased efficacy in producing imitations.

5. Conclusions

Results indicate an intact ability for simple hand imitations in ASD. While the ASD group did not demonstrate decreased activation in any of the core MNS regions, they did show increased activation and functional connectivity in mirror, motor, and visual neural systems. Consistent with previous research, these findings argue against a global deficit in the functioning of the MNS and against an overall deficit in imitation in ASD. However, they also provide evidence that, even when able to perform an imitation task, individuals with ASD demonstrate abnormalities in the neural approach to the task. Although these results demonstrate an intact ability for simple imitations in children and adolescents with ASD, the increased reliance on these neural systems for this task suggests that the ASD group may have experienced greater difficulty when performing these simple hand imitations. Given the simplistic nature of the current imitation task and the unrealistic environment presented during neuroimaging, future studies may provide additional information regarding the degree to which these abnormal processing patterns may affect more complex imitation tasks presented in real-world social environments.

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Table 1. Table displaying scores on neuropsychological measures by group.

IMITATION

	ASD			TD			
Measure	**n = 15**	**Range**	**S.D**	**n = 13**	**Range**	**S.D.**	**p-value**
Age	13	8-17	2.79	12	9-15	1.76	0.17
EQ	24	9-42	7.64	42	23-54	10.25	<0.01
SQ	26	15-50	8.47	29	14-40	7.99	0.42
FSIQ	109	80-126	16.96	103	83-130	15.57	0.33
VIQ	108	75-128	16.88	101	84-134	18.77	0.4
PIQ	108	75-124	15.23	98	84-119	9.13	0.09
SCQ	17	1-31	8.29	3	0-10	3.66	<0.01
VMI	92	66-117	12.4	98	86-110	6.18	0.11
VMI Motor	89	51-110	13.65	92	75-107	11.77	0.6
PANESS	57	39-78	12.04	47	36-71	9.41	0.03
AQ	78	32-135	28.67	32	5-67	23.18	<0.01
RME	19	11-24	3.27	21	17-25	2.36	0.06
Imitation	127	108-130	5.94	129	127-130	1.22	0.21

Table 2. Table displaying areas of activation for each group when imitation conditions are contrasted with a fixation baseline.

Imitation vs. Fixation (TD)

Region	x	y	z	Hem	BA	Cluster	t
Postcentral/Precentral	-34	-24	50	L	3	3282	14.46
Middle/Superior Occipital	32	-64	38	R	19	1894	11.90
Supplementary Motor Area	6	18	50	R	8	1151	10.59
Cerebellum/Fusiform/ITG	30	-54	-22	R	NA	1531	8.60
Inferior Frontal	52	8	28	R	44	747	7.18
Inferior/Middle Occipital	-50	-76	-10	L	19	628	6.81
Lingual/Calcarine	2	-84	-12	R	18	758	6.44
Superior Temporal	-58	14	-8	L	38	206	6.39

Imitation vs. Fixation (ASD)

Region	x	y	z	Hem	BA	Cluster	t
Middle Occipital	-44	-86	-2	L	18	20390	12.84
Precentral/Inferior Frontal	-52	4	28	L	9	1850	8.04
Supplementary Motor Area	-4	0	50	L	24	2298	6.74
Precentral/IFG/Insula	52	6	30	R	9	1803	5.98
Thalamus	-12	-24	-10	L	NA	683	5.79
Inferior/Middle Frontal	56	38	8	R	45	301	5.49

Figure Captions

Figure 1. Figure showing example imitation stimuli. Participants were provided with a high-quality photograph of a hand. They were asked to imitate the hand gesture observed in the image.

Figure 2. The within-group and between-group activation patterns for imitation stimuli (contrasted with a fixation baseline) are shown. For this contrast, analyses were run on $p<0.01$ uncorrected with a voxel threshold of 136 continuous $2mm^3$ voxels (as determined by Monte Carlo simulations).

Figure 3. Graph displaying areas of increased PSC values for the ASD group. The ASD group showed significantly greater PSC in the Left EBA, Right Cerebellum, RIFG, and Right SMA. The TD group did not show any areas of significantly greater PSC.

Figure 4. Graph displaying areas of significantly greater functional connectivity for the ASD group. Results of a network analysis indicated significantly greater functional connectivity between the visual system and both the insula and STS for the ASD group. There were no areas of significantly greater functional connectivity for the TD group.

Figure 5. Scatterplots displaying significant correlations between network-based functional connectivity and scores on neuropsychological measures. These plots display the positive correlation between MNS:Visual Cortex connectivity and PIQ scores. They also display the positive correlation between MNS: Motor Cortex connectivity and PANESS scores.

