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The Biological Bases of Social Deficits: The roles of social motivation, Theory of Mind, and selected genotypes (OPRM1, 5-HTTLPR) in Autism Spectrum Disorder

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"Parents of autistic children know that there is some constitutional infirmity within their children's minds that eventually must be addressed in neurophysiological and neurochemical terms. To what extent I have woven a tapestry of logic or fantasy is to be seen."

- Jaak Panksepp, 1979, pg. 177

Declaration

I hereby declare that this submission is my own work, both in concept and execution, and that to the best of my knowledge and belief it contains no material written by another person or material that has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

Signed by candidate

_____ _09 February 2020

Kate Hamilton Date

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Finally, to all the other Neuropsychology graduates and believers in South Africa: we have so much to give this country, and one day I hope we all achieve the South African Neuropsychology Dream.

List of Abbreviations

Abbreviation	Description
5-HTTLPR	Serotonin transporter promoter length polymorphism
ADHD	Attention Deficit / Hyperactivity Disorder
ADI-R	Autism Diagnostic Interview – Revised
ADOS	Autism Diagnostics Observation Schedule
ADOS2	Autism Diagnostics Observation Schedule – Second Edition
AGRE	Autism Genetic Resource Exchange
ASCQ	Attachment Style Classification Questionnaire
ASD	Autism Spectrum Disorder
BAS	Behavioural Assessment Scale
BSE	Behavioural Summarised Evaluation
CPRS	Childhood Psychiatric Rating Scale
DNA	Deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders
ERP	Event-response potential
ESDM	Early Start Denver Model
HPCSA	Health Professional Council of South Africa
IACC	Interagency Autism Coordinating Committee
IQ	Intelligence Quotient
MRA	Multiple regression analysis
NEPSY-II	Developmental Neuropsychological Assessment, Second Edition
NT	Neurotypical

OPRM1	Mu opioid receptor gene
RELN	Reelin gene
RRB	Restricted Repetitive Behaviours
SA	Social Affect
SCQ	Social Communication Questionnaire
SERT	serotonin transporter protein
SES	Socio-Economic Status
SRS	Social Responsiveness Scale
SSRI	Serotonin specific re-uptake inhibitor
ToM	Theory of Mind
UCT	University of Cape Town
VABS	Vinelands Adaptive Behaviour Scale
VIF	Variance inflation factor
VIQ	Verbal Intelligence Quotient

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Abstract

Autism Spectrum Disorder (ASD) is a diverse disorder, and the heterogenous range of possible presentations hinders our understanding of its aetiology. Recently there has been a surge of genome wide association studies for ASD, while historically psychological theories were relied on to explain the emergence of ASD. These fields continue to provide insights into ASD, but they tend to operate in parallel – genetic studies often lack comprehensive psychological phenotyping and theoretical backing, and psychological studies tend to lack genetic explanations. I propose that moving forward, genotype-phenotype studies should have a strong foundation in both fields and should focus on genes and theories with real-world implications for ASD diagnostics and/or interventions. This approach can be supported by focusing on established, well supported psychological theories, and selected ASD candidate genes that could be implicated in these theories, and ideally the genetic mechanism implicated should be one that can be targeted by existing medications.

I therefore selected two prominent psychological theories, the Social Motivation Theory for ASD (Panksepp, 1979) and the ToM Theory for ASD (Baron-Cohen et al., 1985), and selected an ASD candidate gene that was likely implicated in each of these theories, namely the mu-opioid receptor gene (OPRM1) and the serotonin transporter promoter length polymorphism (5-HTTLPR) respectively. For the first study of this protocol, I assessed the possible relationships between social motivation, OPRM1, and the ASD phenotype. For the second study, I assessed possible relationships between ToM deficits, 5-HTTLPR, and the ASD phenotype. These two studies shared a sample of 153 male children 4-16 years old; 51 boys per group (i.e. non-verbal ASD; verbal ASD; neurotypical). All ASD children completed ADOS2 assessment for ASD phenotyping.

For the Social Motivation Study, attachment was used as a proxy to assess level of social motivation in all participants, and 76 of the 102 children with ASD provided DNA for OPRM1 genotyping. Comparisons across all three groups showed that the ASD samples had significantly lower social motivation than the neurotypical sample, with the non-verbal ASD group displaying the most severely reduced level of social motivation. Reduced social motivation was associated with ASD-related deficits in the non-verbal ASD sample but not the verbal ASD sample. Finally, I was unable to statistically assess the role of OPRM1 as hypothesized, due to an unprecedentedly high rate of the OPRM1 *G* allele, which indicated atypical mu-opioid processes. This clearly implicated OPRM1 in ASD and is the first study to show this so convincingly. Overall, this study's findings led me to suggest that Panksepp's (1979) theory could be updated to include a threshold effect such that more severely reduced social motivation in ASD is associated with little-to-no language acquisition, while those with less severely reduced social motivation are able to develop language and this protects against associations between social motivation and ASD-related deficits in childhood.

For the Theory of Mind Study, the verbal ASD sample and neurotypical sample completed a developmental ToM Battery (i.e. *University of Cape Town Theory of Mind Battery*) and WASI assessment to establish verbal intelligence quotient (VIQ) scores, and 70 of the children with ASD were successfully genotyped for 5-HTTLPR. This study found that verbal male children with ASD tended to be one developmental stage behind age-matched neurotypical peers on ToM tasks. ToM deficits were associated with greater impairment in overall ASD severity and in symptoms from the social communication and interaction domain. For the non-verbal ASD sample, the 5-HTTLPR short allele, which is implicated in atypical serotonergic transmission, was associated with greater impairment overall and in the restricted and repetitive

behaviours and interests symptom domain. No associations between 5-HTTLPR and ToM, or with ASD-related symptoms, was found for the verbal ASD group. This again suggested that language acquisition is an important consideration in genotype-phenotype studies in male children with ASD.

This protocol illustrated the importance of including a range of ASD presentations in research studies. This is especially true in the case of non-verbal participants with ASD, as the possibility of different genotype-phenotype relationships in verbal versus non-verbal children with ASD has not been a major research focus to date, with little research being available. This finding may have implications for diagnostic and intervention practices in ASD, as current knowledge is generally based on research with verbal children with ASD and may therefore have some limitations being generalised to non-verbal children with ASD. Specifically, the genotype-phenotype relationships for these two theories and genes were only found for the non-verbal ASD group, suggesting they may benefit from medications which target these pathways, such as naltrexone and SSRIs, although further research is needed to confirm this. Overall, contextualising these genotype-phenotype studies within prominent psychological theories has provided greater insight into these theories, at least in the context of male children with ASD. This has provided more nuanced knowledge on the roles of these two ASD candidate genes in the male ASD-phenotype, and identified possible treatment roles for targeting these genes in specific children with ASD.

Keywords: Autism Spectrum Disorder; social motivation; SEPARATION-DISTRESS; OPRM1; Theory of Mind; 5-HTTLPR.

Chapter 1: General Introduction to Protocol

This research protocol was compiled and submitted as my dissertation for the degree of Doctor of Philosophy at the University of Cape Town (UCT). This research was conducted under the primary supervision of Associate Professor Susan Malcolm-Smith, with co-supervision by Prof. Kirsty Donald, as part of our Autism Research Team in the ACSENT Laboratory, UCT. Under said supervision, I was able to design this project, collect all data, and finally conduct the analyses and compile this write up. I was involved in all aspects of data collection, and personally conducted all ADOS2 assessments and the bulk of the Autism Spectrum Disorder (ASD) data collection. I co-supervised honours and masters students in our team who assisted with data collection, particularly the parent interviews and data collection for the neurotypical group. All DNA was personally collected and was then processed at the Department of Human Genetics, UCT.

The overarching focus of this dissertation is on genotype-phenotype studies in the field of ASD. There is a large body of research in this field, but I found that psychological research often lacked sufficient biological backing, and conversely, genetic studies lacked sufficient psychological input when compiling ASD phenotypic data. I hope to contribute to the field by showing how the roles of ASD candidate genes can be better understood when investigated in a hypothesis-driven manner, alongside prominent psychological theories underlying our current understanding of ASD.

Currently, we know that ASD affects individuals from the start of development and throughout the lifespan. Yet despite the high prevalence and sometimes devastating effects of this disorder, we do not have a definitive understanding of the causes of ASD; without a full understanding of the causes and contributing factors, our prevention and treatment efforts will

always be limited. This thesis did not aim to find a central or single cause for ASD, but rather sought to assess two prominent psychological theories for ASD alongside appropriate ASD candidate genes. Numerous psychological theories exist to explain the emergence of ASD, and hundreds of ASD candidate genes have been identified. Together these fields should contribute to elucidating the underlying causes of ASD, of core deficits, and how this disorder unfolds across development in this way we can understand the biological and developmental pathways underling ASD and see beyond the confusion that exists due to the heterogenous presentations evident in this disorder.

Due to the sheer volume of research in both fields, I believe that a useful way forward would be to explore the role of selected ASD candidate genes in prominent psychological theories using a hypothesis-driven approach. Psychological theories should be selected based on the level of support from existing research, as well as their ability to meaningfully explain the emergence and development of ASD. Candidate genes should be selected based on their likelihood of being implicated directly in critical elements of said psychological theories, and on outcomes that could result from identifying their role in ASD – that is, we should aim for knowledge that can lead to improvements in diagnostics, interventions, and medical treatments. My thesis therefore reviews our current knowledge of ASD, explores two selected psychological theories with associated candidate genes, and discusses my findings and the validity of this approach to genotype-phenotype research in ASD.

This protocol was therefore written up across five chapters. This first chapter gives an overview of the approach I've taken toward this research. Chapter Two then provides a literature review on our current knowledge of ASD. ASD is complex disorder, with a history of changing psychological theories explaining it, and newer medical research attempting to do the same.

Understanding this developing knowledge sets the foundation for the way forward; taking our existing knowledge and bringing it together cohesively across fields, as I hoped to do, presents the opportunity to understand the biological and psychological aspects of this disorder. In turn, this may open further avenues to improve diagnostics and treatment options.

Chapter Three focuses on the first psychological theory of interest, the Social Motivation Theory for ASD (Panksepp, 1979). This theory proposed that ASD arises when mu-opioid systems are disrupted, either by increased baseline opioid levels or increased activation, and that the effects of these disruptions undermine social interest in the individual such that infants do not find social interaction pleasurable or social separations unpleasant. This psychological theory directly implicated the mu-opioid receptor gene (OPRM1) in biological pathways to ASD, but the exact role of this gene in the ASD phenotypes has yet to be adequately explored within an ASD sample. I selected the Social Motivation Theory for ASD as it has long been researched in animal models and is very well supported at this level but lacked sufficient research in human studies. I also selected this theory because the effects of the implicated gene (i.e. OPRM1) can easily be targeted by existing medications. I therefore explored the level of social motivation deficits in ASD when compared to neurotypical children, how these deficits related to the ASD phenotype, and what role OPRM1 played in reduced social motivation and/or ASD-related deficits.

Chapter Four focused on the ToM Theory for ASD, as Theory of Mind (ToM) has long been an area of interest in ASD research (Baron-Cohen et al., 1985). ToM deficits are common in ASD samples, and their role in the overall phenotype and in specific ASD-related deficits has not been fully elucidated. I therefore aimed to quantify the degree of ToM deficit compared to neurotypical samples, and to explore the role of ToM deficits in the ASD phenotype. Although

the ToM Theory for ASD does not implicate a specific ASD candidate gene, the serotonin transporter promoter length polymorphism (5-HTTLPR) is of interest as it regulates the neurotransmission of serotonin, which is implicated in ToM processing (Bosia et al., 2011; Murphy et al., 2006). Further, 5-HTTLPR has shown atypical allelic distribution patterns in ASD samples (Arieff et al., 2010). To my knowledge, two phenotype-genotype studies for 5-HTTLPR in ASD have been reported (Brune et al., 2006; Tordjman et al., 2001). These studies did not agree on the role of 5-HTTLPR in overall ASD severity, or in whether it was associated with specific aspects of the ASD phenotype. 5-HTTLPR is easily targeted by serotonin-specific re-uptake inhibitors (SSRIs) which are already in use in some ASD treatment regimens (Sugie et al., 2005). However, treatment outcomes are mixed and it is unclear why some children show an alleviation of ASD-related symptoms with this treatment approach, while others do not, and the symptoms which do appear to benefit from treatment are not consistent across cases. I therefore aimed to further explore the role of ToM in the ASD-phenotype, and to explore the role of 5-HTTLPR in the ASD phenotype and in ToM deficits in ASD.

Finally, Chapter Five provides an overview of the results from the above two studies, as well as a general discussion on pertinent findings. This chapter explores the value of this approach to phenotype-phenotype research in ASD. The strengths and weaknesses of the current protocol are also discussed, with suggestions for future research.

While this protocol aimed to clarify associations between selected ASD candidate genes and core aspects of ASD with a male sample, it is noted that this study was conducted in a South African context - that is, in the context of a resource limited developing country. While organic findings could be universal, at least for male children with ASD, their phenotypic outcomes are likely to be influenced by cultural and environmental factors. The context of this protocol should

be seen as a strength, however, as globally we need to increase our focus on ASD in various contexts and move away from our current over-reliance on research and knowledge from first world countries (de Vries, 2016). ASD constantly evades full understanding due to the complexity and variability of its presentations, so exploring a range of ASD presentations across a range of contexts is necessary. I hope the current protocol and its approach to merging psychological and genetic research will aid in moving forward toward a better understanding of this disorder.

Chapter 2: Current Understanding of ASD

ASD is a neurodevelopmental disorder characterised by deficits in social communication and social interaction, alongside restricted and repetitive behaviours and interests (American Psychiatric Association, 2013). ASD is a pervasive disorder with lifelong deficits. Although it is prevalent worldwide, its full aetiology remains poorly understood, and this places limitations on prevention and intervention efforts. As a neurodevelopmental disorder, these deficits present in early development. However, deficits may only become clinically recognised when the child is older and the demands on social communication and interactions skills increase to the point where they exceed the child's capabilities.

Although ASD is a social disorder, it is not a homogenous disorder and the core social difficulties can present in a number of ways, resulting in an unusually diverse range of symptom presentations (American Psychiatric Association, 2013). Several social areas are assessed during the ASD diagnostic process, from the ability to socially modulate eye-contact, to the ability to identify emotions and to understand one's role in social relationships (Lord et al., 2003; Rutter, Le Couteur, et al., 2003). ASD initially had subtypes due to the diverse range of presentations (American Psychiatric Association, 2000), although the new diagnostic criteria have moved away from subtyping and instead focus on severity in each symptom domain and additional descriptors that specify whether common signs, such as language impairment, are present (Appendix A; American Psychiatric Association, 2013). Ongoing research has revealed that some children previously thought to have ASD actually presented with unique genetic disorders (e.g. Rett's syndrome; Caballero & Hendrich, 2005). Although currently all ASD cases are considered to represent one disorder, it is possible that further research may reveal that some

cases represent different genetic disorders It is therefore important to understand the genetic similarities and differences across what are currently the considered ASD phenotype spectrum.

The variance in the ASD phenotype is seen in how the diagnosis of ASD can be given to one child who has significant difficulties with socially modulating their eye-contact, directing facial expression, and with initiation of social overtures on one hand, as well as to another child who presents very differently with numerous but inappropriate social overtures, stereotyped speech, and difficulty reading social cues (Caballero & Hendrich, 2005). There is little clarity on how deficits in different social skills relate to overall level of ASD severity, or how initial social deficits underlie the development of later social deficits.

In addition to varying qualitative presentations, ASD also presents on a spectrum of severity (American Psychiatric Association, 2013). Some children will present with no expressive language and with self-harming behaviours and an inability to conduct self-care, while others may be able to function in mainstream schooling and some even complete tertiary education despite some restricted interests, compulsions, and difficulties relating to others (American Psychiatric Association, 2013). The diversity of possible symptom presentations for ASD and the range of possible outcomes further complicates our understanding of this disorder.

Prevalence

ASD is one of the most prevalent developmental disorders globally with prevalence rates ranging from 13.1 to 27.5 per 1000 (Baio et al., 2018; Taylor & Seltzer, 2011). Worldwide prevalence rates are heavily dependent on reports from Europe and the USA, while the prevalence of ASD in many developing countries remains unknown. A community survey of 1169 children in Uganda identified 8 children with ASD (i.e. 6.8/1000 of the sample) (Kakooza-Mwesige et al., 2014), while a study in a paediatric neurology clinic in Nigeria identified 54

cases of ASD among 2320 patients (i.e. 23.3/1000 in a paediatric neurological sample) (Lagunja et al., 2014). While these samples are small, and the second sample is not from the general community, they show that ASD is prevalent in Africa. There is no official reported prevalence rate for ASD in South Africa, but an estimated average of ten children per week are diagnosed with ASD between three tertiary level state hospitals in the Western Cape (Bateman, 2013). As the Western Cape is only one of nine provinces, this indicates a high prevalence of ASD in South Africa.

ASD is predominantly diagnosed in males, with the ratio of male to female diagnoses being 4:1 (American Psychiatric Association, 2013), although a metanalysis of 54 studies found that the ratio was closer to 3:1 (Loomes et al., 2017). There is some evidence that females with ASD have a different phenotype to males with the same diagnosis, although the nature of these differences requires further investigations (Rivet & Matson, 2011). The prevalence of ASD does not appear to be affected by race or ethnicity, but research indicates that communities of low socio-economic status (SES) are likely to under-identify cases of ASD, and that diagnoses from these areas tend to be made at a later age (Bertrand et al., 2001; Bakare & Munir, 2011b; Fombonne et al., 1994).

The high prevalence of ASD is concerning and there is an increasing global awareness of the burden of this disorder (Baird et al., 2006; Bertrand et al., 2001; Kogan et al., 2009; Malcolm-Smith et al., 2013). The heterogeneous nature of the disorder results in needs for support across various domains, but many of these children will never develop into independently functioning adults. ASD therefore places substantial strain on families and the state. There is generally very little information available locally on ASD and its social and monetary cost (Kogan et al., 2009) and South Africa also lacks sufficient diagnostic and

intervention services, meaning that even when a formal diagnosis is made, very limited services are available to the child and their family.

ASD in South Africa

de Vries (2016, p. 130) commented that "almost everything we know about ASD comes from high-income countries, mainly the USA, UK, and other European countries", and continues to point out that this is despite the reality that 90% of people with ASD live in contexts that are low and middle-income, such as Africa. The paucity of research in South Africa, and Africa in general, means very little is known about ASD diagnostics, prevalence, and phenotypes in South Africa. To date, even prevalence rates are unclear in the African region, as there have yet to be any comprehensive prevalence studies in sub-Saharan Africa at all (Harrison et al., 2014).

However, there is a rising interest in ASD locally, and the last decade has shown an increase in African research, particularly in South Africa and Nigeria (Abubakar et al., 2016). Local studies have focused on issues such as the appropriate use of the ADOS2 in Cape Town, South Africa (Smith et al., 2017), on the role of genetics in ASD (Arieff et al., 2010; Sharma et al., 2013), and on symptom presentations in terms of empathic responses such as cradling bias (Pileggi et al., 2015) and ToM development (Hoogenhout & Malcolm-Smith, 2014, 2016).

ASD Resources in South Africa

Despite this increase in research, South Africa is critically under-resourced to deal with the high incidence of ASD. In the Western Cape, the Red Cross War Memorial Children's Hospital, Lentegeur Hospital, and Tygerberg Hospital report collectively diagnosing 10 children a week with ASD (Bateman, 2013), but there are only 9 schools in the entire country that are specifically tailored to provide the specialised education required for children with ASD. In general, medical and clinical professionals have limited access to diagnostic and intervention

services through the public health sector (Malcolm-Smith et al., 2013). In South Africa there are no standard policies or good practice guidelines for the diagnosis, treatment, support, or education of people with ASD (de Vries, 2016).

South Africa further struggles due to poor access to appropriate ASD assessment measures. When resources can be accessed, many practitioners are not adequately educated about ASD to know to use them (Mitchell & Holdt, 2014). Further, there are concerns about the use of Western tests in South Africa, which is a culturally and linguistically diverse country. Globally, the ADOS2 is the gold standard for ASD diagnosis assessment (Chambers et al., 2017), but at the time of the current research there was no standardised and validated translation of the ADOS2 into a non-English South African language (Smith et al., 2017).

Chambers et al. (2017) conducted a study in urban and peri-urban areas in Pietermaritzburg, South Africa, where they translated several ASD diagnostic measures, including the ADOS2, into isiZulu. Assessment with 26 isiZulu-speaking toddlers (n = 10 ASD participants; n = 16 non-ASD participants) found that the ADOS2 was still able to reliably, and validly, identify ASD "red flags", although a comprehensive validation study was not conducted due to the small sample size. Similarly, Smith et al. (2017) assessed the cultural appropriateness of an Afrikaans translation of the ADOS2 in a Western Cape sample. A pre-pilot study of this translation was conducted with 7 children from a clinical sample with their caregivers in the room for the assessment; caregivers were then given the opportunity to report back on how appropriate they found the measure. The researchers noted some of the subtests were not culturally appropriate for their low-to-middle socioeconomic sample, such as the *Description of a Picture* task, *Demonstration* task, and the *Birthday Party* task. Overall, however, the study found that most of the ADOS2 materials were familiar and appropriate for use with this sample,

and the ADO2 translation was able to identify the participant with ASD, and that the other six children did not meet the criteria for an ASD diagnosis.

The shortage of appropriate tests and access to resources also results in a later age of diagnosis locally compared to the USA and UK. Mitchell and Holdt (2014) conducted a qualitative study with seven families in KwaZulu-Natal, South Africa, where they explored the process of ASD diagnosis. The families reported that the diagnostic process was time-consuming and laborious, and time between initial concern and final diagnosis took over three years on average, with one family reporting a diagnosis time of seven years. Part of this difficulty was the number of clinicians and professionals required to make the diagnosis, with the families reporting an average of seven practitioners being seen before reaching a diagnosis. It is noted that these families had the means to pursue some private services, but the majority of the South African population has limited socioeconomic means, and their dependence on the limited state facilities could result in an even poorer outlook, with many children never receiving a formal diagnosis.

Non-Verbal ASD in South Africa

A further characteristic of ASD locally that needs to be noted is the high reported prevalence of non-verbal children with this disorder. Bakare and Munir (2011 a, b) conducted two literature reviews on the presentation of ASD in Africa, and they found that there was a much higher rate of non-verbal ASD presentations compared to Western presentations, and further, that these children often failed to develop meaningful expressive language as they aged. In their literature search they found that over 50% of cases reported lacked expressive language. They observed that this could be due to poor access to diagnostics and then to intervention

resources. Limited access to resources, however, could also result in a bias such that children with more severe ASD are more likely to be seen and diagnosed.

Springer et al. (2013) conducted a retrospective review of the medical files available at Tygerberg's Children Hospital, South Africa, and found 58 children who met the criteria for an ASD diagnosis. Using the classification of "non-verbal" for children who used fewer than 10 non-echoed words, they found their sample was predominantly non-verbal (72.4%, $n = 42$). One possible reason for the higher rate of non-verbal diagnoses in South Africa is that these children are frequently referred for hearing tests when they fail to develop language, and may therefore be given priority at the clinics compared to verbal children with possible ASD, resulting in children with milder forms of ASD either not presenting to health care facilities or not being referred to the appropriate institutes.

Research findings from the USA and UK cannot be assumed to be appropriate in South Africa without considering our context. In order to understand the occurrence of ASD locally and work toward revealing its aetiology, research needs to be conducted within a local context. While international research has laid the foundation for understanding ASD, the unique local context emphasises the need to conduct further research in South Africa.

ASD Diagnostic Criteria

An ASD diagnosis, as per the DSM-5 (American Psychiatric Association, 2013), requires a child to have deficits in two main symptom dimensions (Appendix A). The first dimension encapsulates deficits in social communication and interaction, and the second dimension refers to deficits related to restricted and / or repetitive behaviours, interests or activities. Deficits in both dimensions must be present in multiple contexts. Current and historical symptoms are assessed during diagnostic processes as symptoms must be present in early development to indicate a

neurodevelopmental disorder. Consideration of historical symptoms is especially important when assessing language development, as delayed language development is often a key symptom of ASD, but a child may have overcome this delay by the time they see a clinician for diagnostic purposes. When assessing symptoms in early development one must be mindful that symptoms may not yet be fully manifest but will become more apparent as the child ages and demands exceed their capabilities in a more apparent manner (Lauritsen, 2013). A child must have symptoms in both core symptom domains to meet the diagnostic criteria, the deficits must cause impairment to their functioning, and their symptoms must be best explained by an ASD diagnosis and not by any other learning or developmental disorders (American Psychiatric Association, 2013). An ASD diagnosis under the DSM-5 can also include specifiers noting whether the ASD diagnosis is with or without comorbid intellectual impairment, language impairment, and / or catatonia, or is associated with a known medical or genetic disorder, an environmental factor, or with another neurodevelopmental, mental, or behavioural disorder.

ASD is generally diagnosed at the age of 3 or 4 years old, or later in developing countries, but atypical development is often noted prior to this age (Chakrabarti & Fombonne, 2005; Chawarska et al., 2007; Mitchell & Holdt, 2014). Some early signs of ASD may be present at 6 months of age if the child fails to make eye-contact or to show anticipatory gestures, or if they show a general disregard for social interaction. Later, at 1 year and older, a failure to respond to their name, or to orient themselves toward someone interacting with them, may be seen. At two years and older, expressive language may fail to develop, may be delayed, or may be atypical. Atypical expressive language may include pronoun reversals, echolalia, or stereotyped speech, and speech is often unusual in rhythm or tone. This can be accompanied by poor joint attention, limited facial expressions and gestures, low social interest in others and poor

empathy (Chawarska et al., 2007; Cox et al., 1999). As the child continues to age, those who do develop language often fail to also develop the gestures and facial expressions that accompany typical speech, and those that do may fail to integrate these with their speech.

ASD is a pervasive disorder that continues to impact the individual throughout their life. Adults with ASD tend to find the transition from the structured school environment into the adult world challenging (Taylor & Seltzer, 2011). Adults with ASD tend to have limited independence, struggle to form adult friendships and relationships, and battle with unemployment and low economic status (Eaves & Ho, 2008; Howlin & Moss, 2012; Taylor & Seltzer, 2011). Adults with ASD who do find employment are often limited to part-time work, and this work tends to be menial in nature. Some studies have found that individuals who were able to find some form of employment or develop relationships tended to be those with better language capabilities by age five, as well as the absence of comorbid intellectual difficulties (Eaves & Ho, 2008). However, it was also found that adults with ASD without comorbid intellectual difficulties had far less support options than those with intellectual difficulties. Overall, adults with ASD continue to struggle with ASD-related symptoms and difficulties, and only a portion manage full independence as adults.

Other Common ASD Characteristics

In addition to the core areas of deficits required for an ASD diagnosis, ASD has many associated features that are common in this disorder, although these are not unique to it (American Psychiatric Association, 2013).

Children with ASD may experience sensory difficulties. As with many aspects of ASD, the heterogenous nature of this disorder is evident: some children may present with hypersensitivity to sensory input, while others can present with hyposensitivity (American

Psychiatric Association, 2013). Hypersensitivity to sound is common and results in great difficulties integrating an otherwise high functioning child into mainstream schooling or daily activities. By contrast, children with hyposensitivity have been reported to injure themselves and show limited pain response, placing them at a higher risk for self-injury. These children also tend to show an unusual interest in sensation, often seeking higher stimulation in firmer touch from caregivers, and showing a fascination with the sounds and smells of objects.

Children with ASD may present with mildly delayed motor milestones or motor symptoms (American Psychiatric Association, 2013; Lam & Yeung, 2012; Manjiviona & Prior, 1995; Noterdaeme et al., 2010; Szatmari et al., 2006; Thede & Coolidge, 2007). Some motor symptoms are included under the motor component of restricted and repetitive behaviours, such as arm flapping, while others are included under the self-injurious behaviour component, such as head-banging. Generally motor symptoms can be present in the form of clumsiness or odd gait, and toe-walking is especially common. Individuals may present with catatonic-like symptoms, but this is rare.

Sleep difficulties are also commonly associated with ASD, including slow sleep onset, irregular sleep-waking patterns, many night awakenings, and early waking (Gabriels et al., 2005; Krakowiak et al., 2008; Richdale, 1999; Springer et al., 2013). Poor sleep can aggravate existing symptoms, and can lead to further difficulties in concentration, in school performance, in behaviour, and additional stress for the child and caregivers.

