An Investigation into the Mirror Neuron System in Autism Spectrum Disorder Utilising Transcranial Magnetic Stimulation

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I am the author of the thesis entitled *An Investigation into the Mirror Neuron System in Autism Spectrum Disorder Utilising Transcranial Magnetic Stimulation*

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<tr>
<td>ADHD</td>
<td>Attention Deficit Hyperactivity Disorder</td>
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<tr>
<td>APA</td>
<td>American Psychiatric Association</td>
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<td>AS</td>
<td>Asperger’s Syndrome</td>
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<td>ASD</td>
<td>Autism Spectrum Disorder</td>
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<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
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<tr>
<td>CS</td>
<td>Corrugator Superciliii</td>
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<td>DSM</td>
<td>Diagnostic and Statistical Manual for Mental Disorders</td>
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<tr>
<td>EEG</td>
<td>Electroencephalograph</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>FDI</td>
<td>First Dorsal Interosseus</td>
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<tr>
<td>fMRI</td>
<td>functional Magnetic Resonance Imaging</td>
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<tr>
<td>HFA</td>
<td>High Functioning Autism</td>
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<tr>
<td>HFA/AS</td>
<td>High functioning autism and Asperger’s Syndrome Group</td>
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<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
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<tr>
<td>IQ</td>
<td>Intelligence Quotient</td>
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<tr>
<td>M1</td>
<td>Primary Motor Cortex</td>
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<td>MEP</td>
<td>Motor Evoked Potential</td>
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<tr>
<td>MMR</td>
<td>Measles, Mumps and Rubella vaccination</td>
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<tr>
<td>MNS</td>
<td>Mirror Neuron System</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MVI</td>
<td>Mirror-Induced Visual Illusion</td>
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<tr>
<td>PDD</td>
<td>Pervasive Developmental Disorder</td>
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<tr>
<td>PDD-NOS</td>
<td>Pervasive Developmental Disorder not otherwise specified</td>
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<tr>
<td>RHI</td>
<td>Rubber Hand Illusion</td>
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<tr>
<td>RMT</td>
<td>Resting Motor Threshold</td>
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<tr>
<td>rTMS</td>
<td>repetitive Transcranial Magnetic Stimulation</td>
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<tr>
<td>TD</td>
<td>Typically Developing</td>
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<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
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<td>ToM</td>
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Abstract

The present dissertation utilised transcranial magnetic stimulation (TMS) to investigate and compare mirror neuron activity in individuals with an autism spectrum disorder (ASD) and typically developing (TD) individuals using three distinct experimental paradigms. Specifically, it aimed to assess whether the mirror neuron system (MNS) was functionally disturbed in individuals with an ASD during tasks requiring emotional and social processing.

Given the well-entrenched conceptual connection between mimicry and social cognition in psychological thought, study one examined automatic mimicry of emotional facial expressions. It was found that TD individuals automatically mimicked emotional facial expressions, as demonstrated by significantly larger motor evoked potentials (MEPs) in emotion conditions compared to neutral conditions. As increased MEP amplitude during action observation is believed to reflect increased premotor mirror neuron activity, this result suggests that during facial emotion processing, mirror neurons in the premotor cortex provide an internal simulation of the observed motoric behaviour that evokes a similar reaction in the corresponding muscles of the observer. In contrast, study one found that participants with an ASD exhibited significantly lower MEP amplitudes when viewing emotional stimuli compared to neutral stimuli, suggesting that when presented with stimuli that are emotional in nature, the MNS actively inhibited an automatic mimicry response.

To further explore the role of the MNS in social cognition, study two examined yawning contagion, as it is believed that contagiousness of yawning depends on mechanisms that develop during childhood in parallel with the empathic capacity to understand mental states of others. Study two’s finding that
TD participants displayed the capacity for yawning contagion, as demonstrated by significantly larger MEPs when viewing yawns compared to control mouth movements, supports this view. Further strengthening this contention was the finding that participants with an ASD demonstrated disturbances in the capacity to yawn contagiously, as evidenced by similar MEP amplitude when viewing both yawns and control mouth movements.

As it is believed that a disturbance at the basic level of self-awareness could contribute to the higher order deficits in social cognition observed in ASD, study three utilised an adapted mirror-induced visual illusion of hand movements’ to investigate differences in self-other processing. In comparison to TD individuals, study three showed that individuals with an ASD displayed a deficit in self-awareness, reflected in ASD participants’ weaker cortical activation, indicative of reduced MNS activation, during a perceptual illusion of kinaesthetic experience, and in their difficulty discerning their experience in subjective measures.

The present thesis provides support that social cognition may, at least in part, be sub-served by the MNS and that disturbances in this system likely contribute to the social communicative disturbances observed in ASD. Although much research is required to elucidate the link between social cognition, the MNS and ASD, the present dissertation raises several questions regarding the possible downstream consequences that may result from imitative deficits and abnormal self-other representations early in development, which appear to be linked to disturbances in the MNS. As such, the present thesis contributes new knowledge on processes that shape social cognition in both the typical and atypical social mind as well as highlighting possible neural substrates that mediate this process.
The term autism spectrum disorder refers to a group of neurodevelopmental syndromes characterised by impairments in socialisation, disturbances in verbal and non-verbal communication, and restrictive and repetitive patterns of behaviour (American Psychiatric Association [APA], 2013). Understanding contemporary conceptualisations of disorders as complex as those on the autism spectrum, requires a degree of insight into the condition’s historical descriptions. Although autism spectrum disorders (ASD) undoubtedly existed prior to the original description of autism published in 1943 by Kanner, his initial report of the disorder demonstrates the first identification of autism as a distinct developmental disorder (Benaron, 2009).

A Brief History of Autism Spectrum Disorders

In 1943 Kanner published a paper containing detailed descriptions of eleven children, eight boys and three girls, exhibiting similar patterns of behaviour. These cases enabled Kanner to synthesise a number of core symptoms of the disorder that he later termed ‘early infantile autism’. Whilst Kanner recognised that the children differed with respect to the severity of their symptoms, their developmental course and their specific interests and behaviours, he was adamant that the defining characteristic of autism was impairment in social functioning. These children demonstrated severe limitations in their awareness of the social environment and a lack of interest in social interactions. All eleven of the children described by Kanner preferred interacting with objects rather than people and would become quite upset or annoyed when others attempted to become involved. As a result the children were typically described as aloof or distant. Although Kanner stressed impairments in social relations as the
core feature of autism, he also noted several other distinctive features. These included; abnormal language development and use; repetitious play - often involving objects that would not interest a typical child; an insistence on sameness; unusually intense reactions to certain stimuli; distinctive motor mannerisms and rapid shifts in mood.

Kanner established the foundation for understanding autism as a disorder principally defined by an inability to interact and relate to people in the typical fashion and, that this was generally accompanied by communication peculiarities and a preference for restricted and repetitive behaviours (Kanner, 1943). Considering the fact that Kanner based all of his conclusions on the observations of only eleven children over 60 years ago, it is remarkable that his core description still remains.

At the same time as Kanner’s initial description of autism, Asperger described another part of what we now call the autism spectrum. In 1944 Asperger published a paper describing four boys exhibiting peculiar social behaviours. As the paper was not brought to the attention of English-speaking countries until the 1980s, Asperger’s contribution was long-overlooked. It was not until 1981 when Wing published a paper based on Asperger’s translated observations and her own clinical experience that Asperger’s work was recognised by the non-German speaking world (Wing, 1981). Wing’s paper described 34 children and adults with autism whose symptomology more closely resembled the descriptions of Asperger than Kanner. As the cases did not easily match the diagnostic criteria for autism that was being used at the time, Wing used the term Asperger’s syndrome (AS) in an attempt to provide a new diagnostic category within the autism spectrum.
Whilst Wing (1981) noted that both those with autism and those with AS shared several fundamental features, such as; unusual eye-contact, restricted facial expressions, vocal oddities and impairments in non-verbal communication, she proposed three key characteristics that differentiate between the two conditions (Wing, 1981). Firstly, the autistic child is likely to be distant or indifferent towards others; whereas the child with AS is more likely to be peculiar or one-sided during social approaches. Secondly, the autistic child is likely to have delayed or abnormal speech, whereas individuals with AS have relatively intact speech but have difficulties understanding complex meanings and often use speech that is inappropriate for the social context. Lastly, Wing proposed that the child with autism tends to develop stereotyped, repetitive routines involving objects or people, while the child with AS becomes fixated on specific topics of interest.

Although Wing successfully brought AS to the attention of researchers in the autism domain, it was over ten years before AS was formally recognised as a separate diagnostic entity in the main diagnostic systems (Benaron, 2009). This led to the conceptualisation of autism existing not as a discrete entity, but rather as a spectrum along which there are multiple disorders of varying severity emerging in the late 1990s, and was reflected in the diagnostic systems, the most widely utilised being the International Classification of Disease, Tenth Edition (ICD-10) and The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR). Thus, autism was conceptualised as one of a spectrum of conditions, all of which were characterised by a triad of impairments in social interaction, disturbances in language and communication and narrow, repetitive patterns of behaviour.
In the DSM-IV-TR, autism and AS are subsumed under the heading pervasive developmental disorders (PDD). The PDD category houses an additional three conditions, those being Rett’s disorder, childhood disintegrative disorder and pervasive developmental disorder not otherwise specified (PDD-NOS). A diagnosis of PDD-NOS is given if there is uncertainty that the individual truly fits within the PDD category or if there is something atypical about the clinical course (APA, 2000). Although these five disorders are grouped together under the PDD category in the DSM-IV, researchers and clinicians generally use the term ASDs when referring to autism, AS and PDD-NOS. This is because children with Rett’s disorder and childhood disintegrative disorder vary from ASDs with respect to their clinical course, pathophysiology and the diagnostic strategies implemented (Levy, Mandell & Schultz, 2009). This is important to note as often researchers have used the terms ASDs and PDDs interchangeably without explicitly stating the disorders they are referring to, thus creating a certain degree of ambiguity around these terms.

The diagnostic differentiation of ASDs is subject to ongoing debate and is currently in a transitional phase with the advent of the DSM V in 2013 (APA, 2013). The DSM-IV-TR attempted to differentiate ASDs on the basis of developmental history, age of onset and cognitive functioning in an effort to improve differential diagnosis and specificity (APA, 2000). However, attempting to draw lines where no clear lines exist does not benefit the system or the individual and makes categorical diagnoses imprecise (Benaron, 2009). This has been especially true for the distinction between individuals with high functioning autism (HFA) and AS. HFA is the term used to describe autistic individuals with normal intelligence (IQ ≥ 70) that had early language delays but went on to
develop functional language skills. The issue of whether HFA and AS are distinct conditions has been a source of continuing debate (Howlin, 2003). Additionally, there has also been considerable disagreement regarding the validity of the diagnostic criteria used in the DSM-IV and ICD-10 to distinguish between the two conditions (Manjiviona & Prior, 1999). Several studies suggest that if the hierarchical guidelines proposed by the DSM/ICD, stating that a diagnosis of autism takes precedence over a diagnosis of AS, are applied then a diagnosis of AS becomes unlikely, or even impossible (Mayes, Calhoun & Crites, 2000). As a result the label ‘Asperger syndrome’ has been used loosely with little agreement as often clinicians may identify that the DSM/ICD criteria for autism are satisfied but use a different diagnostic label, such as AS, to convey their overall clinical impressions (Williams et al., 2008).

Although several studies comparing groups of individuals with HFA and AS have failed to demonstrate reliable differences between the two groups (e.g. Dissanayake, 2004; Howlin, 2003; Manjiviona & Prior, 1999), inadequate group matching, small samples, and, above all, lack of agreement on diagnostic criteria have meant that few of these studies have produced any conclusive results. Regardless of this, the differential diagnosis can have implications for services, with individuals diagnosed as having AS often being deprived of the level of support that is offered to those diagnosed as having autism (Klin & Volkmar, 2000). This has facilitated the removal of the subcategories of autism, AS and HFA in favour of ASD as one all-encompassing diagnosis in the DSM-V. However, this has attracted controversy as many individuals who have been diagnosed with a particular subtype rely on their diagnosis for particular medical or social services (Wing, Gould & Gillberg, 2011). Thus, debate still continues
whether the sub categories can be differentiated diagnostically. Despite the challenges encountered by both clinicians and researchers regarding classification, it is generally agreed upon, that the diagnosis of an ASD identifies children with similar patterns of problems with social interaction, communication and behaviour.

**Clinical Characteristics**

ASDs are extremely heterogeneous in their presentation as there is marked variability in the severity of symptomology across individuals and, their level of intellectual functioning can range from profound mental retardation through to the superior range on standard IQ tests (Filipek et al., 1999). However, all children on the autism spectrum demonstrate impairments that fall into core domains that are reliably measured and generally consistent over time, even though specific behaviours may change with development. Thus, accurate diagnosis requires looking for specific qualitative symptoms and signs within the core domains that characterise ASDs.

In line with the original description of autism by Kanner, difficulties in social domains, especially difficulties developing meaningful attachments and interpersonal reciprocity, are generally still considered the core feature of ASDs (APA, 2013). The behaviours under this rubric range from a total lack of awareness of another person, to eye contact that is present but not used to moderate social interactions. Hence, impairment can manifest in a variety of behavioural symptoms, creating large variation in the clinical presentation. Typically, an individual with an ASD will display atypical patterns of eye-contact, facial expressions, body posture and gestures to regulate social
interactions (APA, 2000). For instance, when compared to normal children, children with an ASD will generally exhibit reduced or abnormal eye contact or fail to vary facial expressions to initiate social contact (Willemsen-Swinkels, Buitelaar, Weijen & van Engeland, 1998). Children with an ASD appear to have difficulties engaging in joint attention behaviours with others, coordinating social cues and orienting to social stimuli, such as consistently responding to the calling of one’s name (Leekam & Ramsden, 2006). A child with an ASD will likely engage in less imitation behaviours, such as mimicking facial expressions, and experience difficulties perceiving and understanding others emotional states (Wetherby, Prizant & Hutchinson, 1998). As children with ASD lack fundamental social skills and often behave in a socially inappropriate manner, they have difficulties developing close, meaningful relationships with peers (Downs & Smith, 2004).

Another area of difficulty is in the acquisition and appropriate use of language for communication. Communicative language function across the autism spectrum spans from completely mute to verbal fluency, although fluency is generally accompanied by various comprehension and communicative errors (Filipek et al., 1999). In early infancy some children with an ASD will not babble or use communicative vocalisations and will also fail to use compensatory gestures or facial expressions (Johnson, 2008). Those children with an ASD who do acquire some speech will almost always have comprehension deficits, especially in understanding higher order complex phrases. They tend to make literal interpretations and have difficulty grasping information that requires an understanding of complex concepts (Attwood, 2007). They are also likely to display deficits in pragmatics, the use of language to communicate effectively.
Some children with an ASD speak relatively fluently but are unable to initiate or sustain a conversation on a topic of mutual interest as their speech tends to be repetitious and self-directed (Filipek et al., 1999). Their language use tends to be stereotyped, repetitive or idiosyncratic. This may include immediate and delayed repetition of another person’s speech, termed echolalia, referring to one’s self in the third person or the usage of fabricated words called neologisms (Volden & Lord, 1991). Their speech may also consist of concrete and poorly constructed grammar, be used functionally rather than communicatively and lack imaginative qualities (Stone & Caro-Martinez, 1990). Additionally, children with an ASD generally exhibit peculiar prosodic expression such as an exaggerated or monotonous tone, atypical pitch and rhythm or even an adopted accent (McCann & Peppe, 2003). Whilst both social and communicative difficulties are still viewed as core features of ASDs, it is important to note that the DSM V has recently collapsed the two previously discrete diagnostic features into a single category. This has attracted controversy as some authors argue that social and communicative deficits are distinct genetically (Happè, Ronald, & Plomin, 2006).

Another defining feature of ASDs is the engagement in restricted, repetitive, and stereotypic patterns of behaviour, interests and activities. Like the previous domains, children with an ASD can display various atypical behaviours in a variety of areas including peculiar mannerisms, odd attachments to objects, obsessions, compulsions, self-injurious behaviours and stereotypies (Volkmar, Chawarske & Klin, 2005). Stereotypies are repetitive, non-functional, atypical behaviours such as hand clapping or flapping, peculiar finger movements and rocking and twirling that children with an ASD tend to engage in when feeling excited or upset (Volkmar et al., 2005). It has been observed that in children with
HFA the stereotypic movements may diminish as they get older, developing into more socially acceptable minimised behaviours, such as finger tapping (Filipek et al., 1999). In non-autistic intellectually disabled individuals the display of stereotyped, repetitive movements is inversely related to IQ (Matson et al., 1997). This has resulted in the widespread assumption that individuals with an ASD who have IQs in the intellectually disabled range engage in higher rates of repetitive behaviour than individuals with high functioning ASDs, however there is no reliable evidence supporting this conjecture (Turner, 1999). Children with an ASD will often engage in highly repetitive play that rarely involves traditional toys and lacks the spontaneity and symbolism evident in the play of typically developing children (Filipek et al., 1999). Additionally, they often form unusual attachments and restricted interests with regards to objects of play or particular topics and facts (APA, 2000). For example, a child with ASD may insist on carrying around a toy cow but rather than play with the toy in an imaginative manner they will be obsessed with the manufacturing of dairy products. Many children on the autism spectrum become preoccupied with ‘sameness’ (Richler, Bishop, Kleinke & Lord, 2007). They develop an inflexible adherence to non-functional routines and rituals that if disturbed, even marginally, cause extreme emotional distress (Richler et al., 2007). Individuals on the autism spectrum may also develop hypo- and hypersensitivities to certain stimuli, such as noise for example. Although this is more prominent in children with ASDs than typically developing children, there is no evidence that sensory symptoms differentiate children with ASDs from children with other developmental disabilities (Rogers & Ozonoff, 2005).
As the historical conceptualisations of autism have evolved so have the criteria utilised for diagnosis. The qualitative behavioural impairments listed above currently form the basis for diagnosing a child with ASD and are the result of decades of past research. While the significant changes in the operational definitions and criteria used to diagnose ASDs have led to improved diagnostic precision, they have made epidemiological estimation of ASDs particularly challenging.

**Epidemiology, Clinical Course and Comorbidity**

Since the first systematic studies estimating the prevalence of autism carried out in the 1960s, to recent prevalence estimations, there has been a perceived increase in the prevalence of autism. The median prevalence rate of autism for 18 studies published between 1966 and 1993 was 4.7 cases per 10 000, while that for the 18 studies published between 1994 and 2000 was 12.7 cases per 10 000 (Fombonne, 2005). This increase has led to the hypothesis that the prevalence of autism is on the rise. However, several factors complicate the interpretation of the perceived increase, including changes in diagnostic practice, the view of autism as being on a spectrum rather than a discrete entity, increased awareness of ASDs, earlier diagnosis and issues of study design and case ascertainment. Furthermore, a common source of confusion has been the lack of differentiation between prevalence, the proportion of individuals in a population who suffer from a defined disorder, and incidence, the number of new cases occurring in a population over a period of time. At present, the recent upward trend in rates of prevalence cannot be directly attributed to an increase in the incidence of the disorder (Fombonne, 2005). Although most of the existing
epidemiological data are inadequate to properly test hypotheses on changes in the incidence of ASDs, meta-analytic reviews of epidemiological studies suggest that unique design features could account, almost entirely, for between-studies variations in prevalence rates and time trend changes (Fombonne, 2005; Wing & Potter, 2002). Further, these reviews suggest that changes in diagnostic practice and the increased awareness and recognition of ASDs are responsible for the higher prevalence figures (Fombonne, 2005; Wing & Potter, 2002). Presently available data suggest that a prevalence rate of 1 in 68 is a reasonable estimate for ASDs (Centre for Disease Control [CDC], 2014).

Studies based on both clinical and epidemiological evidence find a considerable difference in the prevalence of ASD with respect to gender, with boys being affected more than girls in a ratio averaging around 3.5:1 to 4.3:1 (Fombonne, 2005). Gender differences are more pronounced when an ASD is not associated with intellectual disability, with a male to female ratio of 5.5:1. Conversely, when only cases with moderate to severe mental retardation are considered, the sex ratio decreases to approximately 1.95:1 (Fombonne, 2005; Scott, Baron-Cohen, Bolton & Brayne, 2002). The cause of the observed gender differences in relation to intellectual functioning remains a debated topic.

Although the average age of diagnosis for children with an ASD is usually between three and four years of age, there is wide variability in the age of diagnosis from one case to the next (Mandell, Novak & Zubritsky, 2005). For instance, retrospective studies suggest that in some children deficits can be detected in the first year of life (Werner, Dawson, Osterling & Dinno, 2000), while those with less severe symptoms may not be diagnosed until they reach school age, or in the case of higher functioning individuals with an ASD as late as
nine years of age (Howlin & Asgharian, 1999). Given that the literature is showing that signs of ASDs are present in the first year of life, the mean ages of diagnosis are still rather high. One of the main reasons for the delayed diagnosis of ASDs despite their early behavioural manifestations is that fact that the current diagnostic systems focus on behaviours that generally occur later in development (Barbaro & Dissanayake, 2009).

Long term follow up studies predict that the majority of individuals with an ASD will demonstrate a poor prognosis (Nordin & Gillberg, 1998). It appears IQ level at the time of diagnosis and language, particularly the presence of communicative speech before five years of age, are the best predictors of long-term outcome in ASDs (Billstedt, Gillberg & Gillberg, 2007). Although there is wide variability in outcome, most individuals with IQs in the intellectually disabled range and an absence of communicative speech by the age of five years will have severe deficits in social functioning and be unable to live independently (Nordin & Gillberg, 1998). In those with higher IQs it is more difficult to make a reliable prediction regarding outcome however individuals with HFA/AS are certainly more likely to be able to work and live independently (Nordin & Gillberg, 1998). The presence of co-morbid conditions can also have implications on an individual’s prognosis with co-morbidity generally being the rule in ASDs rather than the exception. A large number of medical conditions, psychiatric disorders, and behavioural and motor dyscontrol symptoms are associated with ASDs (Gillberg & Billstedt, 2000). Some of the more common co-morbid conditions include epilepsy, hearing and visual impairments, attention deficit hyperactivity disorder (ADHD), Tourette syndrome and depression (Gillberg & Billstedt, 2000).
Concluding Remarks

The history of conceptualising ASDs is almost as complex as the disorders themselves. In light of this, it is surprising that Kanner’s (1943) original descriptions have stood the test of time so well. Although debate still continues regarding the classification, diagnosis and the true prevalence of ASDs there is currently universal agreement that the ASDs are a heterogeneous group of neurodevelopmental disorders characterised by a recognisable pattern of behaviours and an onset in early childhood. Given the wide variability in language ability, intellect, social skills and the numerous co-morbid conditions, it is not surprising that attempts to identify unified aetiological theories have been unsuccessful. Contemporary research supports the notion that ASDs may be caused by several factors, including genetic vulnerability and environmental influences (Inglese & Elder, 2009). However, the exact causes of the abnormal brain function that leads to the behaviours exhibited in ASDs remains an extremely contentious issue. The following chapter aims to review the contemporary aetiological theories of autism.
Chapter 2: The Actiology of Autism Spectrum Disorders
Given the heterogeneous nature of autism spectrum disorders (ASDs), it is not surprising that the question of causation continues to perplex experts in the field. There is currently an emerging consensus that ASDs are a group of neurodevelopment disorders that are likely to result from a number of causal factors rather than result from a single cause (Bailey et al., 1995; Happe, Ronald, & Plomin, 2006; Ritvo, Freeman, Mason, Mo & Ritvo, 1985). However this is where the consensus ends and the controversy begins, as the exact cause of the abnormal brain functions that lead to the behaviours that define ASD remain unknown. As the aetiology of autism has been examined by a variety of disciplines, there is a substantial number of theories proposed and literature available. The present chapter aims to provide a brief review of various contemporary theories and research that have added to the understanding of ASDs.