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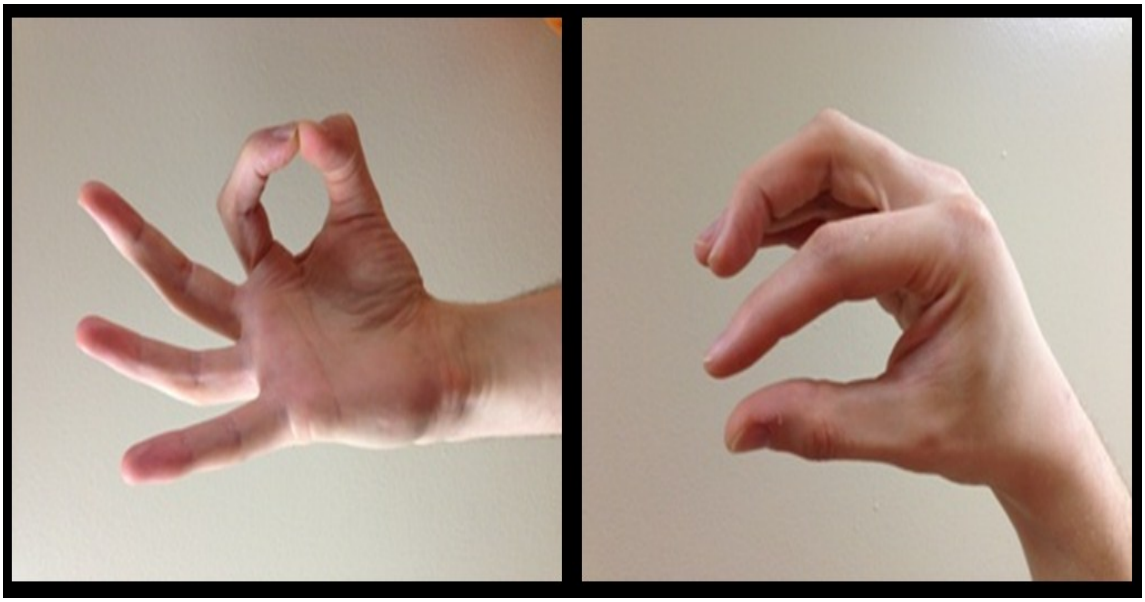

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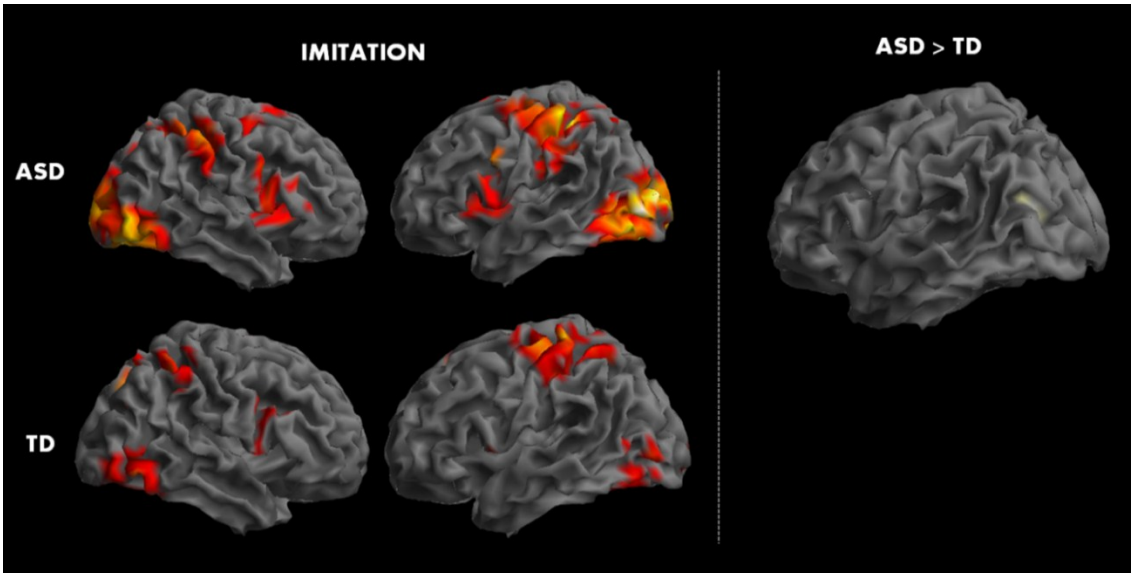