Gastrointestinal symptoms, dietary sensitivities, and difficulty with food intake are commonly noted in children with ASD (Cermak et al., 2010; Chandler et al., 2013; Knivsberg et al., 2002; Kral et al., 2013; Talay-Ongan & Wood, 2000; White, 2003), although gastrointestinal symptoms are not always more common in ASD samples compared to neurotypical samples

(Black, Kaye, & Jick, 2002). These children can be highly selective with food and are often unwilling to try new or unfamiliar foods (Cermak et al., 2010; Kral et al., 2013; Talay-Ongan & Wood, 2000). Children may display odd behaviours with food, such as touching and smelling without eating the food, and ingesting non-food substances is also reported. Some researchers have ascribed these difficulties with food to sensory sensitivity in reaction to the textures, smells and tastes of the foods; this may be why some children are resistant to some foods and, conversely, could also explain intense fascination with certain foods shown by some other children with ASD (Cermak et al., 2010; Talay-Ongan & Wood, 2000). Gastrointestinal symptoms such as vomiting, diarrhoea, abdominal pain, bloating, and constipation are also more common in children with ASD when compared to neurotypical children (Cermak et al., 2010; Chandler et al., 2013; Kral et al., 2013; White, 2003). The above challenges with food and gastrointestinal symptoms combine to create daily challenges in eating routines, and limited food intake can result in inadequate nutrition, stunted development, and may exacerbate other ASD symptoms (Cermak et al., 2010; Knivsberg et al., 2002; Kral et al., 2013; White, 2003).

Children with ASD may also receive comorbid diagnoses (American Psychiatric Association, 2013). Intellectual impairment is one of the most common comorbid diagnoses (American Psychiatric Association, 2013; Baio et al., 2018; Chakrabarti & Fombonne, 2005; Happe & Frith, 2006; Thede & Coolidge, 2007). Intellectual ability can range from severely impaired to above average. However, even individuals with high intellectual functioning can present with a substantial difficulty with adaptive functional skills in social contexts.

Anxiety is very common in ASD, and as these individuals reach adulthood they may be diagnosed with comorbid depression or anxiety disorders (American Psychiatric Association, 2013; Simonoff et al., 2008). Attention Deficit/Hyperactivity Disorder (ADHD) (American

Psychiatric Association, 2013; Peacock et al., 2012; Simonoff et al., 2008) and seizures or Epilepsy (American Psychiatric Association, 2013; Bolton et al., 2011; Peacock et al., 2012; Simonoff et al., 2008) are also frequently diagnosed in children with ASD.

The heterogeneous nature of ASD, as well as the possible associated characteristics and comorbidities, can make it a challenging disorder to characterise and diagnose. The high prevalence of ASD globally underscores the need to further improve our understanding of the disorder, as well as our current diagnostic procedures and interventions.

Social Deficits

At its core, ASD is a disorder of social ability, with striking impairment in communication and interaction (American Psychiatric Association, 2013). From the origin of its conceptualisation, ASD was characterised as a disorder where a child was unable to socially connect with others, including their caregivers, and that even those who developed language struggled to use it effectively to communicate in a social context. Leo Kanner (1943), one of the first clinicians to classify and explore ASD, asserted that the core feature of ASD was a sense of "aloneness", whereby children presented as profoundly disconnected and socially separated from others. He described how the children he worked with struggled to connect and relate themselves to people around them, and that this was evident from the very beginning of life. Based on his work with children with ASD and their families, he concluded that "we must then assume that these children have come into the world with an innate inability to form the usual, biologically provided affective contact with people, just as other children come into the world with innate physical or intellectual handicaps" (Kanner, 1943, p. 250). Further research and a greater understanding of ASD has shown that this apparent lack of social drive is present for many of the children on the spectrum, and we see persistent difficulties in understanding reciprocal

interactions, in relating to and understanding others, and possibly a decreased or absent desire, or atypical ability, to enter social interactions (American Psychiatric Association, 2013).

While some children with ASD desire social relationships, others seem to show no social interest at all. When first conceptualising ASD as a disorder, Kanner (1943) noted that the children he worked with did not simply withdraw from social participation – they appeared to disregard, ignore, and completely shut themselves off from social situations. In many cases social aloofness is not a preference but seems to result from an early failure to develop a social interest, and this in turn leads to a failure to develop social attachments. These children struggle to make and maintain friendships. It is not uncommon for the child to fail to develop friendships at all, and often if they do develop friendships these are shallow and inappropriate for their age (American Psychiatric Association, 2013; Lauritsen, 2013). Interaction is often awkward and can be characterised by socially maladaptive behaviours. In cases where a natural social ability does not develop, some children may develop strategies to navigate social situations, and these strategies can become more complex and refined with age. However, these strategies are typically still too restricted to fully compensate and approximate innate social ability, and the social behaviour of these individuals may therefore come across as scripted and unnatural and places a burden on the individual to perform in a certain way (Hull et al., 2017).

Social deficits present differently as the child develops. As young infants, children with ASD may fail to exhibit anticipatory postures in order to prepare to be picked up, suggesting a failure even at that age to understand the basic intentions of others (Happe & Frith, 1996; Klin et al., 1992). At this age poor attachment with the caregiver is often noted and can be characterised by little to no comfort seeking (Rutgers et al., 2004; Rutgers et al., 2007). Failure to initiate or maintain eye contact often becomes more evident as these children develop, and difficulties in

joint attention mark their social isolation when surrounded by others (Meyer & Minshew, 2002). Many children with ASD struggle to read the non-verbal cues and body language of others, and often respond inappropriately. They may demonstrate unusual body postures and gestures in social situations, as well as inappropriate facial expressions (American Psychiatric Association, 2013). Impaired ToM can be a component of the difficulty in understanding others (Baron-Cohen et al., 1985). The lack of understanding of others inevitably translates into incomprehension of social norms and conventions, and ultimately results in a socially isolated childhood.

While ASD is predominantly diagnosed in males, research with female children with ASD has found several differences across the sexes which could suggest differential phenotypes with different developmental trajectories and outcomes (Van Wijngaarden-Cremers et al., 2014). In particular, female children with ASD may have a social advantage over male children with ASD. Sedgewick et al. (2016) explored friendship experiences and social motivation in a mixed sex sample of children from special education schools, both with and without ASD. They found that female children with ASD displayed similar friendship quality and social motivation to female children without ASD. By contrast, male children with ASD displayed less motivation for social interaction, and their friendships were qualitatively different to the female children with ASD and to the children without ASD. Further, the female children with ASD reported higher levels of relational aggression within friendships when interviewed. Similarly, a qualitative study using semi-structured interviews with a sample of 11 female children with ASD between 11-17 years old found that the participants were interested in having friendships. Female children with ASD appeared to have an understanding of what was required for successful friendships, and derived enjoyment from these social relationships (Cook et al., 2017; Vine Foggo & Webster, 2017). Finally, female children with ASD appear to display more behaviours indicating a desire

and understanding of friendship than male children with ASD, such as a better understanding of empathy, entering multiple social interactions flexibly, and staying in the proximity of their peers when together (Dean et al., 2014; Dean et al., 2017; Head et al., 2014).

In addition to difficulties with understanding social situations and struggling to relate to others, social communication difficulties can also include language deficits which further undermine their ability to function in a social context (American Psychiatric Association, 2013; Lauritsen, 2013).

Language Deficits

Language deficits represent one aspect of a more general deficit in verbal and non-verbal communication in ASD (Verhoeven et al., 2012; Zillmer et al., 2008). The ability to communicate with gestures is regarded as a precursor to the development of language and the skills needed for social interactions, yet children with ASD with impaired language often fail to utilise non-verbal behaviours to compensate when trying to communicate.

Language deficits in ASD can present as impairments in the expression and/or comprehension of language (American Psychiatric Association, 2013; Mody et al., 2013). Approximately 25% of individuals with ASD fail to develop functional language, while those who develop language may have a significant delay in this development (Mody et al., 2013). As mentioned above, the rate of non-verbal children with ASD is even higher in under-resourced and developing countries (Bakare & Munir, 2011b).

When language is present, it is often unusual (Zillmer et al., 2008). It can be characterised by stereotyped content, tangential responses, and replies to questions can be inappropriate or unrelated to the context of the conversation. Pronoun misuse is common and is most often noted in a failure to switch between pronouns referring to the self versus pronouns

referring to others (Happe & Frith, 1996). Children with ASD often have a very literal understanding of language and may therefore fail to use generalised terms. Some therefore struggle to understand the general uses of ‘yes’ or ‘no’, and resort to responding to questions by repeating them as statements. Difficulties with nonliteral language can also result in an inability to understand metaphor, sarcasm and irony (Happe & Frith, 1996; Meyer & Minshew, 2002). Research on language development in ASD has shown that many children have a very high rate of depending on heard sentences and repeating these rather than generating their own sentences; this technique can have mixed results as sentences are sometimes used appropriately and sometimes are not, but in severe cases this can result in echolalia (Rapin & Dunn, 2003).

Language is fundamentally intertwined with cognitive, social, and emotional domains of function, and these areas develop in ways that involve ongoing influencing of one another (Mody et al., 2013). The development of language therefore significantly affects children’s thinking, learning, and their ability to form relationships. As children with ASD often fail to orient to speech from a young age, including when their names are called, it is likely that communication impairment may be, at least in part, secondary to their deficits in social competence and reciprocity (Mody et al., 2013).

Language deficits in ASD could be a direct extension of the deficits in social functioning, as indicated by the shift from the DSM-IV-TR's separate symptom domains of "social interaction" and "communication" into the DSM-5 single diagnostic criteria of impairment in "social communication and social interaction" (American Psychiatric Association, 2000, 2013). A review of the state of research on non-verbal children with ASD found that these children had severe deficits in social motivation, supporting this association between language development and social interest (Tager-Flusberg & Kasari, 2013). A review of the literature on language

acquisition in ASD found that language was impaired in most cases of ASD, even when children were considered fluent (Eigsti et al., 2011).

A direct relationship between social interaction and language development in neurotypical children was shown by a series of experiments by Kuhl (2010). They exposed English speaking toddlers to Mandarin for 12 sessions across 4-5 weeks and then used behavioural and event-related potential (ERP) measures to assess phonetic perception. Toddlers were divided into groups with various levels of social interaction included in the sessions: some children had no social interaction and heard the Mandarin over an audio-tape or television; some children were exposed via an interactive screen; and some children had a person in the room as a live tutor. The children in the first condition showed no language learning at all, and those in the final condition showed equal learning to Taiwanese children who had been exposed to Mandarin for 10 months, while those in the middle condition showed limited learning.

The relationship between ASD-related social deficits and the language difficulties associated with this disorder is unclear. A study with 129 preschool children with ASD found that ASD symptom severity was the greatest contributor to variability in non-verbal communication skills deficits (Kjellmer et al., 2012). They also found an association between ASD symptom severity and verbal communication skills, although this was not as strong as the association between cognitive ability and verbal communication skills.

Despite the intertwined nature of language deficits with other domains, language impairment is in itself a striking feature of ASD and it poses a significant challenge to the quality of life that individuals with ASD can attain. It is also unclear whether the absence of language development results in a qualitatively different ASD presentation to verbal children with ASD, or whether non-verbal children with ASD have the same overall presentation but at a higher

severity. Increased inclusion of non-verbal children with ASD in research may help clarify this issue.

Non-Verbal Children in Research

Language deficits not only influence a child with ASD's ability to function in daily life, but also impact research efforts into understanding ASD. Children with ASD who are unable to understand instructions or communicate a response due to language deficits cannot participate in a great deal of research beyond initial toddler studies. This limiting effect on methodologies has resulted in the abundance of available psychological research being conducted with verbal ASD samples (Tager-Flusberg & Kasari, 2013).

It is not standard practice for studies to report the language fluency of their participants and verbal capabilities must often be inferred from the selection of measures used in each study. Studies that do report verbal capabilities in their samples face a further problem however, as there is no generally accepted definition of what constitutes "non-verbal", so these studies may not be comparable. As non-verbal children with ASD cannot participate in a great deal of research, there is the possibility that conclusions from existing studies cannot be generalised across the ASD spectrum (Tager-Flusberg & Kasari, 2013).

Research methods have evolved to include non-verbal children with ASD, and eye-tracking, physiological, and neuroimaging studies are being conducted; however, these studies still favour verbal children with ASD over non-verbal children with ASD due to higher compliance with instructions. Improvements in technology make it easier to include non-verbal participants in research, and organisations such as Autism Speaks and the Interagency Autism Coordinating Committee (IACC) (Interagency Autism Coordinating Committee, 2011; Tager-

Flusberg & Kasari, 2013) have called for greater research with these participants. Despite this, however, this area remains understudied.

Restricted and Repetitive Behaviours and Interests

Although ASD is a social disorder, children with ASD also have symptoms of restricted and repetitive interests and / or patterns of behaviour (RRB) (American Psychiatric Association, 2013). These symptoms also affect a child's social behaviour, their language, and how others respond to them, contributing to their social isolation. RRB symptoms encompass repetitive motor and/or sensory behaviours, such as repetitive or stereotyped motor symptoms (e.g. hand-flapping) and those that represent a resistance to change and insistence on sameness (e.g. behavioural symptoms such as compulsion or atypical play characterised by the lining up of toys) (Cuccaro et al., 2003). Language is often affected as well, with children with ASD tending to display echolalia, or relying on stereotyped speech by using idiosyncratic phrases (American Psychiatric Association, 2013). Some children with ASD can be highly rigid in their routines, and an insistence on sameness can extend to all elements of their environment (Turner, 1999). This can include how a room is organised, an unusual attachment to a particular object or toy, refusal to change the route taken to school, and even a resistance to changes in the appearance of their family members such that a change in a parent's hairstyle could be upsetting. This inflexibility can extend to thoughts as well, with some children fixating on specific subjects and thinking patterns, often resulting in difficulties understanding that others have different thoughts to their own, and resulting in stilted social interactions as they are unaware of the other's disregard for their topic of interest (American Psychiatric Association, 2013; Baron-Cohen et al., 1985).

Aetiology of ASD

Our understanding of ASD has its roots in psychiatry, with research trends showing an initial focus on psychological explanations for the development of ASD, and more recently a focus on medical and genetic contributions to the disorder. ASD was identified as a unique disorder in the 1940s when American child psychiatrist Leo Kanner (1943) and German scientist Hans Asperger (1944) published their findings from working with children with social disorders and they recognised autism and Aspergers as unique disorders rather than a form of childhood schizophrenia. Despite the decades of research since then, the full aetiology of ASD remains unknown.

Kanner (1943) stated that all the children he observed showed social disinterest from early development and he believed this was an innate inability to form social relationships that the children were born with. He also noted, however, that these children had highly intelligent parents with obsessive tendencies but who lacked warmth. He commented that he was unsure what effect, if any, the absence of parental warmth had on the overall presentation of ASD. Despite clearly stating that ASD was the result of an organic cause, later clinicians latched onto his comments on the parents and hypothesised that ASD arose from cold parenting styles, ultimately leading to the popularisation of the “Refrigerator Mother” theory for ASD (Bettelheim, 1972). Bettelheim (1972) was a strong advocate for removing children with ASD from their parents’ care and argued that the abnormalities seen in these children were the result of cold parenting.

This initial focus on psychological influences being the cause of ASD was challenged by Bernard Rimland (1964) and led to great debate on whether parenting or genetics were the cause of ASD. Rimland (1964) stated that children with ASD presented with brain damage, and the

root of the disorder was therefore neurological, and he became a strong advocate for stopping the removal of these children from their parents.

In the 1970s twin studies pointed to a high heritability statistic if one twin had ASD, which indicated that it was a hereditary condition (Folstein & Rutter, 1977). This research helped shift our understanding of ASD further away from the psychodynamic theories and toward medical explanations. Research then moved to focusing on medical causes for ASD, with psychological studies tending to focus on interventions or better characterising the ASD phenotype. The shift to considering ASD as a medical disorder also resulted in a shift in treatment approaches as it became a disorder that clinicians believed could be treated with medical intervention.

Several medical theories have been proposed, with mixed support. One of the most well-known theories was that of Wakefield (1999) who proposed that the measles, mumps and rubella (MMR) vaccine caused ASD. This theory was ultimately rejected when the research was found to be fraudulent (Godlee et al., 2011). Due to the heterogeneity of the ASD phenotype, research on its aetiology is being conducted in several medical disciplines. Research focusing on the prenatal period has found associations between a higher incidence of ASD and severe viral infections during pregnancy (Libbey et al., 2005), to exposure to certain drugs during pregnancy (Dufour-Rainfray et al., 2011), and to high levels of intrauterine testosterone (James, 2014). Factors considered during the prenatal period and early childhood have included: the role of poor nutrition and/or gastrointestinal issues; the role of environmental and medical toxins; the effects of early infections; the role of metabolic irregularities; and, the role of the neuroendocrine system (Inglese & Elder, 2009). While this research has led to some useful findings in regard to symptom alleviation in ASD, the full aetiology of ASD is still unknown.

While more recent research still considers how the factors above could influence the ASD phenotype, researchers generally now consider ASD a genetic disorder. The specific genetic basis of ASD has remained elusive, but the high heritability estimate and equally high concordance of ASD between identical twins indicate the importance of genetic research for this disorder (Kendler, 2010; Muhle et al., 2004).

While initial studies focused on a singular cause for ASD, more recent research has been marked by a shift toward conceptualizing ASD as a disorder that arises from multiple genetic contributions, with Happe, Ronald, and Plomin (2006) suggesting that different elements of impairment should be studied separately. Current research focuses on establishing which genes contribute to a predisposition to ASD, which genes contribute to specific symptoms in ASD, and the likely outcomes of interactions between gene products (Poelmans et al., 2013). Genome-wide studies have compared the genomes of individuals with ASD to genomes of control subjects and have identified an estimated 400 candidate genes (Cho et al., 2011; Cross-Disorder Group of the Psychiatric Genomics, 2013). A recent review noted that the progress being made in genetic research has led to identifying the genetic etiology of ASD cases in 25-35% of cases (Wisniowiecka-Kowalnik & Nowakowska, 2019), with another review finding the rate to be as high as 40% of cases (Genovese & Butler, 2020). These reviews noted that newer studies included methods such as newer chromosomal SNP microarrays, whole-exome sequencing, and next generation sequencing (NGS). The sheer number of genes implicated in ASD, and the overlapping contributions of numerous genes to specific elements of ASD, add to the argument that no single gene will be found to underlie ASD (Happe et al., 2006).

While genotype-phenotype association studies and genome-wide association studies (GWAS) are the current techniques of choice in research in many medical and psychiatric

disorders, and in ASD research specifically, these studies do have their limitations. Generally, there is concern that GWAS studies are too broad, and will ultimately implicate the entire genome in different disorders, as well as directly implicate genes in disorders where there is no biological mechanism to explain such associations (Tam, 2019). Further, the complexity of psychiatric disorders means that any determinant of predisposition toward that disorder is likely to be subtle (Sher, 2001). SNPs and genotype prevalence are unevenly distributed in different ethnicities (Dvornyk et al., 2004), and research findings are therefore difficulty to replicate (Sher, 2001). However, these difficulties can be overcome by focusing on narrowly defined phenotypes (Sher, 2001), and ensuring ethnicity data is included where appropriate.

Despite the above limitations, further genetic research into the underlying mechanisms of ASD can make valuable contributions to our understanding of this disorder, and to improve diagnostics and refine treatment programmes. The Autism Genetic Resource Exchange (AGRE), a repository for ASD phenotypic and genotypic data, has therefore been established to aid ASD research (Geschwind et al., 2001). AGRE was established in 1997 and is the largest private, open-access repository of clinical and genetic information dedicated to ASD research, with over 150 research groups using the resource (Autism Speaks, 2020). The National Institute of Mental Health has placed an emphasis on phenomic research in mental health, and in response to the growing number of genes implicated in ASD, they support research that will result in improved phenotyping and that will elucidate the underlying neurobiology and mechanisms of ASD specifically (National Institute of Mental Health).

ASD-related genetic research in South Africa is an emerging field in this context and is therefore limited. To my knowledge, only two genes have been assessed for possible roles in ASD, and collectively only four studies have been conducted. Three studies explored the allelic

distribution of 5-HTTLPR in typically developing and ASD samples locally (Arieff et al., 2010; Esau et al., 2008; Hamilton, 2014) and one study explored associations of specific single-nucleotide polymorphisms for the reelin gene (i.e. rs736707 and rs362691) with ASD (Sharma et al., 2013). South African researchers are therefore still heavily reliant on findings from international studies which may consist of primarily Caucasian or European participants and therefore under-represent samples comparable to those in South Africa.

Treatment of ASD

As the genetic and psychological aetiology of ASD is not fully understood, ASD treatments focus on symptom alleviation more than on cure. Treatments regimens can include psychological interventions, medications, or both, and vary depending on the child's phenotype. Psychological interventions tend to focus on social and learning deficits, while medical treatments tend to focus on the amelioration of symptoms associated with ASD.

Psychological interventions are recommended from early childhood for best effects, with some programmes being available to children from as young as 12 months. Most ASD interventions tend to fall into one of two categories, as some take a naturalistic developmental behavioural approach, while others follow a structured, consistent programme format based on educational methods. The naturalistic, developmental approach delivers intervention through social interactions in a common environment (Schreibman et al., 2015). These interventions use child-directed teaching strategies that allow for intervention with familiar materials in a familiar environment, thereby using the child's natural behaviours and interests to build social competence and target other deficits. By contrast, structured interventions typically rely on applied behaviour analysis which aims to alter problematic behaviours by identifying the antecedents and consequences of these behaviours to understand why they occur, and through

altering the consequences of behaviours using positive reinforcement for desired behaviours. More recently some interventions have merged these approaches, such as the Early Start Denver Model (ESDM), and have shown positive long-term outcomes (Dawson et al., 2010; Estes et al., 2015). Outcomes, however, remain varied.

Children with ASD are frequently prescribed medications for their symptoms, and research has found a very high incidence of polypharmacy throughout childhood (Barnette et al., 2019). Medical treatments for ASD often include the use of antipsychotics and antidepressants for anxiety and mood symptoms (Birch et al., 2018; Madden et al., 2017). ADHD medication is also prescribed due to the high comorbidity rate between ASD and ADHD (Hazell, 2007). Doctors also frequently prescribe medication for gastrointestinal symptoms, neurological symptoms and epilepsy, sleep disturbances, skin disorders, and urologic disorders (Jones et al., 2016). While medications can alleviate symptoms to some degree, and can make the overall disorder more manageable, they do not cure core deficits of ASD.

Numerous interventions and medical plans therefore exist to provide support for children with ASD. However, while interventions aim to target core deficits, clinicians cannot always identify which children would best benefit from which type of intervention – understanding the relationships between core deficits and the overall ASD phenotype could assist with this. Further, while medical treatments are common in ASD, they tend to focus on symptoms associated with this disorder rather than with core elements of the disorder itself – by not focusing on core elements, the possible effects of medication on overall ASD severity are limited. Ultimately a better understanding of the development of ASD and the relationships between symptoms and core deficits would help with appropriate targeting of interventions and

understanding which medical processes could support the interventions may greatly improve their efficacy.

Summary and Conclusion

ASD is a neurodevelopmental disorder with a high prevalence rate that has lifelong effects. The widely heterogenous presentations of this disorder have undermined research into its aetiological pathways (both developmental and biological), and we therefore lack a full understanding of the underlying mechanisms of ASD. Many biological theories have been proposed as explanations for the development of ASD and more recently genome-wide association studies have found hundreds of genes that are implicated in this disorder. While progress in research has shifted our understanding of this disorder so that we believe it has an organic cause, we cannot simply dismiss the knowledge gained through psychological research. The next step should therefore be to consider psychological and medical / genetic findings and theories together, and move toward understanding ASD in the context of both of these fields. Well-supported psychological theories should not be disregarded, but should instead be expanded to include genetic components, while well-supported genetic research should include psychological theories when assessing genotype-phenotype relationships.

It is unlikely that a single theory, or a single cause, will fully explain the range of clinical presentations seen in ASD. However, some theories, and some genes or interactions between genes, could account for specific *aspects* of ASD. All these theories and genetic contributions cannot be assessed collectively, but two theories and two genes stand out as having potential to explain, at least in some part, the deficits seen in ASD. The first is the Social Motivation Theory of ASD, and OPRM1 as implicated in this theory, and the second is the ToM Theory of ASD, and

5-HTTLPR as implicated in this theory. This protocol focused on these theories in the two studies that follow.

Chapter 3: The Social Motivation Study

Panksepp (1979) stated that ASD arises due to emotional, rather than cognitive, difficulties. Specifically, he stated that it arose due to an innate dysfunction in the drive to socially engage with others; he called this drive "social motivation". This eminent psychological theory has long been supported by animal research, and has directly implicated the mu-opioid system in the aetiology of ASD. Despite this, however, studies investigating associations between ASD-related deficits, levels of social motivation, and the functioning of mu-opioids have not been adequately explored in human ASD samples. This study provides a first step in rectifying this situation by exploring associations between ASD-related symptoms, social motivation levels, and the mu-opioid receptor gene (OPRM1).

This chapter reviews our current knowledge regarding the Social Motivation Theory of ASD, and the potential role of mu-opioids in ASD. Thereafter I detail my study methodology and results and provide a discussion of my findings.

Literature Review

Humans are social beings and much of our survival depends on our ability to connect with others. Panksepp (1979) referred to social motivation as one of the earliest social behaviours; he believed we have an innate drive to seek social engagement and to avoid social isolation. Social motivation is a drive that develops in response to the experiences of the SEPARATION-DISTRESS system[1]. He proposed that ancient pain pathways evolved to facilitate social bonding through the SEPARATION-DISTRESS system, and that these opiate-based pathways facilitated the development of our earliest social relationships, namely those between

[1] SEPARATION-DISTRESS is capitalized, as are all Panksepp's basic emotion systems, to avoid confusion with general emotion terms, and to indicate that this term refers to a system that is evident across different mammalian species

infants and their caregivers. Panksepp (1979) proposed that the core ASD symptom of poor social interest and attachment could be explained by excessive opioid activity undermining the functioning of this system, which thus undermines our innate social motivation system.

Panksepp (1979) proposed that ASD was essentially a disorder of emotional disturbance, and that it arose from dysfunction of the opioid systems in the brain. He stated that the fundamental problem in ASD is that the child is unable to properly experience the emotions that usually arise from social relationships. He pointed to the earliest signs of ASD, including those of failure to cling to parents, lack of crying, and low desire for social interaction, as indications that a child with ASD does not experience typical emotions arising from social relationships, and that this deficit is present from the start of life. He noted that many of these children not only fail to seek their caregivers, indicating low social motivation, but they often fail to show any signs of comfort when re-united with their caregivers - he took these as signs of a failure of the SEPARATION-DISTRESS system in these children.

Panksepp (1979) hypothesised that children with ASD are born with increased mu-opioid activity, and this undermines their experience of social reward in terms of their need for, and development of, social motivation. As the SEPARATION-DISTRESS system is dependent on the functioning of mu-opioid systems, Panksepp (1979) drew further support for his theory that this system was disrupted by noting that some children with ASD are insensitive to pain, and these children often show higher levels of mu-opioids than neurotypical individuals (Gottfried & Riesgo, 2011; Panksepp, 1979; Panksepp et al., 1978; Sahley & Panksepp, 1987); it is thought that this excessive brain mu-opioid activity may be present as early as the neonatal period, resulting in a very early reduction of social motivation (Gottfried & Riesgo, 2011).

This review will therefore start by outlining Panksepp's (1979) SEPARATION-DISTRESS Theory, and the role of OPRM1 in social motivation within this theory. This is followed by a review of research regarding social motivation in ASD, and the role of mu-opioids in ASD.

SEPARATION-DISTRESS Theory

Panksepp's (1997) overarching basic emotion theory states that core emotion systems have evolved in mammalian species to promote survival. These systems each have unique neurochemical and / or neuroanatomical correlates, with associated patterns of behavioural, physiological, and psychological responses that occur when these systems are activated. These systems are genetically coded and activate in response to events that could affect survival. The affective state that arises from the activation of such a system results in the person (or other mammal) experiencing the event as "good" if it promotes survival and "bad" if it threatens survival. These responses therefore serve to encode value to events in order to promote survival, and the SEPARATION-DISTRESS system does this by supporting social cohesion.

One of the basic emotion systems identified by Panksepp's (1997) core emotion theory is the SEPARATION-DISTRESS system (previously PANIC/GRIEF), which mediates social attachment by making social isolation undesirable and social cohesion desirable, and facilitates the basic drive for social engagement, termed social motivation. This system is thought to have two associated feeling states: contact comfort, associated with positive social experiences, and SEPARATION-DISTRESS, associated with social distance or social loss. SEPARATION-DISTRESS is an adaptive warning system to prevent separations that could prove fatal for those entirely dependent on their caregivers.