**Genetic Predisposition**

For over 20 years, genetic investigations have played a major role in ASD research. As a result, it is now well established that ASDs are highly heritable (Grice & Buxbaum, 2006; Ritvo et al., 1985; Ronald et al., 2006). The relative risk of recurrence among siblings when one is diagnosed with an ASD is 20 to 80 times greater than the population base rate (O’Roak & State, 2008). Dependent on the definition and criteria employed, twin studies suggest that 58-92% of monozygotic twins are concordant for ASDs, compared with approximately 10%-20% for dizygotic twins (Hallmayer et al., 2011; Muhle, Trentacoste & Rapin, 2004; Ronald & Hoekstra, 2011). While these studies suggest a strong genetic base for ASDs, they also highlight that there are likely to be environmental and/or
epigenetic factors influencing both the expression and severity of ASDs (Muhle et al., 2004).

In a minority of cases, 10-15%, ASDs are associated with known genetic causes. The most common include fragile X syndrome, tuberous sclerosis, and various chromosomal abnormalities such as maternal duplications and deletions of identified genes (Kumar & Christian, 2009). It should be noted that none of these causes are specific to the disorder as a whole; rather they are specific to a range of phenotypes, such as intellectual disability. The male predominance also suggests a genetic role in the inheritance of ASDs. Several genetic processes can lead to male predominance, including causative genes located on the X chromosome (Benaron, 2009). However, the view that an uneven sex ratio is the result of damaged genes on the X chromosome is not universally accepted. For instance, Baron-Cohen proposed the theory that ASDs are the result of ‘the extreme male brain’ (Baron-Cohen, 2002). The theory attributes the uneven sex ratio to the fact that male brains are naturally programmed to focus on systematising, while the female brain is oriented towards empathising. The theory proposes that high levels of testosterone in utero result in the brain developing more systematising abilities and less empathising tendencies. Although this theory is not widely accepted, it illustrates that it is important not to assume that the uneven sex ratio is purely the result of damaged genes on the X chromosome and to recognise that there may be other factors in play.

In an attempt to explain the extensive heterogeneity in ASDs, contemporary genetic research is exploring the complex interactions between multiple genes. Consequently, it has recently been proposed that separate genes may contribute to the three core areas of impairment, thus explaining the wide
variations along the autism spectrum (e.g. Happè, Ronald & Plomin, 2006). Neuroscientists have also noted that it is essential to examine genes modulating different biological functions, rather than just genes expressed in the brain (Benaron, 2009; Grice & Buxbaum, 2006). This line of thinking has resulted in the discovery of a variant of the MET gene’s implication in the expression of ASDs. The MET gene produces a product that modulates the nervous system and the gut (Campbell et al., 2009). Evidence from genetic linkage studies suggests that disrupted MET signalling may contribute to an increased risk of developing an ASD (Campbell et al., 2009). The MET gene is just one of the many genes implicated in the development of ASDs. It is important to note that possessing a specific gene that increases an individual’s susceptibility is not sufficient enough to cause an ASD; other genetic or environmental factors must be involved before an ASD develops. Whilst it is generally accepted that environmental factors modulate already existing genetic mechanisms implicated in the phenotypic expression of ASDs, the identification of specific environmental factors involved is an extremely controversial topic.

Environmental Issues

As many of the brain abnormalities associated with ASDs occur in the first two trimesters of pregnancy, it is believed that environmental factors, such as teratogenic exposure, may be implicated in the development of ASDs (Kolevzon, Gross & Reichenberg, 2007). Although the literature suggests that ASDs are linked to various environmental exposures, for example exposure to pesticides, as of yet, none have been linked conclusively (Benaron, 2009). Various perinatal factors, such as low birth weight, duration of gestation and events during the
birthing period have also been investigated (Juul-Dam, Townsend & Courchesne, 2001). However, as findings have been inconsistent no conclusions can be reliably drawn.

Aetiological possibilities occurring after birth have also been investigated. The focus of most of the research has been on the belief that the administration of certain immunisations, namely the measles, mumps and rubella vaccine (MMR) and vaccines containing mercury, causes ASDs in some genetically predisposed children (Inglese & Elder, 2009). After completing a study examining 12 children, it was contended by Wakefield et al. (1998) that the onset of regressive autism and intestinal abnormalities was associated with the MMR vaccination. This conjecture gained a lot of attention from both professionals and the media. Despite criticism concerning the methodological flaws of the study, a retraction of the article from the journal, rigorous follow-up studies and reports from the institute of medicine stating that there is no causal link between the MMR vaccine and ASDs, it still remains a controversial issue (Brown et al., 2012; Institute of Medicine [IOM], 2001; The editors of the Lancet, 2010). Questions have also been raised regarding the effects of mercury-containing vaccines on brain development in ASDs (Blaxill, Redwood & Bernard, 2004). It has been postulated that the preservative thimerosal, which contains mercury, causes neurotoxicity which leads to the development of an ASD (Blaxill et al., 2004). However, recent systematic reviews suggest that, to date, no association has been found between thimerosol containing vaccines and ASDs (Parker, Schartz, Todd & Pickering, 2004). Although most researchers are convinced that genetic vulnerabilities interacting with environmental factors play a significant role in the
development of ASDs, the details remain to be explicated. Thus, it is essential that research continues to evaluate possible genetic and environmental factors.

**Neurobiological Links to Autism**

In recent years, intense research efforts have focused on elucidating the neurobiological origin of ASDs. A growing body of evidence from neuropathology and neuroimaging studies indicate that there are fundamental discrepancies in brain development and organisation between individuals with an ASD and typically developing individuals. In Kanner’s (1943) initial description of autism, it was noted that the children had enlarged heads. Subsequent reports of autism head circumference, magnetic resonance imaging (MRI) and post-mortem brain weight have generally confirmed Kanner’s conjecture (Redcay & Courchesne, 2005). Studies that have failed to report significant increases in brain or head size have generally been criticised on the basis of insufficient statistical power or specific sample characteristics (e.g., Herbert et al., 2003). While a great deal of research and discussion has focused on confirming that brain size is abnormal in autism, studies are only now beginning to address age-related changes. Redcay and Courchesne (2005) conducted a meta-analytic review of 15 studies of head circumference, MRI, and post-mortem brain reports in an attempt to examine age-related changes in brain size in ASDs from birth to adulthood. Redcay and Courchesne found that individuals with ASDs display reduced or normal brain size at birth, followed by an early rapid rate of brain growth. This is then believed to be followed by an abrupt cessation of growth by 2 to 4 years as fewer adolescents and adults with ASDs exhibit increased brain size when compared to controls. However, as the meta-analysis examined cross-sectional
Longitudinal studies are needed to verify these observations.

The identification of the neural factors underlying the perceived rapid brain growth in early years is essential, as it will likely improve understanding of the mechanisms underlying the emergence of ASDs. Thus far, it is not clear whether all neural structures are equally affected by the rapid brain overgrowth or what the functional significance of this may be. To date, studies examining the enlargement of individual lobes have been contradictory. For example, a study completed by Piven et al. (1995) found a selective enlargement of occipital, parietal and temporal lobes, while a similar study found the opposite, i.e. the frontal lobe was enlarged (Carper, Moses, Tigue, & Courchesne, 2002). However, given the heterogeneity of ASDs it is difficult to establish whether the conflicting findings are the result of methodological and participant variations or different neural profiles. Investigations have also attempted to determine if the increase in brain volume is due to an increase in grey matter, white matter, or both (e.g. Herbart et al., 2003; Waiter et al., 2005). Although the research is limited, the data suggests an age related effect. The cross-sectional research indicates that grey and white matter are increased in young children, white matter is increased in older children, and grey but not white matter is increased in adolescents and adults with ASDs (Lainhart, Lazar, Bigler & Alexander, 2005). These changes are likely to result in abnormal neuronal connectivity, thus impairing communication between brain areas. Given the physical constraints on how large the brain can grow while still preserving adequate levels of connectivity, co-occurrence of reduced white matter and reduced functional connectivity appears quite plausible (Ringo, 1991). Thus, changes in grey and white matter with age need to be further
investigated in ASD. The application of new methods, such as cortical pattern mapping, will allow a more detailed, accurate study of the anatomic underpinnings of increased brain volume in ASD and changes across development (Sowell, Thomson & Toga, 2004).

The study of the volume and shape of selected subcortical structures has been of interest given what is known about the modularity of the brain and the conviction that specific brain-behaviour relationships can be identified in ASDs. Post-mortem and MRI studies, mostly of adolescents and adults with ASDs, have found variable evidence of structural abnormalities (Amaral, Schumann & Nordahl, 2008). The most frequently highlighted being the frontal lobes which are involved in complex reasoning; the amygdala which plays a critical role in emotion formation and the cerebellum which plays an important role in attention, language development, motor functioning and affective processing (Amaral et al., 2008). Given the enormous heterogeneity of ASDs, the diverse occurrence of significant co-morbid conditions and the relatively small sample sizes of both MRI and post-mortem studies of ASDs, the detection of these abnormalities are quite noteworthy. However, attempts at defining the neuroanatomy of ASDs are still in their infancy and the findings are still largely inconsistent, thus any links between abnormalities in specific subcortical structures and autism spectrum pathology are still extremely tentative.

Functional magnetic resonance imaging (fMRI) has permitted an unprecedented opportunity to examine patterns of brain activation when individuals are presented with a stimulus or asked to perform a particular cognitive task. However, because of the need for higher functioning, verbal subjects who can understand tasks and cooperate, and the labour intensity
involved in processing and analysing fMRI data, the sample sizes have generally been quite small. None the less, studies are suggesting that brain functioning, in terms of the areas of the brain involved and the degree of involvement, are different in individuals with ASDs than typically developing individuals with respect to a variety of different domains (Lainhart at al., 2005). Functional MRI studies utilising face processing tasks have been particularly informative with respect to the neural circulatory required for normal social interactions. Studies have demonstrated that when typical individuals gaze at faces an area in the frontal lobe, called the fusiform gyrus, and the amygdala are activated (Pierce, Muller, Ambrose, Allen & Courchesne, 2001). However, studies suggest that this may not be the case for individuals with ASDs (e.g. Wang, Dapretto, Hariri, Sigman & Bookheimer, 2004). Several studies have demonstrated that individuals with ASDs have abnormal or weak activation of the regions supporting face processing in typical individuals and utilise some brain areas not used by typical individuals during face processing (e.g. Kleinhans et al., 2008; Pierce et al., 2001). These findings suggest that the normal neural network involved in facial processing is disrupted in individuals with ASD, resulting in an alternative, less effective pathway being formed.

Functional MRI studies have also suggested that a network of visuomotor cells known as mirror neurons may not function optimally in individuals with ASDs (e.g. Dapretto et al., 2005). Mirror neurons are activated by the performance or observation of object or goal related actions. Thus they are believed to be responsible for imitation that occurs automatically. Automatic imitation is an area that individuals with ASD’s have been shown to possess deficits in (e.g. Smith & Bryson, 1994). As multiple areas of the brain, including
the motor and emotional control centres, must be connected through the mirror neuron system (MNS) to allow for the natural coordination of imitation, mirror neuron dysfunction would provide an explanation for many of the problem areas in ASDs (Benaron, 2009). Whilst research examining the MNS is still in its infancy, the theory of mirror neuron dysfunction in autism appears promising.

**Cognitive Theories of Autism**

Psychological models of ASDs play a key role in the search for factors involved in the aetiology and pathogenesis of ASDs. Researchers have conducted numerous experiments examining how individuals with an ASD approach intellectual tasks and perceive the social world around them. As a result, several theories have emerged that attempt to explain the cognitive and social patterns exhibited by individuals with an ASD. Currently, the most prominent theoretical concepts are; weak central coherence, deficits in executive function and deficits in theory of mind.

Central coherence refers to an individual’s ability to integrate information into central concepts and meaningful wholes. As individuals with ASDs have a tendency to focus on details rather than integrated and meaningful wholes they are hypothesised to possess weak central coherence (Happe & Firth, 1996). This has been demonstrated through studies showing an autistic superiority on tasks requiring attention to detail, such as the block design subtest of the Weschler IQ test (Shah & Firth, 1993), and an impairment on tasks requiring integrating fragments of objects and sentences in a paragraph (Jolliffe & Baron-Cohen, 2001). Although the weak central coherence hypothesis has been questioned on the basis of some conflicting findings and the limited range of perceptual and...
cognitive domains studied from this perspective (Volkmar, Lord, Bailey, Schultz & Klin, 2004), it is supported by neurological findings. Abnormal connections between separate brain areas due to white matter abnormalities would likely impair an individual’s ability to synthesise complex information. MRI studies indicate that individuals with ASD display white matter abnormalities, thus providing biological support for the weak central coherence hypothesis (e.g. Herbert et al., 2003; McAlonan et al., 2005; Waiter et al., 2005). For example, a recent MRI study comparing 17 children with high functioning autism (HFA) and 17 age and IQ matched controls found that white matter was reduced bilaterally in the cerebellum by 19% in the children with HFA (McAlonan et al., 2005).

Executive function refers to the brain processes that filter incoming stimuli and direct attention towards the most important output (Hill, 2004). Executive function skills require higher-order thinking processes, thus they are concentrated in the frontal lobes (Benaron, 2009). The executive dysfunction theory postulates that the repetitive, restrictive behaviour exhibited by individuals with an ASD is the result of an inability to shift attention (Hill, 2004). To date, this is the only cognitive theory that attempts to explain this facet of the condition. However, studies demonstrating that an ASD can be present without executive dysfunction, pose a serious challenge to the executive dysfunction hypothesis of ASDs (e.g. Griffith, Pennington, Wehner & Rogers, 1999; Hill & Russell, 2002). For example, a study examining self-monitoring abilities, a key component of executive functioning, found no performance differences between 20 children with an ASD and 20 age matched controls (Hill & Russel, 2002). Thus, it has now been suggested that while some individuals with an ASD may have impairments in executive function, executive dysfunction is not a core feature of ASDs.
The theory of mind (ToM) describes the ability to understand the mental states, beliefs, desires and intentions of others, and to appreciate how these differ from our own (Baron-Cohen, 2001). The ToM hypothesis posits that the social dysfunction in ASDs results from disruptions in the processes that lead to the ability to conceive one’s own mind and the mind of others (Baron-Cohen, 2001). Under-connectivity between separate areas of the brain would be expected to disrupt the complicated neural circulatory that allows this process to take place. The MNS may be an example of one type of complex system that is necessary for effective ToM abilities. Although a theoretical link has been made between mirror neurons and ToM, this hypothesis is yet to be tested directly. Given the large research base identifying ToM deficits among those with ASD and the promising research surrounding mirror neurons it is essential that this link be investigated.

**Concluding Remarks**

Although there is a wealth of literature examining the aetiology of ASDs the question of causation is yet to be answered. While it is generally accepted that environmental factors modulate already existing genetic mechanisms, the identification of both the specific environmental factors and genes involved in the development of ASDs have yet to be elucidated. A growing body of evidence from neuropathology and neuroimaging studies indicate that there are fundamental discrepancies in brain development and organisation between individuals with an ASD and typically developing individuals. Although a definable and reliable, neurophysiological marker has yet to be identified, the cumulative data on brain circulatory indicates that the biologic basis of the brain
dysfunction in ASDs may be the result of abnormal connectivity in neural networks. Promising research suggests that disruptions in the MNS may explain many of the problem areas seen in ASDs. In light of this, the following chapter aims to provide a detailed description of mirror neuron dysfunction in autism spectrum disorders.
Chapter 3: The Mirror Neuron System and Autism Spectrum Disorders
Elucidating the underlying neural basis of the social and communicative deficits that characterise autism spectrum disorder (ASD) has proved challenging. However, the recent discovery of visuomotor cells, known as mirror neurons, in macaque monkeys may provide a basis for explaining various behavioural impairments exhibited by individuals with ASD (Rizzolatti & Craighero, 2004). While mirror neurons are primarily thought to be involved in the perception and comprehension of motor acts, they are also believed to play a fundamental role in higher order cognitive processes such as empathy, imitation, theory of mind (ToM) and language, all of which are known to be impaired in ASDs (Williams, Whiten, Suddendorf & Perrett, 2001). The present chapter aims to review research investigating the mirror neuron system (MNS) and its dysfunction in ASDs.

The Mirror Neuron System

Mirror neurons were originally discovered in the ventral premotor cortex (area F5) and the inferior parietal lobule of the macaque monkey (Rizzolatti & Craighero, 2004). The defining characteristic of these neurons is that they discharge both when the monkey performs a goal related motor action, such as grasping an object, and when the monkey observes a similar motor action being performed by another monkey or a human (Ferrari, Gallese, Rizzolatti & Fogassi, 2003; Rizzolatti & Craighero, 2004). These neurons ‘mirror’ the action performed by another, as though they themselves were performing the action. This allows a direct matching between the visual description of an action and its execution. Thus, this observation-execution system is likely to be involved in the comprehension of action (Gallese & Goldman, 1998).
Although mirror neurons have only been directly observed in humans recently (Mukamel, Ekstrom, Kaplan, Iacoboni and Fried, 2010), the existence of an analogous system in the homologous brain regions has been supported by indirect measures such as transcranial magnetic stimulation (TMS) (e.g. Fadiga, Fogassi, Pavesi & Rizzolatti, 1995), electroencephalograph (EEG) (e.g. Muthukumaraswamy, Johnson & McNair, 2004) and functional magnetic resonance imaging (fMRI) (e.g. Buccino et al., 2001) over the past two decades. Brain imaging studies have demonstrated that the observation of transitive actions completed by others results in an increase in blood oxygen level-dependant signals not only in the visual areas, but also in the inferior parietal lobule, the ventral premotor cortex and the caudal part of the inferior frontal gyrus, which roughly corresponds to the pars opercularis (Fabbri-Destro & Rizzolatti, 2008). The latter three areas have motor properties and closely correspond to the areas containing mirror neurons in monkeys (Rizzolatti, Fabbri-Destro & Cattaneo, 2009). Activation of these areas have been consistently reported not only during the execution and observation of actions but also during the imitation of actions, an ability that is commonly disturbed in ASDs (Iacoboni, 2005).

Mukamel and colleagues (2010) were the first and only researchers to date to provide direct electrophysiological evidence of the existence of mirror neurons in humans. Mukamel and colleagues conducted a single cell study on humans by investigating patients with intractable epilepsy. They reported that the human MNS appears to extend beyond the regions previously identified, as neurons with mirror properties were also observed in the medial frontal cortex supplementary motor area and the medial temporal lobes, namely the hippocampus parahippocampal gyrus and entorhinal cortex. Whilst neurons with mirror
properties were also observed in the amygdala, as well additional areas in the medial frontal cortex, including the pre-supplementary motor area and both rostral and dorsal aspects of the anterior cingulate, the number of such cells did not reach significance levels. Thus, whilst the very existence of mirror neurons in humans is no longer under such scrutiny, the specific distribution of mirror neurons in the human brain requires additional evidence as the prevailing view of a fronto-parietal network circuit homologous to the primate brain may be limited (Keysers & Gazzola, 2010).

Researchers posit that because the same pattern of mirror neuron activation occurs both when performing and observing an action; primates can recognise the goal of a motor act performed by others (Ferrari et al., 2003; Rizzolatti & Craighero, 2004; Tkach, Reimer & Hatsopoulos, 2007). Additionally, in humans it is speculated that mirror neurons have evolved to represent not only the physical aspects of an action but also the underlying intentions, thoughts and feelings motivating that action, possibly through reciprocal connections with other brain regions such as the limbic system or medial prefrontal cortex (Williams et al., 2001). Further, it is believed that this evolutionary process has provided the basis for more complex functions such as imitation, empathy, ToM and language, all of which can be impaired in autism (Williams et al., 2001).

The Functional Significance of the Mirror Neuron System

The ability to imitate the actions of others is usually disturbed in ASDs (Williams, Whiten & Singh, 2004). It is likely that in order to imitate motor acts one must possess the capacity to transform the sensory description of the
observed action into an internal motor representation (Iacoboni et al., 1999). As mirror neurons appear to have the capacity to function as a bridge between higher visual processing and the motor cortex, i.e. between seeing and doing, they are believed to play a key role in imitative abilities. Further, it is suggested that this mirror system might underlie the ability to understand other people’s intentions by providing an automatic simulation of their actions, goals, and intentions (Gallese & Goldman, 1998). Neuroimaging studies examining typically developing individuals have provided evidence supporting the supposition that mirror neurons represent the neural basis for imitation (e.g. Iacoboni et al., 1999).

One study used repetitive TMS (rTMS), a technique that provokes a transient depression of neural activity in the stimulated region, to disrupt functioning in specific cortical regions during imitative tasks. The study demonstrated that when eight participants received rTMS over the left or right pars opercularis their performance was significantly impaired on imitative tasks (Heiser, Iacoboni, Maeda, Marcus & Mazziotta, 2003). Thus, the disruption of an area believed to be populated by mirror neurons resulted in a decrement in imitative performance. It is important to note that the MNS is obviously not sufficient for the implementation of all forms of imitative behaviour. Existing data suggests that large scale interactions between the core circuit for imitation and other neural networks are necessary for imitative learning and social mirroring (Iacoboni, 2005).