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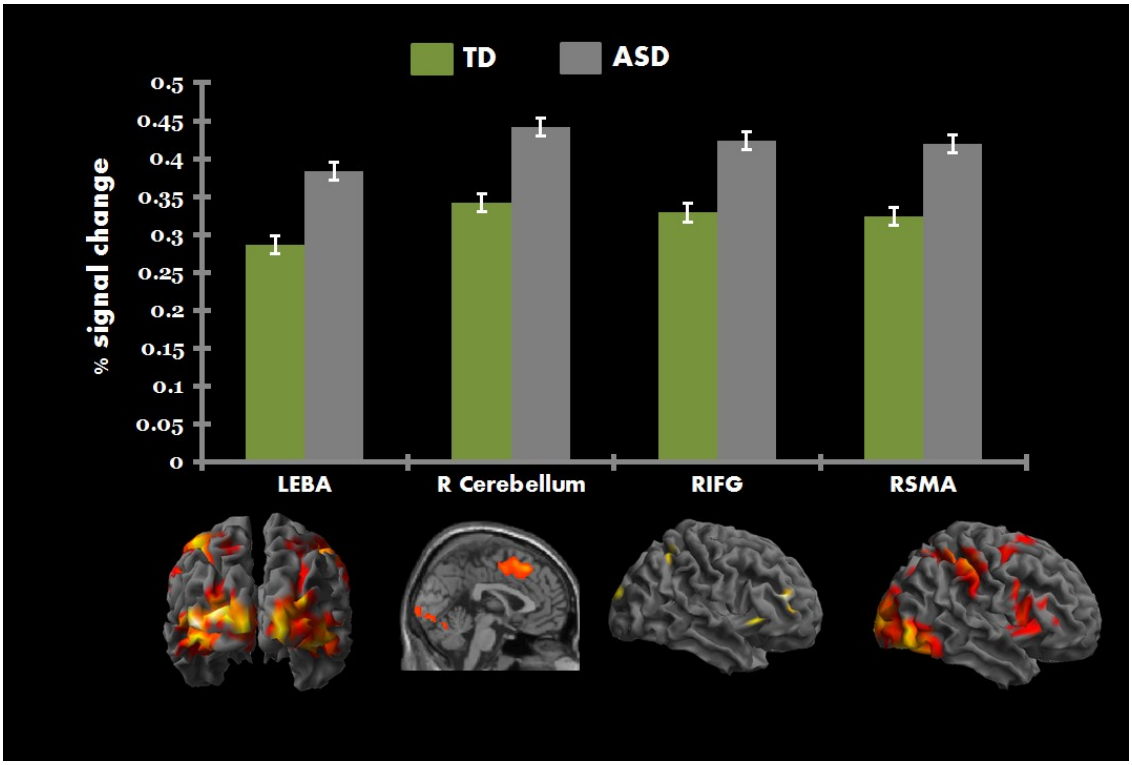

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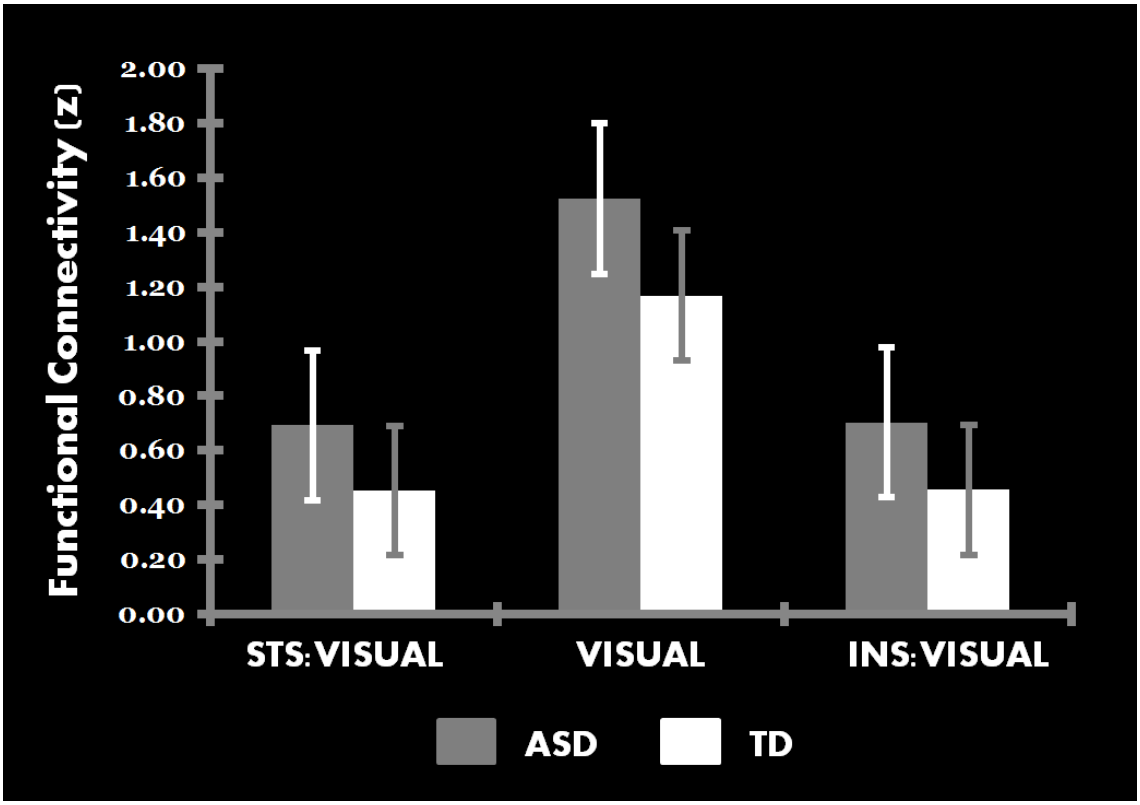