Panksepp's (1997) core emotion theory proposes that the SEPARATION-DISTRESS system evolved from the body's primitive pain and reward mechanisms to reinforce social bonding. The proposed neurochemistry underlying the SEPARATION-DISTRESS system is therefore dominated by endogenous opioids, which are known to reduce the physical sensation of pain (Nelson & Panksepp, 1998; Panksepp et al., 1997). The theory states that events that promote social bonds have an associated mu-opioid release, and those that threaten social bonds have an associated decrease in mu-opioid levels, in oxytocin levels, and an increase in corticotropin releasing hormones (Nelson & Panksepp, 1998; Panksepp et al., 1997; Panksepp & Watt, 2011). In typically functioning mu-opioid systems, an increase in mu-opioid levels is associated with decreased pain sensation and elevated mood. Social contact is experienced as pleasurable when mu-opioids are released and is therefore comforting after a period of separation or isolation. However, if the system is not operating typically, then this release of mu-opioids is either blocked or the body is insensitive to it, preventing one from experiencing pleasure during social interaction. In contrast, social separation is experienced as painful, akin to the state experienced during opioid withdrawal, with an acute panic phase, and this phase is either terminated naturally by social contact, medically through opioid administration, or enters a chronic depressive phase characterised by withdrawal and behavioural shutdown. Infants find separation from a caregiver painful, and as they will have previously experienced the pleasurable mu-opioid release during contact with their caregiver, they will actively attempt to end the separation – this drive is social motivation. Infants therefore instinctively seek their caregiver through crying, calling, or attempting to look for or follow the caregiver. These signs of seeking social engagement are signs of their social motivation, and it is believed that if the mu-opioid system is atypical it will not adequately reinforce the SEPARATION-DISTRESS system. If the

SEPARATION-DISTRESS system is not reinforced, social motivation may fail to develop fully or will present in an atypical or inconsistent manner.

OPRM1 in the SEPARATION-DISTRESS Theory. One of the mechanisms implicated in the SEPARATION-DISTRESS system is OPRM1 due to its role in regulation of mu-opioids (Butler et al., 2014). OPRM1 encodes mu-opioid receptors, which are the primary site for mu-opioids. These receptors bind with natural opioids such as beta-endorphin and enkephalins, and synthetic opioids, including morphine, fentanyl, methadone, and heroine (Moles et al., 2004). The endogenous opioids that bond with mu-opioid receptors are shown to mediate natural rewards by creating a pleasurable mood while also decreasing the experience of negative emotion states and physical pain. These opioids therefore generate the experience of contact comfort as described in the SEPARATION-DISTRESS system. The role of OPRM1 in social motivation and the SEPARATION-DISTRESS system can therefore be assessed through genetic modification of this receptor in animal models, which alters the availability of mu-opioid receptors, through assessing the transmission of mu-opioids, or through manipulation of mu-opioid levels with synthetic opioids, making it an ideal gene for research purposes.

OPRM1 is located in human chromosome 6q24-q25 (Fillingim et al., 2005). This receptor has two common alleles, the *A* and *G* alleles, allowing for three common genotypes, namely: *A/A*, *A/G*, and *G/G*. The *G* allele is less common than the *A* allele, making the *A/A* genotype the most commonly occurring. Having one or more *G* alleles (i.e. the *A/G* or *G/G* genotype) is associated with an increased response to endogenous opioids through mu-opioid receptors (Mura et al., 2013). This allele may be associated with a greater binding affinity at mu-opioid receptor sites, so individuals carrying this allele could have higher opioid "tone" when receptors are activated – that is, once activated, it takes longer for the effect to deplete, and also

makes one less sensitive to fluctuations in mu-opioid levels while in this analgesic state (Bond et al., 1998; Fillingim et al., 2005).

OPRM1 allelic distributions are not associated with sex (Troisi et al., 2011), although different ethnic groups do present with different distribution patterns. A Swedish study with the LifeGene biobank found that 77% of their sample of 18 963 people had the *A/A* genotype, while the remaining 23% carried at least one *G* allele (Persson et al., 2019). By comparison, the *G/G* genotype tends to occur in only 1-4% of samples, with an allelic frequency rate of between 0.07 and 0.22 (Bart et al., 2004; A. C. Chen et al., 2013; Way et al., 2009). The exceptions to these studies are some of those with Asian samples, where the incidence of the *G* allele has been as high 0.35 (A. C. Chen et al., 2013; Way et al., 2009; Wei et al., 2017). A local study with 640 women from the Western Cape (364 African participants and 276 mixed race participants) revealed an incidence of 0.05 for the *G* allele, which is in keeping with the internationally reported prevalence (D. Stein and S. Dalvie, personal communication, May 23, 2019).

OPRM1 is implicated in the SEPARATION-DISTRESS system, and therefore in the pathway to ASD according to Panksepp's (1979) theory that ASD arises from atypical social motivation. The *G* allele is associated with greater sensitivity to endogenous opioids (Mura et al., 2013), but also with strong binding, which could essentially leave one in a state of opioid bondage – that is, as receptors are already activated, any other changes meant to activate them cannot take effect. Following Panksepp's (1979) theory, carriers of the OPRM1 *G* allele would therefore be insensitive to the changes in opioid levels during social interactions or separations. If one is insensitive to the fluctuations in mu-opioid levels associated with the SEPARATION-DISTRESS system, social motivation may fail to fully develop. This could result in the social

behaviour typical of children with ASD. Research regarding the role of mu-opioids in social motivation are reviewed below.

Non-Primate Animal Studies. Evidence supporting the existence of the SEPARATION-DISTRESS system and the role of opioids in this system is primarily drawn from non-primate animal studies. Studies show that when separated from the mother, these animals tend to display signs of "social pain" such as crying. These behaviours are often used to draw the attention of the mother with the intention of initiating a reunion. As predicted by the theory, research shows that opioids are released during positive social interactions, such as during social grooming, during sexual behaviour, during maternal nurturance, and during play (Machin & Dunbar, 2011; Panksepp, 1979; Panksepp et al., 1997).

Studies manipulating opioids have also supported their role in social motivation. Opiates have been administered to infant mice when they show distress after being separated from their mothers, and it was found that administration of a low dose of an morphine effectively reduced the distress calls as long as the drug was active, prompting Panksepp to note that opiates were “neurochemically equivalent to the presence of the mother” (Panksepp, 1979, p. 175). This relationship between opioids and reduction in social motivation, as seen by reduced distress calls in infant animals on separation after administration of opioids, has been consistent across multiple species, including dogs (Panksepp et al., 1978), guinea pigs (Herman & Panksepp, 1978), rats (Carden et al., 1991), and chickens (Warnick et al., 2005).

Warnick et al. (2005) recognised that although research had repeatedly found opioids played a role in mediating social attachment processes, there was a need for more work to identify whether specific opioid agonists and opioid antagonists influenced distress behaviour on separation, and therefore whether there were roles for specific types of opioids. Using chicks

from domestic fowl, they administered various opioid agonists and opioid antagonists to the chicks at 7 days post-hatching. They then placed the chicks in low- or high-stress scenarios using mirrors, which have similar effects to social companionship for chicks. They found that only the mu-opioid receptor played a role in the processes of social attachment as administration of a mu-opioid agonist resulted in a decrease in distress vocalisations. However, administration of a mu-opioid antagonist did not show a significant increase in vocalisations. This led them to suggest that mu-opioids modulate, rather than mediate, social attachment processes.

Genetic knockout of the mu-opioid receptor in mice results in considerable changes in social behaviour in keeping with the Social Motivation Theory. These mice fail to selectively approach their mothers and do not exhibit distress calls when separated from their mothers (Cinque et al., 2012; Moles et al., 2004), juvenile mice have reduced interest in social interaction with other mice (Cinque et al., 2012), young adult male mice showed reduced responsiveness and interest in female vocalisations (Gigliucci et al., 2014; Wöhr et al., 2011), and adult mice of both sexes had reduced social interest and rates of social interaction (Becker et al., 2014).

There is therefore an abundance of support for the existence of the SEPARATION-DISTRESS system in non-primate animals, and for the role of mu-opioids and OPRM1 in this system. However, the above studies were limited to animal models that did not include primates. Social motivation research for primates and humans is reviewed below.

Primate and Human Studies. Despite the clear support for the Social Motivation Theory from non-primate animal studies, research on primates has been less clear. Primates carry a mu-opioid receptor gene (OPRM1 C77G) that has the same functionality as the human mu-opioid receptor gene (OPRM1 A118G), and research in primates allowed for a close approximation of the role of OPRM1 in humans. Although some studies support the role of mu-

opioids in social motivation in a similar manner to that seen in the smaller animal studies reported above, others suggest a reversal of this relationship in human studies (Loseth et al., 2014; Pellissier et al., 2018).

Studies Supporting the Social Motivation Theory. As with previous literature, mu-opioids are sometimes implicated in social motivation in primates and humans. Mu-opioids actively play a role in the processing of pain in the anterior cingulate cortex and insula, and older research with monkeys has illustrated the role of these areas in the SEPARATION-DISTRESS system. Monkeys with lesions to these areas, who therefore could not experience the fluctuations of mu-opioids that result from the SEPARATION-DISTRESS system, showed an indifference to social contact and did not produce distress calls when experiencing a separation (Hadland et al., 2003; MacLean & Newman, 1988).

In a human study, Zubieta et al. (2003) investigated the involvement of mu-opioids in the regulation of affective states in a sample of 14 healthy female participants. Using positron emission tomography they studied neurotransmission of mu-opioids while the participants recalled an autobiographical event of profound social sadness (i.e. sadness state condition) compared to when they were asked to relax (i.e. neutral emotion state condition). Participants showed reduced mu-opioid activity during the sadness state condition, indicating that mu-opioid levels drop during the experience of social pain, which is in keeping with the Social Motivation Theory.

Troisi et al. (2012) assessed the relationship between early maternal care and anxious attachment in 112 Caucasian psychiatric patients. They specifically looked at an anxious attachment style in adults characterised by anxious and avoidant behaviours, as well as increased sensitivity to social rejection; they referred to this as fearful attachment. The avoidant behaviours

and lack of social interest seen in their participants can be equated with those of low social motivation. The OPRM1 allelic distribution in their sample was in keeping with reported population norms: 69% carried the *A/A* genotype; 29% carried the *A/G* genotype; and 2% carried the *G/G* genotype. For analyses they merged the *A/G* genotype and *G/G* genotype groups. They found a crossover interaction between OPRM1 and maternal caregiving: carriers of the *G* allele showed high rates of fearful attachment regardless of the quality of maternal care, while non-carriers of the *G* allele showed that higher levels of maternal care correlated with lower levels of fearful attachment. This finding supports the Social Motivation Theory by indicating that *G* allele carriers were insensitive to the influence of maternal care, possibly due to their indifference to the pain of social separation and the pleasure of social interactions.

Finally, human studies looking at the effects of prolonged elevation of opioid levels have shown that former opiate addicts who were being treated with opioid maintenance (i.e. chronic low dose of opiates) displayed reduced social interaction and had social cognition deficits (McDonald et al. 2013). This suggests that, much like in the non-primate animal models, mu-opioids could substitute for social interaction by providing an alternate cause for increased opioid levels or by making one insensitive the mu-opioid fluctuations underlying the SEPARATION-DISTRESS system.

Studies Contradicting the Social Motivation Theory. Some studies, however, have shown results which appear to contradict the expected role of mu-opioids in the social motivation system. Barr et al. (2008) conducted a study on infant rhesus monkeys that compared attachment behaviour across OPRM1 genotypes. They found that monkeys carrying a *G* allele showed greater persistence of distress calls when they were separated from their mothers and they showed a higher preference for social contact with their mothers when reunited than the monkeys

with the *A/A* genotype. Barr et al. (2008) hypothesised that this was due to a "gain-of-function" of the *G* allele as it would allow greater sensitivity to mu-opioids. This, however, would result in greater social motivation if Panksepp's (1979) theory is correct regarding the role of mu-opioids in the SEPARATION-DISTRESS system.

Similarly, a study by Higham et al. (2011) with free-ranging female rhesus monkeys found that females carrying the *G* allele for OPRM1 tended to prevent separations from their young by restraining them more so than females not carrying the *G* allele. This suggests that these rhesus monkey mothers showed a greater sensitivity to separations if they had the *G* allele, perhaps supporting the "gain-of-function" hypothesis (Barr et al., 2008), rather than the decreased sensitivity consistently seen in non-primate animal models.

Way et al. (2009) investigated the possible relationships between OPRM1, dispositional pain, and neural sensitivity to social rejection. They recruited 122 neurotypical university students to complete self-report inventories regarding dispositional sensitivity to social rejection, genotyped them for OPRM1, and a subsample of 31 participants underwent an fMRI scan while playing Cyberball, a social rejection game. The OPRM1 allelic distribution of their sample was in keeping with reported frequencies: 73 participants with *A/A* genotype (60% of sample), 44 participants with *A/G* genotype (36% of sample), and 5 participants with *G/G* genotype (3% of sample). Although ethnicity data was provided, the OPRM1 genotype distribution across these groups was not reported. They merged the *A/G* genotype and *G/G* genotype groups due to the small size of the latter group. They found that carriers of the *G* allele showed higher reactivity to social rejection than non-carriers; they also found an association between the *G* allele and increased trait-like tendencies to be concerned about social rejection. This does not support a role for OPRM1 in the SEPARATION-DISTRESS system as the theory would predict that having a

G allele would result in insensitivity to social rejection due to higher baseline mu-opioids. However, during the social rejection game the fMRI showed increased activity in the dorsal anterior cingulate cortex, which is known to be involved in the processing of physical and social pain. Way et al. (2009) suggested that this region may mediate the relationship between OPRM1 and social pain sensitivity, rather than a direct relationship existing between the two.

The literature examining the role of mu-opioids in social motivation in primates and humans is therefore mixed. Studies that support the theory are those that show changes in mu-opioid activity when experiencing sadness, which show that lesions preventing typical mu-opioid neurotransmission undermine social motivation, and which show that increased mu-opioid baseline levels indicated by the *G* allele for OPRM1 are associated with an indifference to maternal care. However, some studies indicate a gain of function for OPRM1 *G* allele carriers that makes carriers more sensitive to social separations - which contradicts the Social Motivation Theory. If OPRM1 and the SEPARATION-DISTRESS system are implicated in ASD, it appears that human studies with ASD samples are needed to start clarifying their roles.

Social Motivation Theory and ASD. Despite the mixed support for mu-opioids' role in the Social Motivation Theory in primates and humans, the interest in this theory as an explanation for ASD has persisted. Panksepp (1979) stated that if the neurochemical foundation of SEPARATION-DISTRESS system was disrupted it would undermine the child's social motivation capabilities, which would result in atypical psychosocial development. This reduced social motivation might result in considerable social and language acquisition deficits. He therefore hypothesised that children with ASD may have disruptions in their mu-opioid systems that undermine their social motivation. Some of the studies reviewed above supported the role of mu-opioids in the social motivation system. Further, manipulation of the mu-opioid system in

animals not only resulted in changes in social motivation behaviour, but it resulted in symptoms typically associated with ASD as well.

The research above has highlighted behaviours that are analogous to those required to fulfil the DMS-5 ASD diagnostic criteria for the first symptom domain regarding deficits in social communication and social interaction (Appendix A; American Psychiatric Association, 2013) Most notably, the absence of distress calls during separation in infant animals after receiving mu-opioids is considered to represent an equivalence to the absence of observed distress noted in many children with ASD when separated from their caregivers (Carden et al., 1991; Herman & Panksepp, 1978; Panksepp et al., 1978; Pellissier et al., 2018; Warnick et al., 2005). This behaviour is often accompanied by a lack of desire for social companionship and poor response to cuddling with the caregiver, behaviours typically reported by parents of children with ASD (Vostanis et al., 1998).

Interestingly, OPRM1 knockout mice also showed the behaviours required to fulfil the diagnostic criteria for the second symptom domain for ASD, namely restricted and repetitive patterns of behaviour, interests, or activities (American Psychiatric Association, 2013). The animals in these mu-opioid studies exhibited unusual learning effects characterised by extreme persistence of behaviour in the absence of external rewards, which can be equated to the restricted and repetitive behaviours seen in ASD. A review of the literature on OPRM1 knockout mice by Pellissier et al. (2018) noted the following symptoms similar to those in children with ASD were present in these mice: increased aggression; increased anxiety; poor motor development or clumsiness; poor spatial learning; and higher susceptibility to seizures. Knockout mice also showed an insensitivity to pain, which is frequently reported in ASD, and unusual body posturing such as toe walking was present after young animals received morphine.

Therefore, despite the mixed results for the role of mu-opioids in the SEPARATION-DISTRESS system and typical development of social motivation in primates, there is sufficient evidence from non-primate animal studies to indicate that atypical mu-opioid processes may underlie some aspects of the ASD presentation. There have been very few studies of social motivation within ASD samples, but those that have been conducted have supported the theory. Human studies have approached social motivation and opioid research by studying social motivation behaviours in children with ASD, and in assessing the results of medications that lower mu-opioid levels in these children.

Social Motivation Behaviours in ASD. Positive attachment behaviours may be seen as indications of social motivation and therefore provide further insight into the role of social motivation in ASD. Deficits in attachment in ASD have been noted from as early as two years of age (Van Ijzendoorn et al., 2007), and include behaviours such as failing to cuddle, lack of eye contact, low responsiveness to social interaction, failure to direct smiles socially, and indifference to affection (Rutgers et al., 2004); these behaviours are similar to those described in social motivation research (Panksepp et al., 1979).

It was previously believed that children with ASD cannot develop secure attachment at all, but a review of 40 articles investigating attachment in ASD found that some children with ASD can be securely attached, which could indicate typical social motivation processes (Teague et al., 2017). However, other reviews have found that although secure attachment may be present, children with ASD tended to show less secure attachment than children without ASD, possibly indicating low or atypical social motivation (Kahane & El-Tahir, 2015; Rutgers et al., 2004). Further, even when children with ASD showed secure attachment behaviours, their overall attachment behaviour was disorganised and considered atypical (Buitelaar, 1995; Capps

et al., 1994; Rutgers et al., 2004). It is possible that if mu-opioid systems are atypical, they could undermine functioning of the SEPARATION-DISTRESS system as the opioid release associated with social contact may not always, or consistently, be sufficient to provide the necessary pleasurable sensation during social contact, and this could be an important contributor to the disorganised attachment seen in some children with ASD.

Although a great deal of research has focused on quality of attachment in ASD, there has been less research focusing on the role of attachment in ASD, and specifically the relationship between attachment and ASD deficits. Those that have looked at these relationships have done so from the perspective of how other factors influence attachment in ASD rather than how attachment could impact psychosocial development as part of the manifest spectrum. An early study by Shapiro et al. (1987) found no relationship between ASD deficits and attachment, but a review focused on more recent literature found that attachment behaviours were related to the severity of ASD as well as the severity of comorbidities (Kahane & El-Tahir, 2015). Although this lacks specificity, it is in keeping with the notion that atypical, or indeed low, social motivation would relate to more severe deficits in ASD.

Klintwall et al. (2014) investigated the relationship between a child's social interest, or social motivation, and ASD outcomes. They noted that level of interest during ADOS assessment when the child was 2 years old was a good predictor of deficits at age 3 years. Specifically, lower interest in the assessment and in the person conducting the assessment was related to poor skill acquisition in verbal, non-verbal, and adaptive skills. This low social interest suggests poor social motivation, and as these children would have less desire to engage socially, they are likely to show less improvement with age than the children who engaged with others.

Children with ASD therefore frequently present with low social motivation as indicated by poor or atypical secure attachment. Further, this low social motivation is not only related to ASD deficits but may be a predictor of the severity of some symptoms across development.

Mu-Opioids in ASD. As early as the 1980s, research emerged showing that individuals with ASD had atypical levels of endogenous opioids, and tended to show higher levels of these opioids in their cerebrospinal fluid compared to neurotypical children (Gillberg et al., 1985). Very few human studies are available, but some that use naltrexone, an opioid receptor antagonist that specifically targets the mu-opioid receptor to reduce mu-opioid levels, have shown positive effects in ASD (Bouvard et al., 1995; Leboyer et al., 1992). There are however very few studies of this kind, and a review article noted that while Naltrexone could improve some symptoms seen in ASD, it was not clear that there was a direct influence on core symptoms (i.e. the core diagnostic criteria) (Roy, Roy, Deb, & Unwin, 2015). Instead, the review suggests that a subgroup of children with ASD may show positive responses to Naltrexone treatment, and that identifying such a cohort and understanding the role of mu-opioid processes in this sample could be significant and should be a research priority. As social motivation deficits are not a core diagnostic criterion for ASD, but could be an important feature of this disorder, better understanding the possible relationship between social motivation and mu-opioids in the context of ASD could contribute to our understanding of this disorder.

Bouvard et al. (1995) assessed the response to administration of naltrexone in a pilot study including 10 children with an ASD diagnosis. The participants were between 5-14 years of age, with five female and five male participants. The participants were recruited through a Parisian hospital where they were receiving out-patient treatment. Six of the ten participants had no expressive language (2 male; 4 female), and none of the children exhibited significant current

self-injurious behaviours, although 6 had previously shown mild self-injurious behaviours. Bouvard et al. (1995) conducted a naltrexone-placebo crossover study over four weeks, where the first group of participants received two weeks of placebo treatment followed by two weeks of treatment with naltrexone, and the second group received the trial medication in reverse order. Blood levels were drawn throughout the experiment to measure the whole blood levels of serotonin, dopamine, norepinephrine, and epinephrine, and neuropeptides. The clinical observations for behavioural markers were made with the Childhood Psychiatric Rating Scale (CPRS), the Behavioural Summarised Evaluation (BSE), and the abbreviated Conners Parents Teacher Rating Scale (Conners). They found that only a subset of their sample responded to the active drug with changes in behavioural outcomes, and that these participants showed the most marked normalisation of plasma parameters in the sample. Assessment of the subscales for two of the behavioural measures showed a mixed picture: for the CPRS, the largest improvements were seen in hyperactivity, hostility and restraint; and for the BSE, the largest improvements were seen for sociability, communication, object relations, and attention. ASD-specific diagnostic measures were not used to clinically profile the participants, so it is unclear how these findings relate to the core elements of ASD. However, it would appear that social communication was influenced, which would support Panksepp's (1979) theory that normalisation in opioid levels may promote social motivation. Unfortunately, there was no control group of neurotypical children to assess the general effects of this medication trial.

Herman et al. (1987) described a smaller case series that specifically explored the effects of naltrexone on self-injurious behaviours in three male participants, two of whom had diagnoses of ASD. The participants were in their adolescence and had showed severe self-injurious behaviours for at least five years prior to the study. Each participant was administered naltrexone

in tablet form. The researchers hypothesised that individuals who exhibit self-injurious behaviours do not experience the pain typically experienced from these behaviours due to an opioid analgesic state. They further hypothesised that as naltrexone has an affinity for mu-opioid receptors, the administration of naltrexone would decrease opioid levels and these behaviours would then elicit a pain response, hence reducing their frequency. They found that naltrexone significantly decreased self-injurious behaviours, with the greatest difference found for the participant with ASD with the most severe presentation of self-injurious behaviours: he showed a 50-fold decrease in said behaviours. By contrast, the participant without ASD showed the lowest response, but still showed a 33% decrease in these behaviours. Herman et al. (1987) therefore concluded that a mu-opioid antagonist could be a viable treatment option for cases of self-injurious behaviours, perhaps particularly those seen in ASD.

There is therefore some evidence indicating that decreasing mu-opioids in ASD may result in an improvement in symptoms, suggesting that the elevated baseline levels of mu-opioids may be related to core aspects of ASD. To my knowledge, no studies have assessed OPRM1 directly in an ASD sample, and certainly not in the sub-Saharan context. The possible role of OPRM1 in ASD, and its allelic distribution in this population, are therefore unknown.

Conclusion

Panksepp (1979) proposed that ASD was a social disorder that resulted from disruptions to the SEPARATION-DISTRESS system due to atypical mu-opioid levels, and the undermining of this system resulted in absent or low social motivation developing. He stated that children with ASD had reduced social motivation early in life which led to deficits in later psychosocial development. The role of mu-opioids in the SEPARATION-DISTRESS system is well supported by non-primate animal studies, as is the association between alteration in this system and low

social motivation. These studies showed that the atypical functioning of the mu-opioid is associated with the emergence of an ASD-like presentation in non-primate animal models. Although results from primate and human studies are mixed in their support, with some contradicting the Social Motivation Theory for ASD, the ASD-like presentation in OPRM1 knock-out mice is startlingly similar to behaviours seen in children with ASD - and these behaviours cover both core symptom domains for ASD in the DSM-5. Studies with ASD samples indicate that mu-opioids are implicated in the presentation of ASD and could be linked to specific symptoms or overall severity of ASD; although these relationships are suggested, they are still unclear. Further, given the heterogeneity across ASD presentations, it is possible that reduced social motivation, and by association mu-opioid processes, could contribute to explanations for common symptoms across ASD cases, or perhaps a core deficit identifying a subset of cases.

It would therefore be of interest to explore the main tenets of the Social Motivation Theory for ASD directly in an ASD sample: namely, that children with ASD have lower social motivation than neurotypical children; how social motivation relates to core symptoms in ASD and overall severity of the disorder; and, as strongly suggested by OPRM1 knockout studies, whether OPRM1 is linked to reduced social motivation and / or ASD-related deficits. As medication already exists to alter mu-opioid levels, improving our knowledge in this area could have implications for ASD management. The Social Motivation Theory for ASD suggests that early disruptions social motivation lead to impairment in later psychosocial development. Exploring the strength of this theory could therefore allow for early, targeted medical interventions being developed for children with ASD.

Rationale

ASD is a prevalent neurodevelopmental disorder, but its cause(s) and underlying mechanism(s) are not fully understood. Despite the heterogeneous nature of ASD, social deficits are a core feature of the disorder that appear early in life and may underlie development of later deficits. The overall role of reduced social motivation in ASD, however, is still unknown. ASD is a complex disorder with a diverse range of presentations and is therefore unlikely to arise from a single cause; instead, it is likely to have various mechanisms contributing to specific deficits, which jointly account for the diverse presentations seen. There is strong evidence for a genetic basis for ASD, and it is therefore likely that specific genes underlie specific deficits. OPRM1 is strongly implicated in the development of social motivation and is therefore of interest when assessing the Social Motivation Theory for ASD.

Research exists supporting a role for OPRM1 in the SEPARATION-DISTRESS system and the development of social motivation, and for reduced social motivation in ASD, but there is insufficient human research in ASD samples to assess the exact role of OPRM1 and social motivation in the ASD phenotype. Generally psychological research has omitted organic explorations, and genetic studies rarely feature comprehensive psychological data to provide phenotypic profiles. Further, research to date often excluded non-verbal children with ASD and overlooked the role of expressive language in ASD phenotypes. Several research gaps therefore need to be addressed in genotype-phenotype research for OPRM1, as research needs to directly assess levels of social motivation in human ASD samples, and this must be done alongside assessment of the ASD-phenotype,

Psychological and biological research programmes have often run in parallel, but there is a clear need to converge data from these two research areas and to further study the relationships

between these different levels of investigation. This study aimed to comprehensively assess social deficits in ASD, and specifically reduced social motivation, alongside genetic data for OPRM1. This study is unique in the scope and depth of assessment of social deficits, in its consideration of differences between verbal and non-verbal presentations of ASD, and in its aim to bring together several possibly related lines of existing research in biological and psychological fields to empirically assess the integrity of relationships that have so far remained hypothetical.

Aims and Hypotheses

This study aimed to explore the role of social motivation in ASD, using attachment behaviour as a proxy for social motivation. I hypothesised that the ASD groups would have lower social motivation than the neurotypical group. Within the ASD groups, I anticipated that lower social motivation would relate to greater ASD deficits. I hypothesised that reduced social motivation and ASD-related deficits would be associated with the presence of the OPRM1 *G* allele as this allele is associated with greater bonding and an opioid analgesic state that would undermine the SEPARATION-DISTRESS system. I anticipated differences between the non-verbal ASD group and the verbal ASD group in these relationships due to the role expressive language plays in social abilities but did not have any specific hypotheses for these group differences.

To explore Panksepp's (1979) Social Motivation Theory for ASD, I researched three tenets of his argument. The first is that children with ASD would have lower social motivation than neurotypical children. The second tenet is that reduced social motivations would relate to ASD-related deficits. And finally, that the mu-opioid system would relate to social motivation

levels and ASD-related deficits. I therefore asked the following questions, and addressed them with the associated hypotheses:

Question 1. Do children with ASD exhibit lower levels of social motivation, as indicated by attachment behaviours, than neurotypical children?

Hypothesis 1.1. Children with ASD will have lower secure attachment scores than neurotypical children.

Question 2. Is reduced social motivation related to ASD-related deficits, and does language ability have a role in this relationship?

Hypothesis 2.1. Non-verbal ASD children will have either higher or lower secure attachment scores than verbal ASD children.