Developmental behavioural data demonstrates that imitative behaviour is critical in the development of social cognitive skills, such as empathy (Meltzoff & Prinz, 2002). Empathy refers to the ability to recognise, understand and vicariously experience the emotional states of others. A perceived lack of
empathy is a very early sign of an ASD, and deficits in empathic behaviour have been shown to emerge as early as 20 months (Charman et al., 1997). It is well established that humans are inclined to imitate each other automatically when interacting socially (Chartrand & Bargh, 1999). Additionally, the more they tend to imitate others, the more likely they are to be empathic (Chartrand & Bargh, 1999). Anatomical studies investigating the perceived role of mirror neurons in empathy have generally utilised the paradigm of observation and imitation of facial expressions (e.g. Braadbaart, de Grauw, Perrett, Waiter & Williams, 2014; Carr, Iacoboni, Dubeau, Mazziotta & Lenzi, 2003). These findings suggest that the embodiment of the facial expressions and body postures of others may be one means of eliciting an empathic response. Given the role of mirror neurons in imitation and action understanding, it is logical to posit that the MNS may be involved in the empathic process. Additionally, as empathising also requires emotional processing, the limbic system is also believed to be implicated in the formation of an empathic response (Iacoboni & Dapretto, 2006). Since the MNS and the limbic system are anatomically connected via the insula in the primate brain (Augustine, 1996), it is believed that in the human brain a large-scale network composed of the MNS, the insula and some limbic structures may provide the ability to empathise with others through the representation and inner imitation of the actions of others. Evidence from anatomical studies has provided support for this hypothesis. For example, one study used fMRI to examine 11 typically developing subjects while they were asked to imitate or observe emotional facial expressions (Carr et al., 2003). The study found that during both observation and imitation the MNS, insula and amygdala were activated. Although evidence from anatomical studies implicate the MNS in the process of
forming an empathic response, the methodology of correlating a task to brain activity does not definitively prove the involvement of the MNS.

Another important skill needed for successful social interactions is the ability to understand the mental states, intentions, beliefs and desires of others. This ability is generally termed ToM and as discussed earlier, there is a large body of literature identifying ToM deficits in individuals with ASDs (Baron-Cohen, 2001). It has been theorised that the MNS may underlie part of this ability as it is believed to have the capacity to provide the observer with an automatic internal simulation of the actions, goals and intentions of another (Gallese & Goldman, 1998). Although this theory is yet to be tested directly, research suggests that the human MNS can selectively respond to specific intentions. For example, an fMRI study examining 23 typically developing subjects found that the mirror neuron areas responded differently to the sight of the same grasping action embedded in two different contexts, drinking and cleaning up (Iacoboni et al., 2005). This provides evidence that the MNS codes not only the action but also the intention associated with it. Thus, providing indirect support that the MNS plays a role in ToM abilities.

Although somewhat more tenuous, mirror neurons have also been linked to language development. Subtle language impairment is one of the main diagnostic criteria for autism and experimental and clinical studies demonstrate that a wide array of language impairments can occur in ASDs (APA, 2000). It has been theorised that the evolution of language from an earlier gestural communication system may be the result of the observation-execution system that mirror neurons provide (Arbib, 2005; Rizzolatti & Arbib, 1998). Communicative mirror neurons have been discovered in area F5 of the Macaque monkey (Ferrari
et al., 2003). These neurons discharge during the observation and execution of communicative mouth actions, such as lip-smacking. As the human homologue region to area F5 is Broca’s area, which is believed to be the language centre of the brain, it has been speculated that the evolution of language from an earlier gestural communication system may be the result of the observation-execution system that mirror neurons provide (Arbib, 2005; Rizzolatti & Arbib, 1998). This progression from gestural communication to vocal language is believed to be the result of a need to communicate complex mentalistic concepts such as thoughts, feelings and intentions (Williams, 2005).

As a result of the association between the behavioural deficits seen in ASDs and the theorised functions of the MNS, it has been hypothesised that the MNS may be impaired in individuals with an ASD. It has been proposed that a dysfunctional development of the MNS, possibly the result of a combination of environmental and genetic factors, could result in impaired imitation abilities and self-other representations (Perkins, Stokes, McGillivray & Bittar, 2010). Consequently, this could lead to impairments in social and communicative abilities such as ToM, empathy and language, all of which are defining features of ASDs (Enticott et al., 2012; Fecteau, Lepage & Théoret, 2006; Théoret & Fecteau, 2005; Williams et al., 2001). The following section aims to review some of the evidence pertaining to this theory. However, it is important to note that an impaired MNS is unlikely to account for all the behavioural symptoms seen in ASDs as features of restrictive, repetitive and inflexible behaviour appear to incorporate a degree of imitation from others.
Mirror Neuron Dysfunction in Autism Spectrum Disorders

Likely the result of its explanatory power, the mirror neuron hypothesis of ASDs has been tested frequently in recent years, using various techniques and approaches. One well established EEG observation is that \textit{mu} rhythm, an EEG waveform recorded from the motor cortical areas, is suppressed not only when a person makes a voluntary movement but also when they observe another individual performing a voluntary movement. As the \textit{mu} rhythm is generated by activity in the sensorimotor areas, and mirror neurons are believed to be located in the premotor cortex, it has been hypothesised that the suppression of the \textit{mu} rhythm reflects a downstream modulation of primary sensorimotor areas by mirror neuron activity (Pineda, 2005). Thus, it is believed that \textit{mu} wave suppression to observed actions can be used as a selective measure of activity in the MNS (Oberman et al., 2005). Given this, it would be reasonable to expect that if mirror neurons are abnormal in individuals with an ASD then \textit{mu} suppression will be absent or reduced when they observe another individual performing a motor act.

Oberman et al. (2005) used this methodology to compare \textit{mu} wave suppression in 10 males with an ASD and 10 age and gender matched control subjects while watching a video of a moving hand, a video of a moving non-biological stimulus, and while moving their own hand. The control subjects showed significant \textit{mu} suppression during both the self-performed and observed hand movement, while the ASD group only showed significant suppression during the self-performed hand movement. This finding has recently been replicated by a similar study that utilised both male and female subjects (Martineau, Cochin, Magne & Barthelemy, 2008). On the contrary, one study
utilising a similar paradigm did not find a significant group difference during the observed hand movement when comparing a group of 20 high functioning ASD subjects and 20 aged matched controls (Raymaekers, Wiersema & Roeyers, 2009). Although these results could be viewed as evidence against the mirror neuron hypothesis, comparison of ASD samples across studies is problematic as they are such a diverse group and symptom severity is often not reported in detail. Given the extreme heterogeneity seen in children with an ASD it is important that future research investigates to what extent factors such as social cognition, influence mirror neuron functioning. Ideally, this will provide some clarity regarding the discrepancy in research findings. Despite one differing finding, for the most part this research base provides support for the hypothesis that individuals with an ASD may possess a dysfunctional MNS. However as the tasks were primarily motor based, the social and emotional domain was not tested directly.

It has been hypothesised that the mirror neuron dysfunction reported in individuals with an ASD may be the result of a lack of social relevance in the stimuli employed (Oberman, Ramachandran & Pineda, 2008). In an attempt to explore this, a recent study investigated how familiarity between an observing individual and a person performing an action influences \( \mu \) suppression (Oberman et al., 2008). In this study, 13 children with an ASD and 13 typically developing children observed a video of a stranger, a guardian and themselves performing the same grasping action. The control group showed significant \( \mu \) suppression for all three conditions, with the lowest \( \mu \) suppression when viewing a stranger’s hand. In contrast the ASD group showed significant \( \mu \) suppression when viewing themselves or a familiar person performing the hand
action but not when it was performed by an unfamiliar person. These findings suggest that mirror neuron activity was only disturbed in the ASD group when the stimulus was believed to be socially unfamiliar. However, the results should be interpreted with caution as the mirror neuron activity could be influenced by factors other than familiarity. For example, the results could be due to a compensatory mechanism whereby mirror neuron activity towards a familiar person is altered by other brain regions as the result of an automatic learned response to the familiar person. It has been proposed that the pattern of results found provides an explanation for the clinical observation that an individual with an ASD displays improvements in social and communicative skills when interacting with a parent or sibling as opposed to a stranger (Oberman et al., 2008). When reviewing EEG research it is important to note that it is an indirect indication of mirror neuron activity as it is measuring the modulation of sensorimotor neurons by the premotor cortex. Another method commonly employed to investigate mirror neuron activity in humans is by means of fMRI. Functional MRI studies have been particularly valuable in the assessment of regions of brain activation during tasks requiring emotional processing.

A recent fMRI study investigated the functioning of the MNS in 10 high functioning children with an ASD and 10 age and IQ matched typically developing controls in the context of a socio-emotional task (Dapretto et al., 2006). Subjects were required to imitate and observe facial expressions displaying basic emotions such as anger and sadness. The results showed a markedly weaker activation of areas believed to be populated by mirror neurons, especially the pars opercularis, in the children with high functioning ASD when compared to the control group during both the imitation and observation task. Given that both
groups showed reliable activation of areas implicated in both facial processing, including the fusiform gyrus and the amygdala, the difference cannot be attributed to the high functioning ASD group failing to adequately attend to the facial stimuli. Furthermore, the activity measured in the mirror neuron areas in the children with high functioning ASD during both tasks was negatively correlated with symptom severity as assessed by widely used clinical scales including the autism diagnostic schedule ($R^2=0.49$) and the autism diagnostic observation interview ($R^2=0.72$; Dapretto et al., 2006). The results of this study support the hypothesis that mirror neuron dysfunction is a core deficit in ASD and suggests that activity in mirror neuron areas during socio-emotional tasks could be a neurophysiological marker for ASDs. Whilst fMRI studies have proved valuable in attempts to localise the human mirror system, the demonstration that the motor cortex dynamically replicates observed actions, as if the observer was executing them, can only be achieved by techniques providing fast and focal measurements of cortical activity (Fadiga, Craighero & Olivier, 2005). This is where TMS has proved to be a valuable technique in assessing MNS activation during the perception of actions performed by others, as it can provide a temporally accurate estimate of corticospinal excitability (Fadiga et al., 2005).

TMS is a means of stimulating nerve cells in the motor cortex via the administration of a brief magnetic pulse to the scalp. This pulse produces a motor evoked potential (MEP) in the specific muscle stimulated that can be measured via surface electromyography (EMG). As the premotor cortex extends posteriorly to the primary motor cortex, mirror neurons in the ventral premotor cortex are believed to connect sensory neurons responding to the visual properties of an observed action and corticospinal neurons that discharge MEPs during the
execution of a similar action (Fadiga et al., 2005; Rizzolatti & Craighero, 2004; Zult, Howatson, Kádár, Farthing & Hortobágyi, 2013). Thus, when TMS is delivered during the observation of action within the stimulated muscle, it is believed that premotor mirror neuron activity increases excitability in the motor cortex resulting in enhanced MEP amplitude. This has been supported by several TMS experiments demonstrating that when typically developing individuals view the actions of others their motor system provides a simulation of the observed action in a strictly congruent and temporally aligned manner (Brighina, La Bua, Oliveri, Piazza, & Fierro, 2000; Fadiga et al., 1995; Gangitano, Mottaghy, & Pascual-Leone, 2004; Sartori, Cavallo, Bucchioni & Castiello, 2011).

Whilst this methodology has been frequently adopted to investigate motor facilitation during the observation of action in typically developing individuals, it has not been widely utilised with ASD populations. Despite the limited research base, there is evidence suggesting that this modulation may be abnormal in individuals with an ASD (Théoret et al., 2005). For example, a study completed by Théoret and colleagues (2005) utilised TMS to investigate motor facilitation during the observation of intransitive, meaningless finger movements in 10 individuals with an ASD and 10 age and gender matched controls. The authors demonstrated that observation of movement in TD individuals’ selectively enhanced motor activity specific to the muscles required to reproduce that movement, whereas this modulation was abnormal in ASD participants. Specifically, ASD participants displayed typical patterns of motor activation when performing the observed action and when viewing another completing the same action from the allocentric (other) perspective. However when that same action was viewed from the egocentric (self) perspective, ASD participant’s failed
to show motor facilitation in the specific muscles required to reproduce this action. These findings imply a mirror-related deficit in self-awareness that affects self-other processing in ASDs, thus providing further support for the possible role of the MNS in tasks requiring social and emotional processing, such as our ability to differentiate and compare the self and other.

While there is a growing body of evidence investigating the functional disturbances of the MNS in individuals with an ASD, few studies have investigated anatomical differences in the MNS. Although further research is required, the existing evidence supports the mirror neuron hypothesis of ASDs (e.g. Hadjikhani, Joseph, Snyder & Tager-Flusberg, 2006). For example, an MRI study comparing 14 high functioning adults with an ASD to 14 sex, age and IQ matched typically developing individuals found local decreases of grey matter in the ASD group in areas believed to house mirror neurons, specifically the pars opercularis, superior temporal sulcus and inferior parietal lobule (Hadjikhani et al., 2006). Thus, current evidence suggests that mirror neurons can be both functionally and structurally disturbed in individuals with ASDs.

Conclusions and Future Directions

Although mirror neurons have only been discovered recently, they have already stimulated research programmes in a variety of disciplines such as social and cognitive neuroscience and the neurobiology of disease. The mirror neuron hypothesis of ASDs is beginning to provide further insights into the condition and inspire novel forms of intervention (Rizzolatti et al., 2009). While the research base is still relatively small, there is evidence suggesting that brain regions believed to contain mirror neurons can be functionally and structurally disturbed
in individuals with ASD. Although caution needs to be employed when interpreting findings due to the small number of studies, the research indicates that mirror neuron dysfunction in ASD is marked when information is of a socio-emotional nature. Given that the few studies that directly investigate the socio-emotional domain utilise the observation and imitation of facial expressions as their experimental paradigm it is important, not only that these studies are replicated but also that new experimental paradigms examining different socio-emotional aspects are developed. As studies have demonstrated that the MNS interacts with other brain regions during tasks involving emotional processing in typically developing individuals it is essential that the connectivity between the regions believed to house mirror neurons and regions implicated in emotional processing are explored further in ASD samples. This could help explain the heterogeneity seen in autism as specific problems in connectivity between the mirror neuron network and multiple additional brain regions could result in a multitude of variable symptoms.

When reviewing the hypothesis that individuals with an ASD possess an impaired MNS it is important to note that the theory does not purport to explain all the symptoms of the disorder, such as various aspects of restrictive and repetitive behaviours. Instead it is believed that mirror neuron activity plays a fundamental role in the higher order cognitive processes such as empathy, imitation, ToM and language that are disturbed in ASD. Explicating the role of mirror neuron dysfunction in ASDs will not only improve diagnostic clarity but it will also have substantial treatment implications as the brain is a plastic organ and it function and structure can be modified by training. Thus, it is crucial research continues to explore mirror neuron activity in individuals with an ASD.
As a result of this, the present dissertation aims to investigate and compare mirror neuron activity in individuals with an ASD and typically developing individuals using three distinct experimental paradigms. Achieving this by utilising TMS to assess corticospinal excitability during tasks requiring emotional and social processing.

It is hypothesised that when compared to typically developing individuals, individuals with an ASD will display functional disturbances in the MNS, as evidenced by reduced or absent corticospinal activation when viewing stimuli that is socio-emotional in nature.
Chapter 4: Automatic Facial Mimicry and Autism Spectrum Disorder
Introduction

The ability of typically developing (TD) individuals to automatically mimic the behaviour of those around them is a pervasive and fundamental aspect of social human behaviour. Elementary mimicry, such as tongue protrusion, lip smacking and mouth opening, has been observed in new-born infants as early as 42 hours after birth (Meltzoff & Moore, 1989). These rudimentary imitation abilities are present very early in life and increase in complexity during development with adults displaying a tendency to mimic others’ body postures and gestures (Chartrand & Bargh, 1999), tone of voice, prosody and syntactic constructions (Niedenthal, Barsalou, Winkielman, Krauth-Gruber & Ric, 2005), facial expressions (Dimberg, Thunberg & Elmehed, 2000) and even breathing rates (McFarland, 2001). These automatic mimicry behaviours are believed to facilitate social functioning and communication, including building interpersonal rapport, emotional contagion, empathy and the understanding of other minds (McIntosh, 2006).

In development, these imitative abilities are believed to provide the child with information about the actions and intentions of those around them, which assists the process of social learning, and forms the basis for future social development (Rogers, Hepburn, Stackhouse & Wehner, 2003). Given this, a deficit in imitative abilities could not only impair a child’s ability to understand the emotions of others but, if such a deficit occurred early in development it could significantly impair the child’s ability to form self-other representations (Rodgers, 1999). As a result, the significance of imitation for interpersonal and emotional processes has generated significant interest in recent years with a particular focus
on possible imitative deficits in disorders of social-emotional functioning such as Autism Spectrum Disorders (ASD; Sevlever & Gillis, 2010).

One of the most investigated and robust instances of mimicry is the spontaneous mirroring of emotional facial expressions (McIntosh, Reichmann-Decker, Winkielman & Wilbarger, 2006). That is, the mere observation of another person’s emotional facial expression elicits a corresponding expression in the observer that occurs quickly (Dimberg & Thunberg, 1998) and automatically (Dimberg, Thunberg, & Grunedal, 2002). This spontaneous mirroring has been proposed to facilitate social skills through a process of contagion and an internal simulation of the observed emotion (Oberman, Winkielman & Ramachandran, 2007). Underlining the potential significance of this process for social understanding, recent investigations have shown this facial mimicry effect is associated with greater empathy (Sonnby-Borgström, 2002), emotion recognition (Enticott, Johnston, Herring, Hoy & Fitzgerald, 2008) and emotional reciprocity (McIntosh, 2006).

Given the developmental significance of spontaneous mirroring, including facial mimicry, recent interest has focused on identifying the neural substrates that mediate this process of imitation (Kana, Wadsworth & Travers, 2011). The discovery of specific cortical brain cells that respond to both the execution and observation of motoric behaviour has offered promising insight as to how humans may perceive and perform the actions of others. It is proposed that this system, known as the mirror neuron system (MNS), allows for the simulation of not just another individual’s intentions, but also their state of mind, which is then believed to inform subsequent interactions (Enticott et al., 2008). As a result, it has been speculated that mirror neurons are involved in the development of social
cognition processes that facilitate effective social interactions including the
development of empathy, theory of mind (ToM) and facial emotion processing
(Enticott et al., 2008; Fecteau, Lepage & Théoret, 2006; Uddin, Iacoboni, Lange
& Keenan, 2007). In addition to the empirical evidence supporting the link
between the MNS and social cognition in TD individuals (e.g. Agnew, Bhakoo &
Puri, 2007; Oberman, Pineda, & Ramachandran, 2007; Rizzolatti & Craighero,
2004), there is also evidence suggesting a deficit in this system may be linked to
the social-emotional deficits observed in ASDs (e.g. Buccino & Amore, 2008;
Hadjikhani, Joseph, Snyder & Tager-Flusberg, 2006).

Many researchers believe the MNS directly influences imitative abilities
as it facilitates the appropriate understanding of actions which, appears to be an
important prerequisite for imitation, as one has to form a representation of the
model and then effectively plan and execute the imitative action (Kana et al.,
2011). Specifically, it has been proposed that the spontaneous mimicry of facial
expressions, may in part, be attributable to the MNS, whereby automatic
processing by mirror neurons allows an internal modelling of another’s facial
expression which facilitates an understanding of the observed individual’s mental
and affective state and results in the spontaneous mimicking of the observed
expression (Enticott et al., 2008; Kana et al., 2011). This contention is supported
by several fMRI studies in TD individuals which suggest that the production and,
in some accounts, the mere observation of facial expressions is associated with
enhanced activation in a region of the premotor cortex, a proposed component of
the MNS (Carr, Iacoboni, Dubeau, Mazziotta & Lenzi, 2003; Dapretto et al.,
2006). Despite the theorised importance of automatic emotional mimicry to the
social functioning of TD individuals, little research has been dedicated to
exploring how this process may be impacted in ASDs, particularly with respect to a dysfunctional MNS. Although the exact nature of this impairment remains unknown, the available research does suggest the presence of deficits in spontaneous mimicry in individuals with an ASD.

Contemporary studies have employed automatic mimicry tasks in combination with neurological measures to investigate differences in automatic facial mimicry between TD individuals and individuals with an ASD. In these tests of automatic mimicry, participants are not asked to imitate modelled movements. Instead they are instructed to merely observe actions while the experimenter measures involuntary muscular responses. This protocol provides a measure of the extent to which observing an action is priming its execution through activation of the muscles involved in its execution in a passive observation task. For example, McIntosh et al. (2006) utilised surface electromyography (EMG) to measure muscular activity in specific facial muscles while TD individuals and individuals with an ASD viewed various emotional facial expressions. While TD individuals spontaneously activated facial muscles corresponding to the observed expression, i.e. they exhibited greater activation in muscles involved in smiling when they were presented with a smiling face and greater activation in the muscles involved in frowning when they observed an angry face, individuals with an ASD did not exhibit this pattern. Stel, van den Heuvel and Smeets (2008) observed a similar effect in adolescents and Oberman Winkielman & Ramachandran (2009) in children.

In addition, fMRI studies have also found that when children and adults observe emotional facial expressions, participants with an ASD display reduced activation in the premotor mirror neuron area compared to TD participants.
(Dapretto et al., 2006). In contrast, Press, Richardson and Bird (2010) argue that automatic mimicry abilities in ASD are intact. In Press et al.’s stroop paradigm participants were asked to perform a pre-specified surprised or angry facial expression in response to observed angry or surprised facial actions, measuring the speed of this action with motion tracking equipment. The authors found that both the TD and ASD group responded faster when they were instructed to make the same expression to the one viewed compared to making an incongruent emotional expression. From this finding they contended that there are no deficits in automatic mimicry abilities in individuals with an ASD. Further, the authors posit that this finding suggests there is no impairment in the MNS in ASD and that previous demonstrations of impairments are instead driven by a lack of visual attention to the stimuli or motor sequencing impairments.

It should be emphasised that empirical evidence strongly suggests that individuals with an ASD are successfully able to voluntarily imitate the emotional facial expression of another when specifically instructed to do so (e.g. McIntosh et al., 2006; Oberman et al., 2009). Given this, it is plausible that Press and colleagues (2010) tested the voluntary imitative abilities of their participants as opposed to automatic mimicry as participants were instructed to perform a pre-specified facial expression. Thus, the studies that have observed an absence of automatic facial mimicry in ASD likely reflect a true disturbance in these abilities as opposed to deficits in perception, praxis, motivation or task understanding.

While previous research investigating deficits in automatic facial mimicry in ASD has, for the most part, used neurological techniques such as EMG and fMRI to look at either muscular or cortical responses in isolation and then infer deficits in mirror neuron activity, the research examining cortico-muscular
activity is extremely limited. Given the proposed link between the MNS and automatic mimicry, assessing simultaneous brain and muscular activity would provide important insights (Carr et al., 2003; Kana et al., 2011). In an attempt to assess this, Enticott et al. (2008) utilised transcranial magnetic stimulation (TMS) in conjunction with EMG to investigate the association between mirror neuron activation and facial emotion processing in TD adults.