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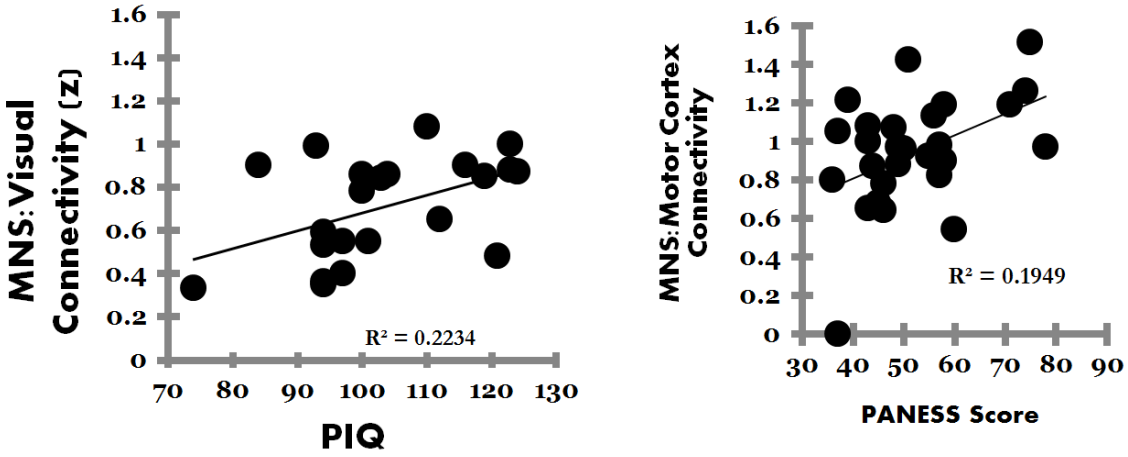

SUMMARY

The current project involved the use of three studies (each targeting components of imitation) to evaluate imitation in children and adolescents with ASD. The studies analyzed behavioral performance on tasks, the integrity of the MNS, and brain-behavior relationships related to each component. Below is the summary of each specific aim and the related findings.

Specific Aim # 1: Investigating Behavioral Performance in ASD

This project evaluated behavioral performance of high-functioning children and adolescents with ASD and TD controls on tasks comprising the various components of imitation. Consistent with study hypotheses, individuals with ASD performed significantly better on the task involving mental rotation of hands. However, further analyses revealed this to be an effect of cognitive level (i.e., PIQ) rather than an effect of group (ASD or TD). This finding, however, is likely still meaningful given that visuospatial ability is an important component of tasks measuring PIQ. Therefore, the ASD group's higher PIQ scores may be reflective of this group's advantage in spatial tasks such as mental rotation. Although it was predicted that the ASD group would be less accurate for tasks involving mental imitation, no group differences in accuracy were found for this study. Consistent with hypotheses, participants with ASD did demonstrate longer reaction times for both conditions (transitive and intransitive actions) compared with TD controls. This finding indicates that, while both groups were able to perform the

task, the ASD group may have experienced greater difficulty. More research is required to determine the extent to which this longer reaction time may influence the use of skills in real-world social environments. Also consistent with study hypotheses, participants with ASD demonstrated an intact ability to perform the simple hand imitations when explicitly instructed to do so. Analysis of the data revealed a ceiling effect for accuracy for both groups, demonstrating the simplistic nature of this task for the particular subjects included in the current study.

Specific Aim # 2: Investigating Integrity of MNS in ASD

This project also examined the functional integrity of different cognitive (visuospatial, attention, motor simulation) and brain (MNS) systems in mediating imitation ability and its dysfunction in children and adolescents with autism. It was predicted that individuals with ASD would show decreased activation in the frontal component of the MNS (IFG) as well as reduced functional connectivity between the posterior (IPL) and anterior (IFG) components of the system. Results instead revealed intact to increased activation and functional connectivity throughout the MNS in ASD. Group differences in activation and functional connectivity were found, however, both within the MNS and other neural regions. Together these studies revealed a number of interesting emerging themes regarding the underlying neural processes of imitation in ASD.

Intact to Increased Activation of the MNS

Contrary to original study hypotheses, results of these studies indicated that individuals with ASD demonstrated aberrant, but not reduced, activation in the core MNS regions. This group difference in MNS activation centered mostly on increased activation of the IFG for the ASD group. Specifically, the ASD group displayed increased activation in the LIFG for the mental imitation task and increased psc in the RIFG for the motor imitation task.

Although not consistent with original study hypotheses, this result is in line with previous findings of increased frontal activation in ASD during tasks involving visual pursuit (Takarae et al., 2007), motor inhibition (Schmitz et al., 2006), observation of human motion (Martineau et al., 2010), and theory-of-mind (Brambilla et al., 2004). Additional LIFG activation has also been found in unaffected siblings of children with ASD while performing an executive functioning task (Spencer et al., 2012). While potentially resulting from increased task complexity (Martineau et al., 2010), previous research has indicated that this additional activation in frontal regions in ASD may also function as a compensatory mechanism. For example, a study performed by Baron-Cohen and colleagues (2006) found that parents of individuals with ASD showed increased activation in the frontal cortex during the Reading the Mind in the Eyes task. They hypothesized that greater activation of the frontal cortex may act as a compensatory strategy for managing underlying perceptual differences. Although no correlations with social functioning were found in the current study, an increase in IFG activation in ASD has been previously found to be associated with better social functioning (Bastiaansen, 2011).