Hypothesis 2.2. Non-verbal ASD children will have either higher or lower ADOS2 scores than verbal ASD children, indicating different symptom profiles.

Hypothesis 2.3. Low social motivation, indicated by low secure attachment score, is related to higher ADOS2 scores.

If hypotheses 2.1 – 2.2 are supported, indicating significant differences in social motivation and/or ASD deficits between ASD groups, hypothesis 2.3 would be extended to look for differences in relationships between social motivation and ASD deficits in the non-verbal ASD sample and the verbal ASD sample separately.

Question 3. Could OPRM1 play a role in social motivation levels and ASD-related deficits, and does the acquisition of language influence these relationships?

Hypothesis 3.1. Children with ASD will show a higher rate of the OPRM1 *G* allele than reported in neurotypical samples.

Hypothesis 3.2. ASD children carrying the OPRM1 *G* allele will present with higher ADOS2 scores than those who do not carry a *G* allele.

Hypothesis 3.3. ASD children carrying the OPRM1 *G* allele will present with lower social motivation (i.e. lower secure attachment scores) than those who do not carry a *G* allele.

Method

Research Design

The Social Motivation Study was the first of two studies conducted in the overall PhD protocol (see Chapter 4: The Theory of Mind Study, pg.125, for second study). This study assessed whether children with ASD had reduced social motivation, whether OPRM1 was implicated in social motivation, and whether social motivation and OPRM1 linked to specific aspects of ASD symptomatology. The protocol reported here constituted a cross-sectional, relational design.

Context

This protocol was conducted in the Western Cape, South Africa, as part of the research work done by the UCT Autism Research Group, ACSENT Lab, University of Cape Town. Data collection was conducted in and around Cape Town. Children with ASD were either assessed at their schools or, in cases where the child was not attending school, at their homes. Children were seen individually for several sessions in distraction-free environments, and care was taken to see the children with ASD in environments in which they were comfortable.

Participants

This study incorporated three groups of male participants with similar SES backgrounds: two clinical groups were of male children with ASD, one with non-verbal children and one with fluently verbal children; and a group of neurotypical male children. A child was considered

neurotypical if they had no medical or psychiatric diagnoses and were typically developing. The neurotypical group served as a control group. ASD participants were divided according to verbal ability for three reasons: first, due to the possible relationship between social motivation and language acquisition; second, to ensure equal inclusion of non-verbal participants as they have previously been overlooked in research; and third, due to the language demands of measures used in the second study of this protocol, the Theory of Mind Study (Chapter 4: The Theory of Mind Study - Method - Measures pg.151).

This protocol recruited a total of 176 male children between 4 and 16 years old. Of these, 108 children had ASD diagnoses and 68 were neurotypical. The ASD participants were grouped by expressive language ability: 53 of these children were classified as non-verbal and 55 children were classified as verbal. Children were considered verbal if they had flexible, fluent speech (as required for the ADOS2 Module 3), while those in the non-verbal group had no expressive language (as required for the ADOS2 Module 1), or limited phrase speech (as required for the ADOS2 Module 2). A subsample of the neurotypical group were selected to form a control group that matched the verbal ASD group on age, language, and SES.

ASD Participants. The ASD sample was built using purposive sampling. These children were recruited from ASD-specific and special needs schools in the Western Cape, and through the UCT Autism Research Group's database of families willing to participate in ASD research. Snowball sampling was also utilised, as parents in the study were encouraged to share the study information with any friends that they thought would also be willing to participate. The neurotypical children were recruited using purposive sampling as well, but through mainstream primary schools in the Western Cape. The study was also featured on the UCT Autism Research

Group blog site, and parents who heard about the study there were able to contact the researcher for more information.

Of the 53 children who were recruited to the non-verbal ASD group, two were excluded due to missing demographic data (i.e. unable to confirm date of birth and family household income). The remaining 51 children were included in the study as all had data for at least two of the three measures, and each research question only required one or two measures. Of the 55 children who were recruited to the verbal ASD group, three were excluded due to new medication use during the study, and one was excluded due to a new diagnosis of conduct disorder. As with the non-verbal ASD group, the remaining 51 children were included in the study as they had sufficient data for at least one of the research questions.

All 51 children in the non-verbal ASD group had complete demographic and ADOS2 datasets. Three of the families refused the parent interview but did consent for DNA collection so they were not excluded. Of the 51 participants, 34 provided DNA samples and all samples were successfully genotyped for OPRM1. This resulted in a sample size of 48 children for Questions 1 and Question 2 (i.e. ADOS2 and parent interview data), and Question 3 had a sample size of 34 for hypotheses 3.1 and 3.2 (i.e. ADOS2 and OPRM1 data) and 31 for hypothesis 3.3 (i.e. OPRM1 and parent interview data).

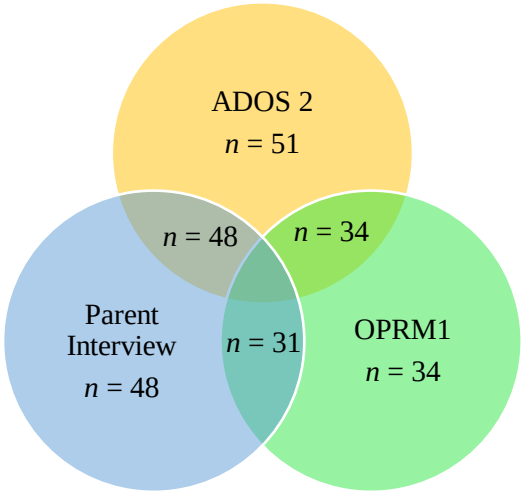

Figure 1. Non-verbal ASD group data collected per measure, and sample available for each research question

The 51 children in the verbal ASD group had complete demographic dataset and had all completed ADOS2 assessment. The parents of three children refused the parent interview, but the children had data for other critical measures, so they were not excluded. Of the 51 children, 42 provided DNA samples; all 42 samples were successfully genotyped for OPRM1. This resulted in a sample size of 48 children for Questions 1 and Question 2 (i.e. ADOS2 and parent interview data), and Question 3 had a sample size of 42 for hypotheses 3.1 and 3.2 (i.e. ADOS2 and OPRM1 data) and 39 for hypothesis 3.3 (i.e. OPRM1 and parent interview data).

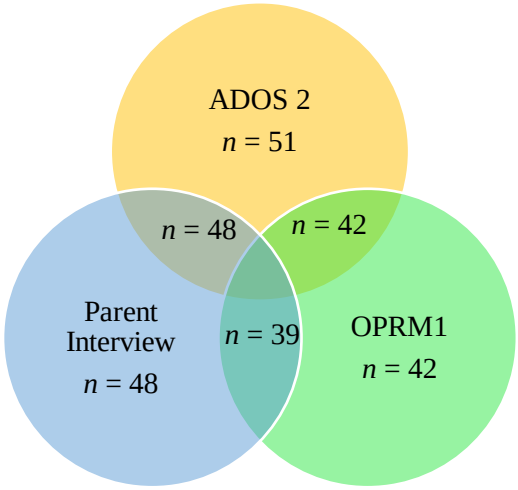

Figure 2. Verbal ASD group data collected per measure, and sample available for each research question.

Neurotypical participants. Neurotypical children were included in the protocol to assess for differences in levels of social motivation between neurotypical and ASD children (Question 1 of this study). There were 68 male children recruited for the neurotypical group. For inclusion in the study, demographic data needed to be complete and parents needed to complete the parent interview. Ten children were excluded from the study (see Figure 3), including two who were familiar with the measures due to recent assessment by an Education Psychologist, and two children with possible medical conditions who were in the process of being diagnosed (1 participant was undergoing speech therapy and one child was being treated for poor muscle tone). This resulted in a sample size of 58 children, none of whom were missing any data. As each ASD group consisted of only 51 children, I opted to have a neurotypical group of 51 children as well. I included all neurotypical children from low to middle income schools (21 participants), as the majority of the ASD children were not from high-income families, and then randomly filled the remaining slots with children from the higher income schools (30 of the remaining 37 children); this resulted in a final neurotypical group of 51 children.

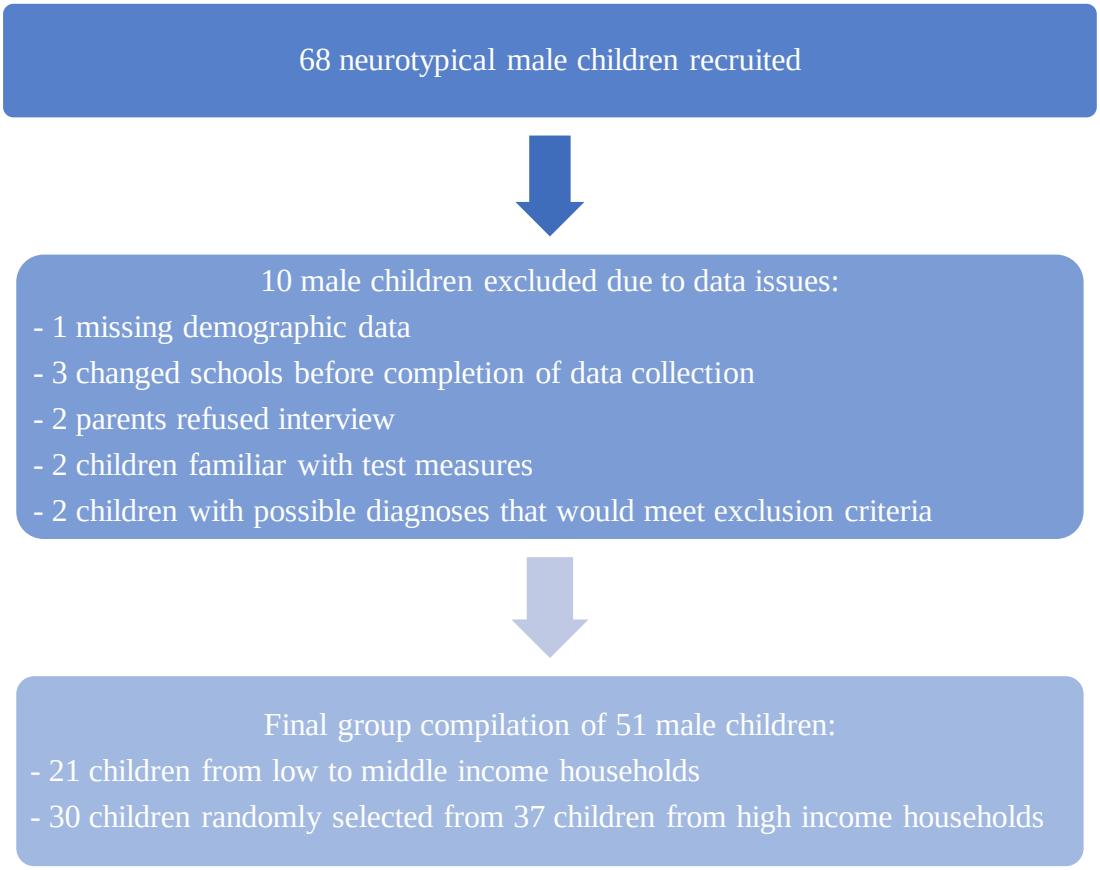

Figure 3. Selection of 51 male neurotypical children for inclusion in this protocol.

Sample size. The sample size for this study was selected based on similar studies which explored genotype-phenotype relationships in ASD. There is a sparsity of research available for genotype-phenotype relationships for OPRM1 in ASD samples, with research on naltrexone use in ASD using samples of ten or fewer participants (Bouvard et al., 1995; Herman et al., 1987), and these have not been replicated or further explored in more recent years. Studies similar to those for the second study of this protocol (see Chapter 4: The Theory of Mind Study – Method – Participants, pg.147) were therefore reviewed; genotype-phenotype studies reviewed had 71 children (Tordjman et al., 2001) to 73 children (Brune et al., 2006). The current protocol

therefore aimed to recruit a minimum of 80 child-parent pairs; a total of 102 ASD child-parent pairs were recruited, and DNA was obtained from 76 children within this sample.

The inclusion of non-verbal children in the protocol was considered essential, as research frequently only represents higher functioning children with ASD, and generally does not include non-verbal children (Tager-Flusberg & Kasari, 2013). The inclusion of children with ASD who had a range of capabilities, including those on the more severe end of the symptom spectrum, was therefore necessary to ensure that my research outcomes could be validly generalised to the ASD population, and not just to a higher functioning subsample. To obtain the largest possible sample, data collection took place over three and a half years, until the lack of response to recruitment requests through schools and the UCT Autism Research Group's database indicated that all families who would be willing to participate in the study in the region had been approached, and finding further participants would be unlikely.

Inclusion and Exclusion Criteria. Only male children were recruited as there is some debate regarding whether females on the spectrum present with a different phenotype to males (Rivet & Matson, 2011). Further, research within ASD has found that female children with ASD appear to have a social advantage relative to male children with ASD (Van Wijngaarden-Cremers et al., 2014). ASD is also universally four times more prevalent in males than females (Kogan et al., 2009). As I wanted to ensure clear phenotyping, I did not want sex to be a confounding variable, and it was not possible to ensure equal recruitment of male and female participants to try control for this statistically. As an exploratory study, it was arguably justifiable to conduct this initial investigation in a cleaner phenotype (i.e. males only) in the hope that later confirmatory studies could expand to include females.

This protocol focused on social impairment in childhood and early adolescence. Recruitment was therefore restricted to children who were in pre-primary or primary school. For neurotypical children, this limited the age range to 4-13 years old, and the children in the ASD groups were between 4 and 16 years of age, although those over 13 years of age had mental ages younger than their chronological ages. All children had to be fluent in English or, in the case of non-verbal children, had to come from English-speaking homes or schools. This was due to the ADOS2 only being standardised in English and not in other South African languages. Parents had to be fluent in English or Afrikaans to complete the interviews.

Children were excluded if they had a history of head injury. Children with ASD were excluded if they had any other neurological or psychological diagnoses, although ADHD was allowed due to the high comorbidity in children with ASD. All measures were administered in such a way that the effects of inattention or hyperactivity were minimized. Neurotypical children could not have any reported medical, neurological, or psychiatric diagnoses. Children with ASD were excluded if they used any medication that could influence opioid or serotonin processing, due to the biological nature of this study; neurotypical children did not use medication as all children with any medical conditions were excluded.

	ASD participants	**Neurotypical participants**
Inclusion criteria	- Male - 4-16 years old - Fluent in English (or from English school or home if non-verbal)	- Male - 4-16 years old - Fluent in English

Exclusion criteria	- Head injury or neurological disorder - Medical disorders - Psychiatric disorders (excl. ADHD) - Medications which influence mu-opioid or serotonin processes (e.g. SSRIs and anti-anxiety medications)	- Head injury or neurological disorder - Medical disorders - Psychiatric disorders - Medications which influence mu-opioid or serotonin processes (e.g. SSRIs and anti-anxiety medications)

Figure 4. Inclusion and exclusion criteria per group for this protocol.

Limitations on Sample Size. This protocol faced several challenges regarding recruitment of children with ASD, which limited the sample size obtained. ASD is a complex neurodevelopmental disorder with several common comorbidities, including depression or anxiety disorders; and seizures or epilepsy (American Psychiatric Association, 2013; Bolton et al., 2011; Peacock et al., 2012; Simonoff et al., 2008). As these comorbidities might have confounded the relationships explored in this protocol, children with comorbidities were excluded, significantly reducing my recruitment pool.

The sample was further limited by the decision to restrict recruitment to only male children. Prevalence rates estimate that 1 out of every 4 children with ASD is female, so excluding all female children meant a possible 20% reduction in the recruitment pool. However, cleaner phenotyping added robustness to analyses, a justified approach for exploratory studies.

As a South African study, I also limited my recruitment pool by having to restrict all recruitment to English speaking families, as the ADOS2 was not standardized in any other common languages spoken in South Africa at the time. The ADOS2 is the gold-standard for research and diagnostics in ASD, and often a necessary measure when seeking to publish ASD research, so it's inclusion in the protocol was considered essential for assessing participants' ASD phenotypes.

Although the collection of DNA did not limit recruitment, it did place restrictions on the sample sizes for aspects of the study using genetic data as DNA collection and processing in ASD faces further limitations. ASD often includes sensory integration difficulties, and this can result in some children being unable to tolerate swabbing. These children's lowered verbal activity had an associated low salivation rate which undermines the volume of DNA collected by swabbing. Further, poor oral hygiene results in high bacteria counts that prevent successful genotyping (Hansen et al., 2007). Non-verbal children who tolerated the swab were, in some cases, later excluded from DNA analyses due to poor DNA yield.

Finally, data collection was restricted to the Cape Town area, and after multiple rounds of recruitment I had exhausted the willing recruitment pool. Further rounds of data collection were unlikely to identify more participants and time and budgetary constraints prevented recruitment outside of Cape Town.

Procedure

Children and their families were recruited through their schools with letters, or via email or telephone if they were recruited through the UCT Autism Research database. All respondents were screened with a demographic and medical history form. Children who were included were then seen for assessment and their primary caregiver was interviewed telephonically or in person.

Recruitment. Children with ASD were recruited via special needs or ASD-specific schools, or through the UCT Autism Research database which had a list of participants from previous studies who were interested in participating in further research. Parents were sent information sheets (Appendix B), a screening questionnaire that collected demographic data and medical information (Appendix C), and a consent form that required consent for the psychological data collection and optional additional consent to participation in the DNA aspect of the study (Appendix D). These forms were sent via schools in sealed envelopes or via email for participants from the database. Parents then returned these forms via the school psychologist or via email as they preferred. Recruitment for participants with ASD was conducted from January 2015 through to September 2017, at which point the low response rate indicated that most possible participants at these schools and on the database had been approached and further recruitment was terminated.

Neurotypical children were all recruited through mainstream schools. They were sent information packs that included an information sheet (Appendix E), the same demographic questionnaire and medical history form as the ASD participants (Appendix C), and a consent form for psychological data collection but not DNA collection (Appendix F). These forms were also returned via email or the school psychologist. Recruitment of neurotypical children began in February 2017 and was terminated in September 2017 as recruitment of ASD participants was terminated and more neurotypical children had enrolled for the protocol than verbal children with ASD (i.e. the group the neurotypical sample would be matched against).

Screening and ADOS2 Assessment. Once parents returned the above forms, screening was conducted based on the medical and demographic data received (see Inclusion and Exclusion Criteria, pg. 58). Children with ASD had further screening: ADOS2 assessment by

this doctoral candidate had to confirm ASD diagnosis, and children were then placed in the non-verbal ASD group or verbal ASD group based on their ability to consistently understand two-stage commands on the Developmental Neuropsychological Assessment, Second Edition (NEPSY-II) *Comprehension of Instructions Task*.

Parent Interview. Parent interviews for all three groups were conducted after screening and concurrently to the child sessions for the verbal ASD and neurotypical groups. These were conducted via telephone at a time selected by the parent, or in the case of home visits for children with ASD, these interviews were conducted at the participant's home on the same day as the ADOS2 assessment.

DNA Collection. DNA samples were collected from ASD participants in November and December 2017. Only children whose parents had given additional consent for DNA collection were approached, and DNA was only collected if the child gave verbal assent in the case of verbal children with ASD, or if the child willingly participated in the process in the case of non-verbal children with ASD. DNA was collected in the form of buccal cells obtained from cheek swabs. Children were allowed to practice the procedure using normal earbuds if they wished, and then the researcher conducted the proper swabs. Two swabs were collected for each child and were delivered to the University of Cape Town Human Genetics Laboratory on the same day as collected. DNA was extracted and preserved, and then processed when all samples were collected. DNA was destroyed after processing.

Measures

This study had three phases of data collection: first children were recruited and screened, then their parents were interviewed, and finally DNA was collected from a subsample of participants. This protocol divided participants into three groups: a non-verbal ASD sample, a

verbal ASD sample, and a neurotypical sample. All three groups completed the screening and parent interview, but only the two ASD groups completed ADOS2 assessment.

Screening. Demographic and medical data was collected for each participant during recruitment for screening purposes (Appendix C). Demographic data included age, sex, home language and socioeconomic status. Additional questions were asked to ascertain whether the child had a history of medical or psychiatric difficulties or had experienced any head traumas. This survey also established if participants were using any medication that could affect their mu-opioid or serotonin functioning. The demographic data was used for aggregate matching of participants across groups and the medical data was used to screen participants according to my inclusion and exclusion criteria.

The demographic forms also asked the ASD participants to list their ethnicity if they felt comfortable doing so. South Africa is a diverse country, but with a history of inequality based on racial injustice. Asking participants their ethnicity is therefore a sensitive topic and only considered appropriate for certain procedures; in this protocol, ethnicity was only considered relevant for the DNA aspects of the protocol. Ethnicity was therefore not considered during recruitment or matching of participants but was reported alongside the allelic frequency of OPRM1 due to the possibility of different ethnic groups having different allelic frequencies for this gene.

Language and Group Allocation. Children who were included in the protocol based on the initial screening from the demographic survey then completed the *Comprehension of Instruction* subtest from the NEPSY-II to screen for difficulties in language comprehension (Brooks et al., 2009). All neurotypical children were able to consistently follow two-stage commands; if any had not been able to, they would have been excluded. Participants with ASD

were divided into ASD sub-groups based on their language ability: children who were non-verbal or had limited language and could not consistently follow two-stage commands were placed in the non-verbal ASD group; participants with ASD who were verbally fluent and were able to consistently follow two-stage commands were placed in the verbal ASD group.

The *Comprehension of Instruction* task was developed for children aged 3-12 years, and has since been adapted for children aged 3-16 years (Brooks et al., 2009). This subtest assesses auditory comprehension of verbal commands and ability to process and then execute these instructions. Children have to point to pictures according to instructions from the examiner. These instructions begin as simple single stage commands, and then increase in complexity. For this protocol ability to follow instructions beyond two-stage commands was not assessed as this level of comprehension was deemed sufficient for the instructions of the ToM battery.

The NEPSY-II has established psychometric properties as a battery, and the *Comprehension of Instructions* subtest derives its validity from the battery as a whole (Brooks et al., 2009). The reliability for the *Comprehension of Instructions* subtest ranges ranging from .71 to .82 depending on the age of the sample. The NEPSY-II has been translated and validated into several languages, although I only administered it in English due to the ADOS2 only being appropriate for use in English-speaking local samples. The first and second editions of the NEPSY showed acceptable psychometric properties in samples from Iran and Zambia (Abedi et al., 2012). These studies with non-Western samples showed that cultural differences could undermine performance on the NEPSY and result in lowered scores compared to Western norms, although this did not significantly undermine the validity of the test. As I only used the *Comprehension of Instructions Task*, and only to assess pass/fail on two-stage commands rather

than overall performance for the task with a norm-based scoring, this bias was unlikely to undermine my protocol and the subtest was considered appropriate for screening purposes.

ADOS2. Children in the ASD groups had their ASD diagnosis confirmed and ASD-related deficits were assessed and characterized with the Autism Diagnostic Observation Schedule, Second Edition (ADOS2; Lord et al., 2012). The ADOS2 is a semi-structured, standardised observation tool for ASD diagnostics and for research purposes that has been adapted for all levels of language ability and ASD severities. The ADOS2 has improved psychometric properties compared to the first edition ADOS and is the gold-standard for use in diagnostics and research (Lord et al., 2012; Lord et al., 1989). The ADOS2 has sensitivity ratings in the upper 90% range and specificity in the upper 80% to lower 90% range. Internal consistency ranges from .47-.96, but all lower scores were for non-social domains. Test-retest reliability is high for social domains, and acceptable for non-social domains.

The ADOS2 has specific modules that are administered to the child based on their age and language ability. There are five modules, with the *Toddler Module* being appropriate for children from 12months of age, *Module 1* for non-verbal children over 30 months of age, *Module 2* for children who have developed phrase speech, *Module 3* for verbally fluent children and adolescents, and *Module 4* for adults. This protocol administered modules 1,2 or 3 based on the child's abilities, and these assessments took between 40 and 60 minutes.

The ADOS2 assesses core symptom domains and key ASD characteristics and returns three main scores: the Comparison score indicates overall level of ASD severity; Social Affect (SA) score indicates level of deficit related to social communication and interaction; and Restricted Repetitive Behaviours (RRB) score indicates level of deficit for the restricted and repetitive behaviours and interests symptom domain. The Comparison score takes age into

account and was comparable across all ADOS2 modules, while the Social Affect and RRB scores are comparable across modules due to having the same scoring range although they did not explicitly take age into account.

The ADOS2 was developed in English and has been translated and validated into several languages internationally, including French, German, and Japanese (Western Psychological Services, 2019). I administered the ADOS2 in English as there were no validated translations for other South African languages at the time of data collection. All ADOS2 assessments were conducted by the PhD candidate, who had completed her ADOS2 training in English and had achieved research reliability (Appendix G).

The ADOS2 is considered the gold-standard for ASD diagnostics, and for research purposes. As such, many journals require the ADOS2 be included in research protocols when seeking publication. As a measure of choice in diagnostics and in research, it is an ideal measure for assessing core and essential aspects of ASD. Other measures exist for identifying and quantifying ASD-related symptoms and behaviours and are often used in research, including the Social Communication Questionnaire (SCQ; formerly the Autism Screening Questionnaire) (Rutter, Bailey, et al., 2003) and Social Responsiveness Scale (SRS) (Constantino & Grber, 2005).

These measures, however, rely on interviews with caregivers and teachers, rather than direct observation of the child. Further, recent research found that both these measures did not perform reliably with families of low SES, low maternal education, or when the child showed lowered developmental functioning and greater behavioural difficulties (Moody et al., 2017). Clinically, it is recommended that these measures be paired with the ADOS (or ADOS2 more recently) for diagnostic purposes (Corsello et al., 2007). As the ADOS2 is a direct observational

measure, and was administered only after receiving clinical and research training, I deemed this measure to be better suited to my phenotyping needs. For phenotyping, I wanted to identify ASD-related behaviours that were considered core characteristics of the ASD phenotype and that align with the overall diagnosis, and the ADOS2 identifies these across both symptom domains.

Parent Interview. The parents of all participants were interviewed telephonically or face-to-face, as they preferred. In this interview parents completed the Attachment Style Classification Questionnaire (ASCQ; Appendix H; Finzi-Dottan et al., 2012). Although the ASCQ is designed to assess attachment styles in children, I used this measure to assess behaviours indicative of social motivation. When this study was designed, there were no standard measures available to assess social motivation in children with ASD, so I relied on indicators of social interest, which are similar to those assessed in measures of attachment. Since then, and to my knowledge, two measures have been developed. The first is a 28 item questionnaire, the Social Motivation Questionnaire, developed as part of a doctoral dissertation from the Palo Alto University (Schapp, 2016). More recently, the Social Motivation Interview was developed (Elias & White, 2019). These measures indicate progress in the field but require further validation. However, had they been available at the start of the current protocol, it is likely that they would also have been adapted to parent interviews as neither are appropriate for use with non-verbal children.

I therefore had to look to other measures that assessed behaviours *indicative* of social motivation, and I found that questions usually indicating secure attachment also reflected social interest. The ASCQ is a 15 item questionnaire that rates a child's level of secure attachment, anxious-avoidant attachment, and anxious-ambivalent attachment, rather than categorising a child into one predominant attachment style. The ASCQ was developed as a self-report measure

for children between 7 and 14 years. In the current protocol the measure was administered to parents in an interview format instead of as a self-report measure for the children. This change was made so that data could be collected for both the ASD groups and the neurotypical group in a consistent manner. The non-verbal children with ASD were unable to complete self-report measures, and because many of the verbal children with ASD lacked insight into social interactions and social norms it would be inappropriate to rely on their self-report on social behaviours. This change also allowed the same measure to be administered to the full group of participants as the initial minimum age for the ASCQ was based on when a child would be capable of reliable self-report, which was no longer an issue. Some of the children with ASD were older than 14, however they were still in a primary school environment and were developmentally delayed, so this measure was considered appropriate for these children.

Ideally direct observation would be used to assess social motivation, but I preferred to have the parents provide this information as they had a long-term overview of behaviours whereas the time and budget constraints of this protocol would have meant that any observation of social motivation would be limited to single observation. I did not think that a single observation would be representative of a child's overall level of social interest, especially in the case of children with ASD who are often anxious when their routines are disrupted or if they are placed in novel situations.

The ASCQ assessed secure attachment based on a child's behaviour with their family and peers. Some questions asked if the child seeks out social contact (Item 6: "My son would like to be really close to some children and always be with them") as well how the child responds when others initiate contact (Item 15: "Usually when anyone tries to get too close to my child, it does not bother him"). While parent-report is open to some bias, my team required parents to support

their answers with examples and often with descriptions of typical behaviours in social situations. For example, when asked Item 5 “Sometimes my son is afraid that other kids won’t want to be with him” we asked for descriptions of how engaging and secure (or anxious) the child was when entering a social group. Ultimately these questions allowed us to assess how interested the child was initiating social interaction, how comfortable they were with this interaction, and whether they engaged socially when given the opportunity. Where students were not sure the answers were accurate, these could be followed up with a further discussion with the parent.