TMS was used, as it is a means of stimulating nerve cells in the motor cortex via the administration of a brief magnetic pulse to the scalp. This pulse produces a motor evoked potential (MEP) in the specific muscle stimulated that can be measured via EMG. As the premotor cortex extends posteriorly to the primary motor cortex, mirror neurons in the ventral premotor cortex are believed to connect sensory neurons responding to the visual properties of an observed action and corticospinal neurons that discharge MEPs during the execution of a similar action (Fadiga, Craighero & Olivier, 2005; Rizzolatti & Craighero, 2004; Zult, Howatson, Kádár, Farthing & Hortobágyi, 2013). Thus, when TMS is delivered during the observation of action within the stimulated muscle, it is believed that premotor mirror neuron activity increases excitability in the motor cortex resulting in enhanced MEP amplitude. Enticott et al. (2008) correlated participants’ performance on a task of visual discrimination, where participants indicated if pairs of emotional expressions were the same and, an emotion recognition task where participants had to identify the emotional expression, with MEP amplitude in a specific muscle in the hand whilst observing movement observation videos of hand actions. Enticott et al. found that increased MEP amplitude during action observation was correlated with better performance on the facial emotion processing tasks. From this, Enticott et al. argues that this
research demonstrates that a marker for mirror neuron activity in the premotor cortex, i.e. TMS during action observation, correlates with social cognition, thus providing empirical support for theoretical accounts concerning the role of the MNS in social functioning.

While Enticott et al. (2008) provides valuable information regarding facial emotion processing and mirror neuron activity, to date this methodology has yet to be employed to directly assess automatic facial mimicry in TD individuals or individuals with an ASD. Further, Enticott and colleagues stimulated and recorded cortico-muscular activity of the hand and used this to infer face-related cortico-muscular mirror neuron activity as opposed to measuring facial activity directly. Ideally, the cortical region of the motor cortex corresponding to the face should be stimulated directly to gain a more accurate measurement of muscular response and mirror neuron activation specific to the face.

Given the well-entrenched conceptual connection between mimicry and social cognition in psychological thought, and in an attempt to progress the above line of enquiry, the present study aimed to investigate automatic mimicry of emotional facial expressions in TD individuals and individuals with an ASD. Achieving this, by employing an automatic facial mimicry paradigm whilst utilising TMS to stimulate the cortical region of the motor cortex corresponding to the face and using EMG to assess spontaneous muscle activation in the corresponding muscles of the face. It was hypothesised that TD individuals would actively engage in automatic mimicry of emotional facial expressions as demonstrated by increased MEP amplitude in emotional conditions compared to neutral conditions. Further, it was also hypothesised that individuals with an ASD would demonstrate a lack of automatic mimicry of emotional facial expressions as
demonstrated by no difference in MEP amplitude between emotion and neutral conditions.

Method

The methods and procedures used in this chapter form the basis for all methods and procedures of data collection for subsequent chapters in this thesis. Therefore, the methods in each experimental chapter are an abridged version of the current chapter, with only the most appropriate sections reproduced. All 11 typically developing (TD) participants and 10 participants with an autism spectrum disorder (ASD) attended a single session where they partook in each of the three experimental paradigms. Experimental order was counterbalanced between participants’ to account for any potential order effects and each participant was tested at the same time of day. Due to equipment malfunctions not all participants are included in each of three studies.

Participants.

Participants were ten typically developing male adults (TD) and ten male adults with a diagnosis of either high functioning autism (HFA) or Asperger’s syndrome (AS). One TD participant was excluded due to equipment malfunction. Participants with HFA/AS (M=23.3) were age stratified to TD participants (M=24.7) within 3 years (age range 18-34). The mean age and SD of all participants was 24 and 3.9 respectively. TD participants were recruited by word of mouth and at Deakin University. Participants with HFA/AS were recruited from Autism Victoria’s research database, schools such as the Western Autistic
School, word of mouth, and advertisements and mail outs from paediatric clinics. An experienced Clinical Psychologist who has worked extensively in the autism field confirmed diagnosis using DSM-IV-TR (APA, 2000) criteria.

All participants were right handed and had normal or corrected-to-normal vision. Prior to the experimental procedures, participants completed the Adult Safety Screening Questionnaire to determine their suitability for transcranial magnetic stimulation (TMS; Keel, Smith & Wassermann, 2001). The TMS safety screen was used to exclude participants who had any pre-existing medical conditions (such as epilepsy or stroke), or any implanted devices (such as cardiac pacemakers), which may have resulted in adverse effects from TMS (see Appendix A).

The Deakin University Human Research Ethics Committee (DUHREC 2009-135) granted ethics approval in accordance with the National Statement on Ethical Conduct in Human Research (2007) prior to the commencement of this research (see Appendix B). All participants were provided with the plain language statement (PLS) and gave written, informed consent prior to engaging in the experimental procedures (see Appendix C).

**Stimuli.**

The stimuli presented were eight happy, eight angry and eight neutral static pictures of facial expressions sized 10 cm by 10 cm presented on a 13-inch laptop computer screen placed approximately 60 cm away from the participant. The eight individual faces chosen in each condition were selected from the NimStim stimulus set consisting of 192 photographs of different facial expressions (Tottenham et al., 2009). The photographs utilised in the present
study comprised of eight male actors matched across conditions and were selected from this set based on their emotional identification reliability estimates of > .8 for happy and angry facial expressions and > .7 for neutral expressions. Each photograph was displayed for 20 seconds, followed by a 15 second inter-stimulus interval where a black screen with a white central fixation cross was presented.

Coordinated with the timed presentation of the photographs was a pre-recorded sound that was elicited once each photograph had been displayed for five seconds and then again when the photograph had been displayed for 15 seconds. The sound indicated to the experimenter to deliver a TMS pulse and allowed for consistency of TMS delivery across all stimuli presentation. The sound was delivered to the experimenter through headphones to prevent the sound from being heard by the participant.

**Experimental Design.**

The experiment consisted of two blocks. In the Zygomaticus Major (ZM) block participants’ engagement in automatic mimicry towards happy facial expressions was examined, specifically, activation of the ZM muscle, which pulls up the cheek when making a smiling facial expression (Fridlund & Cacioppo, 1986). In the Corrugator Supercilii (CS) block participants’ engagement in automatic mimicry towards angry facial expressions was examined, specifically, activation of the CS muscle, which furrows the brow when making a frowning facial expression (Fridlund & Cacioppo, 1986). The ZM and CS blocks were counterbalanced between participants to account for any potential order effects.

Within both the ZM and CS blocks were three conditions: baseline, control and experimental. In all conditions within the ZM block, surface
electromyography (EMG) electrodes were placed on the ZM muscle and the identified optimal position on the primary motor cortex (M1) to elicit motor evoked potentials (MEPs) in the ZM was identified. Similarly, in all conditions within the CS block, surface EMG electrodes were placed on the CS muscle and the identified optimal position on the M1 cortex to elicit MEPs in the CS was identified. Before each condition participants were instructed simply to ‘watch the pictures as they appear on the screen’.

In the baseline conditions participants were presented with a black screen with a white central fixation cross. During these conditions 16 MEPs were taken while the participant simply viewed the screen. The baseline conditions examined individual participants resting MEP amplitude in the ZM and CS muscles. Thus each participant completed this condition twice, once in the ZM block and once in the CS block.

In the control conditions participants were presented with eight neutral facial expressions displayed for 20 seconds each. During these conditions 16 MEPs were taken while the participant viewed the photographs, one after each photograph had been displayed for five seconds and one after each photograph had been displayed for 15 seconds. Each photograph was followed by a 20 second inter-stimulus interval where a black screen with a white central fixation cross was presented. Again, each participant completed this condition twice, once in the ZM block and once in the CS block.

Similarly, in the experimental conditions participants were presented with eight happy facial expressions in the ZM block and eight angry facial expressions in the CS block, with 16 MEPs taken in the same manner as the control conditions.
The baseline, control and experimental conditions were counterbalanced within each ZM and CS block to account for any potential order effects.

**Transcranial Magnetic Stimulation and Electromyography.**

TMS is a safe, non-invasive technique that depolarises corticospinal neurons at the level of M1. A coil is placed on the surface of the skull, which passes a weak electrical current through the scalp to stimulate neuron pools. Thus, TMS allows MEPs to be measured at the muscle when the corresponding part of M1 is stimulated.

Prior to using TMS, measurements of each participant’s skull dimensions were taken. A tape measure was used to measure the subject’s head in reference to nasion-inion and inter-aural lines. A tight fitting cap was then placed on the subject’s head, and was dotted with sites at 1 cm intervals in a latitude-longitude matrix.

Focal TMS was used to measure corticospinal excitability of specific facial muscles, ZM in the block of happy experimental conditions and CS in the angry block of experimental conditions. Specifically, TMS was applied over the left M1 using a BiStim unit attached to two Magstim 200² stimulators (Magstim Co, Dyfed, UK) to produce MEPs in the left ZM and CS muscles. A circular coil, with an external loop diameter of 90 mm, was held over the left M1 at the optimal position to elicit MEPs in the desired facial muscle for the appropriate experimental block. The coil was placed over the vertex of the head and held tangential to the skull in an antero-posterior orientation. Sites near the estimated centre of the ZM and CS were explored to find the optimal site at which the largest MEP amplitude was obtained, and this area was marked with a small “x”
in permanent marker. Care was taken by the researcher to ensure that the coil was held over the same position on the scalp so that the same area of the M1 was stimulated for all conditions within the relevant experimental block (Figure 4.1).

Figure 4.1 Experimental set up. Participants wore a tight fitted cap with markings of 1 cm distance in both antero-posterior and medio-lateral directions. A circular coil (90 mm) was held tangential to the skull in an antero-posterior orientation, so that the current flowed in a counter-clockwise direction for activating the left M1.

Once each subject’s optimal spot was identified, their resting motor threshold (RMT) was tested. For this study, RMT was defined as the minimum intensity required to elicit an MEP (i.e. a peak-to-peak amplitude of 50 μV). Once RMT was identified, MEPs in all conditions were taken at 120% of RMT, which ensured a reliable signal. All MEP trials that contained any pre-stimulus EMG (100 ms before stimulation) were discarded from analysis. MEP amplitudes were
measured peak-to-peak in each individual trial. The process outlined above was completed independently for both the trials utilising the ZM and the CS muscles.

Surface EMG activity was recorded from the ZM muscle (cheek) during the conditions within the ZM block and from the CS muscle (brow) during the conditions within the CS block. Bipolar Ag-AgCl electrodes were placed on the respective muscle belly whilst a reference electrode was placed on the bony prominence of the participant’s clavicle. All cables were fastened with tape to prevent movement artefact. The area of electrode placement was shaved to remove fine hair, rubbed with an abrasive skin rasp to remove dead skin, and then cleaned with 70% isopropyl alcohol. EMG signals were amplified (x1000) with band pass filtering between 20 Hz and 1 kHz and digitised at 2 kHz for 500 ms, recorded and analysed using PowerLab 4/35 (ADInstruments, Australia).

**Data Analysis.**

Although research suggests TMS obtains moderate to good reliability of MEP amplitude (Kamen, 2004), given the inherent variability of MEP amplitude, steps need to be taken to minimise the impact of both inter-individual and intra-individual variation (Burke, Hicks, Stephen, Woodforth, & Crawford, 1995; Wassermann, 2002). Inter-individual variation, i.e. the variation between MEP amplitude from trial to trial, was addressed in the present study by evoking a number of MEPs within each experimental condition in order to gain a more representative measurement of MEP amplitude within that muscle for each participant (McDonnell, Ridding, & Miles, 2004).

To account for intra-individual variation, data was standardised (i.e expressed as z scores) within each participant and within each muscle group (ZM,
CS), attenuating the impact of highly reactive individuals on group scores and allowing for meaningful comparisons. As commonly employed in experimental paradigms utilising TMS (e.g. Funase, Tabira, Higashi, Liang, & Kasai, 2007; Garry, Loftus & Summers, 2005), transformation of the data to z-scores was made relative to each participant’s individual baseline for ZM and CS instead of standard z-scores in order to reflect a ratio of individual change. This was undertaken as standard z-scores reflect data in terms of their standard deviation (SD) points from the mean and thus would themselves have a mean of zero, yielding no effects. Further standardising to each individuals’ baseline for ZM and CS reflected their own change in MEP strength relative to their respective baseline. Thus, meaningful comparisons on the same scale in terms of SD points from their baseline measures were possible. The z-score transformations of individual MEPs relative to their respective baseline were calculated by taking the appropriate mean baseline score from each individual MEP in the two control and two experimental conditions for each muscle group and then dividing that by the standard deviation of their respective baseline (See Equation 1).

\[
Z = \frac{x - \mu_{\text{Baseline}}}{\sigma_{\text{Baseline}}} \quad \text{(Eqn. 1)}
\]

**Statistical Analysis.**

Two ANOVA designs were used to compare Z score MEP amplitudes across condition and group; both being 2x2 mixed model ANOVAs (between subjects factor group in both: TD and ASD; while the within subject factor varied by condition: ZM neutral and ZM Happy in design one, and: CS neutral and CS
happy in design two). Where appropriate, planned comparisons were carried out to compare MEP amplitude across conditions within the same group.

Results

Data Screening.

Data was cleaned, screened and analysed using SPSS Version 18 (SPSS Inc.) with no missing values found. Seven univariate outliers were identified using z-scores with a criterion of $\alpha < .001, (z = \pm 3.3$ cut-off). Hypothesis-related analyses were run with and without these outliers and as no meaningful difference was evident, the values were retained (Tabachnick & Fidell, 2007).

The Kolmogorov-Smirnov statistic indicated that all conditions were significantly non-normal. However an examination of the Q-Q plot for each condition did not suggest any significant deviations from normality. Thus, given the number of samples was large ($n=320$ MEPs per condition) it is likely that the significant Kolmogorov-Smirnov statistic was due to the large number of samples rather than a true deviation from normality (Field, 2009).

Zygomaticus Major Analysis.

A 2x2 mixed model ANOVA was conducted in order to compare the TD and ASD group mean Z score MEP amplitudes across the conditions ZM-Neutral and ZM-Happy. Box’s test of equality of covariance’s matrices was violated ($p<.001$) and Levene’s test of equality of error variances was violated for the condition ZM-Neutral. Given the large sample with equal group sizes the analysis
was still believed to be appropriate, however in light of the violations a more stringent alpha of $\alpha < .001$ was adopted (cf. Tabachnick & Fidell, 2007). There was a significant interaction between group and condition ($F_{(1,318)}=65.64$, $p<.001$, $\eta^2_p = .17$). The main effects for condition ($F_{(1,318)}=43$, $p>.05$, $\eta^2_p < .01$) and group ($F_{(1,318)}=99$, $p>.05$, $\eta^2_p < .01$) were not significant (see Figure 4.1 for plot).

![Figure 4.2 TD and ASD mean Z score MEP amplitudes across the conditions ZM-Neutral and ZM-Happy](image)

*Figure 4.2 TD and ASD mean Z score MEP amplitudes across the conditions ZM-Neutral and ZM-Happy*

Simple main effects comparing condition within groups were undertaken using independent samples t-tests, adjusted for unequal variances where necessary. These indicated that within the TD group Z score MEP amplitude in the condition ZM-Happy was significantly higher than in the condition ZM-Neutral ($t_{(318)}=-5.25$, $p<.001$, $\eta^2_p = .09$). In contrast, the ASD groups Z score MEP amplitude in the condition ZM-Neutral was significantly higher than in the
condition ZM-Happy \( t(304)=-5.16, p<.001, \eta_p^2=0.08 \); see Table 4.1 for means and Figure 4.3 for sample MEPs).

Table 4.1

Mean Z Score MEP Amplitudes and Standard Deviations Normalized to Baseline for TD and ASD Groups for the Conditions ZM-Neutral and ZM-Happy

<table>
<thead>
<tr>
<th>Condition</th>
<th>TD</th>
<th>ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>ZM-Neutral</td>
<td>-.21</td>
<td>1.52</td>
</tr>
<tr>
<td>ZM-Happy</td>
<td>.75</td>
<td>1.74</td>
</tr>
</tbody>
</table>

\( n = 160 \) MEPs per condition and 10 participants per group
Figure 4.3 Sample overlaid MEP amplitude sweeps recorded from one TD participant and one participant with an ASD for the conditions ZM-Neutral and ZM-Happy
Corrugator Supercilii Analysis.

A 2x2 mixed model ANOVA was conducted in order to compare the TD and ASD group mean Z score MEP amplitudes across the conditions CS-Neutral and CS-Angry. Box’s test of equality of covariance’s matrices was violated ($p<.001$) and Levene’s test of equality of error variances was violated for the condition CS-Neutral. Given the large number of samples, with equal group sizes, the analysis was believed to be appropriate; however, in light of the violations a more stringent alpha of $\alpha<.001$ was adopted (cf. Tabachnick & Fidell, 2007). There was a significant interaction between group and condition ($F_{(1,318)}=65.08$, $p<.001$, $\eta_p^2=.17$) and a significant main effect for group ($F_{(1,318)}=33.93$, $p<.001$, $\eta_p^2=.10$). The main effect for condition ($F_{(1,318)}=.52$, $p>.05$, $\eta_p^2<.01$) was not significant (see Figure 4.4 for plot).

Figure 4.4 TD and ASD mean Z score MEP amplitudes across the conditions CS-Neutral and CS-Angry
Simple main effects comparing condition within groups were undertaken using independent samples t-tests adjusted for unequal variances where necessary. These indicated that within the TD group Z score MEP amplitude in the condition CS-Angry was significantly higher than in the condition CS-Neutral ($t_{268}=-4.13$, $p<.001$, $\eta^2_p=0.06$). In contrast, the ASD groups Z score MEP amplitude in the condition CS-Neutral was significantly higher than in the condition CS-Angry ($t_{318}=-4.27$, $p<.001$, $\eta^2_p=0.05$; see Table 4.2 for means and Figure 4.5 for sample MEPs).

Table 4.2
Mean Z Score MEP Amplitudes and Standard Deviations Normalised to Baseline for TD and ASD Groups for the Conditions CS-Neutral and CS-Angry

<table>
<thead>
<tr>
<th>Condition</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TD</td>
<td></td>
<td></td>
<td>ASD</td>
<td></td>
</tr>
<tr>
<td>CS-Neutral</td>
<td>.48</td>
<td>1.46</td>
<td></td>
<td>.29</td>
<td>2.36</td>
</tr>
<tr>
<td>CS-Angry</td>
<td>1.37</td>
<td>2.32</td>
<td></td>
<td>-.78</td>
<td>2.12</td>
</tr>
</tbody>
</table>

$n = 160$ MEPs per condition and 10 participants per group
Figure 4.5 Sample overlaid MEP amplitude sweeps recorded from one TD participant and one participant with an ASD for the conditions CS-Neutral and CS-Angry
Discussion

The present study utilised transcranial magnetic stimulation (TMS) in an automatic mimicry paradigm to investigate automatic facial mimicry in typically developing (TD) individuals and individuals with an autism spectrum disorder (ASD) with respect to the mirror neuron system (MNS) as a possible neural substrate mediating this process. In line with theoretical tenets and previous findings (Enticott et al., 2008; McIntosh et al., 2006; Oberman et al., 2009), it was anticipated that TD individuals would actively engage in automatic mimicry of emotional facial expressions. The results supported this hypothesis, as demonstrated by a significant increase in motor evoked potential (MEP) amplitude in emotional conditions compared to neutral conditions. In contrast, it was anticipated that individuals with an ASD would demonstrate a lack of automatic mimicry of emotional facial expressions. Thus it was hypothesised that in participants with an ASD there would be no significant difference in MEP amplitude between emotion and control conditions. While the results supported the hypothesis that individuals with an ASD would not demonstrate automatic mimicry of emotional expressions, the results also suggested that individuals with an ASD might have an automatic mimicry response actively inhibited when viewing emotional stimuli. This was demonstrated by significantly lower MEP amplitude in both the happy and angry emotional conditions when compared to their respective neutral conditions.

It is important to note that the addition of TMS to electromyography (EMG) measures in the present study demonstrated the ability to not only capture and record rapid and automatic facial muscle reactions, but also obtain an understanding of the corticospinal changes believed to be a result of premotor
mirror neuron activity. Thus taken together, the results of this study provide valuable insight into the role of the MNS in both TD individuals and individuals with an ASD with respect to automatic mimicry.

The finding that TD individuals automatically mimic emotional facial expressions, as demonstrated by significantly larger MEPs in emotional conditions compared to neutral conditions, supports previous research and the view that social cognition is, at least partially, sub-served by the MNS (McIntosh et al., 2006; Oberman et al., 2009). Specifically the results suggest that during facial emotion processing, mirror neurons in the premotor cortex provide an internal simulation of the observed motoric behaviour that evokes a similar reaction in the corresponding muscles of the observer. It is believed that this simulation process allows TD individuals to understand the observed individual’s mental and affective state (Enticott et al., 2008), which in turn facilitates social functioning and communication (Uddin et al., 2007). This notion is further supported by empirical evidence demonstrating that in TD individuals, automatic mimicry is associated with greater levels of empathy, perspective taking and the understanding of other’s minds (McIntosh, 2006; Sonnby-Borgström, 2002).

Further strengthening the argument that automatic mimicry is important for social cognition and, at least in part, sub-served by the MNS, is the present finding that participants with an ASD, a disorder of social functioning, exhibited deficits in automatic mimicry of emotional facial expressions. In line with previous research, the present study found that when presented with emotional facial expressions participants with an ASD did not display the tendency to automatically mimic the expression with which they were presented (McIntosh et al., 2006; Stel, van den Heuvel & Smeets, 2008). Further, the present study found
that participants with an ASD exhibited significantly lower MEP amplitude when viewing emotional stimuli compared to neutral stimuli. This suggests that when presented with stimuli that are emotional in nature an automatic mimicry response is actively inhibited in individuals with an ASD, supporting the notion that individuals with an ASD likely possess a disturbance in the MNS. Specifically, the present results support the inference that when individuals with an ASD view emotional facial expressions, reduced synaptic efficacy between premotor mirror neurons and corticospinal neurons suppresses activity in the facial region of the primary motor cortex (M1), in turn resulting in an inhibited response at the muscular level as evidenced by reduced MEP amplitude. This is supported by functional magnetic resonance imaging (fMRI) research demonstrating that when observing emotional facial expressions, children and adults with an ASD display reduced activation in the perceived premotor mirror neuron area compared to TD participants (Dapretto et al., 2006).

The present findings raise important questions regarding the possible downstream consequences of a deficit in automatic mimicry in ASD. As discussed, it has been established that TD new born babies exhibit rudimentary forms of facial mimicry (Meltzoff & Moore, 1989). Further, in a normal developmental trajectory these behaviours increase in complexity and form the basis for future social development by providing the individual with information about the actions and intentions of others (Rodgers et al., 2003) Thus, if a mimicry deficit occurred early in the developmental trajectory of a child with an ASD not only would automatic mimicry behaviour be significantly impaired but this could consequently impair the child’s ability to co-experience other’s emotional states (McIntosh et al., 2006). This deficit in emotional contagion may
subsequently prevent the child with an ASD from developing a sense of intersubjectivity and emotional correspondence which are essential for social learning and the understanding of other minds (Bandura, 1977; Meltzoff, 1999). As a consequence, this in turn could impair the development of higher order socio-communicative abilities such as empathy, emotional reciprocity and theory of mind (ToM), disturbances of which characterise ASD (APA, 2013).