The current findings of intact MNS activation and intact ability for tasks are also compatible with previous findings indicating that individuals with ASD can override these automatic top-down processes and activate their MNS by intentionally deeming some actions as important, such as when tasks are presented in an explicit manner (McIntosh et al., 2006; Shields, 2012). When stimuli are not automatically deemed as relevant, aberrant top-down control processing may lead to atypical recruitment or the lack of recruitment of the MNS (Cook & Bird, 2012; Oberman et al., 2008).

Intact to Increased Functional Connectivity in ASD

In addition to examining activation of the MNS in ASD, these studies also assessed the integrated functioning of the system and the functioning of the system with related neural regions. While there were also no findings of decreased functional connectivity in ASD, participants with autism demonstrated increased connectivity of the insula with IFG, motor, parietal, and visual cortex regions. Aberrant functional connectivity patterns also included increased connectivity between occipital and temporal regions during imitation tasks. While several previous studies have implicated underconnectivity in autism (e.g., Just et al., 2004; Courchesne & Pierce, 2005; Villalobos et al., 2005; Cherkassky et al., 2006; Kana et al., 2006), many of these studies were conducted with adults with ASD. Recent research examining children with ASD has instead suggested patterns of aberrant connectivity, with many neural regions displaying increased functional and anatomical connectivity (e.g., Billeci et al., 2012; Di Martino et al., 2011; Uddin, Supekar, & Menon, 2013; Uddin et al., 2013; Supekar et al., 2013). This has led to the suggestion of "hyper connected" brains in children with ASD

that become less connected over time (Uddin, Supekar, & Menon, 2013).It is believed that this increased connectivity may create an excess of neural "noise". It is possible that this increased activation or connectivity in the MNS for ASD may be related to the observed deficits in imitation and other social skills during real-world social interactions.

Increased Reliance on Other Neural Networks in ASD

However, aberrant activation and connectivity patterns in the ASD group were not confined to the MNS. Overall, results from these three studies show that the ASD group demonstrated an increased reliance on a wider neural network, which may represent a compensatory neural approach to the tasks. Abnormal activation and connectivity may also contribute to abnormal modulation of the MNS. While participants were still able to perform the tasks involved in the current set of studies, aberrant responses of neural systems interacting with the MNS may result in abnormal imitation in situations outside of the study environment. Specifically, these abnormal interactions may lead to decreased social motivation and, thus, decreased occurrences of spontaneous imitation (Ingersoll, 2008; Oberman et al., 2008; Wang, Ramsey, & Hamilton, 2011).

One particular region consistently showing increased connectivity and activation in ASD during all tasks included in the current studies was the insula. As a component of the salience network (SN), the insula is involved in attention focusing based on proper identification of relevant stimuli (Menon & Uddin, 2010). Hyperconnectivity of the SN is a fairly consistent finding in ASD research (Uddin et al., 2013; Supekar et al., 2013; Toyomaki & Murohashi, 2013). The insula is connected to the frontal component of the MNS (the IFG) and is thought to modulate the system by assisting in the determination of

relevant stimuli (Craig, 2010). Therefore, abnormal connectivity with or activation of the insula in ASD may influence functioning of the MNS. Increased activation of the insula in ASD has been hypothesized to result in subsequent difficulty identifying relevant stimuli (Di Martino et al., 2011; Uddin et al., 2013). Given the insula's role in switching between default mode network and active processing (Menon & Uddin, 2010; Goulden et al., 2014), additional activation in this region may also indicate that this is not as fluid or automatic in ASD.

Another component of aberrant neural activation in ASD is the activation of memory-related processing regions in ASD for both meaningful and meaningless gestures, as well as both transitive and intransitive gestures. Research suggests that memory related mechanisms are typically implicated in imitation of transitive or meaningful gestures (Mozaz, 2002). Consistent with current results, however, previous findings suggest that individuals with ASD lack this flexible approach to imitation and indicate that they tend to use a similar strategy for both forms of imitation (Wild et al., 2012). While studies propose that the use of a common approach for both forms of imitation in ASD is the result of increased difficulty with imitation of meaningless gestures (Williams et al., 2004; Gowen, 2012; Vivanti & Rogers, 2011), the exact nature of the deficit remains debated. Researchers have hypothesized that deficits in attending to and using kinematic information may contribute to difficulties with imitation of meaningless gestures. Gowen (2012) suggested that less attention to kinematic aspects (thru altered top-down control of attention) may result from a lack of motivation in ASD to attend to and imitate kinematic details of an action, which often contains socially relevant information. While the goal of an action may be motivating, socially based

information is often not naturally reinforcing for individuals with ASD (Dawson et al., 2004; Chevallier et al., 2012).