The ASCQ was initially designed in Hebrew, and an English version has since been translated and adapted. The ASCQ has test retest reliability ratings between .87-.95, internal consistency ratings between .69-.81, and validity has been consistently established in clinical and non-clinical samples (Al-Yagon & Mikulincer, 2004; Finzi-Dottan et al., 2012).

DNA Collection and Genotyping. All ASD participants in this protocol were approached to provide DNA but this was an optional aspect of the study. Of the 102 children with ASD who participated, 76 children provided assent and had parental consent to provide DNA samples. These samples were collected at the end of the child sessions, when the children were more comfortable with the researcher. All DNA was processed by the UCT Human Genetics Department.

Neurotypical children were not approached for DNA samples for two reasons. First, this protocol had limited funding and I was not able to cover the costs of DNA processing for the full study sample. As the primary interest was in the ASD phenotype, I chose to focus on collecting samples from the ASD participants for comparison to their phenotypes. The neurotypical sample was not phenotyped – they did not complete ADOS2 assessment (i.e. the assessment used to

quantify ASD-related deficits for the ASD phenotype), as they were unlikely to have sufficient variability in their scoring on this measure, and were likely instead to show a floor effect. Second, I was interested in social motivation levels and expected that while low levels were likely prominent in the ASD samples, they were unlikely to be common in the neurotypical sample. The possible absence of scoring variability in the variables of interest in the neurotypical sample would therefore undermine any possible genotype-phenotype analyses. I chose instead to use the funding only for the ASD samples to ensure a larger number of DNA samples for inclusion in the ASD analyses, and to have a comparable sample size to other genotype-phenotype studies with ASD samples

DNA Extraction. Buccal cells were collected with sterile check swabs. DNA was extracted from samples by a modified salting-out method (Freeman et al., 2003; Meulenbelt, Droog, Trommelen, Boomsma, & Slagboom, 1995), quantified with a NanoDrop 1000 (Thermo Fisher Scientific, Johannesburg, South Africa) and normalised for genotyping.

OPRM1 Genotyping. All reagents and kits were used according to the manufacturer's instructions. OPRM1 genotyping was performed with a customised TaqMan™ assay (assay ID c_8950074_1) and TaqMan™ Genotyping Master mix (Thermo Fisher Scientific, Johannesburg, South Africa) on a Bio-Rad CFX96 real-time PCR system (Bio-Rad Laboratories, Johannesburg, South Africa). Selected genotypes were confirmed by direct sequencing.

Direct (Sanger) Sequencing. Selected OPRM1 samples were confirmed by direct sequencing. All reagents and kits were used according to the manufacturer's instructions. OPRM1 was amplified with primer sequences from (L. K. Chen et al., 2013) using GoTaq G2 Taq polymerase (Promega, Madison, WI, USA); aliquots were used to confirm PCR quality by agarose gel electrophoresis. Amplicons were digested with 1U each of *Exo*I and FastAP (Thermo

Fisher Scientific, Johannesburg, South Africa). Cycle sequencing was performed with the BigyDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Johannesburg, South Africa). Sequencing was performed on an Applied Biosystems 3130*xl* Genetic Analyzer (Thermo Fisher Scientific, Johannesburg, South Africa), using standard conditions. Sequencing traces were analysed with Sequencing Analysis v5.4 software (Thermo Fisher Scientific, Johannesburg, South Africa).

Ethical Considerations. This protocol was conducted in line with the ethical guidelines for human subjects as per the Health Professional Council of South Africa (HPCSA), the University of Cape Town (UCT) Codes for ethical research, and the International Declaration of Helsinki (World Medical Association, 2013). Ethical approval was obtained from the UCT Psychology Department Ethics Board (ref: PSY2014-024; Appendix I), and from the UCT Faculty of Science Research Ethics Committee (ref: HREC 076-2014; Appendix J). Further ethical approval for DNA collection and analyses was obtained from the UCT Faculty of Health Sciences Human Research Ethics Committee (ref: HREC 346/2017; Appendix K).

Permission to approach schools for participation in the study was obtained from the Western Cape Government Education Department (ref: 20150422-46598; Appendix L). Informed consent was obtained in writing from parents for participation in the study, with separate consent for the collection of psychological data and DNA data for ASD participants (Appendix D) and only for psychological data for the neurotypical participants (Appendix F). Verbal participants provided oral assent for participation in the study at the start of each session. Assent was considered obtained for non-verbal ASD participants if they willingly engaged in the protocol as the behavioural rigidity inherent in ASD makes it unlikely that these children would unwillingly complete any tasks or social interactions (American Psychiatric Association, 2013).

All data collected in this study was only used for research purposes and access was restricted to members of the UCT Autism Research Group, and confidentiality was maintained at all times. All physical psychological data was stored in sealed boxes in a locked storage area in an access-controlled room in the UCT Psychology Department. Electronic data was stored in a password protected file and all identifying information was removed when participants were allocated a participant number, with only the doctoral candidate having access to the master file linking identifying data with the allocated participant number. All DNA was processed by the UCT Human Genetics Department and was immediately destroyed after processing; this department only received buccal samples marked with participant numbers, ensuring complete confidentiality.

This protocol did not harm the participants in any way. Children from the ASD groups were seen once to complete the *Comprehension of Instructions* task and ADOS2 assessment. The ADOS2 is designed for use with children with ASD and is therefore appropriate. The children were given breaks where necessary to prevent fatigue, and children were able to end the session and/or withdraw from the study at any time. Neurotypical children did not need to complete any child measures for this study. Parents were given contact details for the doctoral student, as well as for the relevant ethics boards and for the UCT Psychology Department so they could withdraw from the study at any time, or lodge a complaint if necessary. Schools were also able to contact the researcher at any time if they wished to withdraw from the study or limit access to their students.

ASD participants whose parents gave consent for DNA collection also had to give assent to this DNA collection in the same way as with the psychological data collection. If a child was

not completely comfortable, they were able to "practise" the collection procedure with a cotton earbud and could withdraw at any point. The actual collection was painless and rapid.

On completion of data collection each ASD child received an individualised research report that parents could share with their child's school or clinician, and I was available to answer any queries. This report provided information from the ADOS2 assessment. Neurotypical participants received a research report as part of their participation in the Theory of Mind Study (Chapter 4: The Theory of Mind Study - Methods, p. 73).

Analyses

All statistical analyses were run using IBM SPSS Statistics, Version 25 (IBM, 2017). Participant characteristics were first compared across groups to ensure they were appropriately matched on relevant demographic variables. The questions for the Social Motivation Study were then addressed. For all the following analyses, assumptions were tested and the analyses were only conducted if they were upheld; or, if assumptions were violated, tests were only continued if the analyses would be robust against this violation, such as when homogeneity of variance was violated for ANOVA with equal group sizes (Field, 2013).

This study was an exploratory analysis of the relationships between ASD-related deficits, social motivation, and OPRM1 alleles, and was the first of its kind. As an exploratory study, there were a high number of analyses, which could increase the probability of a Type 1 error. However, given the novelty of the research, especially in terms of combining psychological and genetic fields, I wanted to find possible relationships that could then be further investigated in later confirmatory studies. To ensure any possible relationships could emerge, alpha was kept at 0.05 in all analyses. Although my approach may increase the risk of spurious findings, effect sizes were included in all analyses to provide an additional estimation of the associations present.

Participant Characteristics. This protocol had three participants groups: a non-verbal ASD group, a verbal ASD group, and a neurotypical group. All participants were male and first language English speakers (or in the case of non-verbal participants they came from English speaking homes and schools). ANOVA was used to assess whether all three groups were aggregate matched on SES and age. It was anticipated that the non-verbal ASD group could be younger than the other two groups, so post-hoc tests were used to assess whether the verbal ASD and neurotypical groups were matched on age. Ethnicity was recorded for use in reporting DNA results for the ASD subsamples that provided DNA samples, but was not used in recruiting or reported for the neurotypical group, so no analyses were conducted on this data.

Social Motivation Study Questions. The Social Motivation Study had three main questions, each with their own analyses. For this study, secure attachment scores were continuous and were a proxy for level of social motivation such that higher scores indicated higher levels of social motivation. ADOS2 scores indicated ASD-related deficits and were also continuous, but here higher scores indicated greater deficits. OPRM1 genotypes and alleles were reported for a subsample of the ASD participants, and the presence of one or more *G* alleles indicated higher sensitivity to, and binding of, mu-opioids, likely generating an analgesic state that would undermine the SEPARATION-DISTRESS system.

For the first question on whether children with ASD had lower social motivation than neurotypical children, hierarchical regression was conducted with age, SES, and group (in two dummy variables) as predictors of secure attachment scores (i.e. the proxy for level of social motivation).

The second question related to whether social motivation related to ASD-deficits. From the above analyses, the dummy variable coding for group would also reveal whether there were

group differences in social motivation between the non-verbal ASD group and the verbal ASD group. As there was, I also assessed for ADOS2 score differences between the groups using ANCOVA for three analyses, where age was the covariate; these analyses compared ADOS2 Comparison scores, ADOS2 Social Affect scores, and ADOS2 RRB scores across the two ASD groups. As group differences were found for both social motivation and ADOS2 scores across the ASD groups, further analyses for this question were conducted separately for the two ASD groups. Multiple regression analyses (MRAs) were conducted for each group with age, SES, and secure attachment scores as predictors for each of the ADOS2 outcome scores (i.e. Comparison score, Social Affect score, and RRB score).

The third question of this study assessed the role of OPRM1 in ASD-related deficits and in social motivation levels. The OPRM1 genotype and allelic frequencies were reported for each ASD group for comparison against reported rates for neurotypical samples; these were also reported for each ethnic group in these samples. I planned to use MRA to assess for relationships between OPRM1 and ASD-related deficits, where age, SES, and OPRM1 genotypes were assessed as predictors of ADOS2 scores, but these analyses could not be conducted due to the unanticipated low incidence of the *A* allele. Similarly, MRA was going to be conducted for each ASD group with age, SES, and OPRM1 genotypes as predictors of secure attachment scores, but was abandoned due to the same limitation.

Results

Participant Characteristics

This research protocol included 153 male children, divided equally into three groups of 51 children (i.e. non-verbal ASD, verbal ASD, neurotypical). To limit potential bias, the groups were assessed for differences in age and socio-economic status (SES; total annual household

income) with a one-way MANOVA. As all children were male, sex would not be a confounding factor. For age there was a significant difference across groups, $F(2,150) = 14.65$, $p < 001$, with a large effect, $\omega^2 = .151$(Kirk, 1996). Post-hoc comparisons using Games-Howell showed that while the non-verbal ASD group was significantly younger than the verbal ASD group, $p < .001$, and the neurotypical group, $p < .001$, the verbal ASD group and the neurotypical group did not differ significantly on age, $p = .599$. It was anticipated that the non-verbal ASD group might be slightly younger than the verbal ASD group and the neurotypical group, as children with ASD often have delayed speech development (Mody et al., 2013), so where the non-verbal ASD group was included in further analyses, age was considered as a covariate. For SES, the 3 groups did not differ, $F(2,150) = 2.01$, $p = .138$, $\omega^2 = .013$. The groups were therefore equal in size and matched on sex and SES. The verbal ASD group and neurotypical group were also matched on age.

Table 1.

Demographic Characteristics Across Groups

Characteristics	Group			*F*	*p*	Omega Squared
	Non-Verbal ASD (*n*=51)	Verbal ASD (*n*=51)	Neurotypical (*n*=51)			
Age (years):						
$\bar{X}$ *(SD)*	7.57 (3.47)	10.29 (2.64)	9.86 (1.82)	14.65	.001	.151
Range	3.33-15.83	6.08-16.92	6.58-13.50			
SES:						
$\bar{X}$ *(SD)*	30156.17 (13523.20)	25336.50 (14889.48)	31126.55 (18108.39)	2.01	.138	.013
Range	1499.50 – 45875.50	4649.50 - 45875.50	4649.50 - 45875.50			

Notes. ASD = Autism Spectrum Disorder. SES = Socio-economic status (total annual household income in Rands per year). $\bar{X}$ = Mean. *SD* = Standard deviation.

My demographic questionnaire also recorded ethnic data for the sample. This data was not used in participant recruitment or matching but was recorded for the ASD samples for inclusion in the DNA analyses. My non-verbal and verbal ASD samples showed similar ethnic distributions, as Fisher's Exact test was non-significant, $p = .703$.

Table 2.
Ethnic Group Distributions for ASD Groups

Ethnicity	Group		
	All ASD (*n*=102)	Non-Verbal ASD (*n*=51)	Verbal ASD (*n*=51)
Caucasian	34 (33.33%)	16 (31.37%)	18 (35.29%)
Mixed race	52 (50.98%)	26 (50.98%)	26 (50.98%)
African	10 (9.80%)	6 (11.76%)	4 (7.84%)
Asian	5 (4.90%)	2 (3.92%)	3 (5.88%)
Other	1 (0.98%)	1 (1.96%)	0 (0.00%)

Notes. ASD = Autism Spectrum Disorder.

Question 1: Do Children with ASD Exhibit Lower Levels of Social Motivation than Neurotypical Children?

The Social Motivation Study started by exploring the integrity of Panksepp's (1979) theory by assessing whether children with ASD have lower levels of social motivation than

neurotypical children. Hierarchical regression was conducted to assess the relationship between group and social motivation. As language in ASD was of interest, the grouping variable had three levels: neurotypical, non-verbal ASD, and verbal ASD.

Hypothesis 1.1 Children with ASD Will Have Lower Social Motivation Scores than Neurotypical Children. This study investigated the role of secure attachment as an indication of level of social motivation, starting by comparing secure attachment levels in ASD to a neurotypical sample. Descriptive statistics indicated that the group means for social motivation as indexed by secure attachment scores showed the predicted pattern with the neurotypical sample having higher levels of secure attachment, $M = 8.25$, $SD = 1.59$, than the ASD samples. The verbal ASD group, $M = 6.56$, $SD = 2.39$, followed, with the non-verbal ASD group showing the lowest levels of secure attachment, $M = 4.77$, $SD = 2.68$.

Table 3.

Secure Attachment Scores Across Groups

Group	Secure Attachment Score		
	Mean	SD	Range[a]
Non-Verbal ASD (n = 48)	4.77	2.68	0-10
Verbal ASD (n = 48)	6.56	2.39	0-10
Neurotypical (n = 51)	8.35	1.59	4-10

Notes. ASD = Autism Spectrum Disorder. *SD*=Standard deviation.

[a] Possible score range of 0-10

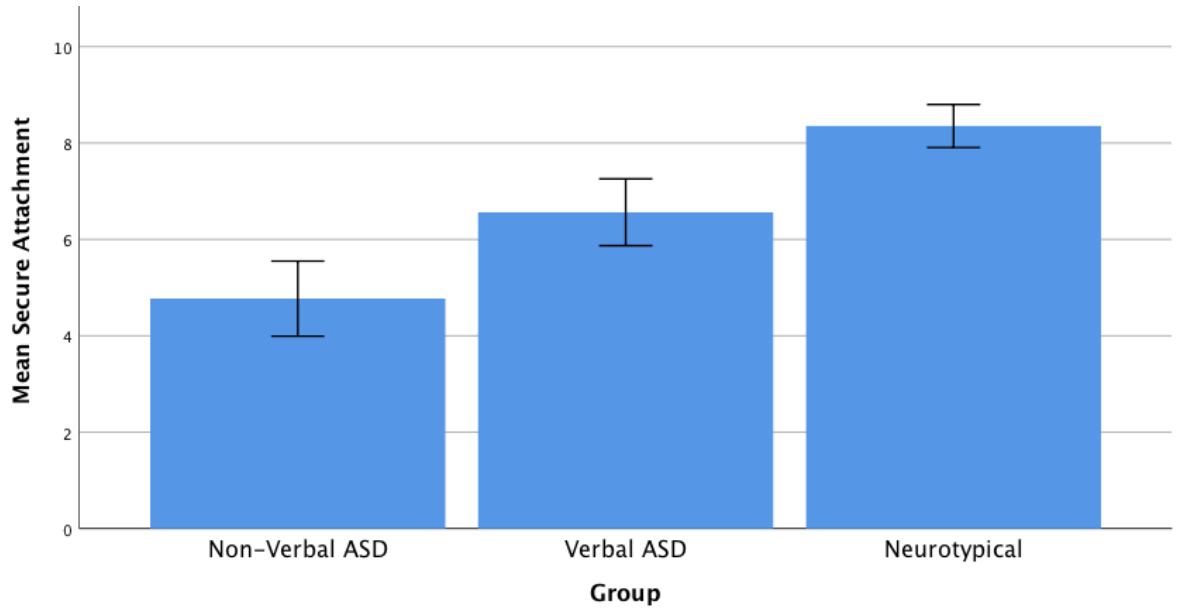

Figure 5. Mean secure attachment scores across groups with 95% confidence intervals.

To assess whether the grouping variable had a significant association with levels of secure attachment, a hierarchical regression analysis was conducted. Due to the grouping variable having three levels, two dummy variables were used. Group was placed subsequent to age and SES in the regression model to ensure that any association seen was over and above the influence of the demographic variables.

The variables were assessed for correlations. The outcome variable, secure attachment score, was significantly correlated with the two group dummy variables as predicted. No issues with multicollinearity were noted (Table 4). All tolerance scores and variance inflation factor (VIF) scores were acceptable, confirming no multicollinearity (Field, 2013).

Table 4.

Zero-Order Correlation Matrix for All Participants: Secure Attachment Score

	1	2	3	4	5
1. Age (Months)	-				
2. SES	-.20**	-			
3. Group: Neurotypical v ASD	-.17*	-.08	-		
4. Group: Neurotypical &Verbal v Non-Verbal ASD	-.43**	.07	.51**	-	
5. Secure Attachment Score	.05	.05	-.48**	-.48**	-

Notes. SES = Socio-Economic Status (total annual household income). ASD = Autism Spectrum Disorder.

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The overall model was significant, $F(4,142) = 17.28$, $p < .001$, and the adjusted R_2 value showed that the model explained 30.8% of the variance in secure attachment, and the R^2 change value of .32 showed that this was accounted for by the group variable (i.e. non-verbal ASD, verbal ASD, or neurotypical; Table 5). This model had a large effect size, $f_2 = .49$ (Selya et al., 2012), and an observed power of .95. Table 6 showed that age was a significant predictor of secure attachment. It also showed that both dummy variables for group were significant predictors for secure attachment as well.

The first dummy variable for group, which indicated ASD diagnosis by giving neurotypical participants a score of zero and all ASD participants a score of one, had a significant negative beta value, indicating that the difference between neurotypical versus ASD participants was such that ASD participants had lower secure attachment scores. The second

dummy variable, which considered language deficit by allocating a zero to neurotypical and verbal ASD participants and a one to non-verbal ASD participants, also showed a significant negative beta value, indicating that having a language deficit was associated with lowered secure attachment scores compared to both the verbal ASD group and the neurotypical group. This supported the hypothesis that children with ASD have lower secure attachment scores, indicating lower levels of social motivation, than neurotypical children.

Table 5.

Predictors of Secure Attachment Scores: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	*R Square Change*	*F Change*	Change Statistics *df 1*	*df 2*	*Sig. F Change*
1	.08	.01	-.01	2.69	.01	0.42	2	144	.66
2	.58	.33	.31	2.23	.32	33.94	2	142	<.001

Notes.
Model 1: Constant, Total Annual Family Income (SES), Age
Model 2: Constant, Total Annual Family Income (SES), Age, Group (Dummy variables used)

Table 6.

Coefficients for Model 2: Secure Attachment Scores

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*			Collinearity Statistics	
		B	Std. Error	*β*	*t*	*p*	Tolerance	VIF
2	(Constant)	9.77	0.91		10.81	<.001		
	Age (months)	-0.01	0.01	-0.17	-2.19	.030	.78	1.28
	SES	<0.01	<0.01	0.02	0.26	.796	.95	1.06
	Group (NT v ASD)	-1.71	0.45	-0.30	-3.77	<.001	.73	1.38
	Group (NT & Verbal v NonVerbal)	-2.26	0.50	-0.40	-4.53	<.001	.62	1.62

Notes. SES = Socio-Economic Status (total annual household income). NT = Neurotypical. ASD = Autism Spectrum Disorder.

Question 2: Is Reduced Social Motivation Related to ASD-Related Deficits, and Does Language Acquisition Play a Role in This Relationship?

The hypothesis for Question 1 was supported, indicating that the two ASD groups had lower social motivation scores than the neurotypical group. Question 2 focuses on social motivation in ASD; this focus is on both the role of language in social motivation, and possible links between social motivation levels and ASD deficits. As the focus of these analyses was on ASD-related deficits, the neurotypical sample was excluded.

Hypothesis 2.1 Non-verbal Children with ASD Will Have Higher or Lower Social Motivation Scores Than Verbal Children with ASD. Under hypothesis 1.1, the multiple regression model presented above compared secure attachment scores across the three groups

(i.e. neurotypical vs verbal ASD vs non-verbal ASD). In addition to showing that the neurotypical group had higher secure attachment (i.e. social motivation) than both ASD groups, the second dummy variable also showed a significant difference between the two verbal groups (i.e. neurotypical group and verbal ASD group) compared to the non-verbal ASD group. The non-verbal ASD group therefore had significantly lower secure attachment scores, $M = 4.77$, $SD = 2.68$, than the verbal ASD group, $M = 6.56$, $SD = 2.39$.

Hypothesis 2.2 Non-Verbal Children with ASD Will Have Higher or Lower ADOS2 Scores Than Verbal Children with ASD. ASD deficits quantified by ADOS2 scores were assessed across the ASD groups to ascertain what role, if any, verbal abilities played in ASD presentations. As all ASD participants completed ADOS2 assessments to confirm ASD diagnosis, all 102 ASD participants (i.e. 51 non-verbal ASD, 51 verbal ASD) were included in these analyses. ANCOVAs were utilised to assess for differences across all three of the ADOS2 outcome scores, namely: Comparison score, Social Affect score, and RRB score.

For ADOS2 Comparison scores, age was not significant as a covariate, $F(1,99) = 3.75$, $p = .056$. ASD group had a significant association with ADOS2 Comparison scores over and above the role of age, $F(1,99) = 4.42$, $p = .038$, $\omega^2 = .032$, although this was a small effect. The non-verbal ASD group therefore scored significantly higher for ADOS2 Comparison scores, $M = 6.18$, $SD = 1.45$, than the verbal ASD group, $M = 5.69$, $SD = 2.00$.

For ADOS2 Social Affect scores, age was not significant as a covariate, $F(1,99) = 2.54$, $p = .114$. ASD group had a significant association with ADOS2 Social Affect scores over and above the role of age, $F(1,99) = 68.27$, $p < .001$, with a large effect, $\omega^2 = .391$. The non-verbal ASD group therefore scored significantly higher for these deficits, $M = 13.49$, $SD = 3.95$, than the verbal ASD group, $M = 7.55$, $SD = 3.27$.

For ADOS2 RRB scores, age was not significant as a covariate, $F(1,99) = 2.69$, $p = .104$. ASD group had a significant association with ADOS2 RRB scores over and above the role of age, $F(1,99) = 11.34$, $p = .001$, with a moderate effect $\omega^2 = .093$. Once again, the non-verbal ASD group scored significantly higher for these deficits, $M = 3.02$, $SD = 2.12$, than the verbal ASD group, $M = 1.96$, $SD = 1.15$.

Table 7.

ANCOVA Controlling for Age: ADOS2 Scores Across ASD Groups

Characteristics	Group		*F*	*p*	Omega Squared
	Non-Verbal ASD (*n*=51)	Verbal ASD (*n*=51)			
Comparison score[a] $\bar{X}$ (SD)]	6.18 (1.45)	5.69 (2.00)	4.42	.038	.032
Social Affect score[b] $\bar{X}$ (SD)]	13.49 (3.95)	7.55 (3.27)	68.27	<.001	.391
RRB score[c] $\bar{X}$ (SD)]	3.02 (2.12)	1.96 (1.15)	11.34	.001	.093

Notes. ASD = Autism Spectrum Disorder. $\bar{X}$ = Mean. *SD* = Standard deviation. RRB = Restricted and Repetitive Behaviours and Interests Symptom Domain.

[a] Possible score range 1-10; [b] Possible score range 0-20; [c] Possible score range 0-8.

Summary of Hypotheses 2.1-2.2: Group Differences. The above analyses showed that the non-verbal ASD group had lower secure attachment scores, indicating lower social motivation, compared to the verbal ASD group. The non-verbal ASD group also showed greater severity of all areas of ASD-related deficits, namely, ADOS2 Comparison score, ADOS2 Social Affect scores, and ADOS2 RRB scores, than the verbal ASD group. All these differences were

significant, and although age was considered, it did not play a significant role when considering any group differences in social motivation or ADOS2 scores. As group differences were supported (hypotheses 2.1-2.2), relationships between social motivation levels and ASD deficits were assessed for each ASD group individually as these groups presented as qualitatively different.

Hypothesis 2.3 Low Social Motivation is Related to Higher ADOS2 Scores. To assess whether social motivation levles, as indicated by level of secure attachment, was related to ASD-related deficits, hierarchical regression analyses were conducted. I was also interested in whether language acquisition within ASD, as defined in my study as those with fluent speech (i.e. verbal) versus those with little-to-no expressive speech (i.e. non-verbal), played a role in these relationships as Hypotheses 2.1 and 2.2 showed significant differences in ASD presentations between the non-verbal ASD group and the verbal ASD group. I therefore ran separate regression models for the non-verbal ASD group and the verbal ASD group to avoid multicollinearity issues, and to allow any differences in the relationship between secure attachment and ADOS2 scores between the groups to be exposed that may otherwise not have been revealed through full group regression analyses.

Social Motivation and ADOS2 Comparison Scores. To assess whether secure attachment was associated with ADOS2 Comparison score, which indicated overall ASD severity, hierarchical regression analyses were conducted. Each model therefore had age and SES in the first block, followed by secure attachment, and with ADOS2 Comparison score as the outcome.

For the non-verbal ASD group (n = 48) multicollinearity was not an issue, as all tolerance and VIF scores were acceptable (Field, 2013). The outcome variable, ADOS2 Comparison score, was significantly and inversely correlated with secure attachment, r = -.48, p <.001.

Table 8.

Zero-Order Correlation Matrix for Non-Verbal ASD Participants: ADOS2 Comparison Score

	1	2	3	4
1. Age (Months)	-			
2. SES	-.18	-		
3. Secure Attachment	-.23	.01	-	
4. ADOS2 Comparison Score	.20	-.17	-.48**	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The first model, with only age and SES as predictors, was not significant, $F(2,45)= 1.35$, $p = .271$. When the second block containing secure attachment was added, the model became significant, $(3,44) = 5.19$, $p = .004$. This model had a large effect size, $f_2 = .35$, and an observed power of .92.The adjusted R_2 value showed that the final model explained 21.1% of the variance in ADOS2 Comparison score, and the R^2 change value of .20 showed this variance was mainly accounted for by secure attachment scores (Table 9).

Table 10 shows that in the final model, only secure attachment had a significant association with ADOS2 Comparison score. The inverse relationship indicated that lower levels of secure attachment (i.e. lower social motivation) related to higher levels of ASD deficits, as was predicted.

Table 9.

Predictors of ADOS2 Comparison Score for Non-Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	Change Statistics				
					R Square Change	*F Change*	*df 1*	*df 2*	*Sig. F Change*
1	.238	.056	.015	1.47	.056	1.35	2	45	.271
2	.511	.261	.211	1.32	.201	12.22	1	44	.001

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, Secure Attachment

Table 10.

Coefficients for Model 2: ADOS2 Comparison Score for Non-Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*	*t*	*p*	Collinearity Statistics	
		B	Std. Error	*β*			Tolerance	VIF
2	(Constant)	7.75	0.86		9.04	<.001		
	Age (months)	<0.01	0.01	.06	0.47	.643	0.92	1.09
	SES	<0.01	<0.01	-.15	-1.15	.255	0.97	1.03
	Secure Attachment	-0.26	0.07	-.47	-3.50	.001	0.95	1.06

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

The above hierarchical regression was repeated for the verbal ASD group (n=48). Zero-order correlations, tolerance scores, and VIF scores indicated no issues with multicollinearity (Field, 2013). The outcome variable, ADOS2 Comparison score, did not correlate with the predictor secure attachment, r = .04, p = .407, suggesting that a relationship would not emerge.

Table 11.