When interpreting the findings of the present study, a number of limitations should be considered. As the sample size was modest, replication in larger samples is needed to more firmly establish the present findings. Further as the present sample only included male adults the results are therefore limited in their generalizability, thus any developmental inferences drawn are done so speculatively. Thus, given the developmental significance of automatic mimicry behaviours, future research needs to further investigate these abilities in child and adolescent TD and ASD populations in order to establish the developmental trajectory of these behaviours with respect to the MNS. Another limitation of the present study is the narrow range of emotions utilised. While the present study utilised one positive and one negative emotion, happiness and anger respectively, which are easily identified by the TD and ASD populations, future research should employ this methodology to examine a wider range of emotions.

Another potential limitation of the present study is that an alternative explanation for the results could be posited. Specifically it could be argued that participants with an ASD did not engage in automatic mimicry of facial expressions due to either inattention to the stimuli presented or by differential face processing, reflected in a failure to attend to the eye region (Gross, 2004). However there are several reasons as to why these explanations are unlikely.
Firstly, automatic mimicry of facial expressions has been shown even when the stimuli was presented outside of awareness, and thus before participants could consciously attend to it (Dimberg et al., 2000). Secondly, research has shown that the presence of automatic mimicry of facial expressions is not dependent on the participant directing their attention to the gaze of the stimulus model, as mimicry has been observed to occur even when the participant was observing a model attending to an emotional stimulus, as opposed to the observer (McIntosh, 2006). Further, one of the emotional expressions utilised in the present was smiling. As smiling requires the observer to attend to the lower regions of the face, which individuals with an ASD do attend to (Klin, Jones, Schultz, Volkmar, & Cohen, 2002), it is very unlikely that the results are solely due to participants with an ASD failing to attend to the eye regions of the face (Gross, 2004; McIntosh, 2006). Given this, it is posited that mimicry is an innate or fairly automatized response. Thus the differences obtained between TD participants and participants with an ASD in the present study are likely a true reflection of differences in the functioning of the MNS as opposed to differences in attention or face processing. However future research should utilise eye-tracking equipment in order to entirely rule out this possibility.

In conclusion, the present findings converge with previous research highlighting that automatic mimicry, a basic feature of social interaction, is impaired in ASD. Further, the results provide support that social cognition may, at least in part, be sub-served by the MNS and that disturbances in this system likely contribute to the social communicative disturbances observed in ASD. Although more research is required to elucidate the link between social cognition, the MNS and ASD, the present study raises several questions regarding the possible
downstream consequences that may result from imitative deficits early in development and the role disturbances in the MNS may play in this. As such the present study sheds light on processes that shape social cognition in both the typical and atypical social mind as well as highlighting possible neural substrates that mediate this process.
Chapter 5: Contagious Yawning and Autism Spectrum Disorder
Introduction

Spontaneous yawning is a phylogenetically old, yet complex stereotyped motor behaviour that is observed in most vertebrate species from foetal stages to old age. Although the act of yawning spontaneously is widespread among species (Baenninger, 1997), the onset of a yawn triggered by seeing, hearing or even thinking about yawning has only been observed consistently in species that demonstrate the capacity for empathy and self-awareness, such as humans (Provine, 1986) chimpanzees (Anderson, Myowa-Yamakoshi, & Matsuzawa, 2004) and stumptail macaques (Paukner & Anderson, 2006). In contrast to spontaneous yawning, in humans, yawning contagion may not appear until at least the second year after birth (Anderson & Meno, 2003; Piaget, 1951) and typically occurs in approximately 40%-60% of the population (Platek, Critton, Myers, & Gallup, 2003). Thus suggesting not only a higher level of complexity but also a high degree of both evolutionary and developmental specialisation. In light of this, current hypotheses on the evolution and function of contagious yawning focus on its potential role in social interaction, communication and the development of empathy (Platek et al., 2003). Specifically it is posited that the contagiousness of yawning depends on mechanisms that develop during childhood in parallel with the empathic capacity to understand mental states of others (Guggisberg, Mathis, Schnider, & Hess, 2010).

Despite the unique opportunity contagious yawning offers to explore the neurological roots of social behaviour, empathy and imitation, little research has been undertaken to elucidate the origin of this phenomenon. A thorough understanding of the neural underpinnings of contagious yawning is necessary as it could provide considerable insight into the behavioural and communicative
systems of both human and non-human species, and may be particularly relevant to revealing the possible pathology of these processes in disorders of social and communicative functioning such as autism spectrum disorders (ASD).

In line with the contention that contagious yawning reflects a basic capacity for empathy, recent studies have linked individual differences in empathic tendencies to differences in yawning contagion (Platek et al., 2003). For example, Platek and colleagues demonstrated that increased susceptibility to contagious yawning was associated with greater self-recognition abilities and better performance on theory of mind (ToM) tasks. This is further supported by cross species research in chimpanzees, finding that greater yawning contagion was associated with greater self-recognition and increased grooming behaviour, both of which are viewed as an index for the capacity of empathy (Anderson et al., 2004).

Consistent with the link between contagious yawning and its potential role in social and communicative abilities such as mental state attribution, automatic mimicry and the development of empathy, is the observation that individuals with an ASD, who exhibit impairments in these processes, also demonstrate disturbances in the tendency to yawn contagiously (Giganti, & Ziello, 2009; Helt, Eigsti, Snyder, & Fein, 2010; Senju, Maeda, Kikuchi, Hasegawa, Tojo, & Osanai, 2007). For example, several studies have demonstrated that although there is no difference in TD individuals and individuals with an ASD in the tendency to yawn spontaneously, when observing videos of others yawning, TD individuals exhibit significantly more yawns in comparison to control mouth movements. In contrast, studies have shown that often children and adolescents with an ASD display an absence of contagious yawning when viewing others yawn (Giganti, &
Ziello, 2009; Helt et al., 2010; Senju, et al., 2007). It is important to note that it has been suggested that the observed absence of a contagious effect when individuals with an ASD view others yawn, could be due to their difficulty in establishing reciprocal gaze (Volkmar & Mayes, 1990). However, as research has demonstrated that listening to the sound of others yawning also elicits contagious yawning in TD individuals but not individuals with an ASD, this suggests the relative absence of contagious yawning in ASD is more than just atypical orienting to social stimuli (Senju, Kikuchi, Akechi, Hasegawa, Tojo, & Osanai, 2009). Instead, likely reflecting a possible impairment in the neural mechanism relevant to the capacity for contagious yawning.

Although different neuroimaging studies have reported divergent areas to be implicated in contagious yawning, all appear to be part of a distributed neural network related to empathy and social behaviour. For instance, Platek, Mohamed and Gallup (2005) found that viewing someone yawn evoked unique neural activity in the posterior cingulate and precuneus, both of which are linked to self-processing abilities and believed to be part of the neural network involved in empathy. Additionally, ventromedial prefrontal cortex activation, an area previously implicated in empathic processing (Shamay-Tsoory, Tomer, Berger & Aharon-Peretz, 2003), has been associated with the urge to yawn when viewing another yawn in a recent functional magnetic resonance imaging (fMRI) study (Nahab, Hattori, Saad & Hallett, 2009). Activity in core components of the social brain, including the superior temporal sulcus and peri-amygdalar regions (Brothers, 2002), have also been reported in response to yawning susceptibility during the observation of others yawns (Schürmann et al., 2005). Lastly, although controversial, specific cortical cells that respond to both the execution and
observation of motoric behaviour, known as mirror neurons, (Rizzolatti & Craighero, 2004) have also been linked to contagious yawning (Haker, Kawohl, Herwig, & Rössler, 2012).

The mirror neuron system (MNS) is postulated to be involved in yawning contagion as it provides a basis for action understanding (Rizzolatti & Craighero, 2004) and may form part of the neural substrate that underlies imitative actions (Uddin, Iacoboni, Lange & Keenan, 2007), both of which are capacities relevant to the phenomenon of contagious yawning. Further, as mirror neurons are believed to allow for the simulation of not just another individual’s intentions, but also their state of mind, it has been speculated that mirror neurons are involved in social cognition processes such as the development of empathy and ToM skills (Enticott, Johnston, Herring, Hoy & Fitzgerald, 2008). Again, supporting a putative link between the MNS and yawning contagion, as contagious yawning is believed to be part of the neural network involved in empathic skills (Platek et al., 2005). In addition, research also suggests that a faulty MNS may underlie many of the social deficits observed in ASD, a population that demonstrates disturbances in both empathic abilities and contagious yawning (Enticott et al., 2008; Giganti, & Ziello, 2009).

Despite the theorised importance of the MNS, neuroimaging evidence to date is mixed and consequently there is disagreement in the literature as to the possible role of the MNS in contagious yawning. For example, several fMRI studies have found areas of activation that are believed to comprise the MNS not only when viewing videos of other’s yawning but also during control conditions (Nahab, et al., 2009; Platek et al., 2005; Schürmann et al., 2005). As a result, the authors generally concluded that given mirror neuron activity was not specific to
yawning, instead occurring the same amount during other movements, contagious yawning must therefore occur independently of the MNS. However, it should be highlighted that these studies employed control conditions that were arguably mirror neuron stimulating in nature such as laughter and ‘non-nameable mouth movements’ (Cooper et al., 2012). Thus, the design of the control conditions likely masked any specific contribution of the MNS to contagious yawning. To date, only two neuroimaging studies have demonstrated MNS involvement specific to contagious yawning (Arnott, Singhal, & Goodale, 2009; Haker et al., 2012). In contrast to previous fMRI studies, Arnott and colleagues (2009) employed an auditory paradigm where they compared yawn sounds with electronically scrambled versions of the same sounds. The authors found greater activation in the right inferior frontal gyrus, a core area of the MNS, during the yawn condition compared to the control condition. Further, the urge to yawn was also positively correlated with individual’s empathy scores. In a recent fMRI study, Haker and colleagues (2012) also found greater activation in the right inferior frontal gyrus during the yawn condition compared to the control conditions. However their paradigm compared videos of yawning faces to neutral facial expressions. Thus, the results of both these studies suggest that the lack of specific mirror neuron activation in response to yawn stimuli in previous studies was likely the result of experimental design rather than a lack of involvement of the MNS in contagious yawning.

In a further attempt to address the discrepancies in neuroimaging investigations of contagious yawning, Cooper and colleagues utilised electroencephalography (EEG) as it affords higher temporal resolution than fMRI, as well as measuring mu wave suppression, a readily identifiable index of MNS
activation (Cooper et al., 2012). *Mu* rhythm is an EEG waveform recorded from the motor cortical areas that is suppressed not only when a person makes a voluntary movement but also when they observe another individual performing a voluntary movement. As the *mu* rhythm is generated by activity in the sensorimotor areas, and mirror neurons are believed to be located in the premotor cortex, it is thought that the suppression of the *mu* rhythm reflects a downstream modulation of primary sensorimotor areas by the MNS (Pineda, 2005). Thus, it is believed that *mu* wave suppression in response to observed actions can be used as a selective measure of activity in the MNS (Oberman et al., 2005). In light of this, Cooper et al. (2012) completed several experiments where they exposed participants to yawning and control stimuli whilst measuring changes in *mu* activation via EEG. The authors found greater *mu* suppression over the right motor and premotor areas when participants viewed videos of yawns compared to control stimuli, particularly in individuals who scored higher on empathy measures. They also reported a similar effect when audio recordings of yawns were presented and compared to electronically scrambled versions of the same yawn, observing greater *mu* suppression over the right lateral premotor areas when yawns were presented compared to control stimuli. The results from this study support the notion that the MNS is, at least in part, involved in yawning contagion and emphasises the link between contagious yawning and empathy.

While Cooper et al. (2012) provides valuable insights into the possible role of the MNS in contagious yawning, their finding also highlights the need for future research to employ alternative methods to neuroimaging. Given the putative link between empathy and the MNS and the deficits observed in both contagious yawning and empathy in ASDs, it is essential that future research
examine the possible role of the MNS in contagious yawning not just in TD individuals but also in individuals with an ASD. In an attempt to address these gaps, the present study aimed to investigate the role of the MNS in contagious yawning in both TD individuals and individuals with an ASD utilising an alternative means to neuroimaging, namely transcranial magnetic stimulation (TMS).

TMS is a means of stimulating nerve cells in the motor cortex via the administration of a brief magnetic pulse to the scalp. This pulse produces a motor evoked potential (MEP) in the specific muscle stimulated that can be measured via surface electromyography (EMG). As the premotor cortex extends posteriorly to the primary motor cortex, mirror neurons in the ventral premotor cortex are believed to connect sensory neurons responding to the visual properties of an observed action and corticospinal neurons that discharge MEPs during the execution of a similar action (Fadiga, Craighero & Olivier, 2005; Rizzolatti & Craighero, 2004; Zult, Howatson, Kádár, Farthing & Hortobágyi, 2013). Thus, when TMS is delivered during the observation of action within the stimulated muscle, it is believed that premotor mirror neuron activity increases excitability in the motor cortex resulting in enhanced MEP amplitudes.

The present paradigm utilised TMS to stimulate the cortical region of the motor cortex corresponding to the facial muscles engaged during a yawn, and using surface EMG to assess spontaneous muscle activation in the corresponding muscles of the face. It was hypothesised that TD individuals would display the capacity for contagious yawning as demonstrated by increased MEP amplitude when viewing others yawn compared to control mouth movements. Further, it was also hypothesised that individuals with an ASD would exhibit a lack of
yawning contagion as demonstrated by no difference in MEP amplitude between yawn and control conditions.

**Method**

**Participants.**

Participants were ten typically developing male adults (TD) and ten male adults with a diagnosis of either high functioning autism (HFA) or Asperger’s syndrome (AS). One TD participant was excluded due to equipment malfunction. Participants with HFA/AS (M=23.3) were age stratified to TD participants (M=24.4) within 3 years (age range 18-34). The mean age and SD of all participants was 23.8 and 3.9 respectively. TD participants were recruited by word of mouth and at Deakin University. Participants with HFA/AS were recruited from Autism Victoria’s research database, schools such as the Western Autistic School, word of mouth, and advertisements and mail outs from paediatric clinics. An experienced Clinical Psychologist who has worked extensively in the autism field confirmed diagnosis using DSM-IV-TR (APA, 2000) criteria.

All participants were right handed and had normal or corrected-to-normal vision. Prior to the experimental procedures, participants completed the Adult Safety Screening Questionnaire to determine their suitability for transcranial magnetic stimulation (TMS; Keel, Smith & Wassermann, 2001). The TMS safety screen was used to exclude participants who had any pre-existing medical conditions (such as epilepsy or stroke), or any implanted devices (such as cardiac pacemakers), which may have resulted in adverse effects from TMS (see Appendix A).
The Deakin University Human Research Ethics Committee (DUHREC 2009-135) granted ethics approval in accordance with the National Statement on Ethical Conduct in Human Research (2007) prior to the commencement of this research (see Appendix B). All participants were provided with the plain language statement (PLS) and gave written, informed consent prior to engaging in the experimental procedures (see Appendix C).

**Stimuli.**

The stimuli utilised were two videos with sound, one consisting of male actors completing a yawn and one consisting of the same actors completing a control mouth action where they continually said the sound ‘oww’. The control mouth action was selected as the sound and mouth movement was similar to that of a yawn. During the recording of the yawn stimuli, yawns were induced by talking about yawning and pretending to yawn, until natural yawns occurred. Those genuine yawns were then selected as stimuli. Four male actors completed the mouth actions in both videos. Each mouth action segment was eight seconds in duration and was repeated twice. Thus each video contained eight mouth actions. Following each mouth action segment was a 15 second inter-stimulus interval where a black screen with a white central fixation cross was presented. The videos appeared on a 10 cm by 10 cm display presented on a 13-inch laptop computer screen placed approximately 60 cm away from the participant.

Coordinated with the timed presentation of the mouth action segments was a pre-recorded sound that was elicited once each segment had been running for four seconds. The sound indicated to the experimenter to deliver a TMS pulse and allowed for consistency of TMS delivery across all stimuli presentation.
Experimental Design.

There were three conditions within the experiment: baseline, control and experimental. In all conditions electrodes were placed on the masseter muscle on the left side of the jaw and the identified optimal position on the primary motor cortex (M1) to elicit motor evoked potentials (MEPs) in this muscle was stimulated. Before each condition participants were instructed simply to ‘watch the screen’.

In the baseline condition participants were presented with a black screen with a white central fixation cross. During this condition eight MEPs were taken while the participant simply viewed the screen. The baseline condition examined individual participants resting MEP amplitude in the masseter muscle.

In the control condition participants were presented with the video containing eight control mouth movements lasting for eight seconds each. During this condition eight MEPs were taken while the participant viewed the video. Each mouth action was followed by a 15 second inter-stimulus interval where a black screen with a white central fixation cross was presented.

Similarly, in the experimental condition participants were presented with eight yawning mouth actions, with eight MEPs taken in the same manner as the control condition.

The order of conditions was always baseline, followed by control followed lastly by the experimental condition. The yawning condition was always last as residual yawns could interfere with subsequent conditions.
Transcranial Magnetic Stimulation and Electromyography.

Surface electromyography (EMG) activity and TMS evoked motor evoked potentials (MEPs) were recorded from the left masseter muscles in the jaw using bipolar Ag-AgCl electrodes. Please refer to the transcranial magnetic stimulation and electromyography section in chapter four (pages 58-60) for a more detailed description of the methods employed.

Data Analysis.

Data was standardised (i.e. expressed as z scores) within each participant, attenuating the impact of highly reactive individuals on group scores and allowing for meaningful comparisons. Please refer to the data analysis section in chapter four (pages 60-61) for the equation utilised and a more detailed description of the statistical transformation employed.

Statistical Analysis.

A 2x2 mixed model ANOVA (within subjects factor condition: control and experimental and between subjects factor group: TD and ASD) was used to compare Z score MEP amplitudes across condition and group. Where appropriate, planned comparisons were carried out to compare MEP amplitude across conditions within the same group and between groups within the same condition.
Results

Data Screening.

Data was cleaned, screened and analysed using SPSS Version 18 (SPSS Inc.) with no missing values found. Two univariate outliers were identified using z-scores with a criterion of $\alpha < .001$, ($\pm 3.3$ cut-off). Hypothesis-related analyses were run with and without these outliers and as no meaningful difference was evident, the outliers were retained (Tabachnick & Fidell, 2007).

The Kolmogorov-Smirnov statistic indicated that all conditions were normally distributed.

Analysis of Motor Evoked Potential Amplitudes.

A 2x2 mixed model ANOVA was conducted in order to compare the TD and ASD group mean Z score MEP amplitudes across the control mouth movement condition and the yawning mouth movement condition. Box’s test of equality of covariance’s matrices and Levene’s test of equality of error variances were within acceptable limits. There was a significant interaction between group and condition ($F_{(1,158)}=30.46, p<.001, \eta^2_p = .16$) and a significant main effect for condition ($F_{(1,158)}=10.03, p<.002, \eta^2_p = .06$) and group ($F_{(1,158)}=21.65, p<.001, \eta^2_p = .12$; see Figure 5.1 for plot).
Figure 5.1 TD and ASD mean Z score MEP amplitudes across the control mouth movement and yawning mouth movement conditions.

Simple main effects comparing condition both within groups and between groups were undertaken using independent samples t-tests. These indicated that within the TD group, mean Z score MEP amplitude in the yawning condition was significantly higher than in the control mouth movement condition, \((t_{158}) = -5.43, p < .001, \eta^2_p = .16\). Although within the ASD group, the mean Z score MEP amplitude in the control mouth movement condition was higher than in the yawning condition, this difference was not significant \((t_{158}) = 1.72, p > .05, \eta^2_p = .018\).

Within the control mouth movement condition, Z score MEP amplitude did not differ significantly between groups \((t_{158}) = -25, p > .05, \eta^2_p = .00\). However, within the yawning condition Z score MEP amplitude for the TD group was significantly higher than for the ASD group \((t_{158}) = 7.52, p < .001, \eta^2_p = .26\); see Table 5.1 for means and Figure 5.2 for sample MEPs.)
Table 5.1

*Mean MEP Amplitudes and Standard Deviations Normalised to Baseline for TD and ASD Groups for the Control Mouth Movement and Yawning Conditions*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-.59</td>
<td>2.32</td>
<td>-.50</td>
<td>1.83</td>
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<tr>
<td>Yawning</td>
<td>1.25</td>
<td>1.95</td>
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<td>1.84</td>
</tr>
</tbody>
</table>

\(n = 80\) MEPs per condition and 10 participants per group
Figure 5.2 Sample overlaid MEP amplitude sweeps recorded from one TD participant and one participant with an ASD for the control mouth movement and yawning mouth movement conditions.
Discussion

The present study utilised transcranial magnetic stimulation (TMS) to investigate contagious yawning in typically developing (TD) individuals and individuals with an autism spectrum disorder (ASD) with respect to the mirror neuron system (MNS) as a possible neural substrate facilitating this phenomenon. In line with theoretical tenets and previous findings, it was anticipated that TD individuals would display the capacity for contagious yawning. The results supported this hypothesis, as demonstrated by a significant increase in motor evoked potential (MEP) amplitude when viewing yawns compared to control mouth movements. In contrast, it was anticipated that individuals with an ASD would demonstrate a lack of yawning contagion. Again, the results supported this hypothesis, as the MEP amplitude when viewing yawns and control mouth movements did not differ significantly in participants with an ASD.

It is important to note that the addition of TMS to electromyography (EMG) measures in the present study demonstrated the ability to not only capture and record rapid and automatic facial muscle reactions, but also obtain an understanding of cortical changes in the primary motor cortex (M1) believed to be a result of premotor mirror neuron activity. Taken together, the results of this study provide valuable insight into the role of the MNS in both TD individuals and individuals with an ASD with respect to yawning contagion.

Firstly, the finding that TD individuals displayed the capacity for yawning contagion, as demonstrated by significantly larger MEPs when viewing yawns compared to control mouth movements, supports previous research and the view that the MNS plays a role in contagious yawning (Cooper et al., 2012; Haker et al., 2012). Specifically the results suggest that when viewing another’s yawn,
mirror neurons in the premotor cortex provide an internal simulation of the observed motoric behaviour, evoking a similar reaction in the corresponding muscles of the observer. Thus, it is believed that this system may underlie the contagious aspects of the phenomenon. However previous neuroimaging research examining the role of the MNS in contagious yawning has resulted in mixed findings.