Group differences in activation and functional connectivity across studies also indicated increased reliance on visual processing regions in ASD. Consistent with the findings of aberrant activation in visual perception regions in the current study, previous research has suggested abnormal bottom-up sensory processing in ASD (Amso et al., 2014) with several studies demonstrating atypical sensory processing in autism (Marco et al., 2011; Wiggins et al., 2009; Coskun et al., 2009). Increased reliance on bottom-up visual processing strategies in ASD could negatively impact both social and cognitive development (Amso et al., 2014). Enhanced bottom-up processing may lead to weak central coherence (Noens & van Berckelaer-Onnes, 2008) and, thus, the need for increased activation of frontal regions to create a "balance" in processing. Appropriate modulation of the MNS probably relies on integration of the top-down and bottom-up processing components and it is likely this balance that is abnormal in ASD.

Specific Aim #3: Addressing Brain-Behavior Relationships

The third main aim for the current set of studies was to explore the relationship between behavioral characteristics of participants and neural functioning in response to tasks involving different aspects of imitation. Several interesting relationships emerged from these analyses. More specifically, correlations were found between neural activation/connectivity and autism symptomatology, attention, motor skills, visual-motor integration, and empathy.

Relationship between psc in the LSTS and Empathy/ Autism Symptoms

One interesting finding was a negative correlation between autism symptoms (as measured by the AQ) and psc in the LSTS during mental rotation. The LSTS has been found to be involved in the integration of visuospatial and social processing (Hoffman & Haxby, 2000; Pelphrey & Morris, 2006). Therefore, increased activation in this region may represent deficits in this integration and, thus, assist in explaining the dichotomy of a visuospatial advantage and social impairment in ASD. Empathy scores (as measured by the EQ) were instead found to be positively correlated with psc in the LSTS for the mental rotation task. This correlation both provides evidence of the potential role of this region in visuospatial processing and empathy (Decety & Lam, 2007; Iacoboni & Dapretto, 2006) and suggests that utilization of an egocentric approach to mental rotation of body parts is related to empathy (Thakkar & Park, 2010; Gronholm et al., 2012). Together these findings suggest that autism symptoms and empathy are related even to the neural approach to mental rotation.

Influence of Attention and Motor Functioning on Neural Approach

Results of the current studies also demonstrate a possible influence of attention on the neural approach to imitation in ASD. Positive correlations between activation in frontal regions and ANT reaction times in ASD suggest that greater difficulty switching attention is related to increased activation in these regions. If increased frontal activation is necessary to maintain attentional control (despite the regulated nature of the imaging environment), then it is likely that the attention control necessary during real-world social situations may result in increased difficulty.

Results of the current set of studies also support a potential role of motor deficits in the neural activation and connectivity patterns observed. Despite being able to perform the simple imitation tasks, individuals with ASD consistently displayed greater motor difficulties as assessed by the PANESS when compared with TD controls Scores on the PANESS were related to a range of behavioral and neuroimaging data across the studies. For example, during the visuospatial rotation task, PANESS scores were positively correlated with psc in the RIPL and with functional connectivity between the MNS and motor systems. During motor imitation, PANESS scores were positively correlated with psc in the left EBA. There also continued to be a positive correlation with functional connectivity between the MNS and motor systems during motor imitation tasks.

There was also a significant correlation between visual-motor integration (as measured by the VMI) and neural activation patterns. More specifically, individuals with lower VMI scores showed significantly greater LIFG and Left Precentral Gyrus psc during the mental imitation task. Together with the findings of motor skill correlations, this suggests that increased task difficulty may be leading to increased recruitment of neural regions. While this did not lead to group differences in ability for any of the current tasks, it may have greater impact in more complex, real-world social environments. However, given the complexity of imitation and the results from the current study, it seems doubtful that motor or visual-motor deficits could independently account for the imitation deficits in ASD. It is instead likely that both motor deficits and lack of social responsivity (due to altered top-down/ attentional control) have an impact on imitation in young children with autism.

Limitations and Future Directions

Several limitations to the current set of studies may also have influenced the results obtained. One limitation affecting the majority of neuroimaging studies is the need for participants to meet certain cognitive requirements. The exclusion of participants who are lower functioning (below IQ requirements) leads to limitations in the degree to which results can be generalized to other populations. Given the higher-functioning nature of participants, all subjects were able to perform the imitation tasks. As such, it is difficult to determine if the neural activation patterns obtained result from the ASD symptomology or represent a compensatory mechanism resulting in their increased ability for the tasks. Further evaluation of neural mechanisms underlying these tasks in lower functioning populations would assist in teasing apart these issues. The current studies also included smaller sample sizes which may have affected power and, subsequently, the ability to find differences that may be present.

Characteristics of the neuroimaging tasks may also limit the degree to which findings can be interpreted. Excessive motion, for example, created the need to remove different participants from each study's data analysis. As such, the participants included in the analyses for the three studies were slightly different. Additionally, although the tasks were designed to separate out the component skills as closely as possible, the complete separation of cognitive skills required for each task was not possible. For example, tasks designed to be non-motor (e.g., visuospatial task, mental imitation task) still required motor performance to answer the question presented. Also, the scanning environment likely eliminates potential external factors that may be influencing results. This includes the removal of distractors, which may make attentional control more

difficult in real-world environments. The explicit nature of the tasks also removes factors related to social motivation that may drive deficits in implicit imitation (McIntosh et al., 2006; Shields, 2012; Ingersoll, 2008). While allowing examination of deeper brain structures, poor temporal resolution of fMRI also creates difficulty determining the temporal sequence of activation patterns obtained.