Zero-Order Correlation Matrix for Verbal ASD Participants: ADOS2 Comparison Score

	1	2	3	4
1. Age (Months)	-			
2. SES	-.22	-		
3. Secure Attachment	-.09	<.01	-	
4. ADOS2 Comparison score	.25*	-.17	.04	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The first model, with only age and SES as predictors, was not significant, F (2,45)= 1.89, p = .163. When the second block containing secure attachment scores was added the model was still not significant, F (3,44)= 1.29, p = .291. Table 13 confirmed that there were no significant predictors in the final model. The absence of a relationship between secure attachment and ASD deficits was unexpected and revealed a difference in the association between secure attachment and ADOS2 Comparison score for the non-verbal ASD group compared to the verbal ASD group.

Table 12.

Predictors of ADOS2 Comparison Score for Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	Change Statistics				
					R Square Change	*F Change*	*df 1*	*df 2*	*Sig. F Change*
1	.278	.077	.036	2.02	.077	1.89	2	45	.163
2	.284	.081	.018	2.03	.003	0.15	1	44	.701

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, Secure Attachment

Table 13.

Coefficients for Model 2: ADOS2 Comparison Score for Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*	*t*	*p*	Collinearity Statistics	
		B	Std. Error	*β*			Tolerance	VIF
2	(Constant)	3.97	1.70		2.33	.024		
	Age (months)	0.02	0.01	.23	1.55	.128	0.94	1.06
	SES	<.01	<0.01	-.12	-.80	.427	0.95	1.05
	Secure Attachment	.05	0.13	.06	.39	.701	0.99	1.01

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Social Motivation and ADOS2 Social Affect Scores. To assess whether secure attachment was associated with ADOS2 Social Affect scores, hierarchical regression analyses were conducted for each ASD group. The models had age and SES in the first block, followed by secure attachment score, and with ADOS2 Social Affect score as the outcome.

The model for the non-verbal ASD group (n = 48) showed no issues with multicollinearity, as shown by acceptable tolerance and VIF scores (Field, 2013). Table 14

shows the zero-order correlations. The outcome variable, ADOS2 Social Affect score, was significantly correlated with my predictor, secure attachment, as hypothesised, $r = -.54$, $p < .001$. The direction of this correlation was also as hypothesised: lower social motivation (i.e. lower secure attachment) correlated with increased deficit in ASD, shown by higher ADOS2 Social Affect scores.

Table 14.

Zero-Order Correlation Matrix for Non-Verbal ASD Participants: ADOS2 Social Affect Score

	1	2	3	4
1. Age (Months)	-			
2. SES	-.18	-		
3. Secure Attachment	-.23	.01	-	
4. ADOS2 Social Affect Score	.10	-.05	-.54**	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The first regression model, with only age and SES as predictors, was not significant, $F(2,45) = 0.23$, $p = .794$. When secure attachment was added, the model became significant, $F(3,44) = 17.25$, $p < .001$. The adjusted R_2 value for this final model showed that it explained 24.0% of the variance in ADOS2 Social Affect score, and the R^2 change value of .28 showed this variance was mainly accounted for by secure attachment. This model had a large effect size, $f_2 = .41$, and an observed power of .96. Table 16 shows that in the final model only secure attachment

had an association with ADOS2 Social Affect score. The inverse relationship, indicating that lower levels of secure attachment related to higher levels of ASD deficits, was predicted.

Table 15.

Predictors of ADOS2 Social Affect Score for Non-Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	*R Square Change*	*F Change*	Change Statistics *df 1*	*df 2*	*Sig. F Change*
1	.101	.010	-.034	4.10	.101	0.23	2	45	.794
2	.538	.289	.240	3.52	.279	17.25	1	44	<.001

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, Secure Attachment

Table 16.

Coefficients for Model 2: ADOS2 Social Affect Score for Non-Verbal ASD

Model	Predictors	Unstandardized coefficients *B*	Std. Error	*Standardized coefficients β*	*t*	*p*	Collinearity Statistics Tolerance	VIF
2	(Constant)	18.15	2.29		7.92	<.001		
	Age (months)	-0.01	0.01	.04	0.28	.781	0.92	1.09
	SES	<-0.01	0.00	-.05	-0.39	.702	0.97	1.03
	Secure Attachment	-0.82	0.20	-.54	-4.15	<.001	0.95	1.06

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

The above analysis was repeated for the verbal ASD group (n = 48). Zero-order correlation, VIF and tolerance scores showed that multicollinearity was not an issue (Field, 2013). The outcome variable, ADOS2 Social Affect score, did not correlate with secure attachment, r = -.01, p = .481. It was therefore unsurprising that the regression model was not

significant, $F(3,44) = 1.45$, $p = .240$. The relationships found in the non-verbal ASD group analysis did not show for the verbal ASD group, and the hypothesis regarding a relationship between social motivation and ADOS2 Social Affect scores was not supported for the verbal ASD group.

Table 17.

Zero-Order Correlation Matrix for Verbal ASD Participants: ADOS2 Social Affect Score

	1	2	3	4
1. Age (Months)	-			
2. SES	-.22	-		
3. Secure Attachment	-.09	<.01	-	
4. ADOS2 Social Affect score	.29*	-.15	-.01	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

Table 18.

Predictors of ADOS2 Social Affect Score for Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	Change Statistics				
					R Square Change	*F Change*	*df 1*	*df 2*	*Sig. F Change*
1	.300	.090	.049	3.29	.090	2.22	2	45	.120
2	.300	.090	.028	3.32	<.001	0.02	1	44	.903

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, Secure Attachment

Table 19.

Coefficients for Model 2: ADOS2 Social Affect Score for Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*			Collinearity Statistics	
		B	Std. Error	*β*	*t*	*p*	Tolerance	VIF
2	(Constant)	4.52	2.78		1.63	.110		
	Age (months)	0.03	0.02	.23	1.80	.080	0.94	1.06
	SES	<-0.01	0.00	-.09	-0.64	.527	0.95	1.05
	Secure Attachment	0.03	0.20	.02	0.12	.903	0.99	1.01

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Social Motivation and ADOS2 RRB Scores. The final analyses for relationships between social motivation and ASD-related deficits were for ADOS2 RRB scores. Hierarchical regression was run for each ASD group, with age and SES placed first, followed by secure attachment, with ADOS2 RRB score as the outcome.

The regression model for the non-verbal ASD group (n = 48) showed no issues with multicollinearity, as shown by the correlation table (Table 20), VIF scores, and tolerance scores (Field, 2013). The outcome variable, ADOS2 RRB score, was significantly correlated with my predictor secure attachment, r = -.41, p =.002. The direction was as hypothesised: lower social motivation (i.e. lower secure attachment) correlated with increased deficit in ASD, shown by higher ADOS2 RRB scores.

Table 20.

Zero-Order Correlation Matrix for Non-Verbal ASD Participants: ADOS2 RRB Score

	1	2	3	4
1. Age (Months)	-			
2. SES	-.18	-		
3. Secure Attachment	-.23	.01	-	
4. ADOS2 RRB Score	.28*	-.30*	-.41**	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The first model with age and SES as predictors was significant, $F(2,45) = 3.78$, $p = .030$, with SES as the significant predictor although with a small influence. When secure attachment was added the model remained significant, $F\ (3,44) = 5.63$, $p = .002$. This final model explained 22.8% of the variance in ADOS2 RRB score as per the adjusted R_2 value. This model had a large effect size, $f_2 = .38$, and an observed power of .95. The R^2 change value of .13 showed that just under half the variance was accounted for by secure attachment, with SES remaining a contributor (Table 21).

Table 22 shows that in the final model secure attachment had a significant association with ADOS2 RRB score. SES also had an association with ADOS2 RRB score, although this relationship had a smaller standardised beta value than for secure attachment. The inverse relationship between secure attachment and ADOS2 RRB score, indicating that lower levels of secure attachment related to higher levels of ASD deficits, supported my hypothesis.

Table 21.

Predictors of ADOS2 RRB Score for Non-Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	Change Statistics				
					R Square Change	*F Change*	*df 1*	*df 2*	*Sig. F Change*
1	.379	.144	.106	2.06	.144	3.78	2	45	.030
2	.527	.277	.228	1.91	.133	8.12	1	44	.007

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, Secure Attachment

Table 22.

Coefficients for Model 2: ADOS2 RRB Score for Non-Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*	*t*	*p*	Collinearity Statistics	
		B	Std. Error	*β*			Tolerance	VIF
2	(Constant)	5.22	1.25		4.19	<.001		
	Age (months)	0.01	0.01	.15	1.09	.283	0.92	1.09
	SES	<-0.01	0.00	-.27	-2.10	.042	0.97	1.03
	Secure Attachment	-0.31	0.11	-.38	-2.85	.007	0.95	1.06

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

The above hierarchical regression was repeated for the verbal ASD group (n = 48). Zero-order correlations showed that the outcome variable, ADOS2 RRB score, was not correlated with secure attachment r = .01, p =.484 (Table 23). The absence of a correlation indicated that there was no relationship between social motivation and ADOS2 RRB scores for the verbal ASD group, and this was confirmed as the hierarchical regression model was not significant. The first model with age and SES as predictors was not significant, $F(2,45) = 0.49$, p = .615. When the

second block, containing secure attachment, was added the model remained non-significant, $F(3,44) = 0.32$, $p = .810$. This finding did not support the hypothesis that lower social motivation would relate to higher ASD deficit, and again shows a different pattern of associations in the verbal vs non-verbal ASD groups.

Table 23.

Zero-Order Correlation Matrix for Verbal ASD Participants: ADOS2 RRB Score

	1	2	3	4
1. Age (Months)	-			
2. SES	-.22	-		
3. Secure Attachment	-.09	.01	-	
4. ADOS2 Social Affect Score	-.02	-.14	.01	-

ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

Table 24.

Predictors of ADOS2 RRB Score for Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	Change Statistics				
					R Square Change	*F Change*	*df 1*	*df 2*	*Sig. F Change*
1	.146	.021	-.022	1.52	.021	0.49	2	45	.615
2	.146	.021	-.045	1.54	<.001	0.00	1	44	.994

ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, Secure Attachment

Table 25.

Coefficients for Model 2: ADOS2 RRB Score for Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*			Collinearity Statistics	
		B	Std. Error	*β*	*t*	*p*	Tolerance	VIF
2	(Constant)	2.69	1.28		2.10	.041		
	Age (months)	<-0.01	0.01	-.05	-0.35	.732	0.94	1.06
	SES	<0.01	0.00	-.15	-0.97	.337	0.95	1.05
	Secure Attachment	0.01	0.09	.01	0.01	.994	0.99	1.01

ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Summary of Findings for Social Motivation and ASD-Related Deficits. The above analyses focused on relationships between social motivation, as assessed through secure attachment behaviours, and ASD-related deficits. A further interest was whether the verbal abilities of a child with ASD, specifically differences between non-verbal children with ASD and verbal children with ASD, played a role in the relationships between these variables as these two groups showed significant differences in attachment scores and ADOS2 scores.

For the non-verbal ASD group, there were significant relationships between secure attachment and all three ADOS2 scores, while for the verbal ASD group no significant relationships between secure attachment and ADOS2 scores emerged. The hypothesised relationship between low social motivation, indicated by low secure attachment, to higher intensity of ASD deficits was therefore upheld for the non-verbal ASD group but not for the verbal ASD group.

Question 3: Could OPRM1 Play a Role in Social Motivation and/or ASD-Related Deficits?

This study aimed to assess for relationships between OPRM1 genotypes, low social motivation and increased ASD deficits. I predicted that the *G* allele would be linked to increased

deficits as it is associated with increased mu-opioid levels, and this would undermine the SEPARATION-DISTRESS system. The questions and associated hypotheses are addressed below.

Hypothesis 3.1 Children with ASD will present with higher rates of the OPRM1 G allele than reported neurotypical samples. Of the 102 children with ASD in this protocol, 76 provided DNA samples for genotyping. All 76 of these samples were successfully genotyped for OPRM1; 34 were non-verbal ASD children and 42 were verbal ASD children.

For the full ASD group, 2 children had the *A/A* genotype, 22 children had the *A/G* genotype, and 52 had the *G/G* genotype (Table 26). When considering the ASD groups individually, the non-verbal ASD group had 7 children with the *A/G* genotype, and 27 children with the *G/G* genotype. The verbal ASD group had 2 children with the *A/A* genotype, 15 children with the *A/G* genotype, 25 children with the *G/G* genotype. This study therefore found a considerably higher rate of the *G* allele compared to neurotypical samples reported in the literature, where the *G/G* genotype tended to only occur in 1-4% of the population (Bart et al., 2004; A. C. Chen et al., 2013; Way et al., 2009), with the highest incidence being reported at 16% in a female Taiwanese sample (L. K. Chen et al., 2013). This is also a higher incidence of the *G* allele compared to the rate of 0.05 found for another local sample of 640 African and mixed race women from the Western Cape (D. Stein and S. Dalvie, personal communication, May 23, 2019). This finding therefore supported the hypothesis that there would be a higher incidence of the *G* allele in the ASD population.

Table 26.

OPRM1 Genotypes and Allelic Distribution in ASD

	Genotypes			*Allelic Distribution*	
	A/A	*A/G*	*G/G*	*A*	*G*
Total (*n* = 76)	2 (2.63%)	22 (28.95%)	52 (68.42%)	.17	.83
Non-Verbal ASD (*n* = 34)	0 (0.00%)	7 (20.59%)	27 (79.41%)	.10	.90
Verbal ASD (*n* = 42)	2 (4.76%)	15 (35.71%)	25 (59.52%)	.23	.77

Notes. ASD = Autism Spectrum Disorder.

The genotype and allelic frequencies for OPRM1 across ethnic groups in the ASD samples are shown below (Tables 27-29). The increased rate of the *G* and of the *G/G* genotype is consistent across all ethnic groups, although some samples are too small for their findings to be representative of the population. No obvious disparities in allelic or genotype distributions across ethnicities were noted.

Table 27.

OPRM1 Genotypes and Allelic Distribution in ASD Across Ethnic Groups

	Genotypes			*Allelic Distribution*	
	A/A	*A/G*	*G/G*	*A*	*G*
Caucasian (*n* = 27)	1 (3.70%)	7 (25.93%)	19 (70.37%)	.17	.83
Mixed race (*n* = 39)	1 (2.56%)	13 (33.33%)	25 (64.10%)	.19	.81
African (*n* = 6)	0 (0.00%)	0 (0.00%)	6 (100%)	.00	1.00
Asian (*n* = 3)	0 (0.00%)	2 (66.67%)	1 (33.33%)	.33	.67
Other (*n* =1)	0 (0.00%)	0 (0.00%)	1 (100.00%)	.00	1.00

Notes. ASD = Autism Spectrum Disorder.

Table 28.

OPRM1 Genotypes and Allelic Distribution in Non-Verbal ASD across Ethnic Groups

	Genotypes			*Allelic Distribution*	
	A/A	*A/G*	*G/G*	*A*	*G*
Caucasian (*n* = 12)	0 (0.00%)	2 (16.67%)	10 (83.33%)	.08	.92
Mixed race (*n* = 16)	0 (0.00%)	4 (25.00%)	12 (75.00%)	.13	.88
African (*n* = 4)	0 (0.00%)	0 (0.00%)	4 (100.00%)	.00	1.00
Asian (*n* = 1)	0 (0.00%)	1 (100%)	0 (0.00%)	.50	.50
Other (*n* = 1)	0 (0.00%)	0 (0.00%)	1 (100.00%)	.00	1.00

Notes. ASD = Autism Spectrum Disorder.

Table 29.

OPRM1 Genotypes and Allelic Distribution in Verbal ASD Across Ethnic Groups

	Genotypes			*Allelic Distribution*	
	A/A	*A/G*	*G/G*	*A*	*G*
Caucasian (*n* = 15)	1 (6.67%)	5 (33.33%)	9 (60.00%)	.23	.77
Mixed race (*n* = 23)	1 (4.35%)	9 (39.13%)	13 (56.52%)	.24	.76
African (*n* = 2)	0 (0.00%)	0 (0.00%)	2 (100%)	.00	1.00
Asian (*n* = 2)	0 (0.00%)	1 (50.00%)	1 (50.00%)	.25	.75
Other (*n* = 0)	-	-	-	-	-

Notes. ASD = Autism Spectrum Disorder.

Hypothesis 3.2 Children with ASD Carrying the OPRM1 *G* Allele Will Present with Higher ASD-Related Deficits than Those Who Do Not Carry This Allele. I had initially

hypothesised that ASD deficits, as measured by the ADOS2 assessment, would be greater for children carrying the *G* allele. However, due to the entirely unanticipated finding of only two of my participants being A/A genotype carriers, I was unable to assess this hypothesis. I have presented the descriptive statistics for the ADOS2 scores across genotypes below.

All 76 of the participants who provided DNA samples also had ADOS2 assessments. Their ADOS2 scores across OPRM1 genotypes are shown below (Tables 30-32), and no clear patterns of association emerge even when the low frequency of the *A* allele is considered.

Table 30.

OPRM1 Genotypes and ADOS2 Scores for All ASD Participants

	A/A (*n* = 2)	*A/G* (*n* =22)	*G/G* (*n* = 52)
Comparison score[a] $\bar{X}$ *(SD)*	7.00 (1.41)	5.59 (1.76)	5.87 (1.87)
Social Affect score[b] $\bar{X}$ *(SD)*	10.00 (2.83)	8.91 (4.32)	10.27 (4.84)
RRB score[c] $\bar{X}$ *(SD)*	1.50 (0.71)	2.41 (1.82)	2.75 (2.05)

Notes. ASD = Autism Spectrum Disorder. $\bar{X}$ =Mean. *SD*=Standard deviation. RRB = Restricted and Repetitive Behaviours and Interests symptom domain.

[a] Possible score range 1-10; [b] Possible score range 0-20; [c] Possible score range 0-8.

Table 31.

OPRM1 Genotypes and ADOS2 Scores for Non-Verbal ASD Participants

	A/A (*n* = 0)	*A/G* (*n* = 7)	*G/G* (*n* = 27)
Comparison score[a] $\bar{X}$ *(SD)*	-	5.71 (1.254)	6.37 (1.445)
Social Affect score[b] $\bar{X}$ *(SD)*	-	13.14 (4.319)	13.11 (3.856)
RRB score[c] $\bar{X}$ *(SD)*	-	2.43 (2.070)	3.67 (2.075)

Notes. ASD = Autism Spectrum Disorder. $\bar{X}$ =Mean. *SD*=Standard deviation. RRB = Restricted and Repetitive Behaviours and Interests symptom domain.

[a] Possible score range 1-10; [b] Possible score range 0-20; [c] Possible score range 0-8.

Table 32.

OPRM1 Genotypes and ADOS2 Scores for Verbal ASD Participants

	A/A (*n* = 2)	*A/G* (*n* =15)	*G/G* (*n* = 25)
Comparison Score[a] $\bar{X}$ *(SD)*	7.00 (1.414)	5.53 (1.995)	5.32 (2.135)
Social Affect Score[b] $\bar{X}$ *(SD)*	10.00 (2.828)	6.93 (2.576)	7.20 (3.851)
RRB Score[c] $\bar{X}$ *(SD)*	1.50 (0.707)	2.40 (1.765)	1.76 (1.508)

Notes. ASD = Autism Spectrum Disorder. $\bar{X}$ =Mean. *SD*=Standard deviation. RRB = Restricted and Repetitive Behaviours and Interests symptom domain.

[a] Possible score range 1-10; [b] Possible score range 0-20; [c] Possible score range 0-8.

Hypothesis 3.3 Children with ASD Who Carry the OPRM1 *G* Allele Will Present with Lower Social Motivation than Those Who Do Not Carry This Allele. As per Panksepp's (1979) Social Motivation Theory, I hypothesised that children carrying the OPRM1 *G* allele would have lower social motivation than those without a *G* allele; that is, the *G* allele carriers

would have lower secure attachment scores than non-carriers. Of the 76 ASD children who provided DNA samples, 70 of these children had complete data sets for attachment and were included in this analysis; 31 of these children were in the non-verbal ASD group, and 39 were in the verbal ASD group.

Due to the unexpectedly low number of children in my sample who were homozygous for the *A* allele, I only presented descriptive statistics for secure attachment across OPRM1 alleles and genotypes. Descriptively, Table 33 shows that the carriers of the *A/A* genotype tend to have higher secure attachment scores than carriers of the *G* allele. Carriers of the *G* allele showed similar scores for secure attachment regardless of whether they were heterozygous (i.e. *A/G* genotype) or homozygous (i.e. *G/G* genotype). It therefore appears that carrying a *G* allele is related to lower social motivation, as hypothesised, although I again note caution due to the small group size for the *A/A* genotype group, and due to the large standard deviations.

Table 33.

OPRM1 Genotypes and Secure Attachment Scores for ASD Participants

	A/A	*A/G*	*G/G*
Full ASD Sample[a] $\bar{X}$ *(SD)*	8.00 (2.83)	5.35 (2.74)	5.79 (2.59)
Non-verbal ASD[a] $\bar{X}$ *(SD)*	-	5.33 (3.08)	4.76 (2.67)
Verbal ASD[a] $\bar{X}$ *(SD)*	8.00 (2.83)	5.36 (2.71)	6.91 (2.02)

Notes. ASD = Autism Spectrum Disorder. $\bar{X}$ =Mean. *SD*=Standard deviation.

[a] Range: 0-10

Summary of Findings for Question 3. The above analyses were limited by the unexpectedly small number of carriers of the *A/A* genotype. A higher incidence of the *G* allele in the ASD population was suggested by Panksepp's (1979) Scial Motivation Theory, however this

has never been assessed in human ASD samples. My study found an unprecedentedly high rate of the *G* allele, which suggests a role for OPRM1 in ASD. Indeed, the rate of the *G* allele was such that I lacked sufficient carriers of the *A/A* genotype for statistical investigations into the possible relationships between OPRM1 and ASD deficits. I was therefore unable to test my hypotheses that the *G* allele would be linked to greater ASD deficits or to lowered social motivation, as would be indicated by low secure attachment scores.

Summary of Findings

The above analyses found that the ASD samples had lower levels of social motivation than the neurotypical sample. Further, I found that the non-verbal ASD group showed lower social motivation than the verbal ASD group. The non-verbal ASD group also showed greater ASD-related deficits in all domains, namely, ADOS2 Comparison scores, ADOS2 Social Affect scores, and ADOS2 RRB scores.

In the non-verbal ASD group, associations were evident between secure attachment scores and all three ADOS2 outcomes, although this effect was smallest for ADOS2 RRB scores. My hypothesis that low social motivation, as indicated by low secure attachment, would link to greater ASD-related deficits were therefore upheld for the non-verbal ASD group. The verbal ASD group showed no relationships between attachment scores and ADOS2 score, indicating a difference in social functioning between non-verbal ASD and verbal ASD samples, both of which were male, to the extent that none of the hypothesised relationships between social motivation levels and ASD-related deficits were upheld. The extent of the differences in the role of social motivation between ASD groups is a novel finding.

Exploration of the role of the *G* allele for OPRM1 in ASD was limited by the small incidence of the *A/A* genotype. I confirmed a higher incidence of the *G* allele in my ASD sample

compared to reported incidence rates for neurotypical samples, but the extremely high rate of the *G* allele in my sample was unprecedented. The proposed link between the *G* allele and greater ASD deficits and lowered social motivation could not be explored in this sample due to the unforeseen finding of too few children with the *A/A* genotype.

Discussion

The Social Motivation Study provided mixed support for Panksepp's (1979) Social Motivation Theory for ASD. His theory has three main tenets, which I explored individually. The first tenet is that children with ASD will have reduced social motivation compared to neurotypical children, and this was supported by this study. The second tenet is that lower social motivation will be associated with the higher severity of ASD-related deficits. I found this to be true for non-verbal children with ASD, but not for verbal children with ASD. The final tenet of Panksepp's (1979) theory is that mu-opioids underlie social motivation and are therefore associated with ASD-related deficits. The remarkably high rate of the OPRM1 *G* allele in my ASD sample supports a role for the mu-opioid system in ASD, and possibly in social motivation, but the low rate of the *A* allele meant I could not clarify the role of OPRM1 in ASD.

The most remarkable finding in this study was that male children with ASD have a substantially higher incidence of the *G* allele for OPRM1 when compared to the reported prevalence of this allele in neurotypical samples. Further, only the non-verbal ASD group performed as predicted by the Social Motivation Theory. To date, the role of mu-opioids in the social motivation system has not been examined directly in ASD samples in such a comprehensive manner (i.e. generally only one tenet of the theory is explored). This study therefore provides important direct insight into the role of social motivation in an ASD sample with consideration of the role of OPRM1.

Overall, I sought to contribute to a shift in ASD research, one where psychological theories and genetic theories are not researched in isolation but are rather explored together to support valid results. I emphasise the need to consider the phenotypes of research participants, especially in terms of language capabilities, and to ensure that human studies are conducted following proof-of-concept work in animal studies.

Participant Characteristics

One of the most significant trends throughout my protocol was that the all-male non-verbal ASD sample and the all-male verbal ASD sample showed different relationships between variables of interest. These differences between the two groups, which are discussed in detail later, indicated that the groups were qualitatively different. The categorical difference between these groups was language ability, which could suggest that language acquisition and development is an important phenotypic, and possibly diagnostic, element of ASD. My study reports evidence strongly suggesting that the ability to develop language has ongoing developmental implications, at least for male children with ASD. My research could indicate the need to differentiate ASD subtypes by language ability as one cannot generalise research based on one group to the other, and hence that ignoring language ability within ASD samples could confound results.

Participant Grouping. My protocol had three participant groups, and these groups were closely matched on several demographic variables to avoid sampling bias. Participants were grouped as follows: 51 fluently verbal male children with ASD; 51 male children with ASD who were fully non-verbal or unable to use phrase speech; and 51 neurotypical male children who had no medical or psychiatric diagnoses. All three groups were matched on SES and were all male. Ethnicity data was collected for the ASD groups due to the possible relationships between

ethnicity and different allelic distribution for the gene of interest, and the ASD groups showed a similar ethnic composition. Ethnicity data was not collected for the neurotypical group as it was not deemed necessary, and ethnicity is a sensitive issue in South Africa due to our history of race-based oppression. The verbal ASD group and neurotypical group were matched on age, but the non-verbal ASD group had a younger mean age than these two groups. The younger age for the non-verbal ASD group is likely due to the nature of language development in ASD as many children have delayed language development rather than permanent deficits, so it is more common to have non-verbal children with ASD in younger samples (American Psychiatric Association, 2013; Mody et al., 2013). It is possible then that some of my non-verbal ASD participants could develop language at a later age, but as the mean age for this group was over 7 years old this is unlikely for most of these participants. My participants were therefore matched on several demographics, and as age could be controlled for statistically when the non-verbal ASD group was included in analyses, any group differences noted in this study are unlikely to be due to sampling bias.

Inclusion of Non-Verbal ASD Participants. The inclusion of a non-verbal ASD group and of ASD participants with severe deficits increased my protocol's generalisability to other male children with ASD, and helped reduce the current underrepresentation of these children in research. I included all male children with ASD without comorbidities other than ADHD who scored three or above on the ADOS2 Comparison score, which indicates ASD severity or intensity of symptoms, regardless of language capability. Research tends to exclude children with severe ASD presentations and/or with limited language ability as these children are more challenging to work with and frequently used measures are not always appropriate for them (Tager-Flusberg & Kasari, 2013).

I found that the language capability of my ASD participants was associated with the severity of their ASD as well as the nature of the interactions between variables of interest in my protocol. This expands our current understanding of the relationships between language and other social and cognitive domains by indicating that language may be a foundational skill upon which later developmental interactions between domains depend. If in ASD, the development of language results in different interactions between social and cognitive domains throughout development, rather than simply influencing ASD severity, it means that research based on verbal children with ASD cannot be generalised to non-verbal children with ASD, and vice versa. Recognising these as two distinct groups could mean a great deal of existing research should only be generalised to verbal children with ASD rather than all children with ASD, at least in the case of male children with ASD.

Secure Attachment as a Proxy for Social Motivation

I approached this study in a novel way by using attachment behaviours as a proxy for social motivation. Panksepp et al. (1997) describe social motivation behaviours as those that facilitate social engagement. Behaviours typical of secure attachment can be considered to indicate positive social motivation as securely attached children are consistently pleased by social interaction, they seek out their caregiver appropriately, and they are aware of, and displeased by, separation from the caregiver (Rutgers et al., 2004). As children age, however, their attachment behaviours and indicators of social motivation become more complex. Children develop friendships, and signs of social interest and pleasure extend beyond crying when alone, or seeking out their primary caregiver throughout the day. There is limited research available on social motivation in children with ASD, and there is not a standard measure available for assessing social motivation, especially in the context of ASD and beyond toddler age for these

children. By using attachment as a proxy for social motivation I was able to identify behaviours indicative of social motivation.