The present results align with Arnott et al.’s (2009) and Haker et al.’s (2012) findings, who both found greater activation in specific mirror neuron areas in yawning conditions compared to control conditions. Further, the present results also support the only study to date to utilise EEG to investigate contagious yawning, again finding evidence of MNS activity during yawn conditions but not control conditions (Cooper et al., 2012). As suggested previously, the present results support the argument that the neuroimaging studies that failed to demonstrate MNS activation specific to yawning stimuli were likely the result of experimental design rather than the lack of involvement of the MNS in contagious yawning. Specifically it was proposed that the control conditions utilised in these studies were arguably mirror neuron stimulating in nature, resulting in MNS activation in both yawn and control conditions (Nahab, et al., 2009; Platek et al., 2005; Schürmann et al., 2005). As both the present study and that of Cooper and colleagues (2012) demonstrated MNS activity specific to contagious yawning and given both studies utilised alternative methods to neuroimaging it is likely that this was because EEG and TMS measure different expressions of MNS activation compared to neuroimaging techniques. This again suggests that the previous fMRI studies that failed to observe the specific involvement of the MNS in contagious yawning was the result of the neuroimaging paradigm employed rather
than a true reflection of the MNS perceived lack of specific involvement in yawning contagion.

Further strengthening the argument that the MNS is important in contagious yawning is the present studies finding that participants with an ASD, a population theorised to possess a deficit in this system, demonstrated disturbances in the capacity to yawn contagiously. In line with previous research, the present study found that when viewing others yawns, individuals with an ASD displayed a disturbance in the ability to yawn contagiously (Giganti & Esposito, 2009; Helt et al., 2010; Senju et al., 2007). Specifically, the present findings support the inference that when individuals with an ASD view the yawn of another, reduced synaptic efficacy between premotor mirror neurons and corticospinal neurons, fail to activate the corresponding facial region of the M1 cortex, in turn failing to elicit a corresponding response at the muscular level as evidenced by similar MEP amplitude when viewing both yawns and control mouth movements.

It is important to highlight that it has been suggested that the observed lack of yawning contagion in ASD could be the result of a failure to fixate on the eye region when viewing a yawn as opposed to a true deficit in the neural substrate mediating yawning contagion (Senju et al., 2009). However, given the present paradigm utilised yawning stimuli that included sound as well as the visual image and, previous studies have demonstrated that listening to the sound of others yawning elicits contagious yawning in TD individuals but not individuals with an ASD (Giganti & Ziello, 2009), this alternative explanation is unlikely.

Taken together, the results of the present study provide tentative support for the notion that contagious yawning and the capacity for empathy share
common neural mechanisms. As described earlier, the results of the present study support the claim that the MNS plays a role in yawning contagion and that a deficit within this system may, at least in part, explain why individuals with an ASD display an absence of contagious yawning when viewing others yawn. Additionally, given individuals with an ASD also exhibit disturbances in empathic processing, the present study further supports previous literature linking contagious yawning to empathic abilities (Cooper et al., 2012) as well as research correlating empathy with MNS activation (Kaplan & Iacobonni, 2006). Specifically the present findings not only tentatively support the belief that yawning contagion reflects an index for empathic abilities but also that these processes are, at least in part, linked to the MNS. In addition to the inferences made from evidence in ASD populations, this notion is further supported by cross species research suggesting that mirror neurons are only suspected in certain populations and that these appear to be the same populations that exhibit both the tendency to yawn contagiously and possess the capacity for empathy (Anderson et al., 2004; Paukner & Anderson, 2006).

When interpreting the findings of the present study, a number of limitations should be considered. As the sample size was modest, replication in larger samples is needed to more firmly establish the present findings. Further, as the present sample only included male adults, the results are therefore limited in their generalizability and developmental inferences can only be done so speculatively. Thus, given the differential developmental of contagious yawning compared to spontaneous yawning, future research needs to investigate the developmental trajectory of contagious yawning in line with the development of the MNS by utilising child and adolescent, as well as female populations. Another
limitation of the present study was the likelihood that the control stimuli utilised was still not optimal and may have also activated the MNS, possibly diluting the findings. The development of an ideal control condition for yawns remains elusive and needs to be addressed in future research.

In conclusion, the present findings support the notion that the MNS is, at least in part, involved in yawning contagion and emphasises the link between contagious yawning and empathy. Further, the present study also supports the putative link between abnormalities of the MNS in individuals with an ASD and observed impairments in social and communicative functioning such as disturbances in empathic abilities. Although more research is required to elucidate the developmental properties of contagious yawning and the possible neural mechanisms involved, the present study raises the possibility that contagious yawning could be utilised as a simple measure of empathic abilities and perhaps even a clinically important neurological characteristic used in the diagnosis of ASD.
Introduction

A sense of body ownership and self-awareness is imperative not only for the development of essential motor skills such as navigating one’s environment, but it also plays a fundamental role in the social and cognitive abilities that necessitate both the differentiation of self from other, as well as comparisons between self and other (Decety & Sommerville, 2003; Meltzoff, 2007; Piaget, 1952). This ability to identify, differentiate and compare the self and other is perceived to be an important condition for the understanding of others as intentional agents, subsequently giving rise to the ability to make inferences regarding others' emotions, thoughts and intentions (Cascio, Foss-Feig, Burnette, Heacock & Cosby, 2012). These skills are in turn believed to be foundational for the development of social relational skills such as imitation and empathy (Gallese, Keysers, & Rizzolatti 2004). Thus, the distinction between the self and other is an essential prerequisite in human social interaction, and disturbances in this process can result in a number of social and communicative deficits such as those seen in autism spectrum disorders (ASD; Williams, 2008).

In recent years attempts to understand how one differentiates between the self and others has received increasing interest from the fields of neurobiology and neuroscience (Uddin, Iacoboni, Lange & Keenan, 2007; van Veluw & Chance, 2013). While the underlying neural mechanisms still remain unclear, the recent discovery of specific cortical brain cells that respond to both the execution and observation of motoric behaviour has offered promising insights into the possible neural mechanisms by which the brain distinguishes and compares the self and others (Rizzolatti & Craighero, 2004). It is proposed that this system, known as the mirror neuron system (MNS), facilitates action understanding by
allowing for the simulation of not just another individual’s intentions, but also their state of mind, which is then believed to inform subsequent interactions (Enticott, Johnston, Herring, Hoy & Fitzgerald, 2008). As a result, it has been speculated that mirror neurons are involved in the development of social cognition processes that facilitate effective social interactions including the development of self-other representations, empathy, theory of mind (ToM) and imitation (Enticott et al., 2008; Fecteau, Lepage & Théoret, 2006; Uddin et al., 2007).

In addition to the empirical evidence supporting the link between the MNS and social cognition in typically developing (TD) individuals (e.g. Agnew, Bhakoo & Puri, 2007; Oberman, Pineda, & Ramachandran, 2007; Rizzolatti & Craighero, 2004), there is also evidence suggesting a deficit in this system may be linked to the social and communicative deficits that form the hallmark of the ASD (e.g. Buccino & Amore, 2008; Hadjikhani, Joseph, Snyder & Tager-Flusberg, 2006; Théoret et al., 2005). For example, a study completed by Théoret et al. (2005) demonstrated that observation of movement in TD individuals’ selectively enhanced motor activity specific to the muscles required to reproduce that movement, whereas this modulation was abnormal in ASD participants. Specifically, ASD participants displayed typical patterns of motor activation when performing the observed action and when viewing another completing the same action from the allocentric (other) perspective. However when that same action was viewed from the egocentric (self) perspective, ASD participant’s failed to show motor facilitation in the specific muscles required to reproduce this action. These findings imply a mirror-related deficit in self-awareness that affects
self-other processing in ASDs, thus providing support for the possible role of the MNS in our ability to differentiate and compare the self and other.

Whilst there have been recent advances in our conceptual understanding of how the brain processes information attributed to the self, compared to another, as the experience of one's own body differs from that of others' bodies with respect to viewpoint, morphological features, familiarity, and the hallmark feature of kinaesthetic experience, these hypotheses have proved exceedingly difficult to empirically investigate. Despite these challenges, several researchers have attempted to manipulate body ownership by utilising perceptual illusions such as the rubber hand illusion (RHI; Botvinick & Cohen, 1998; Schütz-Bosbach, Mancini, Aglioti, & Haggard, 2006).

The RHI relies on the premise that the sense of self versus other is dependent on converging input from the proprioceptive, somatosensory and visual systems and that this process can be disrupted when inputs from two or more of these systems are in conflict (Botvinick, 2004; Schütz-Bosbach, Musil & Haggard, 2009). Specifically, in the RHI, the sense of body ownership is altered by delivering synchronous tactile stimulation to both an artificial limb placed in full view of the participant and their real hand, which is hidden. After a short period of synchronous touches the participant experiences a drift in perceived position of the tactile stimulation from their actual hand to the virtual limb, consequently resulting in feelings of ownership (Botvinick & Cohen, 1998). In TD individuals the illusion is believed to occur as the result of the interaction between vision, touch and proprioception (position sense) and the dominance of vision over proprioception (Botvinick & Cohen, 1998).
Given the RHIs ability to manipulate the distinction between self and other, one would theorise that populations with disturbances in self-other representations, such as those with ASD, would likely display differences in RHI susceptibility when compared to TD populations. However, despite the perceived utility of the RHI in the investigation of self-other processes in ASD, few studies have utilised this paradigm. Despite the constraints of the literature, several studies suggest that individuals with ASD demonstrate diminished susceptibility to the illusionary experience of the RHI (Cascio et al., 2012; Paton, Hohwy, & Enticott et al., 2012). Further, the experience of the RHI in individuals with ASD has also been correlated with empathic abilities, with individuals who rate low on this ability being less likely to experience the illusion (Cascio et al., 2012). Thus, understanding the neural mechanisms involved in perceptual illusions such as the RHI, will not only improve our understanding of the mechanisms involved in cognitive abilities such as empathy and intention understanding, but it will also assist in elucidating how these processes are disturbed in ASD.

Insights into the possible neural underpinnings and the role of the MNS in illusions of body ownership have come from closely related work by Ramachandran and colleagues examining phantom limb sensations in amputees (Ramachandran, Rogers-Ramachandran, & Cobb, 1995). Specifically, Ramachandran et al. (1995) asked phantom limb patients to view their intact arm in a mirror, so that their amputated arm appeared to have been restored. Several subjects reported than when viewing the reflection of the intact arm being touched, they felt the touch in the amputated limb. In addition to its therapeutic use to relive pain in phantom limb patients, this mirror paradigm has also been adapted to complete a modified version of the RHI in TD populations as well as
amputees, where the mirrored reflection functions as a virtual limb as opposed to the use of an artificial rubber limb in the traditional RHI (Bertamini, Berselli, Bode, Lawson, & Wong, 2011; Giummarra, Georgiou-Karistianis, Nicholls, Gibson & Bradshaw, 2010).

As stated previously, the sensation of feeling a foreign limb as belonging to the self during perceptual illusions such as the RHI and mirror-induced visual illusions (MVI), is believed to be the result of visual information taking preference over incongruent tactile and proprioception senses. Moreover, Ramachandran and colleagues take this one step further by postulating the neural mechanisms that facilitate this process (Ramachandran & Brang, 2009). Specifically they purport that the reason we do not experience this feeling simply through the observation of tactile stimulation in the absence of any illusionary conditions is that negative feedback created from touch senses in the arm causes a ‘dampening’ of the MNS from reaching a threshold for feeling (Ramachandran & Brang, 2009). However, during perceptual illusions they posit that this signal is vetoed, allowing mirror neuron activity to reach threshold, subsequently giving rise to the perception of feeling a foreign limb as belonging to the self. Support for this contention comes from fMRI investigations in TD individuals, which suggest a linear relationship between the strength of the RHI experience and activity in the premotor cortex, a proposed component of the MNS (Ehrsson, Spence, & Passingham, 2004). Further, although indirect, evidence from converging lines of research, suggesting that ASD individuals display both decreased susceptibility to the RHI (Cascio et al., 2012; Paton, et al., 2012) and disturbances within the MNS (Buccino & Amore, 2008; Hadjikhani, et al., 2006; Théoret et al., 2005), provides additional support for the MNS possible
involvement in the mechanism by which the brain distinguishes between the self and others.

Although pursuing a different line of inquiry, evidence for the involvement of the MNS in self-other processes has come from studies utilising transcranial magnetic stimulation (TMS) whilst individuals participate in a MVI (Funase, Tabira, Higashi, Liang, & Kasai, 2007; Garry, Loftus, & Summers, 2005). TMS is used as a means of stimulating nerve cells in the motor cortex via the administration of a brief magnetic pulse to the scalp. This pulse produces a motor evoked potential (MEP) in the specific muscle stimulated that can be measured via surface electromyography (EMG). As the premotor cortex extends posteriorly to the primary motor cortex, mirror neurons in the ventral premotor cortex are believed to connect sensory neurons responding to the visual properties of an observed action and corticospinal neurons that discharge MEPs during the execution of a similar action (Fadiga, Craighero & Olivier, 2005; Rizzolatti & Craighero, 2004; Zult, Howatson, Kádár, Farthing & Hortobágyi, 2013). Thus, when TMS is delivered during the observation of action within the stimulated muscle, it is believed that premotor mirror neuron activity increases excitability in the motor cortex resulting in enhanced MEP amplitude.

It is important to note that unlike the traditional RHI, the aforementioned studies (Funase et al., 2007; Garry et al., 2005) utilised a MVI that facilitated the illusion of kinaesthetic sensation (movement) of a virtual limb as opposed to proprioception (position). This was achieved by placing one of the participants’ arms behind a mirror that was situated along their midline and instructing them to view this mirror image whilst moving the index finger of their opposite arm. Since the mirror image of the moving limb is superimposed on the participants’
real hand it appears as though the hidden, static limb is also moving. TMS was used to measure excitability of the motor cortex with respect to the static hand and it was found that MEPs were significantly enhanced during the illusion when viewing the movement of the virtual limb compared to when the limb was at rest (Funase et al., 2007; Garry et al., 2005). Further, participants generally reported feeling the sensation that the static hand was actually moving. In accordance with the MNS as a possible mechanism for the experience of a foreign limb as belonging to the self in the RHI, as reflected in the experience of proprioceptive drift, the MNS could also contribute to the attribution of the movement of a foreign limb to the self during the MVI. Specifically, the increase in MEP amplitude during the movement condition likely reflects an increase in mirror neuron activity in the premotor cortex that resulted in the sensation of the movement of the virtual limb being attributed to the self. Thus providing empirical support for theoretical accounts concerning the role of the MNS in perceptual illusions and how we attribute actions to the self, compared to others. Given the ability to plan and initiate movement and the proprioceptive feedback that follows represents a hallmark of bodily self-awareness (Dummer, Picot-Annand, Neal & Moore, 2009), the incorporation of movement in perceptual illusions is an invaluable tool for experimental paradigms attempting to elucidate the neural underpinnings of how we distinguish between the self and other. Despite the potential utility of this methodology to examine possible disturbances in these mechanisms in ASD, to date, this paradigm has yet to be employed to explore self-other processes in populations with an ASD.

In light of this, the present study aimed to investigate the role of movement in perceptual illusions in TD individuals compared to individuals with
an ASD. Specifically the present study utilised TMS to measure motor cortex excitability during a variation of the MVI designed to elicit both the illusion of movement and proprioceptive drift. It was hypothesised that individuals with an ASD would display reduced susceptibility to the illusion compared to TD individuals as demonstrated by significantly lower MEP amplitude when observing both a static and moving hand during the MVI when compared to the MEP amplitude of TD individuals. Further, it was also predicted that this difference would extend to participants subjective experience of the illusion. Specifically, it was hypothesised that individuals with an ASD would report lower scores on the illusion items of the RHI questionnaire adapted for the present study, when compared to TD individuals.

Method

Participants.

Participants were ten typically developing male adults (TD) and nine male adults with a diagnosis of either high functioning autism (HFA) or Asperger’s syndrome (AS). One TD participant and one participant with a diagnosis of HFA/AS was excluded due to equipment malfunction. Participants with HFA/AS (M=22.8) were age stratified to TD participants (M=24.6) within 3 years (age range 18-34). The mean age and SD of all participants was 23.7 and 3.9 respectively. TD participants were recruited by word of mouth and at Deakin University. Participants with HFA/AS were recruited from Autism Victoria’s research database, schools such as the Western Autistic School, word of mouth, and advertisements and mail outs from paediatric clinics. An experienced Clinical
Psychologist who has worked extensively in the autism field confirmed diagnosis using DSM-IV-TR (APA, 2000) criteria.

All participants were right handed and had normal or corrected-to-normal vision. Prior to the experimental procedures, participants completed the Adult Safety Screening Questionnaire to determine their suitability for transcranial magnetic stimulation (TMS; Keel, Smith & Wassermann, 2001). The TMS safety screen was used to exclude participants who had any pre-existing medical conditions (such as epilepsy or stroke), or any implanted devices (such as cardiac pacemakers), which may have resulted in adverse effects from TMS (see Appendix A).

The Deakin University Human Research Ethics Committee (DUHREC 2009-135) granted ethics approval in accordance with the National Statement on Ethical Conduct in Human Research (2007) prior to the commencement of this research (see Appendix B). All participants were provided with the plain language statement (PLS) and gave written, informed consent prior to engaging in the experimental procedures (see Appendix C).

**Experimental Set Up.**

The present study used a permutation of the rubber hand illusion (RHI; Botvinick & Cohen, 1998) in which a mirror was used to create a situation where the mirrored reflection functioned as a virtual limb as opposed to the use of an artificial rubber limb in the traditional RHI. To achieve this, the mirror was placed between the subjects’ hands in such a way that the reflection of their left hand functioned as a virtual limb appearing to be their right hand. Importantly, their right hand was behind the mirror, and therefore not visible.
Whilst previous studies utilising this mirror-induced visual illusion (MVI) (e.g. Funase et al., 2007; Garry et al., 2005) have positioned the right hand such that the mirrored reflection is superimposed on the position of participants real right hand, in the present study the location of the reflected hand and the participants’ right hand was spatially different. Specifically, the location of the reflected limb and participants’ right hand were approximately 15 cm apart, see Figure 6.1. This variation was introduced in an attempt to facilitate proprioceptive drift via the incongruence of visual, tactile and proprioceptive information (Ehrsson et al., 2004). To establish this illusion an experimenter seated opposite the participant used a pair of identical soft paintbrushes to synchronously stroke both the visible left hand and the hidden right hand, in a proximo-distal direction between the second and third knuckles of the index, middle and ring fingers for 90 seconds. Brushstrokes were delivered manually by an experimenter at a rate of approximately 1 Hz. This functioned as an induction phase prior to the administration of any TMS pulses in the experimental conditions.
Figure 6.1 Experimental set up of the illusion with a mirror box. The participants’ right hand was placed in the far right corner of the box, out of view. Their left hand was placed as close to the mirror as possible, in their field of view. Subjects focused their attention on the mirror reflection of their right hand.

Experimental Design.

There were three conditions within the experiment: baseline, illusion-movement, and illusion-static. In all conditions electrodes were placed on the first dorsal interosseus (FDI) muscle of the right hand and the identified optimal position on the primary motor cortex (M1) to elicit motor evoked potentials (MEPs) in this muscle was stimulated. Before each condition the experimenter provided a brief explanation of what it entailed. In all conditions participants were seated at a table with their hands in a neutral position and the ulnar sides of their hands and forearms resting on the table surface directly in front of them. The conditions were counterbalanced between participants to account for any possible order effects.
Prior to participants engaging in the experimental conditions they undertook a brief training phase. This included a scripted description of the illusion and a demonstration, instructions as to how their hands should be placed with respect to the mirror, and practice completing the index finger contractions required for the illusion-movement condition. Following the successful completion of this, participants then went on to complete the experimental conditions.

In the *baseline* condition participants were presented with a black screen with a white central fixation cross appearing on a 10 cm by 10 cm display presented on a 13-inch laptop computer screen placed approximately 60 cm away from the participant. During this condition 10 MEPs were taken while the participant focused upon the screen, whilst a wooden divider occluded participants’ right hand from view. The baseline condition examined individual participants resting MEP amplitude in the FDI muscle of their right hand.

In the *illusion-movement* condition the MVI was established as described in the experimental set up section above. Following the 90-second induction phase was an experimental phase lasting approximately three minutes. During this phase the experimenter continued to stroke the participants’ fingers in a manner identical to that of the induction phase. Over the course of the three minutes the participant was given 10 verbal prompts to ‘move’. When instructed to move, participants performed a simple contraction of their left index finger and an MEP was taken at the apex of this contraction. The participant would relax their left hand following this movement until they received another verbal prompt to move. In order to achieve consistency, verbal prompts were timed by 10 pre-recorded sounds that were elicited every 15 seconds and delivered to the experimenter.
through headphones to prevent the sound from being heard by the participant. During this condition an additional experimenter monitored participants right hand to ensure it remained completely still throughout.

The *illusion-static* condition was identical to the illusion-movement condition described above, except both hands remained completely still. In order to keep the conditions as similar as possible, participants were still given a verbal prompt every 15 seconds however the prompt ‘move’ was replaced with ‘don't move’. Similarly, MEPs were taken following each of the 10 verbal prompts.

At the conclusion of the experimental protocol participants were given a version of the RHI questionnaire (Botvinick & Cohen, 1998), which was adapted for the present study (see Appendix D), to gauge their overall subjective experience of the illusion. Eight of the nine questions from the original questionnaire were chosen, as they could be reasonably adapted to the present study by substituting ‘rubber hand’ for ‘reflected hand’. Each question was rated on a seven-point likert scale ranging from disagree strongly (-3) to agree strongly (3). Of the eight questions, four were statements consistent with the illusionary experience, items 1, 2, 5 and 7, and four were control statements not typical of the illusion experience, items 3, 4, 6 and 8. For the illusion statements a score above zero reflects an endorsement of the illusionary experience, where as a score below zero indicates that the illusion was not experienced. With regard to the control statements, a score below zero indicates that participants were able to distinguish between experiences typical of the illusion and those not. In contrast a score above zero on the control statements suggests difficulty discerning experiences typical of the illusion with those not.
Transcranial Magnetic Stimulation and Electromyography.

Surface electromyography (EMG) activity and TMS evoked motor evoked potentials (MEPs) were recorded from the FDI muscle of the right index finger using bipolar Ag-AgCl electrodes. These electrodes were also placed on the participants’ left hand, however no measurements were taken from these electrodes, rather their function was to ensure both hands looked and felt as similar as possible. Please refer to the transcranial magnetic stimulation and electromyography section in chapter four (pages 58-60) for a more detailed description of the methods employed.

Data Analysis.

Data was standardised (i.e. expressed as z scores) within each participant, attenuating the impact of highly reactive individuals on group scores and allowing for meaningful comparisons. Please refer to the data analysis section in chapter four (pages 60-61) for the equation utilised and a more detailed description of the statistical transformation employed.

Statistical Analysis.

A 2x2 mixed model ANOVA (within subjects factor condition: illusion-static and illusion-movement and between subjects factor group: TD and ASD) was used to compare Z score MEP amplitudes across condition and group. Where appropriate, planned comparisons were carried out to compare between groups within the same condition.
Results

Data Screening.