Future directions should include analysis of anatomical (DTI) data. This data was obtained for all participants of these studies and analysis of this information may assist in determining the degree to which functional connectivity findings may map onto findings of anatomical connections. The fact that both activation and functional connectivity findings differed across tasks supports the proposal that previous results (e.g., results indicating decreased activation in the IFG in ASD) may result from task specific effects rather than underlying differences in anatomical structures or inability to use specific neural regions in ASD. These findings, thus, support the theory of abnormal modulation of the MNS in autism and provide evidence for the importance of further analyzing intrinsic connectivity patterns in ASD to determine the degree of functional connectivity between regions that is independent of task characteristics. Additionally, current results add support for intact and increased functional connectivity in ASD. However, previous studies have also consistently implicated decreased functional connectivity in the disorder (e.g., Just et al., 2004; Cherkasssky et al., 2006; Kana et al., 2006). Recent proposals have suggested that this inconsistency may be due to connectivity patterns in ASD shifting over time (Via et al., 2011; Amaral, 2011; Supekar et al., 2013). While research indicates a possible shift around puberty (Peper et al., 2011; Uddin et al., 2013), the overall developmental trajectory of this shift remains unclear.

Future studies utilizing a longitudinal design are necessary to obtain a clearer understanding of this developmental process. Future research should also continue to focus on the MNS in the context of the larger brain, with a focus on the potential modulatory and cyclical effects of the interaction of the system with other brain networks and regions required for successful imitation in a real-world environment.

Potential Importance of Findings/ Clinical Implications

Along with results from other recent studies, current findings suggest the need to stop focusing on a global under or over connectivity in ASD. Aberrant activation patterns are likely, due to abnormal interactions between neural networks which may result in different activation or connectivity patterns depending on characteristics of the experimental task. Given the current evidence of the importance of external yet related neural networks to appropriate activation of the MNS, focus of interventions should involve improving the functioning of these other networks as a means to improve imitation skills in autism. This should also include neurofeedback methods currently being evaluated as a method of normalizing mirror neuron function in ASD (e.g., Pineda, Friedrich, & LaMarca, 2014; Friedrich et al., 2014). One area of intervention that may be particularly important is the strengthening of executive functioning skills, including work to improve cognitive flexibility. Another important area of intervention appears to be assisting the appropriate functioning of low-level perceptual processes. While focus on details of a visual scene may result in improved visual search, this perceptual strategy will likely have a negative impact on imitation (Neuhaus, Beauchaine, & Bernier, 2010). Therefore, rather than only focusing on imitation, interventions may be more effective by

including a component aimed at improving perception of items from a gestalt perspective.

Results from the current set of studies also indicate the need to focus on appropriate function of the SN as a means to improve attentional control necessary. Recent research has suggested that, in addition to behavioral techniques targeting repetitive behavior, aberrant SN connections may respond well to medical interventions targeting proteins which are specifically increased in ASD (Tang et al., 2014). Thus, combination of a medical intervention approach combined with behavioral interventions aimed at increasing imitation skills may result in even greater improvement. Another way to potentially influence top-down control of the MNS is through instruction techniques aimed at teaching individuals with ASD to focus on learning the "style" of the action. More explicit teaching of imitation of the style of actions may result in subsequent increased amounts of attention to more socially relevant components of imitation.

Current results also suggest that individuals with ASD can perform at least simple imitation tasks when explicitly instructed to do so. Evidence of intact ability for imitation and intact ability for MNS activation in this setting supports the idea that imitation deficits in autism are likely heavily influenced by decreased intrinsic motivation for the social task. Therefore, interventions designed to target motivational aspects and the automatic use of this system in social settings will be particularly important. Interventions should thus continue to work to increase social motivation, potentially by pairing these tasks with external stimuli which are more rewarding. This finding may shed light on the "active ingredients" in therapies known to be effective, including the

Early Start Denver Model (EDSM) approach and Pivotal Response Training (PRT), both of which focus on these aspects of imitation and, more broadly, social learning (Vivanti & Rogers, 2014).

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APPENDIX A

DESCRIPTION OF MEASURES

Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999)

The WASI was developed as a means of obtaining a brief, but accurate measure of intelligence. It has been standardized nationally and results in measures of Verbal, Performance, and Full Scale IQ scores. The WASI consists of four subtests: Vocabulary, Similarities, Block Design, and Matrix Reasoning. Scores on the WASI have been linked to the Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV) and the Wechsler Adult Intelligence Scale – Third Edition (WAIS-III). It has been standardized for use with children and adults ages 6 years and above.

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

The child version of the "Reading the Mind in the Eyes Test" was utilized for the current set of studies to provide information about subjects' theory-of-mind (ToM) ability. This skill was of particular interest to the current studies since ToM has been linked to simulation and MNS functioning (Pineda & Hecht, 2009). This measure assessed the ability of participants to decipher the mental states of other people based on photographs showing the eye region of male and female actors. These photographs were accompanied by four descriptive words (e.g., "serious", "alarmed", "happy", or "scared") and participants were asked to identify the word that best described what the person in the picture was thinking or feeling. Previous research has established that this measure reliably distinguishes individuals with high-functioning autism from healthy controls (Baron-Cohen et al., 2001).