Further, I wanted to assess social motivation, through attachment, in childhood rather than at a younger age. The attachment measure I used, the ASCQ (Finzi-Dottan et al., 2012), asked parents about their son's behaviour around other children, as well as around people in general. This does not focus on the relationship to the primary caregiver, unlike most of the literature, but does allow for an age-appropriate measure of a child's social interest. However, it was based on parent responses, which could be limited in their insight into what their child feels about social relationships. This limitation is particularly true for the non-verbal children with ASD, who have never been able to discuss their social experiences with their parents.

When conducting the interviews, my team members prompted parents to give examples and reasons for their answers, and in this way we tried to ensure that answers reflected accounts of observable behaviours and to maintain consistency across participants. I was therefore able to assess social motivation levels in children with ASD based on behaviour patterns in their daily environment and used a measure that was appropriate for my full sample despite their varying abilities. The ASCQ is typically administered to children and adolescents directly, but this was not possible as a third of my sample was non-verbal. I also had concerns regarding the children with ASD's abilities to answer the questions due to the social deficits inherent in ASD. I therefore considered parent interview to be more appropriate than self-report from the children in my study. An interview also allowed us to query parents' answers to ensure consistency in the ratings assigned to specific responses.

I was able to assess attachment behaviours in children rather than toddlers, and without exposing children with ASD to novel environments. Observation is generally preferable for

assessing attachment and some researchers have found that children with ASD can complete the Strange Situation Procedure or an adaption thereof (Rutgers et al., 2004). However, as children with ASD may struggle with changes in routine and/or new environments (American Psychiatric Association, 2013), I was concerned that an observational measure would be confounded by increased anxiety and would possibly lead to the exclusion of the more severely affected participants (Kahane & El-Tahir, 2015). Further, observation measures like the Strange Situation Procedure would not be appropriate for older children and would likely offer a restricted range of scores for the neurotypical sample. Therefore, although the ASCQ did have these limitations, it was able to assess social motivation behaviours across my three samples in a comparable way that was appropriate for my study.

Social Motivation in ASD vs. Neurotypical Samples

My study found that male children with ASD had considerably lower social motivation levels than male neurotypical children, and that non-verbal male children with ASD had lower social motivation than verbal male children with ASD. Lowered social motivation in ASD has been theorised, and research has been conducted noting specific behaviours indicative of low social motivation (Klintwall et al., 2014; Pellissier et al., 2018). As standardised measures to quantify levels of social motivation are not available, this is the first study to my knowledge to measure overall levels of social motivation in a male ASD sample and directly compare it to a neurotypical sample. Using this continuous measure for social motivation also allowed us to assess confounding variables such as SES, age, and even verbal ability. I found that male children with ASD showed significantly lower social motivation than neurotypical male children over and above all these variables.

My study also found that reduced social motivation was present throughout childhood in ASD. I found that social motivation was lower for both the ASD groups compared to the neurotypical group over and above the effect of age. Further, there was no correlation between level of social motivation and age. To date research has noted behaviours indicative of low social motivation in ASD samples from toddler age through to early childhood (Rutgers et al., 2004), but this protocol expanded this with a more comprehensive assessment of social motivation. The current study showed that low social motivation is still present in later childhood. The absence of a negative correlation between age and social motivation also suggests that one will not "out grow" these deficits, and these deficits may be a core aspect of ASD.

As I used attachment as a proxy for social motivation, it is worth noting that my research is consistent with the attachment literature which generally shows that children with ASD present with reduced secure attachment when compared to neurotypical samples (Kahane & El-Tahir, 2015; Rutgers et al., 2004; Teague et al., 2017). Attachment literature also notes that children with ASD present with a different quality of attachment compared to neurotypical children (Teague et al., 2017). Reviews of the literature have noted that even those children with ASD who met the criteria for secure attachment still presented with less secure attachment behaviours than neurotypical children, and their attachment behaviour tended to be more disorganised (Kahane & El-Tahir, 2015; Rutgers et al., 2004). In keeping with this, my study found that children with ASD did sometimes display behaviours indicating positive attachment / positive social motivation, but parents reported these were not consistent. Further, the ASD samples were not heterogenous in their presentations for social motivation, as some presented with far greater lower social motivation than others. My study is therefore aligned with the general attachment research regarding attachment in ASD, but also highlights that low social motivation and

attachment deficits present like most other ASD deficits: these deficits present on a spectrum and may not be conducive to categorial ratings.

Social Motivation and ASD-Related Deficits

I found the hypothesised relationship between reduced social motivation and ASD-related deficits was present in ASD, but only for non-verbal male children with ASD. Low social motivation was associated with both social and non-social symptom domains. My findings also support a critical role for language acquisition in the relationship between social motivation and ASD-related deficits.

In the non-verbal ASD group social motivation was strongly associated with ADOS2 Social Affect score, but was significantly associated with ADOS2 Comparison score and ADOS2 RRB score as well. This indicates that low social motivation could be a core element of ASD and may underlie development primarily in the social domain, but also extends to non-social domains. Reduced social motivation in ASD may not have a set phenotype, but instead could have a pervasive influence across all levels of functioning. This is in keeping with research on OPRM1 knockout mice, as these mice not only showed disinterest in social engagement, but also showed repetitive behaviours analogous to the restricted and repetitive behaviours and interests symptom domain in ASD (Pellissier et al., 2018). To date much of the research on social motivation has focused on social interest and / or disinterest, but these results indicate that a broader frame should be adopted for future work in this area.

I found that language ability was a critical variable when assessing social motivation in ASD. The presence of an association between social motivation and ASD-related deficits for the non-verbal male ASD group and not the verbal male ASD was not explained by any group difference other than language ability. As the non-verbal ASD group was matched to my verbal

ASD group on SES and the groups had a similar ethnic breakdown, and all participants were male and came from English speaking homes or schools, the only group differences were language ability and that the non-verbal group was younger overall. I found that age did not correlate with social motivation level, and the ADOS2 scoring takes age into account to some degree by having different modules for different ages. Further, I did consider age as a possible covariate when assessing possible associations between social motivation levels and ASD-related deficits and found that age did not have any significant effects. It thus seems reasonable to conclude that the group difference that emerged was associated with language ability.

The asssociation between social motivation and ADOS2 Comparison score, indicating overall ASD severity, aligns with a previous study with toddlers. Klintwall et al. (2014) found a relationship between interest in ADOS assessment at 2 years old and level of ASD-related deficits at age 3, which is in keeping with my findings. They found associations between low interest and lower non-verbal mental age, lower verbal mental age, and reduced adaptability skills. The young age of their sample and assumed limited language ability at that age makes their sample comparable to my non-verbal ASD group rather than my verbal ASD group.

Although a relationship between social motivation levels in ASD and language ability is supported by my study, the exact nature of this relationship is unclear. My non-verbal ASD group had lower social motivation than my verbal ASD group, but the presence low social motivation in this latter group indicates that language deficits alone cannot account for low social motivation. Attachment literature for ASD has found correlations between ASD severity and level of secure attachment. The review by Teague et al. (2017) noted that attachment deficits were higher for ASD samples compared to neurotypical children, children with intellectual disability without ASD, and children with language delay without ASD. The inclusion of

comparisons between children with ASD and children with language delay without ASD shows that the decreased attachment in the ASD samples, indicative of low social motivation, was not due to language deficits alone. This aligns with my study and suggests that within ASD there could be an additional relationship between social motivation and language development.

A possible explanation for my group difference regarding social motivation levels in ASD is that social motivation levels may have a threshold effect that may prevent language acquisition. Panksepp (1979) proposed that lowered social motivation could play a role in language acquisition deficits. My study supports this hypothesis, as the non-verbal ASD group had lower social motivation than the verbal ASD participants, and both ASD groups showed lower social motivation than the neurotypical group. The decreased levels of social motivation in the verbal group could have allowed or facilitated language acquisition. The absence of associations between social motivation levels and ASD-related deficits in the verbal ASD group could also indicate that when language is able to develop in ASD, it serves a protective function and limits the effects of reduced social motivation. Social motivation levels may therefore be an essential target for interventions for non-verbal children with ASD.

OPRM1 in ASD

I found very strong evidence for OPRM1, and by extension the mu-opioid system, being implicated in ASD in this study sample. I had a startling allelic rate of .83 for the OPRM1 *G* allele in my ASD sample, and while this unexpectedly high rate of this allele strongly implicates OPRM1 in ASD, the relatively low rate of the *A* allele meant I was limited in my ability to explore associations between OPRM1 and ASD-related deficits in this group. The Social Motivation Theory for ASD suggests that mu-opioids play a key role in social motivation in ASD, and that mu-opioid levels would relate to the level or type of deficit presented. There is a

shortage of literature assessing the Social Motivation Theory for ASD in human samples, and those that do explore the role of opioids, tend to do so by recording the outcomes of administering opioid-antagonists (Bouvard et al., 1995; Herman et al., 1987). To my knowledge, this is the first study to assess the role of OPRM1 specifically in an ASD sample. Further, the allelic frequency of OPRM1 in an ASD sample has not been assessed before, and a frequency this high has never been reported for any neurotypical samples. This therefore provides exceptionally strong support suggesting a role for OPRM1 in ASD as this finding is unprecedented.

Allelic Distribution of OPRM1 in an ASD Sample. My sample of 76 male children with ASD presented with an allelic frequency of 0.83 for the OPRM1 *G* allele. The non-verbal ASD sample had a higher rate of this allele at 0.90 and had no instances of the *A/A* genotype, while the verbal ASD group had an allelic rate of 0.77 for the *G* allele and only 2 of the 42 children presented with the *A/A* genotype. The presence of the OPRM1 *G* allele is associated with increased transcription of mu-opioids, so the increased frequency of this allele supports Panksepp's (1979) assertion that atypical mu-opioid transmission is implicated in ASD.

The allelic rate of the OPRM1 *G* allele in my ASD sample far surpasses even previously "high" rates of the *G* allele and strongly supports the potential role of OPRM1 as a candidate gene for important biological pathways in males with ASD. In my sample of 76 male children with ASD, 52 (68.42%) of the boys presented with the *G/G* genotype and the sample had an allelic frequency of 0.83 for the *G* allele. The *G* allele for OPRM1 is reported as rarer than the *A* allele in the literature, and the *A/A* genotype is the most commonly occurring genotype in non-ASD samples (Fillingim et al., 2005). The *G/G* genotype is reported as only occurring in 1-4% of samples, with the allelic frequency of the *G* allele ranging from 0.07 to 0.22 (Bart et al., 2004; A.

C. Chen et al., 2013; Way et al., 2009). Some studies have found a higher frequency of the *G* allele in samples of Asian descent (L. K. Chen et al., 2013; Wei et al., 2017), although the rate of the *G* allele did not exceed 0.35, which is substantially less than in my samples.

My sample was racially diverse due to the diversity inherent in the South African population. The ethnic breakdown of my sample of ASD participants was 33% Caucasian, 51% mixed race, 10% African, 5 Asian, and 1% reported themselves as "other". The mixed race sample (n = 39; allelic rate of .81) has mixed heritage and some cases could include Asian heritage. Although the literature found higher rates of the *G* allele in Asian samples compared to non-Asian samples, I do not believe the inclusion of mixed race participants accounts for the high rate of this allele in my sample. If I restrict my sample to the Caucasian (n = 27; allelic rate of .83) and African participants (n = 6; allelic rate of 1.00), who are not of Asian descent, they still showed a much higher rate of the *G* allele than is reported in the literature. Further, in a local study of 640 mothers in the Western Cape (57% African; 43% Mixed race), the allelic frequency for the *G* allele was within the typical range at 0.05 (D. Stein and S. Dalvie, personal communication, May 23, 2019). As my sample was also drawn from the Western Cape, this serves to emphasize the novelty of my finding in a local sample of children with ASD.

A further consideration is sex, but again this does not account for the high rate of the *G* allele in my sample. A different allelic frequency for OPRM1 based on sex has not been suggested in the literature, and a study that did consider the role of sex found that there was no association between sex and OPRM1 alleles (Troisi et al., 2011). Indeed, it is noted that the two studies with Asian samples that showed allelic rates of above 0.30 for the *G* allele were both female samples (L. K. Chen et al., 2013; Wei et al., 2017). I found three studies that reported OPRM1 allelic distributions separately for male and female participants, and two showed a

slightly lower rate of the *G* allele in the male participants compared to the female participants (Fillingim et al., 2005; Way et al., 2009), and one found the reverse (Bond et al., 1998), although the differences were not significant. The sex of my sample therefore does not explain the high frequency of this allele.

The surprisingly high rate of the OPRM1 *G* allele is therefore not fully accounted for by sex or ethnicity. As this allele is associated with increased mu-opioid transcription my study strongly supports the role of mu-opioids as an important biological pathway in ASD, and therefore offers some support for the Social Motivation Theory for ASD. As the first study to explore the allelic distribution of OPRM1 in an ASD sample, this finding shows the importance of this gene as an ASD candidate gene.

OPRM1 and Social Motivation in ASD. In terms of descriptive statistics, my study appeared to support the hypothesis that lowered social motivation is linked to atypical mu-opioid systems, but an exploration of possible relationships between OPRM1 and social motivation was limited by the low incidence of the OPRM1 *A* allele, preventing genotype-phenotype analyses. Descriptively, it appeared that social motivation was lower for carriers of the *G* allele. This would provide support for Panksepp's (1979) Social Motivation Theory for ASD by supporting the hypothesis that disruptions in the mu-opioid system underlie, at least to some degree, the development of ASD. However, with only two carriers of the *A/A* genotype it is impossible to know if this relationship is valid. A larger study is needed, both to make comparisons across genotypes, and to confirm the high incidence rate of the *G* allele. It would also be useful to assess the relationship between social motivation levels and OPRM1 distribution in a neurotypical sample, although this is likely to face the reverse limitation: an underrepresentation of the *G* allele.

My non-verbal ASD sample showed no incidence of the *A/A* genotype, and had significantly lower social motivation and higher ASD-related deficits. I found that social motivation levels were good predictors of ASD-related deficits as measured by all three of the ADOS2 scores for the non-verbal ASD group. The higher allelic frequency of the *G* allele for the non-verbal ASD group alongside their higher level of deficits compared to the verbal ASD group also supports the theory, possibly suggesting that this theory is more appropriate for non-verbal male ASD samples. However, I again note the limitation of the sample as it was not possible to compare social motivation across genotypes.

OPRM1 and ASD-Related Deficits. I faced the same limitation for genotype-phenotype analyses when assessing relationships between OPRM1 and ASD-related deficits. The over-representation of the *G* allele, and therefore an underrepresentation of children with the *A/A* genotype, did not allow me to assess how non-carriers of this allele would possibly differ from the *G* allele carriers. For my sample, ADOS2 scores did not appear to differ across genotypes, and no pattern was apparent for either of the ASD groups. DNA was not collected for the neurotypical group due to financial constraints, and because assessing this group with the ADOS2 was deemed inappropriate as it was unlikely to result in any variance in scores across these participants, resulting in a floor effect.

My ASD samples both showed social deficits, and almost all of the participants presented with the *G* allele. Although the two non-carriers of the *G* allele in my study also showed high ADOS2 scores, the high incidence of the *G* allele is sufficient to warrant further investigation and any interpretation of the two *A/A* carriers must be considered cautiously as there is a large possibility of a sampling bias where these two children do not adequately represent all non-carriers of the *G* allele. Non-primate animal research found that administration of opioids

resulted in ASD-like behaviours, and OPRM1 knockout mice presented with the full behaviour repertoire required for a DSM-5 diagnosis for ASD (Becker et al., 2014). Primate and human studies were mixed: some found a gain-of-function for carriers of the *G* allele that made them appear more socially motivated rather than less so (Barr et al., 2008; Way et al., 2009), while other studies found that this allele was associated with an indifference to the effects of maternal care (Troisi et al., 2012) and long term administration of opiates resulted in ASD-like deficits in social interaction and social cognition (McDonald et al., 2013). Despite the limitations I faced in having so few non-carriers of the *G* allele, my results can contribute to the current understanding of this topic.

OPRM1 in Future ASD Research. The high incidence of the *G* allele in my sample indicates that OPRM1 must be involved in at least some aspect of ASD. The failure to link genotypes or alleles to specific symptom domains or overall severity of ASD in my sample could be due to the low rate of the *A* allele preventing us from seeing group differences. Similarly, although my findings suggest that the OPRM1 *G* allele is related to reduced social motivation, this needs to be further explored. I therefore suggest two important directions for future research – first, better specificity in phenotyping, and second, aiming to recruit a mixed sample with a higher representation of the OPRM1 *A* allele.

This study was exploratory in nature and I lacked specificity in how I measured ASD-related deficits. I aimed to establish which aspects of ASD, in terms of diagnostic domains, related to OPRM1, and whether there was a link to overall ASD severity. OPRM1 may be linked to very specific aspects of ASD that were seen in animal models, such as the reduced responsiveness to others, poor clinging to a caregiver, pain insensitivity, or restricted behaviour.

Future studies should identify specific behaviours of interest (e.g. poor eye-contact, failure to respond to name, etc.) and include them in their phenotyping.

The largest limitation of my study was the low representation of the OPRM1 *A* allele, and future studies will need to overcome this. I suggest that studies include children with and without ASD in their samples. This can be done by including genotyping for neurotypical controls and non-ASD clinical samples, which I could not afford in the current study. Given the low representation of the *G* allele in neurotypical samples, studies only comparing ASD and neurotypical samples may lead to samples with extremes that prevent statistical analyses – that is, the ASD group may present with a high deficit level in the ASD-related area of interest and a high incidence of the *G* allele, while the neurotypical sample would have the reverse. I think more could be learnt by including children without ASD, but who share phenotypic features of interest (e.g. include children with low social interest, atypical eye-contact, etc., based on the study aims), alongside children with ASD and neurotypical children. In this way, each trait and level of social motivation can be measured on a continuum and compared across OPRM1 alleles and genotypes rather than across categorical samples. This shift to a continuous measure of the trait of interest, rather than a categorical division of groups into ASD vs. non-ASD, could provide meaningful data on the role of OPRM1 social development, and then in ASD by extension.

Expanding the Social Motivation Theory for ASD: A Threshold Effect. As all children with ASD in this protocol presented with lower social motivation than the neurotypical sample, and had an increased rate of the OPRM1 *G* allele, but only the non-verbal ASD group showed a relationship between social motivation levels and ASD-related deficits, I propose that the Social Motivation Theory for ASD could perhaps be expanded by considering a threshold effect

between social motivation and language development. Panksepp (1979) hypothesised that lowered social motivation could result in language acquisition deficits, although this was not further elaborated but rather included in his general expectation that social abilities would be undermined. I propose that the effects of reduced social motivation go beyond social and language deficits, as I found that social motivation levels also predicted ADOS2 RRB deficits. Again, however, the link between social motivation levels and ASD-related deficits was only found in the non-verbal ASD group, despite deficits in both these areas being present in the verbal ASD group.

I propose that within ASD there are different levels of social motivation, and children with very low social motivation may then present with language deficits across development, while those with higher, but still reduced, social motivation may be able to develop language more efficiently. There is a direct relationship between social interaction and the development of language (Kuhl, 2010). Very low social motivation, and the associated reduction in social engagement, could underlie atypical language acquisition, thereby increasing the challenges these children have to face. The development of language may allow further psychosocial learning and development, which with time reduces the relationship between social motivation and ASD-related deficits. Whether language development weakens the relationships between social motivation and ASD-related deficits, or whether the relationship just becomes more complex as one needs to consider other cognitive and social domains as contributing, or confounding, factors, is unclear. The absence of an association between social motivation and ADOS2 RRB scores in the verbal ASD group indicates that there is an advantage of language development in both social and non-social domains.

This is a novel idea, and there is little research to help clarify why the non-verbal ASD group in this study showed associations between social motivation and severity of ASD-related deficits whereas the verbal ASD group did not. Klintwall et al. (2014) found a relationship between social motivation and both non-verbal and verbal skills which could indicate why I found a group difference based on language ability. My non-verbal ASD group showed significantly lower social motivation and higher ASD-related deficits scores compared to my verbal ASD group. If, as Klintwall et al. (2014) found, lower social motivation is associated with greater deficits in language abilities, it could account for the increased severity of deficits in my non-verbal ASD group. Perhaps, as I proposed, the verbal ASD group was less hindered by reductions in social motivation deficits, which allowed language development, and ultimately due to the integral nature of language in development of other social and cognitive domains (Mody et al., 2013), they were able to better overcome the influence of low social motivation. This could suggest that language acquisition in ASD provides a considerable advantage in developing other social skills and reducing the lifelong impact of initial lowered social motivation on psychosocial development.

Summary of Findings for the Social Motivation Study

This study investigated Panksepp's (1979) Social Motivation Theory for ASD by individually exploring the three main tenets of his argument: that children with ASD lower social motivation than neurotypical children; that social motivation levels would link to deficits in ASD; and, that a disruption to the mu-opioid system, in this case having increased transcriptional efficacy due to the *G* allele for OPRM1, would relate to reduced social motivation and to ASD-related symptoms. My study was unique in that it attempted to assess all three of these tenets directly in an ASD sample, and in that this sample included non-verbal children. However, while

I was able to show that OPRM1 is most likely implicated in ASD due to the remarkably high incidence of the *G* allele, this also meant I was not able to comprehensively explore the third tenet of the argument – I was not able to adequately compare the phenotypes of *G* carriers with non-carriers. My findings were surprising as the overall theory was supported for the non-verbal ASD group, while the verbal ASD group showed decreased social motivation and an increased rate of the *G* allele, but no associations between social motivation and ADOS2 scores, and the role of OPRM1 could not be assessed. I also found that low social motivation persisted across development as this was present in children up to 16 years old, and for the non-verbal group the association between social motivation levels and ASD-related deficits was present over and above the effect of age.

I propose that the Social Motivation Theory for ASD may need to be expanded to consider a threshold effect whereby those with very low social motivation are unable to develop language efficiently, while those with higher social motivation, although still less so than neurotypical children, do develop language and the effect this then has on development in other social and cognitive domains reduces the impact of their social motivation levels and reduces or eliminates the direct link between social motivation and ASD symptoms. This proposal would need to be empirically tested in future studies,

The main limitation of my study was an unexpected one: that the ASD sample had an unprecedentedly high rate of the *G* allele, and therefore the *A* allele was under-represented. I was therefore unable to make statistical comparisons across OPRM1 genotypes. A larger sample size could perhaps help overcome this limitation, although I was restricted in my sample size due to my inclusion criteria (i.e. no other psychiatric or medical conditions, and no use of medication that could influence the neurochemical pathways of interest) and by many families not being

comfortable providing DNA samples. Again, a larger study would be useful in overcoming these limitations, although it is possible that there would still be an underrepresentation of the *A/A* genotype if the sample does not genotype neurotypical participants and other children without ASD but with shared phenotypic characteristics of interest. It would also be interesting to conduct longitudinal studies to assess whether the threshold effect is present, which would require assessing infants and then tracking their progress and seeing if social development does differ across OPRM1 genotypes, and ideally along with other measures of mu-opioid activity and language development.

The aim to establish a clean phenotype in this protocol came at a cost to overall generalisability of this study, as well as the study in the next chapter, and is discussed in Chapter 5 (pg. 208). Consideration of the inclusion of only male children, and therefore the exclusion of female children, is important given the advantage female children have in language acquisition and social development (Adani & Cepanec, 2019). The exclusion of children with ASD with comorbities also limits the generalisability, Further, within the South African context, limiting participants to those from English-speaking homes or schools does not reflect the diversity of our country.

Despite these limitations, my study does provide insight into the relationships between core ASD symptoms and the role of social motivation. Supporting the Social Motivation Theory of ASD shows that social motivation levels are a critical aspect of ASD and that as they appear early in life, they should be addressed early as well. Perhaps if interventions focus on social motivation in younger children, we can reduce the severity of ASD symptoms across development.

Chapter 4: The Theory of Mind Study

The presence, and importance, of ToM deficits in ASD have been recognised for decades (Baron-Cohen et al., 1985), but the underlying biological mechanisms for ToM deficits in ASD are unknown. Similarly, serotonin is implicated in ASD, but it's role in the ASD phenotype is unclear. As serotonin is implicated in social functioning, and in ToM specifically, I hypothesised that an ASD candidate gene involved in the serotonin system would be a good gene for inclusion in my study. The serotonin transporter promoter length polymorphism (5-HTTLPR) is implicated in social functioning, and serotonin specific reuptake inhibitors (SSRIs) are well described in ASD treatment regimens. I therefore explored the relationships between core ASD symptoms and ToM performance, and a possible role for 5-HTTLPR genotypes in the ASD phenotype and in ToM performance in ASD. This chapter reviews our current knowledge regarding ToM and serotonin in ASD, followed my study details and a discussion of my findings.

Literature Review

The ToM Theory for ASD suggests that ASD is primarily a disorder in relating to and understanding others, and this is possibly due to ToM deficits that undermine the ability to understand others' thoughts, feelings, and intentions (Baron-Cohen et al., 1985). Although these deficits have been consistently noted in ASD, and some links between ToM deficits and ASD-related deficits have been explored, the exact role of ToM deficits in ASD is, as yet, not fully explored. Further, the mechanism underlying these ToM deficits is also unknown. As a social function, ToM may be sub-served by to serotonergic systems such that atypical serotonergic processes may relate to atypical ToM development or functioning. Serotonin is implicated in numerous social functions, and recent research has shown an increased interest in the role of the serotonin transporter promoter length polymorphism (5-HTTLPR) in social ability and in ASD as

a disorder, with a focus on links to ASD severity (Brune et al., 2006; Tordjman et al., 2001). Typical development of ToM and the known deficits in this area in ASD are explored below, followed by research exploring a potential role for serotonin (and 5-HTTLPR), in both ToM functioning and ASD-related deficits.

Theory of Mind

ToM refers to one's ability to understand that other people possess their own thoughts, feelings, and beliefs, that these states in others are independent of one's own mental states, and that these mental states influence other people's behaviour (Baron-Cohen et al., 1985). ToM underlies the ability to understand social situations and predict others' actions, making it crucial for adaptive social functioning (Leslie, 1987). Positive correlations between social competence and ToM have been noted in neurotypical children (Bosacki & Astington, 1999), and ToM deficits in children with ASD have been suggested as a central area of impairment that characterises this disorder (Baron-Cohen, 2002; Hoddenbach et al., 2012).

One of the most striking features of ASD is typically the impairment in areas of social communication and interaction (American Psychiatric Association, 2013; Macintosh & Dissanayake, 2006; Prior et al., 1998). An aspect of social development that appears integral to adaptive social functioning from early development and throughout the lifespan is the ability to understand one's own and others' emotions and mental states. Clinicians have consistently found that children with ASD have an impaired ability to relate to others, as well as an impaired ability to understand reciprocal social interactions (American Psychiatric Association, 2013). There is thus a particular interest in the role of ToM in ASD. Research with neurotypical children showing the expected developmental pattern of ToM, as well as its importance in social functioning, helps us understand the implications of ToM deficits in ASD.

Theory of Mind in Neurotypical Samples. The ability to understand the relationships between beliefs, emotions, intentions, and behaviours, both within oneself and in others, is an innate ability that facilitates adaptive social functioning throughout the lifespan. ToM encompasses a range of social abilities that are noted from 14 months of age (ToM precursors; Low & Perner, 2012) and then continue to develop throughout childhood (Wellman et al., 2001).

ToM development has been comprehensively studied in neurotypical samples, and a hierarchical developmental pattern has emerged (Filippova & Astington, 2008; Wellman & Liu, 2004). At as young as 14 months, children tend to engage in pretend play and to show joint attention with others, skills and behaviours considered as prerequisites for later development of ToM, and early indications of a sense of awareness of others' thoughts (Wellman et al., 2001). By 2 years of age, children will spontaneously start to talk about their own mental states and can show an understanding of others' desires (Frith & Frith, 2003). Children then develop the ability to identify the difference between appearance and reality, as well as start to understand false beliefs (i.e. that others believe something to be true that is actually false) for themselves and others, between ages 3 and 5 years old (Low & Perner, 2012; Naito et al., 1994). The ability to understand second-order beliefs, meaning one's ability to understand having beliefs about other people's beliefs, emerges between 5 and 7 years old (Liddle & Nettle, 2006). Between 6- and 10-years old, children begin to understand complex non-literal language forms such as sarcasm, irony, and metaphors, and can start to differentiate between lies and jokes based on recognition of the other person's intentions (Ackerman, 1981; Brune & Brune-Cohrs, 2006). Finally, the ability to recognise social *faux pas* develops between ages 9 and 11 years (Baron-Cohen et al., 1999). While these trajectories are generally recognised, a meta-analysis in 2008 did note that the development of false-belief reasoning in non-Western samples could differ from Western

samples by up to two years (Liu et al., 2008). These cultural variances make it important to include neurotypical groups for comparison to clinical groups when assessing ToM.