Data was cleaned, screened and analysed using SPSS Version 18 (SPSS Inc.) with no missing values found. Four univariate outliers were identified using z-scores with a criterion of $\alpha < .001$, ($\pm 3.3$ cut-off). Hypothesis-related analyses were run with and without these outliers and as no meaningful difference was evident, the outliers were retained (Tabachnick & Fidell, 2007).

The Kolmogorov-Smirnov statistic indicated that both conditions were significantly non-normal. However an examination of the Q-Q plot for each condition did not suggest any significant deviations from normality. Thus, given the number of samples was large ($n=190$ MEPs per condition) it is likely that the significant Kolmogorov-Smirnov statistic was due to the large number of samples rather than a true deviation from normality (Field, 2009).

Analysis of Motor Evoked Potential Amplitudes.

A 2x2 mixed model ANOVA was conducted in order to compare the TD and ASD group mean Z score MEP amplitudes across the conditions illusion-static and illusion-movement. Box’s test of equality of covariance’s matrices was violated ($p<.001$) and Levene’s test of equality of variances was violated for the condition illusion-static. However, in light of these violations a more stringent alpha of $\alpha<.001$ was adopted (cf. Tabachnick & Fidell, 2007). The main effects for condition ($F_{(1,188)}=8.054, p<.01, \eta_p^2=.04$) and group ($F_{(1,188)}=4.698, p<.05, \eta_p^2=.02$) reached conventional levels of significance however with the adoption of a more stringent alpha of $\alpha<.001$ both became non-significant. There was no
significant interaction between group and condition ($F_{(1,188)}=0.004$, $p>.05$, $\eta^2_p<.01$; see Figure 6.2 for plot).

**Figure 6.2** TD and ASD mean Z scores for MEP amplitudes across the conditions illusion-static and illusion-movement.

There was no significant interaction effect as both groups displayed parallel trends, however the means for both groups for both conditions differed. Thus to assess the significance of this difference independent samples t-test were undertaken to compare both groups across conditions, adjusted for unequal variances where necessary. Within the condition illusion-static the Z score MEP amplitude for the TD group was significantly higher than for the ASD group ($t_{(147)}=2.04$, $p<.05$, $\eta^2_p=.03$). Similarly, within the condition illusion-movement the Z score MEP amplitude for the TD group was significantly higher than for the ASD group ($t_{(188)}=2.02$, $p<.05$, $\eta^2_p=.02$; see Table 6.1 for means and Figure 6.3 for sample MEPs).
Table 6.1

*Mean MEP Amplitudes and Standard Deviations Normalised to Baseline for TD and ASD Groups for the Conditions Illusion-Static and Illusion-Movement*

<table>
<thead>
<tr>
<th>Group</th>
<th>TD</th>
<th>ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Illusion-Static</td>
<td>0.54</td>
<td>3.47</td>
</tr>
<tr>
<td>Illusion-Movement</td>
<td>0.97</td>
<td>2.81</td>
</tr>
</tbody>
</table>

\[ n = 100 \text{ MEPs per condition and 10 participants in TD group} \]

\[ n = 90 \text{ MEPs per condition and 9 participants in ASD group} \]
Figure 6.3 Sample overlaid MEP amplitude sweeps recorded from one TD participant and one participant with an ASD for the conditions Illusion-Static and Illusion-Movement
Analysis of Rubber Hand Illusion Questionnaire Responses.

Participants overall subjective experience of the illusion was assessed by a version of the RHI questionnaire (Botvinick & Cohen, 1998), which was adapted for the present study (see Appendix D). Figure 6.3 depicts the mean scores for the four illusion items and the four control statements for both TD participants and participants with an ASD. As shown in Figure 6.3, on average TD participants reported a stronger illusionary experience than participants with an ASD, as evidenced by TD participant’s higher endorsement of the illusion items. With respect to the control statements, TD participants averaged a score under 0, demonstrating that they were able to distinguish between experiences consistent with the illusion and those not. In contrast, participants with an ASD averaged a score slightly above 0 with respect to the control statements, demonstrating that they had difficulty clearly distinguishing between experiences consistent with the illusion and those not.

Figure 6.4 Mean responses for the illusion items and control statements on the adapted RHI for TD and ASD groups.
Independent samples t-tests were conducted in order to compare the ratings on the illusion items and control statements within each group as well as between each group. These indicated that the TD groups mean ratings on the illusion items were significantly higher than their ratings on the control statements ($t_{(78)}=8.55$, $p<.001$, $\eta_p^2=.48$). This trend was similar in the ASD group as again the ASD groups mean ratings on the illusion items were significantly higher than their ratings on the control statements ($t_{(70)}=3.26$, $p<.01$, $\eta_p^2=.13$). Although the TD groups mean score for the illusion items was higher than the ASD groups, this difference just failed to reach significance ($t_{(74)}=-1.93$, $p>.05$, $\eta_p^2=.05$). However, the difference between the two groups ratings on the control statements was significantly different, with the ASD group having significantly higher ratings on the control statements compared to the TD group ($t_{(74)}=-2.39$, $p>.05$, $\eta_p^2=.07$; see Table 6.2 for means).
Table 6.2

*Mean Responses for the Illusion Items and Control Statements of the Adapted RHI Questionnaire for TD and ASD Groups*

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>TD</td>
<td>ASD</td>
<td></td>
</tr>
<tr>
<td>RHI Responses</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Illusion Items</td>
<td>2.03</td>
<td>1.14</td>
<td>1.39</td>
</tr>
<tr>
<td>Control Statements</td>
<td>-.95</td>
<td>1.89</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*n = 4 items and 10 participants in TD group

*n = 4 items and 9 participants in ASD group

**Discussion**

The present study utilised an adapted mirror-induced visual illusion (MVI) of hand movements to investigate differences in self-other processing in individuals with an autism spectrum disorder (ASD) compared to typically developing (TD) individuals. Additionally, the paradigm incorporated transcranial magnetic stimulation (TMS) as a means of examining the mirror neuron system (MNS) as a possible neural substrate mediating this process. In line with theoretical tenets and previous findings, it was anticipated that individuals with an ASD would display decreased susceptibility to both the illusion of a static and moving hand as belonging to the self, when compared to TD participants. The results supported this hypothesis, as demonstrated by mean motor evoked
potential (MEP) amplitudes in both the illusion-static and the illusion-movement conditions that were significantly lower for participants’ with an ASD compared to TD participants. This hypothesis was further supported by participants’ subjective ratings of the illusion on the illusion items of the adapted rubber hand illusion (RHI) questionnaire utilised for the present study. Specifically, it was found that individuals with an ASD rated their overall experience of the illusion as lower than TD individuals, however it should be noted that this difference did not reach significance.

Before the implications of the above findings and the role of the MNS in this process can be elucidated, several additional findings need to be discussed. Firstly, no interaction between the groups was identified, with both groups demonstrating MEPs of greater magnitude in the illusion-movement condition compared to the illusion-static condition. However, whilst MEP amplitude was above baseline for both conditions in TD participants only MEPs in the illusion-movement condition were greater than baseline for participants with an ASD. Given it is believed that premotor mirror neuron activity increases excitability in the motor cortex, resulting in enhanced MEP amplitude, it is likely that MEP amplitude above baseline in the illusion-static condition reflects a feeling of ownership over the reflected limb. This is in line with previous research demonstrating a relationship between the subjective rating of the illusion and the level of neural activity in the premotor cortex (Ehrsson et al., 2004) and theoretical accounts suggesting that during perceptual illusions, mirror neuron activity reaches threshold, subsequently giving rise to the perception of feeling a foreign limb as belonging to the self (Ramachandran & Brang, 2009). Thus, MEP amplitude above baseline in the illusion-static condition in TD participants is
indicative of a feeling of ownership of the foreign limb. On the contrary, the MEP amplitude below baseline observed in participants with an ASD, is suggestive of an absent or weaker illusionary experience, likely a consequence of reduced mirror neuron activation.

For both groups, MEP amplitude in the illusion-movement condition was above baseline and also above the MEP amplitude of the illusion-static condition. In agreement with previous research, this indicates that not only did an increase in mirror neuron activity give rise to the perception of feeling a static foreign limb as belonging to the self in TD participants; the movement of a foreign limb was also attributed to the self (Funase et al., 2007; Garry et al., 2005). It should be noted that some studies suggest that simply performing certain hand movements can increase excitability in both the contralateral motor cortex controlling the movement and the ipsilateral motor cortex controlling the hand at rest (Garry et al., 2005; Liepert, Dettmers, Terborg, & Weiller, 2001). Consequently, the specific contribution of the MNS to the attribution of the movement of a foreign limb to the self in the present study is difficult to distinguish from interhemispheric processes during unilateral movements.

Ideally the present study would have also included a condition where participants simply performed the index finger movement and observed it whilst the motor cortex of their resting hand was stimulated. However this was omitted to minimise the burden on participants in this complex paradigm. Despite the possible constraints this poses, the literature suggests that low force movements, such as the index finger contractions performed in the present study, do not increase excitability bilaterally in the motor cortices, thus the possible impact of this is likely attenuated (Liepert et al., 2001). Further, it should be highlighted that
when compared to TD individuals, individuals with an ASD do not show
differential bilateral motor cortex activation when simply performing and
observing their own hand movements (Théoret et al., 2005). Thus, the significant
group difference in MEP amplitude in the illusion-movement condition likely
reflects a true difference in the attribution of a movement to the self during the
illusion, rather than simply an artefact of bilateral activation when performing a
unilateral finger movement.

The present finding that individuals with an ASD display reduced
susceptibility to both the illusion of proprioceptive drift and the illusion of
kinaesthetic experience is further supported by participants’ subjective reports. It
was found that, whilst ASD participants did endorse illusionary items on the
adapted RHI questionnaire, on average they reported a weaker illusionary
experience when compared to TD individuals. It is also interesting to note that
individuals with an ASD endorsed the control statements, not typical of the
illusion experience, on the adapted RHI questionnaire whereas TD participants
did not. This suggests that whilst participants with an ASD did experience the
illusion, not only was it to a weaker degree than TD participants but they were
also less able to discern experiences typical of the illusion with those not.

Given the ability to plan and initiate movement and the proprioceptive
feedback that follows, represents a hallmark of bodily self-awareness (Dummer,
Picot-Annand, Neal & Moore, 2009), ultimately the findings of the present study
support previous theory and research suggesting that self-other processing is at
least in part, sub-served by the MNS (Enticott et al., 2008; Uddin et al., 2007).
Further strengthening the argument that the MNS is important in self-other
processing, is the present studies finding that participants with an ASD displayed
a mirror-related deficit in self-awareness (Hadjikhani et al., 2006; Théoret et al., 2005). This deficit in self-awareness was reflected in both ASD participants weaker cortical activation, indicative of reduced MNS activation, during a perceptual illusion of kinaesthetic experience, and their difficulty discerning their experience in subjective measures. The deficits in self-awareness in individuals with an ASD indicated in the present study could not only have significant implications for self-other processing, it could also contribute to many of the disturbances in socio-communicative abilities that form the hallmark of ASD. Given self-awareness is fundamental to our ability to identify, differentiate and compare the self and other, a disturbance at this level could subsequently give rise to difficulties understanding others as intentional agents. Consequently, this could contribute to the observable difficulties individuals with an ASD have making inferences regarding others emotions, thoughts and intentions (Cascio, Foss-Feig, Burnette, Heacock & Cosby, 2012). As these skills are in turn believed to be foundational for the development of social relational skills such as imitation and empathy, (Gallese, Keysers, & Rizzolatti 2004) it is clear that a disturbance at the basic level of self-awareness could contribute to the higher order social and communicative deficits observed in ASD.

When interpreting the findings of the present study, a number of limitations should be considered. As the sample size was modest, replication in larger samples is needed to more firmly establish the present findings. Further as the present sample only included male adults the results are therefore limited in their generalizability. Another limitation of the present study was that a measure of empathic abilities was not utilised. Given research suggests that higher empathic abilities are associated with greater susceptibility to perceptual illusions
(Cascio et al., 2012; Asai, Mao, Sugimori & Tanno, 2011) the incorporation of an index of empathy would have allowed for a better understanding of the link between disturbances in self-awareness and the impact this has on social relational skills. Thus, future research should include an index of empathic abilities in order to explore this further. Due to the design of the present study an additional limitation is that no inferences with regard to temporal dynamics can be made. Previous research suggests that children with ASD display a delayed onset of the illusion relative to TD children (Cascio et al., 2012). This raises the question of how the neural mechanisms change over the duration of the illusion in TD individuals compared to individuals with an ASD. Therefore, future studies are needed to examine changes in the neural mechanisms over time in order to provide a more detailed understanding of the differences in the underlying processes for TD individuals compared to individuals with an ASD during perceptual illusions.

In conclusion, the present study supports the notion that self-other processing is, at least in part, sub-served by the MNS. Further the present study also suggests that individuals with ASD possess a mirror-related deficit in self-awareness that affects self-other processing. This supports the putative link between abnormalities of the MNS in individuals with an ASD and observed impairments in social and communicative functioning such as deficits in social relational skills. Although further research is required to elucidate the link between self-other processing, the MNS and ASD, the present study raises several questions regarding the possible downstream consequences that may result from basic disturbances in self-awareness and the role the MNS may play in this.
Chapter 7: General Discussion
Introduction

Elucidating the neural basis of the social and communicative deficits that characterise autism spectrum disorder (ASD) has proved challenging. However, the recent discovery of visuomotor cells, known as mirror neurons, in macaque monkeys may provide a basis for explaining various behavioural impairments exhibited by individuals with ASD (Rizzolatti & Craighero, 2004). While mirror neurons are primarily thought to be involved in the perception and comprehension of motor acts, they are also believed to play a fundamental role in higher order cognitive processes such as empathy, imitation, self-other processing and theory of mind (ToM), all of which are known to be impaired in ASD (Fecteau, Lepage & Théoret, 2006; Williams, Whiten, Suddendorf & Perrett, 2001).

In light of this, the present dissertation aimed to investigate and compare mirror neuron activity in high functioning individuals with an ASD and typically developing (TD) individuals using three distinct experimental paradigms. This was achieved by utilising transcranial magnetic stimulation (TMS) to infer mirror neuron activation during tasks requiring emotional and social processing. TMS was used, as it is a means of stimulating nerve cells in the motor cortex via the administration of a brief magnetic pulse to the scalp. This pulse produces a motor evoked potential (MEP) in the specific muscle stimulated that can be measured via surface electromyography (EMG). As the premotor cortex extends posteriorly to the primary motor cortex, mirror neurons in the ventral premotor cortex are believed to connect sensory neurons responding to the visual properties of an observed action and corticospinal that discharge motor evoked potentials during the execution of a similar action (Fadiga, Craighero & Olivier, 2005; Rizzolatti & Craighero, 2004; Zult, Howatson, Kádár, Farthing & Hortobágyi, 2013). Thus,
when TMS is delivered during the observation of action within the stimulated muscle, it is believed that increased synaptic input from premotor mirror neurons onto corticospinal neurons, increases excitability in the motor cortex, resulting in enhanced MEP amplitude (Enticott, Johnston, Herring, Hoy & Fitzgerald., 2008; Zult et al., 2013). Collectively, the results of this thesis suggest that mirror neuron system (MNS) activation differs between TD individuals and individuals with an ASD during tasks requiring emotional and social processing.

Specifically, the results suggest that when presented with stimuli of a socio-emotional nature, TD individuals display greater MNS activation compared to control conditions. This was reflected in TD individuals’ increased MEP amplitude. Further, the results also suggest that individuals with an ASD display marked disturbances in MNS activity during tasks requiring emotional and social processing. These disturbances being that individuals with an ASD displayed a response that was either inhibited, absent or reduced compared to TD individuals. This suggests that in individuals with an ASD, the synaptic efficacy between premotor mirror neurons and corticospinal neurons may be reduced, resulting in a response that is markedly lower than that of TD individuals. Thus, this chapter will outline how the major findings of each study contribute to the notion that social cognition is, at least partially, sub-served by the MNS, and that disturbances in this system likely contribute to the social communicative disturbances observed in ASD.

**Social Cognition and the Mirror Neuron System**

Collectively, the results of the present thesis support the putative link between the MNS and various aspects of social cognition. Firstly, given the well-
entrenched conceptual connection between mimicry and social cognition in psychological thought (McIntosh, 2006; Rogers, Hepburn, Stackhouse & Wehner, 2003), study one investigated automatic mimicry of emotional facial expressions. In line with theoretical tenets and previous findings (McIntosh et al., 2006; Oberman, 2009), study one found that TD individuals automatically mimic emotional facial expressions, as demonstrated by the significantly larger MEPs when viewing emotional compared to neutral facial expressions (McIntosh et al., 2006; Oberman, 2009). In this context, it can be inferred that during facial emotion processing, mirror neurons in the premotor cortex provide an internal simulation of the observed motoric behaviour that evokes a similar reaction in the corresponding muscles of the observer. It is believed that this simulation process allows TD individuals to understand the observed individual’s mental and affective state (Enticott et al., 2008), in turn facilitating social functioning and communication (Uddin et al., 2007).

To further explore the role of the MNS in social cognition, study two examined yawning contagion, as it is believed that contagiousness of yawning depends on mechanisms that develop during childhood in parallel with the empathic capacity to understand mental states of others. Study two’s finding that TD participants displayed the capacity for yawning contagion, as demonstrated by significantly larger MEPs when viewing yawns compared to control mouth movements, supported this view. In a similar vein to when viewing an emotional facial expression, the results of study two suggest that when viewing another yawn, mirror neurons in the premotor cortex provide an internal simulation of the observed motoric behaviour, evoking a similar reaction in the corresponding
muscles of the observer. Thus, it is believed that this system may underlie the contagious aspects of the phenomenon.

Lastly, as the ability to identify, differentiate and compare the self and other is believed to play a fundamental role in social cognition, study three investigated this process. Specifically, study three utilised an adapted mirror-induced visual illusion (MVI) of hand movements to investigate the role of the MNS in self-other processing. The illusion attempted to elicit both the feeling of ownership over a foreign limb, as well as the attribution of the movement of a foreign limb to the self. The results of study three suggest that this was achieved in TD participants as not only did their subjective reports indicate that they experienced the illusion; MEPs in the illusion conditions were greater than baseline levels. As it is believed that premotor mirror neuron activity increases excitability in the motor cortex, resulting in enhanced MEP amplitudes, it is likely that MEP amplitude above baseline during the illusion reflects a feeling of ownership over the reflected limb. Thus, given the ability to plan and initiate movement and the proprioceptive feedback that follows, represents a hallmark of bodily self-awareness (Dummer, Picot-Annand, Neal & Moore, 2009), ultimately the findings of the present study support previous theory and research suggesting that self-other processing is at least in part, sub-served by the MNS (Enticott et al., 2008; Uddin et al., 2007).

Together, the findings of the three studies support the putative link between the MNS and various aspects of social cognition. Specifically, the results support the notion that mirror neurons might form a critical link in the chain from behaviour observation to understanding others mental and emotional states, whereby automatic processing by mirror neurons allows an internal modelling of
another’s motoric behaviour that evokes a similar reaction in the observer. In turn facilitating an understanding of the observed individual’s mental and affective state. Thus, via the MNS, physiological and associated emotional states of two individuals can be shared based on perceived motor patterns (Enticott et al., 2008; Uddin, Iacoboni, Lange & Keenan, 2007). This purported motor empathy or empathic resonance is one component within a multi-component model of human empathy that is adjacent to and underlies the development of higher order cognitive processes such as cognitive and emotional empathy and ToM abilities (Williams et al., 2001). The finding that the MNS and the limbic system are anatomically connected via the insula in the primate brain (Augustine, 1996), further adds weight to this idea. From experiments using primates and fMRI studies in humans, it is believed that in the human brain, a large-scale network in which the insula acts as an interface between the frontal components of the MNS and the limbic system, enables the translation of an observed or imitated motoric action, such as a facial expression, into its internally felt emotional significance (Carr et al., 2003; Iacoboni et al., 1999; Rizzolatti & Craighero, 2004).

To surmise, in line with theoretical tenets and previous findings, the collective results of this thesis suggest that during tasks requiring socio-emotional processing, TD individuals display an increased motoric response compared to baseline conditions, which is likely reflective of increased premotor mirror neuron activity (Enticott et al., 2008). This provides further evidence for the perceived importance of the MNS in social cognition.

As a result of the association between the theorised function of the MNS outlined above and the behavioural deficits seen in ASD, it has also been hypothesised that the MNS may be impaired in individuals with an ASD.
Accordingly, in addition to the empirical evidence supporting the link between the MNS and social cognition in TD individuals (e.g. Agnew, Bhakoo & Puri, 2007; Oberman, Pineda, & Ramachandran, 2007; Rizzolatti & Craighero, 2004), there is also evidence suggesting a deficit in this system may be linked to the social-emotional deficits observed in ASD (e.g. Buccino & Amore, 2008; Hadjikhani, Joseph, Snyder & Tager-Flusberg, 2006). The results of the present thesis provides further support for this argument as it was found that participants with an ASD displayed marked disturbances in MNS activity during tasks requiring emotional and social processing. Given this, with respect to the present findings, the following section will discuss the role of the MNS in disturbances of various aspects of social cognition in ASD and the implications of these.

The Role of the Mirror Neuron System in Disturbances of Social Cognition in Autism Spectrum Disorder

In line with previous research, study one found that when presented with emotional facial expressions participants with an ASD did not display the tendency to automatically mimic the expression with which they were presented (McIntosh et al., 2006; Stel, van den Heuvel & Smeets, 2008). In addition, participants with an ASD exhibited significantly lower MEP amplitudes when viewing emotional stimuli compared to neutral stimuli. This suggests that when presented with stimuli that are emotional in nature, an automatic mimicry response is actively inhibited in individuals with an ASD, supporting the notion that individuals with an ASD likely possess a disturbance in the MNS. Specifically, the present results support the inference that when individuals with an ASD view emotional facial expressions, reduced synaptic efficacy between
premotor mirror neurons and corticospinal neurons suppresses activity in the facial region of the primary motor cortex (M1), in turn resulting in an inhibited response at the muscular level as evidenced by reduced MEP amplitude. This is further supported by fMRI research demonstrating that when observing emotional facial expressions, children and adults with an ASD display reduced activation in the premotor mirror neuron area compared to TD participants (Dapretto et al., 2006).

In a similar vein to study one, study two found that when viewing others yawns, individuals with an ASD displayed a disturbance in the ability to yawn contagiously. Specifically, the findings of study two support the inference that when individuals with an ASD view the yawn of another, reduced synaptic efficacy between premotor mirror neurons and corticospinal neurons, fail to activate the corresponding facial region of the M1 cortex, in turn failing to elicit a corresponding response at the muscular level as evidenced by similar MEP amplitude when viewing both yawns and control mouth movements. Although the present thesis examined adults with a diagnosis of ASD, it is important to highlight the possible developmental consequences of the imitative and emotional contagion deficits identified in study one and two.