Empathizing Quotient/ Systemizing Quotient (EQ/SQ) (Baron-Cohen et al., 2003)

Parents of participants also completed the parent-report EQ/SQ. This measure follows a likert format and contains a list of statements about real-life situations, experiences, and interests where either empathizing or systemizing skills are required. The questionnaire is combined and consists of a total of 55 items, with four answer choices for each question. Parents rate how true each statement is about their child. The EQ/SQ measure was completed to provide an interesting comparison for both behavioral and neuroimaging data since empathizing has previously been linked to the functioning of the MNS (Buccino & Amore, 2008).

Social Communication Questionnaire, Lifetime Version (SCQ) (Rutter et al., 2003)

The SCQ is a parent-report measure that is used to screen for the presence or the absence of ASD related symptoms. The lifetime version of the SCQ consists of 40 yes/no questions addressing whether a child has ever displayed specific symptoms of ASD. The SCQ has been standardized for use with children ages 4 and older and takes approximately 5-10 minutes to administer. The SCQ was used during the current set of studies to screen TD participants to rule out an ASD diagnosis. If a TD participant had scored above 15 on the SCQ, they would have been excluded from the study.

Repetitive Behavior Scale – Revised (RBS-R) (Bodfish et al., 1995, 1999)

The RBS-R is a parent completed checklist/ rating scale. Questions address the presence or absence of repetitive-stereotyped behaviors across three subscales:

Stereotyped Behavior, Compulsive Behavior, and Self-Injurious Behavior. In addition to assessing the presence or absence of these symptoms, parents are asked to rate the degree to which these symptoms interfere with everyday life.

Physical and Neurological Examination for Subtle (Motor) Signs (PANESS) (Denckla, 1985)

The PANESS is a standardized motor assessment for children. The assessment includes measures of both gross and fine motor movements including walking a line, hopping on one foot, and tapping fingers and feet rhythmically. Higher scores on the measure indicate worse motor performance. The PANESS was designed to be sensitive to developmental changes and to also minimize the need for both equipment and time for the assessment. It can be completed in 15-20 minutes. The subtle signs assessed by this measure have been found to be effective at differentiating early signs of developmental disabilities such as those found in ADHD (Morris et al., 2001; Mostofsky et al., 2003). It has also been previously utilized to examine motor functioning in autism (Mandelbaum et al., 2006; Jansiewicz et al., 2006) and has been found to accurately discriminate boys with autism from controls (Jansiewicz et al., 2006).

Edinburgh Handedness Inventory (Oldfield, 1971)

The Edinburgh Handedness Inventory is a 10-item measurement scale that is designed to assess the level of dominance of a person's left or right hand when performing everyday activities. Each item presents an everyday activity and the person is asked to identify if they prefer to use their left hand, right hand, or have no preference

regarding which hand they would use to complete that task. It yields a motor laterality index. This measure was used to ensure that all participants showed right-hand dominance.

Attention Networks Task (ANT)
(Fan et al., 2002)

The ANT is a computerized measure aimed at examining multiple aspects of attention. The ANT combines the cued reaction time task (Posner, 1980) and a flanker paradigm (Eriksen & Eriksen, 1974) which allows the examination of alerting, orienting, and executive control attentional networks in a single, integrated task. The task involves presenting participants with flanker stimuli in which a target item is presented surrounded by congruent, incongruent, or neutral flankers. The stimulus presentation is also preceded by different cue conditions. The ANT has been previously utilized in both adult and child populations, including populations with attentional difficulties (Johnson et al., 2008; Keehn et al., 2010).

The Developmental Test of Visual-Motor Integration (VMI)
(Beery, 1997)

This measure allows for the assessment of an individual's ability to integrate their visual and motor abilities. Subjects are presented with drawings of geometric forms which are arranged according to increasing difficulty and are asked to copy each of the figures in the space provided below the form. Norms are provided for 2-18 years of age. Administration takes approximately 10-15 minutes to complete. The goal of this measure

is to provide a convenient method for screening of visual-motor deficits which may impact learning, neuropsychological, and behavioral difficulties.

APPENDIX B

IRB PROTOCOL

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator:	WADSWORTH, HEATHER MARIE
Co-Investigator(s):	
Protocol Number:	**F120803009**
Protocol Title:	*Integrated Functioning of the Mirror Neuron System and Its Role in Imitation Deficits in Autism*

The IRB reviewed and approved the above named project on 10/29/2014. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received FULL COMMITTEE review.

IRB Approval Date: 10/29/2014

Date IRB Approval Issued: 11/3/14

IRB Approval No Longer Valid On: 10/29/2015

Identification Number: IRB00000726

Ferdinand Urthaler, MD

Ferdinand Urthaler, M.D.
Chairman of the Institutional Review Board
for Human Use (IRB)

Partial HIPAA Waiver Approved?: Yes

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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