In development, imitative and emotional contagion abilities are believed to provide the child with information about the actions and intentions of those around them, which assists the process of social learning and forms the basis for future social development (Rogers, Hepburn, Stackhouse & Wehner, 2003). Given this, a deficit in these abilities could not only impair a child’s ability to understand the emotions of others but, if such a deficit occurred early in development it could significantly impair the child’s ability to form self-other
representations (Rodgers, 1999). In light of this, study three explored self-other processing in individuals with an ASD. Study three showed that individuals with an ASD displayed a deficit in self-awareness, reflected in both ASD participants weaker cortical activation, indicative of reduced MNS activation, during a perceptual illusion of kinaesthetic experience, and their difficulty discerning their experience in subjective measures. Given self-awareness is fundamental to our ability to identify, differentiate and compare the self and other, a disturbance at this level could subsequently give rise to difficulties understanding others as intentional agents. Consequently this could contribute to the observable difficulties individuals with an ASD have making inferences regarding others emotions, thoughts and intentions (Cascio, Foss-Feig, Burnette, Heacock & Cosby, 2012).

In summary, the results of this thesis show that during tasks requiring socio-emotional processing, individuals with an ASD demonstrate disturbed motoric responses, likely reflective of reduced synaptic efficacy between premotor mirror neurons and corticospinal neurons, and deficits in aspects of social cognition such as automatic mimicry, emotion contagion and self-awareness. As these skills are believed to be foundational for the development of social relational skills such as imitation and empathy, (Gallese, Keysers, & Rizzolatti 2004) it is clear that a disturbance with in these aspects of social cognition could contribute to the higher order social and communicative deficits observed in ASD. Thus, together, the results provide support for both the notion that social cognition may, at least in part, be sub served by the MNS and that disturbances in this system likely contribute to the social communicative disturbances observed in ASD.
Future Directions

The present findings raise important questions regarding the possible downstream consequences of deficits in aspects of social cognition that are believed to be foundational for effective social and communicative skills. For example, it has been established that TD newborn babies exhibit rudimentary forms of facial mimicry (Meltzoff & Moore, 1989). Further, in a normal developmental trajectory, these behaviours increase in complexity and form the basis for future social development by providing the individual with information about the actions and intentions of others (Rogers et al., 2003) Thus, if a mimicry deficit occurred early in the developmental trajectory of a child with an ASD not only would automatic mimicry behaviour be significantly impaired, this could consequently impair the child’s ability to co-experience other beings emotional states (McIntosh et al., 2006). This deficit in emotional contagion may subsequently prevent the child with an ASD from developing a sense of intersubjectivity and emotional correspondence which are crucial for social learning and the understanding of other minds (Bandura, 1977; Meltzoff, 1999). As a consequence, this in turn could impair the development of higher order socio-communicative abilities such as empathy, emotional reciprocity and ToM, disturbances of which characterise ASD (APA, 2013).

However as the present thesis and most previous research has examined only adult or adolescent populations in a cross-sectional design, any developmental inferences theorised or drawn are done so speculatively. Thus, given the developmental significance of social cognition, future research needs to investigate these abilities in child and adolescent TD and ASD populations over
time in order to establish the developmental trajectory of these behaviours with respect to the MNS. In addition to the question of developmental trajectory of social cognition and the role of the MNS in this, is the question of whether the proposed mirror mechanism is innate or acquired through experience. Whilst it is known that the MNS is extremely plastic and specific motor experience can modify its experience (Rizzolatti & Fabbri-Destro, 2008), the extent of mirror neuron activity at birth and in the first few months of life, still remains unknown. Therefore, future research should develop experimental paradigms to investigate these mechanisms from birth in order to determine whether this process is learned or innate.

It is important to note that in both the present thesis and the extant literature, the issue of causation remains unresolved, with MNS dysfunction being either a cause or a consequence of the social and communicative disturbances that form the hallmark of ASD. However this is not unique to research examining the MNS in ASD. Instead this is common to most areas examining cortical activity as the correlational nature of neuroimaging and neuropsychological data ensures that caution must be taken when attempting to infer causality.

It should also be emphasised that at no point does the present thesis attempt to posit that a deficit in the MNS is solely responsible for the social and communicative deficits observed in ASD. As studies have demonstrated that the MNS interacts with other brain regions during tasks involving emotional processing in TD individuals (e.g. Carr et al., 2003; Iacoboni, 2005) it is instead posited that the MNS is part of a large-scale neural network involved in social and emotional information processing. Thus, given several studies suggest that functional connectivity is disturbed in ASD (e.g. Rudie et al., 2012; Wass, 2011)
it is essential that connectivity between regions believed to possess mirror neurons, and other regions implicated in emotional processing are explored further in ASD samples. This could help explain the heterogeneity seen in ASD, as specific problems in connectivity between the mirror neuron network and additional brain regions could result in a multitude of variable symptoms. Determining the neurological source of an impairment within the MNS could help decide whether it should be pursued in relation to developing new ways of diagnosing and treating ASD, or whether it should simply be considered a consequence of impaired social relating.

Whist this section highlighted various gaps in the extant literature with respect to the possible role of the MNS in the social and communicative disturbances observed in ASD, when interpreting the findings of the present dissertation, a number of limitations should be considered before the concluding remarks are drawn.

**Limitations**

A potential important limitation of the present thesis is that no formal IQ measures were completed. Intelligent quotient measures are commonly assessed, then covaried out to determine if group differences between TD individuals and individuals with an ASD are independent of underlying differences in intelligence. Thus, the lack of formal IQ testing in the present thesis means that the possible influence of intelligence upon the results could not be ruled out. However, as the present study utilised individuals with a confirmed diagnosis of high functioning autism or Asperger’s disorder, limiting the range in which IQ
could vary between groups to 70 or above, the potential influence of this was perceived to be minimal.

Another limitation of the present thesis was that no formal measures of autistic symptom severity were utilised. Given that research suggests that greater symptom severity in ASD is associated with more significant impairments in social cognition (Dapretto et al., 2006) the inclusion of a measure assessing this could have provided additional insights. A further limitation of the present thesis was that a measure of empathic abilities was not utilised. Given research suggests that the aspects of social cognition examined in the present dissertation are linked to empathic abilities (Cascio et al., 2012; Platek et al., 2003; Sonnby-Borgström, 2002) the incorporation of an index of empathy would have allowed for a better understanding of the link between disturbances in social cognition and the impact this has on social relational skills. Thus future research should include an index of empathic abilities in order to explore this further.

Conclusion

Given its intuitive appeal, the mirror neuron hypothesis of ASD has been tested frequently in recent years, using various techniques and approaches. The present thesis sought to further this line of inquiry by utilising TMS to investigate and compare mirror neuron activity in individuals with an ASD and TD individuals during tasks requiring emotional and social processing. Collectively, the results of the present thesis suggest that MNS activation increases when TD individuals are presented with stimuli of a socio-emotional nature. By comparison, individuals with an ASD displayed marked disturbances in MNS
activity during tasks requiring emotional and social processing, exhibiting a response that was either inhibited, reduced or absent.

Thus the present thesis supports the notion that social cognition may, at least in part, be sub-served by the MNS and that disturbances in this system likely contribute to the social communicative disturbances observed in ASD. Although much research is required to elucidate the link between social cognition, the MNS and ASD, the present dissertation raises several questions regarding the possible downstream consequences that may result from imitative deficits and abnormal self-other representations early in development, which appear to be linked to disturbances in the MNS. As such, the present thesis contributes new knowledge on the processes that shape social cognition in both the typical and atypical social mind as well as highlighting possible neural substrates that mediate this process. It is crucial that research continues to explore mirror neuron activity in TD individuals as well as individuals with an ASD, as explicating the role of mirror neuron dysfunction in ASDs will not only improve diagnostic clarity but it will also have substantial treatment implications as the brain is a plastic organ and its function and structure can be modified by training.
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Appendices

Appendix A: Transcranial Magnetic Stimulation Safety screen

Transcranial Magnetic Stimulation† (TMS) Adult Safety Screen

<table>
<thead>
<tr>
<th>Name:</th>
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<td>Date:</td>
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<td>Age:</td>
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Please answer the following:

Have you ever:

- Had an adverse reaction to TMS? ☐Yes ☐No
- Had a seizure? ☐Yes ☐No
- Had an electroencephalogram (EEG)? ☐Yes ☐No
- Had a stroke? ☐Yes ☐No
- Had a serious head injury (include neurosurgery)? ☐Yes ☐No
- Had any other brain-related condition? ☐Yes ☐No
- Had any illness that caused brain injury? ☐Yes ☐No

Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips, or fragments from welding or metalwork? ☐Yes ☐No

Do you have any implanted devices such as cardiac pacemakers, medical pumps, or intracardiac lines? ☐Yes ☐No

Do you suffer from frequent or severe headaches? ☐Yes ☐No

Are you taking any medications? ☐Yes ☐No

Are you pregnant, or is it possible that you may be pregnant? ☐Yes ☐No

Does anyone in your family have epilepsy? ☐Yes ☐No

Do you need further explanation of TMS and its associated risks? ☐Yes ☐No

If you answered yes to any of the above, please provide details (use reverse if necessary):

_________________________________________________________________

† For use with single-pulse TMS, paired-pulse TMS, or repetitive TMS.
SUBJECT INFORMATION

Subject Details

Subject Name: _____________________________________________________
Address: __________________________________________________________
Ph: ___________________________ Sex: ___________________________
DOB: ___________________________ Occupation: _______________________
Ethnic Background:________________________

Background information

Do you suffer from any known neurological disorders?

Are you currently taking any medication which influences nerve conduction or blood clotting? If so, what?

Do you regularly drink beverages containing caffeine? If so, how many cups per day?

Which hand do you use for most daily activities when using only one?

Are you involved in regular physical activity requiring the use of your hands/arms? If so, what is the activity/activities, the intensity and time commitment?

Activity: __________________________________________________________
Intensity: _________________________________________________________
Hours per week: ___________________________________________________
Months per year: ___________________________________________________
Appendix B: Ethics Approval

DEAKIN UNIVERSITY

Human Ethics Research
Office of Research Integrity
Research Services Division
70 Eiger Road Bunwool Victoria
Postal: 221 Bunwool Highway
Bunwool Victoria 3125 Australia
Telephone 03 9251 7123 Facsimile 03 9244 6581
research-ethics@deakin.edu.au

Memorandum

To: A/Prof Mark Stokes
   School of Psychology

B

cc: Tom Perkins & Kayleigh Young

From: Deakin University Human Research Ethics Committee (DUHREC)

Date: 11 March, 2011

Subject: 2009-135
Mirror neuron deficit and reduced neural connectivity in high functioning autism

Please quote this project number in all future communications

The modification to this project, submitted on 3/03/2011 has been approved by the committee executive on 11/03/2011.

Approval has been given for A/Prof Mark Stokes, School of Psychology, to continue this project as modified to 31/03/2013.

The approval given by the Deakin University Human Research Ethics Committee is given only for the project and for the period as stated in the approval. It is your responsibility to contact the Human Research Ethics Unit immediately should any of the following occur:

- Serious or unexpected adverse effects on the participants
- Any proposed changes in the protocol, including extensions of time.
- Any events which might affect the continuing ethical acceptability of the project.
- The project is discontinued before the expected date of completion.
- Modifications are requested by other HRECs.

In addition you will be required to report on the progress of your project at least once every year and at the conclusion of the project. Failure to report as required will result in suspension of your approval to proceed with the project.

DUHREC may need to audit this project as part of the requirements for monitoring set out in the National Statement on Ethical Conduct in Human Research (2007).

Human Research Ethics Unit
research.ethics@deakin.edu.au
Telephone: 03 9251 7123

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DEAKIN UNIVERSITY
PLAIN LANGUAGE STATEMENT AND CONSENT FORM

TO: Participants

Plain Language Statement

Date: April 21 2011

Full Project Title: Mirror neuron deficit and reduced neural connectivity in high functioning autism

Principal Researcher: Associate Professor Mark Stokes

Student Researchers: Tom Perkins and Kayleigh Young

This Plain Language Statement and Consent Form is 6 pages long. Please make sure you have all the pages.

1. Your Consent

You are invited to take part in this research project.

This Plain Language Statement contains detailed information about the research project. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project so that you can make a fully informed decision whether you are going to participate.

Please read this Plain Language Statement carefully. Feel free to ask questions about any information in the document. You may also wish to discuss the project with a relative or friend or your local health worker. Feel free to do this.

Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research project.

You will be given a copy of the Plain Language Statement and Consent Form to keep as a record.

2. Purpose and Background

The purpose of this project is to investigate an aspect of the brain that is believed to function differently in high functioning autism compared to non-autistic persons. This will use Transcranial Magnetic Stimulation (TMS).

In this study, we will ask you to participate in two trials. In the first trial, we will brush your hand lightly with a brush in order to elicit an illusion. In the second trial, we will ask you to
observe a series of different facial expressions. During these two trials, you will be exposed to a TMS pulse. TMS is a non-invasive, painless and safe technique that has been in use world-wide in clinical, and laboratory settings, for more than 25 years, and is used routinely to investigate the human nervous system with very low risk. The TMS utilises a coil which is held over the subjects scalp, and employs a magnetic field to activate the brain. A brief pulse of a magnetic field activates the brain tissue beneath the coil. From your perspective, all you would notice is possibly a very light tap or touch on the head (not all participants report this) and an audible click sound.

This research is being conducted as part of a PhD (Psychology) thesis.

A total of **at least 24** people will participate in this project.

These tasks will be undertaken to explore two prominent theories relating to brain differences between those with HFA and controls (mirror neuron hypothesis and cortical under-connectivity).

You are invited to participate in this research project because you are 16 years of age or above, and/or have a diagnosis of HFA.

The results of this research may be used to help researcher **Tom Perkins** to obtain a **Doctorate of Philosophy**.

The results of this research may be used to help researcher - **Kayleigh Young** to obtain a **Doctorate in Clinical Psychology**

3. **Procedures**

The study will be conducted at the Burwood campus of Deakin University and will take approximately an hour and a half. You will come into the lab, and meet the research team. We will tell you a bit about TMS and answer any questions you may have. You will then fill out a short questionnaire measuring traits associated with autism. Following this, we will begin setting up for the TMS. We ask that you do not wear hair product, and are clean shaven for this research. The reason for this is that hair on the skin can interrupt the signal of the TMS. During the set-up for the TMS, we will measure your head with measuring tape, and place the electrodes on your hand/face. As part of this set-up, an experimenter will take a number of TMS pulses in order to identify the minimum strength of the TMS required to get a response from your brain. Following this, we will begin the experimental trials.

In trial one of the study, you will take part in an illusion. For this illusion, you will be seated at a table with your right arm in a wooden box. The positioning of this box will mean you cannot see your right arm. You will place your left arm on the table so that you can see it. The left wall of the box has a mirror, so that you will be able to see the reflection of your left arm in this mirror. An experimenter will be seated on the other side of the table in front of you. This experimenter will use two acrylic paint-brushes to gently stroke your right hand (which you cannot see) and your left hand (which you can see). In some subjects, this creates an illusion where you experience the touch of the brush on your right hand as occurring in the reflection of the mirror, rather than your actual right hand. During this trial, we will ask you to make small index finger movements with your left hand. While you are completing this, a second experimenter will stimulate you with a TMS pulse to see how your brain activates during this illusion. We will also take TMS pulse measures while you watch another experimenter move their hand, whilst you move your own hand, and whilst you keep your hand still. Following the experiment, we will ask you to fill out a very short questionnaire which asks whether you experienced the illusion or not.

In trial two of the study, stimulation of parts in the brain involved in different facial expressions will be assessed by recording responses produced in facial muscles. These
responses are recorded with electromyography (EMG) pads. These pads are taped to the skin, and measure the level of activity in the brain that is associated with that muscle. During this experiment, you will simply be asked to observe a short video of people with an angry expression, people with a happy expression, people with a neutral expression, then people yawning.

4. Possible Benefits
Benefits include potentially identifying brain regions which are associated with autistic symptoms. Moreover this research provides an opportunity to integrate two prominent theories of autism related to brain pathology. It is hoped this research will benefit diagnostic clarity to autism. We cannot guarantee or promise that you will receive any direct benefits from this project.

5. Possible Risks
A potential risk may be that you will experience a slight headache following the TMS stimulation. TMS has been in use world-wide in clinical, and laboratory settings, for more than 25 years, and is used routinely to investigate the human nervous system with very low risk. TMS is a safe technique, and does not lead to any long lasting effects. In very rare circumstances, seizures have been reported using TMS. Prior to any TMS, you will undertake a safety screen to ensure you are appropriate for this technique. If you have a history of seizures, this research will not be appropriate for you to participate in.

Another issue to note is the possibility of a non-autistic participant receiving a high score on the autistic trait questionnaire. It is important to recognise that a high score on this questionnaire does not mean you are autistic. The questionnaire measures trait’s associated with the disorder, but does not mean you are autistic.

6. Privacy, Confidentiality and Disclosure of Information
The data in this study you provide will be identifiable such that your responses to the questionnaire can be matched to your TMS responses. However, you will not be identifiable by name, as we will use subject numbers to connect the information together. A separate list will contain information holding your subject number and your name; enabling us to remove your data from the study should you wish it. Thereafter, only aggregated data will be reported in a thesis.

The information collected during the study will be stored in hard-copy and computer files in secure storage for a minimum of 6 years, in accordance with Deakin University guidelines. Following this period the hard copy files will be destroyed and the computer files deleted. A report of the study may be submitted for publication to a psychological journal, however individual participants will not be identifiable in such a report as only aggregate data will be reported.

7. Results of Project
You are encouraged to contact the researcher at the completion of the study to be informed of the aggregate research findings. Aggregate results will be published in a thesis and it is anticipated that they will also form part of a publication in a psychology journal.

8. Participation is Voluntary
Participation in any research project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any. Any information obtained from you to date will not be used and will be destroyed.
Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your relationship with Deakin University or through which you have been invited to participate.

Before you make your decision, a member of the research team will be available to answer any questions you have about the research project. You can ask for any information you want. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please do not submit your questionnaire.

9. Ethical Guidelines
This project will be carried out according to the *National Statement on Ethical Conduct in Human Research* (2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

The ethics aspects of this research project have been approved by the Human Research Ethics Committee of Deakin University.

10. Complaints
If you have any complaints about any aspect of the research, the way it is being conducted or any questions about your rights as a participant, then you may contact The Manager, Office of Research Integrity, Deakin University, 221 Burwood Highway, Burwood Victoria 3125, Telephone: 9251 7129, Facsimile: 9244 6581, research-ethics@deakin.edu.au.

Please quote project number EC 135 -2009.

11. Reimbursement for your costs
You will be paid $10 to reimburse costs incurred.

12. Further Information, Queries or Any Problems
If you require further information, wish to withdraw your participation or if you have any problems concerning this project (for example, any side effects), you can contact the principal researcher, Associate Professor Mark Stokes, or the student researchers Tom Perkins and Kayleigh Young.

The researchers responsible for this project are:

*Tom Perkins (student researcher), Deakin University, Faculty of Health, Medicine, Nursing, and Behavioural Sciences, School of Psychology, 221 Burwood Hwy, Burwood, 3125, Ph: 9251 7235. For after hours contact, please call 0400 128 098.*

*Kayleigh Young (student researcher), Deakin University, Faculty of Health, Medicine, Nursing, and Behavioural Sciences, School of Psychology, 221 Burwood Hwy, Burwood, 3125, Ph:0433337847.*

*Associate Professor Mark Stokes (principal researcher), Deakin University, Faculty of Health, Medicine, Nursing, and Behavioural Sciences, School of Psychology, 221 Burwood Hwy, Burwood, 3125, Ph: 9244 6865.*
TO: Participants

Consent Form

Date:

Full Project Title: Mirror neuron deficit and reduced neural connectivity in high functioning autism

I freely agree to participate in this project according to the conditions in the Plain Language Statement.

I have been given a copy of the Plain Language Statement and Consent Form to keep.

The researcher has agreed not to reveal my identity and personal details, including where information about this project is published, or presented in any public form.

Participant’s Name (printed) ..............................................................

Signature ................................................................. Date .........................

Tom Perkins
School of Psychology, Faculty of Health, Medicine, Nursing and Behavioural Sciences
Deakin University
221 Burwood Highway
Burwood
3125, Victoria.

Kayleigh Young
School of Psychology, Faculty of Health, Medicine, Nursing and Behavioural Sciences
Deakin University
221 Burwood Highway
Burwood
3125, Victoria.
TO: Participants

Revocation of Consent Form

(To be used for participants who wish to withdraw from the project)

Date:

Full Project Title: Mirror neuron deficit and reduced neural connectivity in high functioning autism

I hereby wish to WITHDRAW my consent to participate in the above research project and understand that such withdrawal WILL NOT jeopardise my relationship with Deakin University.

Participant’s Name (printed) .................................................................

Signature .................................................................Date .................

Please mail this form to:

Tom Perkins
School of Psychology, Faculty of Health, Medicine, Nursing and Behavioural Sciences
Deakin University
221 Burwood Highway
Burwood
3125, Victoria.

Kayleigh Young
School of Psychology, Faculty of Health, Medicine, Nursing and Behavioural Sciences
Deakin University
221 Burwood Highway
Burwood
3125, Victoria.
Appendix D: Adapted Rubber Hand Illusion Questionnaire

During the experiment there were times when:

1. It seemed as if I were feeling the touch of the paintbrush in the location where I saw the reflection of my hand touched.

<table>
<thead>
<tr>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>
   Disagree Strongly | Neutral | Agree Strongly

2. I felt as if the reflection of my left hand was actually my right hand.

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<th>1</th>
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<th>3</th>
</tr>
</thead>
</table>
   Disagree Strongly | Neutral | Agree Strongly

3. It felt as if my own hand were drifting towards the left (towards the reflection of my hand).

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<thead>
<tr>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>
   Disagree Strongly | Neutral | Agree Strongly

4. It seemed as if I might have more than one right hand or arm.

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<th>3</th>
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<th>1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>
   Disagree Strongly | Neutral | Agree Strongly

5. I felt as if the reflected hand were my hand.

<table>
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<tr>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>
   Disagree Strongly | Neutral | Agree Strongly

6. It seemed as if the touch I was feeling came from somewhere between my own hand and the reflected hand.

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<th>3</th>
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<th>2</th>
<th>3</th>
</tr>
</thead>
</table>
   Disagree Strongly | Neutral | Agree Strongly
7. It seemed as though the touch I felt was caused by the paintbrush touching the reflection of my hand.

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<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disagree Strongly</td>
<td>Neutral</td>
<td>Agree Strongly</td>
<td></td>
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</tr>
</tbody>
</table>

8. It appeared (visually) as if the reflection of my hand were drifting towards the right (towards my hand).

<table>
<thead>
<tr>
<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
<th>1</th>
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<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disagree Strongly</td>
<td>Neutral</td>
<td>Agree Strongly</td>
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</tbody>
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