ToM abilities are well researched in samples of children, but there is relatively little focus on its possible ongoing development in adulthood (Apperly et al. 2009; Apperly, 2013). One of the possible reasons for this lack of research is that there are few ToM tasks that are appropriate for adults. As discussed above, most ToM abilities are developed in childhood, which often results in a ceiling effect when these tasks are presented to adults. Researchers can address this problem by making tasks more complex, as seen in a study looking at third-order false belief that found age was associated with performance when children, adolescents, and adults were compared across the task (Valle et al., 2015). Research can also assess accuracy in tasks, such as the study that compared ToM performance across four groups (child 7.3-9.7 years; child 9.8-11.4 years; adolescent 11.5-13.9 years; adults 19.1-27.5 years) (Dumontheil et al., 2010). Age was associated with improved ToM performance, but perhaps more interestingly, when the adolescent and adult groups were compared, they showed similar performances in the control conditions of the task, but the adults had higher accuracy in the ToM tasks. ToM performance can be influenced by executive function, but this finding suggests that ToM itself became more refined with age. Another interesting finding from a meta-analysis is that a female advantage over men exists in judging emotions or mental states exists into adulthood, at least to the degree assessed by the *Reading the Mind in the Eyes Test* (Kirkland et al., 2013). Therefore, although we do not have an exact developmental trajectory of ToM skills through adulthood, it does appear that adults have more refined ToM abilities than children, suggesting continued development to some degree, and that childhood differences across sex may continue into adulthood.

Within neurotypical samples, ToM abilities have positively correlated with social competence in children (Liddle & Nettle, 2006). A positive association between the ability to maintain friendships and ToM abilities has been noted in child and adult samples (Caputi et al., 2012; Peterson & Siegal, 2002; Slaughter et al., 2015). In adults better ToM skills were associated with a larger network of friends (Stiller & Dunbar, 2007) as well as with better employability and with career success (Hogan et al., 2013). The development of ToM in neurotypical samples is well researched, and the relationship between ToM and social competence is generally accepted.

Theory of Mind in ASD. The ToM Theory for ASD posits that the social and communication difficulties seen in ASD arise from core deficits in ToM abilities (Baron-Cohen, 2002; Baron-Cohen et al., 1999; Hoddenbach et al., 2012). When discussing social impairment in ASD, it is noted that these children often struggle to develop appropriate friendships, and their interaction is typically awkward and contains maladaptive behaviours (American Psychiatric Association, 2013). This behaviour may be explained by impairment in relating to and understanding others due to ToM deficits. ToM deficits in ASD can be seen across development from the initial absence of pretend play, to the later inability to understand the intentions and behaviours of others.

Like other symptoms characteristic of ASD, ToM deficits present on a spectrum of severity within ASD. In general, children with ASD struggle to understand differences between appearance and reality. Problems with pretend, imaginative or symbolic play include the failure to initiate these types of play, or only engaging in them minimally (Lam & Yeung, 2012). When play is entered into, children will often resort to repetitive actions that illustrate a lack of comprehension of the symbolic meaning of toys (Zillmer et al., 2008). As language develops,

these children struggle to recognise mental state words such as “think” and “know”, and often do not understand non-literal speech such as irony and metaphor (Happe, 1993; Zillmer et al., 2008).

Formalised tests have confirmed ToM deficits in children with ASD (Baron-Cohen et al., 1985; Fombonne et al., 1994; Hoddenbach et al., 2012). Many children with ASD fail false belief tasks, although between 15-55% are able to pass these tasks (Happe & Frith, 1996; Ozonoff & McEvoy, 1994). Children with ASD who are able to pass false belief tasks tend to have higher verbal IQs and Happé (1995) found that children with ASD needed a verbal mental age of 11 years 9 months to pass first-order false belief tasks. As neurotypical children start to understand false beliefs at between three and five years of age, Happé (1995) argued that children with ASD may be more dependent on language skills than neurotypical children when solving ToM problems.

The nature and degree of ToM deficits, specifically in ASD, is unclear, as some children appear to have delayed but otherwise typical ToM development (Scheeren et al., 2013; Steele et al., 2003), while others appear to have a plateau in their ToM development, although the point at which this occurs is not known (Holroyd & Baron-Cohen, 1993). A local study by Hoogenhout and Malcolm-Smith (2014) found that both theories could be true, as children with ASD who classified as high functioning in their samples showed delayed ToM development while those classified as low functioning showed atypical development, or appeared not to have developed ToM capabilities beyond a certain point. It is currently not known why some children with ASD would develop ToM abilities while others do not.

Findings on the relationships between ToM and other ASD characteristics and abilities have been inconsistent. Fombonne et al. (1994) used the Vinelands Adaptive Behaviour Scale

(VABS) to assess real life competence in an adolescent and adult ASD sample in France. They found that higher mental age, as indicated by verbal and full IQ scales, and higher chronological age were related to better performance on ToM tasks. The VABS subscale "Maladaptive Behaviours" assessed dysfunctional behaviours in everyday life, and they found that participants who were better able to understand the mental states of others showed less of these dysfunctional behaviours. A similar study in an ASD sample by Frith et al. (1994) used the VABS and supplemented it with items designed to distinguish between social behaviours that necessitated ToM and those that could be learned. They found that only those participants who could pass false belief tasks were capable of insightful interactions in daily life. These participants also exhibited better verbal and communication abilities.

More recently, Lerner et al. (2011) assessed ASD symptoms and their relationship to ToM scores on the Theory of Mind Inventory. They found ToM ability correlated positively with improved social skills, and negatively with ASD-related social impairments and ASD symptoms, indicating that deficits in ToM could underlie impairment in ASD. However, a previous study by Joseph and Tager-Flusberg (2004), which examined whether ToM ability and executive functions could explain the variance in ASD symptom severity, found that ToM did not explain the variance in impairment in social interaction or in repetitive behaviours.

The association between ToM deficits and ASD severity is therefore controversial, as is the manner of ToM testing in ASD. ToM task performance may overestimate a child's spontaneous ToM abilities (Hutchins et al., 2012; Scheeren et al., 2013). Tasks are often presented in a controlled environment and only assess certain behaviours and skills that would otherwise be needed collectively for spontaneous ToM. For instance, in a task a child may only be provided with what a character said in a story and told to infer from that what the character

was thinking. In a real-life scenario the child would also be presented with the experience of the situation, the character's facial expression, body language, tone of voice, and their own impression of, and previous experiences with, that person. The simplified, focused nature of the ToM tasks may therefore not be capable of accurately measuring real-life functional ability.

The reason for ToM deficits remains unclear, and these deficits cannot be fully explained by cognitive ability. Hill and McCune-Nicolich (1981) found a poverty of pretend play in children with ASD, but appropriate levels of pretend play in children with intellectual disability in the absence of ASD. Baron-Cohen et al. (1985) compared ToM test performance between children with ASD, children with intellectual disability without ASD, and typically developing children; they found the children with ASD were significantly more impaired compared to either of the other two groups. Although ToM deficits are not fully explained by cognitive deficits, children with ASD with better intelligence quotient (IQ) scores do tend to perform better on these tasks, and it is possible that children with ASD rely more on compensatory cognitive strategies to solve ToM tasks than neurotypical children do (Bauminger & Kasari, 1999; Tager-Flusberg, 2007).

A further limitation of ToM research in ASD is an over-reliance on false belief testing (Liddle & Nettle, 2006; Peterson, Wellman, & Slaughter, 2012). Neurotypical children are generally able to pass first-order false belief testing from 5-7 years old, so samples of older children may not show variability in their scores for this task. Although these tests have shown a clear delay and/or deficit in ToM development in older children with ASD, the possible absence in score variability for neurotypical children is a limitation in studies wishing to compare ASD and neurotypical children's performances. This binary assessment of ToM ability where one either fails or passes false belief tasks is also far too simplistic given the recognition of ongoing

ToM development in neurotypical children that shows far more complex ToM skills in later childhood.

ToM deficits are therefore often present in ASD, although this is not always the case. However, their high prevalence in ASD suggests that ToM deficits could be a core element of ASD. Clarity is therefore needed regarding the relationships between ToM abilities and ASD-related deficits. As the aetiology of both ToM deficits and of ASD are unclear, further investigations are needed into the possible foundations of these deficits. As ASD is likely to arise from multiple genetic contributions, and ToM deficits appear inherent in ASD, it is possible that a shared genetic foundation exists; that is, it is possible that one or more ASD candidate genes directly underlie the ToM deficits seen in this disorder.

Aetiology of Theory of Mind Deficits. Studies of typical and atypical development of ToM have revealed several factors that can affect ToM abilities. ToM development may be influenced by culture (Shahaeian et al., 2014) and tracking ToM development globally is challenging as not all ToM tests are appropriate across cultures (Hamilton et al., 2016). Socio-economic status (SES) has also been linked to ToM abilities (Liddle & Nettle, 2006), and developing ToM abilities are intertwined with executive function, general intelligence, and language capabilities (Hamilton et al., 2016).

Deficits in ToM are not unique to ASD and have been found in numerous other disorders, but the aetiology of these deficits is still poorly defined. ToM deficits have been noted in psychiatric conditions such as mood disorders (Wolkenstein et al., 2011), psychotic disorders (Pos et al., 2015), and schizophrenia (Brune, 2003). There is also a growing body of research noting ToM deficits in degenerative disorders such as Parkinson's Disease (Bodden et al., 2010)

and Huntington's Disease (Brüne et al., 2011), as well as after traumatic brain injury (Bibby & McDonald, 2005).

Attempts to localise ToM functioning have shown numerous neural regions are implicated this ability, possibly due to the involvement of both affective and cognitive elements (Abu-Akel & Shamay-Tsoory, 2011). Regions that have consistently been associated with ToM processing include: frontal regions (dorsal medial prefrontal cortex, dorsal lateral prefrontal cortex, inferior lateral frontal cortex); posterior regions (the temporoparietal junction, posterior cingulate cortex, superior temporal sulcus); and, limbic and paralimbic regions (orbitofrontal cortex, ventral medial prefrontal cortex, anterior cingulate cortex, temporal pole, amygdala, striatum) (Ahmad Abu-Akel, 2003; Boomsma et al., 2008; Brunet-Gouet & Decety, 2006; Carrington & Bailey, 2009; Frith & Frith, 2006; Saxe, 2006).

A purely neuroanatomical model for ToM faces limitations as these regions are not all implicated in the various disorders characterised by ToM deficits, and they do not explain the variations in ToM deficits seen within each disorder (Ahmad Abu-Akel, 2003). A "ToM Network" has been proposed that includes the medial prefrontal cortex, the posterior cingulate cortex, and bilateral temporoparietal junctions (Abu-Akel, 2003; Kana, 2015). Disruptions to serotonergic and dopaminergic systems have been associated with ToM deficits, and it is possible that differences in the transmission of these neurotransmitters within this network (i.e. between implicated anatomical areas) could explain the variance in ToM deficits within and across disorders. Serotonin is therefore of interest when assessing possible aetiologies of ToM deficits.

Serotonin and Theory of Mind. There is some evidence that serotonin system may be directly involved in ToM processes. A functional brain imaging study found a relationship between a specific allele for the serotonin 1A receptor (5-HT1A-R) and poor performance on

ToM tasks in a sample of 119 clinically stable schizophrenic patients (Bosia et al., 2011). This allele is associated with disruptions between dopamine and serotonin in the prefrontal cortex. This study utilised a neuropsychological battery with a ToM picture sequencing task that assessed first-order false belief reasoning, second-order false belief reasoning, and tactical deception abilities to provide a global ToM score indicating overall ability. Serotonin may therefore be associated with disruptions in multiple aspects of ToM.

Murphy et al. (2006) found a significant reduction in cortical binding of another serotonin receptor, the 5-HT2A receptor, in the anterior cingulate cortex, posterior cingulate cortex, and right frontal cortex in eight men with ASD. Using the Autism Diagnostic Interview – Revised (ADI-R) to rate ASD-related deficits, they found significant correlations between this reduced cortical binding and deficits in reciprocal social interactions, which included skills requiring ToM processing. This suggested that decreased serotonin levels would be associated with reduced ToM capabilities in reciprocal social interactions, which included skills requiring ToM processing. This suggested that decreased serotonin levels might be associated with reduced ToM capabilities

Nakamura et al. (2010) assessed serotonin transporter binding in 22 men with ASD using serial PET scans. They reported lower serotonin transporter binding throughout the brain when compared to controls. They also found an association between impairment on a ToM *faux pas* task and this reduced transporter binding in the anterior cingulate cortex and in the posterior cingulate cortex. As with the previous studies, these results suggested that decreased serotonin may be associated with ToM abilities of varying complexities.

There is therefore emerging evidence that serotonin is implicated in ToM processes. As ToM deficits appear to be a critical element of the ASD symptom presentation, it is worth

investigating the role of serotonin in ASD, and possibly a joint role for serotonergic transmission and ToM deficits as contributing factors to ASD.

Serotonin and ASD

Serotonin is implicated in ASD, and within the serotonergic system the serotonin transporter promoter length polymorphism (5-HTTLPR) has been suggested as an ASD candidate gene (Arieff et al., 2010; Brune et al., 2006; Tordjman et al., 2001). This polymorphism regulates the transcriptional efficacy of serotonin in the central nervous system. Serotonin is also linked to social functioning in neurotypical children and adults and has been implicated in ToM processing (Abu-Akel, 2003). Serotonin may therefore underlie the poor social functioning characteristic of ASD, and may in particular underlie the ToM deficits seen in this disorder. Two studies have investigated genotype-phenotype relationships for 5-HTTLPR in ASD (Brune et al., 2006; Tordjman et al., 2001), but further research is needed to include ToM ability in such studies.

Atypical functioning of the serotonergic system is strongly implicated in ASD. Approximately one third of individuals with ASD present with hyperserotonemia (i.e. elevated serotonin levels) when whole blood is assessed (Hanley et al., 1977; Kolevzon et al., 2006). First degree relatives of individuals with ASD also present with hyperserotonemia in 25-50% of cases (Burgess et al., 2006; Cook & Leventhal, 1996). The elevated serotonin levels in ASD are typically detected in platelets but not in the plasma (Cook & Leventhal, 1996) or in cerebrospinal fluid (Adamsen et al., 2010). This may indicate that although excessive serotonin is being produced by some individuals with ASD, the neurotransmission is undermined at some point in the serotonergic system, resulting in different concentrations being detected in the plasma and cerebrospinal fluid.

Despite the hyperserotonemia seen in the platelets in ASD cases, ASD presentations are more in keeping with that of hyposerotonemia. ASD shares symptomatology with disorders associated with decreased serotonin levels, including depression, anxiety disorders, shyness, and social phobias (Klauck et al., 1997). ASD symptoms are also aggravated when serotonin transmission is decreased through tryptophan depletion (Cook & Leventhal, 1996; McDougle et al., 1996).

The paradoxical picture of elevated whole blood levels of serotonin but a symptom presentation more in keeping with decreased serotonin levels, as well as the difference in serotonin levels between platelets, plasma, and cerebrospinal fluid, might be explained by poor transcriptional efficacy. As the 5-HTTLPR genotypes regulate the transcriptional efficacy of the serotonin transporter protein, this gene is therefore of interest in ASD.

5-HTTLPR. 5-HTTLPR is a variable repeat sequence in the promoter region of the serotonin transporter protein (SERT, 5-HTT). SERT is located on the pre-synaptic membranes of serotonergic neurons (Sen et al., 2004) and acts to reabsorb serotonin by moving it from the synaptic space back into the presynaptic neuron (Kolevzon et al., 2006). The transcriptional efficacy of SERT is moderated by 5-HTTLPR genotypes which determine the different expressions of SERT in the pre-synaptic axonic membranes (Adamsen et al., 2010; Arbelle et al., 2003).

The transcriptional efficacy of 5-HTTLPR differs depending on its alleles. 5-HTTLPR has several rare allele variants, but the common alleles are the long (*L*) allele and the short (*S*) allele (Huang & Santangelo, 2008). The three most common genotypes are therefore *L/L*, *L/S*, and *S/S*. The long allele consists of 528 base pairs and 16 repeat elements, while the short allele consists of 484 base pairs and 14 repeat elements. The short allele has reduced transcriptional

efficacy compared to the long allele and can reduce the availability of serotonin by up to 50% (Huang & Santangelo, 2008). It is not clear whether the long allele or the short allele, if either, have a dominant effect.

Although the allelic distribution of 5-HTTLPR differs to some degree across ethnicity groups due to different genetic heritages, the long allele is usually the most common in neurotypical populations (Arbelle et al., 2003; Esau et al., 2008; Jacob et al., 2004; Klauck et al., 1997; Reneman et al., 2006). In a global comparison of the allelic distribution of 5-HTTLPR, Esau et al. (2008) reported that the *S/S* genotype tended to be present in less than 20% of neurotypical samples, although some Asian samples showed a higher rate of the *S/S* genotype. Asian samples reported in the Esau et al. (2008) review presented with the *S/S* genotype at the following rates: a Japanese sample at 65%; a Chinese sample at 54%; and an Indian sample at 47%. More recent studies have replicated these high rates of the short allele for a Japanese sample (Endo et al., 2010) and a Korean sample (Cho et al., 2007).

5-HTTLPR in ASD. The role of 5-HTTLPR in ASD is not clear. The 5-HTTLPR allelic distribution for ASD populations is not generally known, although limited research has reported that ASD samples have a higher prevalence of the *S/S* genotype than the 20% reported in most neurotypical samples. A local study also found a higher incidence of the short allele for an ASD sample compared to neurotypical samples (Arieff et al., 2010), and this was replicated in a master's study (Hamilton, 2014). Table 34 below shows the 5-HTTLPR genotype and allelic distribution from four studies with ASD samples, including two local studies; ethnicity has been included where available.

Table 34.

Allelic and Genotype Distribution of 5-HTTLPR in ASD Samples

Studies	5-HTTLPR Genotype Distribution (%)			5-HTTLPR Allelic Frequencies		Sample
	L/L	*L/S*	*S/S*	*L*	*S*	
Arieff et al. (2010)						South African; 96 males, 13 females.
Total (*N*=109)	56 (51.38%)	20 (18.35%)	33 (30.28%)	.61	.39	
African (*n*=21)	13 (61.90%)	1 (4.76%)	7 (33.33%)	.64	.36	
Caucasian (*n*=40)	17 (42.50%)	7 (17.50%)	16 (40.00%)	.51	.49	
Mixed / Coloured (*n*=48)	26 (54.17%)	12 (25.00%)	10 (20.83%)	.67	.33	
Brune et al. (2006)						American; 59 males, 14 females; Racially
Total (*N*=73)	21 (28.77%)	36 (49.32%)	16 (21.92%)	.53	.47	diverse (60 Caucasian, 5 African
						American, 6 Asian, 2 Hispanic).
Hamilton (2014)						South African; 49 males, 5 females.
Total (*N*=54)	18 (33.33%)	22 (40.74%)	14 (25.93%)	.54	.46	
African (*n*=5)	1 (20.00%)	4 (80.00%)	0 (0.00%)	.60	.40	
Caucasian (*n*=22)	5 (22.73%)	10 (45.45%)	7 (31.82%)	.45	.55	
Mixed / Coloured (*n*=18)	9 (50.00%)	4 (22.22%)	5 (27.78%)	.61	.39	
Indian (*n*=5)	2 (40.00%)	2 (50.00%)	1 (20.00%)	.60	.40	
Other (*n*=4)	1 (25.00%)	2 (50.00%)	1 (25.00%)	.50	.50	
Tordjman et al. (2001)						French; 46 males; 25 females; All
Total (*N*=71)	26 (36.62%)	35 (49.30%)	10 (14.08%)	.61	.39	Caucasian

Caucasian (*n*=71)	26 (36.62%)	35 (49.30%)	10 (14.08%)	.61	.39

Notes. ASD = Autism Spectrum Disorder.

While genetic studies to date have tended to focus on whether ASD samples showed a different allelic distribution for 5-HTTLPR from neurotypical samples, some studies have moved toward understanding what role, if any, 5-HTTLPR genotypes play in the ASD phenotype. To my knowledge, only three studies have been conducted in this area: one study explored the link between 5-HTTLPR and brain region volumes (Wassink et al., 2007), and two have specifically assessed for genotype-phenotype relationships (Brune et al., 2006; Tordjman et al., 2001).

The first study explored possible associations between 5-HTTLPR genotypes and cortical volume in ASD but did not attempt to assess genotype-phenotype relationships. Wassink et al. (2007) conducted a longitudinal MRI study with 44 male, Caucasian children with ASD, and assessed brain region volumes in these children across the 5-HTTLPR genotypes. They found that the short allele was associated with increased frontal lobe grey matter volume and total cortical volume. Increased cortical volume could indicate poor synaptic pruning processes, which undermine neuroprocessing across brain regions. The Wassink et al. (2007) study could therefore suggest a role for 5-HTTLPR in brain development in ASD. Although this study did not include a control group of neurotypical children, its findings are aligned with the literature as other studies have also noted increased grey matter volumes in ASD participants (Cauda et al., 2011; Duerden et al., 2012).

Tordjman et al. (2001) investigated whether 5-HTTLPR genotypes were associated with ASD severity in a sample of 71 Caucasian French children with ASD. They used the Autism Diagnostic Interview-Revised (ADI-R) to rate severity of impairment in the social communication and interaction symptom domain. They noted similar ADI-R scoring between the children with the *L/S* genotype and the *S/S* genotype, and therefore combined these groups for statistical comparisons to the *L/L* genotype group, with the hypothesis that there was a dominant

effect for the short allele and therefore one or more short alleles would undermine serotonergic transmission and affect ASD outcomes. ADI-R scores showed that children in the short allele group presented with severe impairment, while those in the *L/L* genotype group showed scores in the mild to moderate impairment range. Tordjman et al. (2001) posited that their findings supported a role for 5-HTTLPR in modifying the overall severity of deficits seen in ASD, specifically in the symptom domain of social communication and interaction.

Brune et al. (2006) furthered this research by assessing whether 5-HTTLPR genotypes were associated with specific ASD-related deficits in a sample of 73 children with ASD. They used the ADI-R and the ADOS to rate ASD-related deficits. Their genotype distribution was similar to that of the Tordjman et al. (2001) study (see Table 34, pg.139), and they also merged all short allele carries (i.e. *L/S* genotype carriers and *S/S* genotype carriers) into a reduced transmission group for comparison to the *L/L* genotype group. Despite these similarities to the Tordjman et al. (2001) study, they were unable to replicate the finding that ASD severity indicated by overall ADI-R scores was associated with the short allele. Instead they found that children in the reduced transmission group had more severe impairment in the "failure to use non-verbal communication to regulate social interaction" ADI-R domain. By contrast, the children in the *L/L* genotype group showed greater impairment in the ADI-R domain "stereotyped and repetitive motor mannerism" and "aggression", as well as for "failure to direct facial expressions" and for increased unusual sensory interests as assessed by the ADOS. Brune et al. (2006) posited that rather than the short allele being implicated in overall severity, the different 5-HTTLPR alleles were associated with different aspects of ASD. They suggested that the short allele was specifically associated with impairment in non-verbal aspects of social interaction.

In the absence of further studies which directly assessed ASD-related symptoms across 5-HTTLPR genotypes, studies which use medications that target 5-HTTLPR in ASD samples were consulted to provide indirect support for these relationships. Many selective serotonin-reuptake inhibitors (SSRIs) alter serotonin levels by increasing the time serotonin spends in the synaptic cleft by influencing the function of 5-HTTLPR (Kolevzon et al., 2006). Although SSRIs are often included in treatment programmes for patients with ASD, there is inconsistency in the results regarding the efficacy of these medications in this condition. When these medications do show a favourable outcome in ASD patients, they are associated with improvement in verbalisations, increased communicative gestures, and improved motor skills (Adamsen et al., 2010; Kolevzon et al., 2006). The effects of SSRIs on symptoms in the restricted and repetitive behaviours and interests symptom domain have also been mixed, with some studies supporting treatment (Namerow et al., 2003; Hollander et al., 2005), but another found no effect for this treatment (King et al., 2009). A review of the literature found that while SSRIs can have a general positive effect in ASD, and sometimes a specific effect on restricted and repetitive behaviours and interest, but the effect is usually small (Carrasco et al., 2012).

Sugie et al. (2005) compared the effects of SSRI treatment across 5-HTTLPR genotypes in a sample of 18 Japanese children with ASD. Random assignment was used to allocate ten children to the experimental group, where they were prescribed the SSRI fluvoxamine, and nine children were placed in the placebo group; these groups were then reversed to form a two-way crossover study design. They utilised the Behavioural Assessment Scale (BAS) to rate ASD-related deficits. Genotyping found that one child had the *L/L* genotype, 7 children had the *L/S* genotype, and 10 had the *S/S* genotype. Due to the low incidence of the *L/L* genotype, participants were grouped according to the presence of a long allele (i.e. *L/L* genotype and *L/S*

genotype were grouped), rather than according to the presence of a short allele as was done in the previously described genotype-phenotype studies. Sugie et al. (2005) found that the long allele group showed greater global improvement than the *S/S* genotype group. When specific symptoms were assessed, they found that the long allele group improved in emotion expression while the *S/S* genotype group showed improved eye movement.

The number of studies investigating the exact role of 5-HTTLPR in ASD presentations is limited, and results are inconsistent or unclear due to different methodologies. Genetic studies tend to support a higher incidence of the short allele for 5-HTTLPR in ASD, indicating a mechanism that would undermine serotonergic transmission in this group. Phenotype studies have found links between this short allele and social impairment in ASD, but further research is needed to elucidate the role of this polymorphism.

Conclusion

The ToM Theory for ASD proposed that individuals with ASD have ToM deficits, and these deficits explain the impairment in social functioning. ToM deficits are often noted in ASD, although the degree of these deficits varies. Some research supports an association between these deficits and social competence, particularly in neurotypical samples, and this could extend to ASD samples. The mechanisms underlying ToM deficits are not understood, but serotonin is implicated in ToM and in social functioning, with 5-HTTLPR being a polymorphism of specific interest in ASD. To my knowledge, research assessing ToM abilities and possible associations with 5-HTTLPR genotypes in the context of ASD has not been conducted, but phenotypic studies have found associations between these genotypes and social functioning in ASD. More research is needed on the link between specific aspects of ASD symptom profiles and 5-

HTTLPR genotypes, and it would be of interest to include ToM in the assessment of ASD-related deficits as it may be a core aspect of this disorder.

Rationale

ASD remains a prevalent but complex disorder with an incompletely understood aetiology and high variability in clinical presentations. Further research into underlying mechanisms and into the development of deficits is required to allow for the development of more targeted management strategies. ToM has been proposed as a core area of deficit in ASD, but the degree of deficit compared to neurotypical peers, and the relationship between ToM deficits and other ASD-related deficits, remain inadequately explored. The exact role of ToM skills in overall ASD social competence must be established, and this must be done in a developmentally-sensitive manner that not only accounts for the role of age across childhood, but which also considers the hierarchical development of ToM, rather than simply focusing on a single aspect of ToM, such as false belief reasoning.

Further, our understanding of the causes of ToM deficits in ASD and other disorders is also incomplete. As ToM deficits are so common in ASD, it is possible that ASD and ToM deficits (in ASD and other disorders) share, to some extent, a neurobiological underpinning. Numerous neuroanatomical areas and neurochemical pathways are implicated in ToM, and hundreds of genes have been implicated in ASD, but the serotonin system is implicated in both ASD and ToM processes, and 5-HTTLPR genotypes are specifically implicated. It is therefore important to explore the role of ToM deficits in the ASD-phenotype, and to explore what role specific aspects of the serotonin system, including 5-HTTLPR genotypes, may play in ToM and ASD-related deficits. As medications already exist to target 5-HTTLPR, a better understanding of its phenotypic role may have direct implications for management plans.

Aims and Hypotheses

This study therefore aimed to explore the role of ToM in ASD, and whether 5-HTTLPR genotypes were associated with ASD deficits and/or ToM deficits. Due to the verbal nature of the ToM battery, this study only included the verbal ASD group in the analyses for ToM, although all ASD participants who provided DNA were included when assessing for relationships between ASD symptoms and 5-HTTLPR. I predicted that the ASD group would have less developed ToM capabilities than the neurotypical group. I also hypothesised that ASD children carrying one or more short allele for 5-HTTLPR would have greater ASD-related deficits, as measured by the ADOS2, and would perform more poorly on ToM tasks than ASD children who did not carry any short alleles. I therefore asked the following questions, and addressed them with the associated hypotheses:

Question 1. Do children with ASD exhibit lower levels of ToM than neurotypical children?

Hypothesis 1.1. Children with ASD will have lower ToM scores than neurotypical children.

Question 2. Do ToM deficits relate to greater ASD-related deficits in children with ASD?

Hypothesis 2.1. Lower ToM scores will predict higher ADOS2 scores.

Question 3. Does 5-HTTLRP play a role in ASD-related deficits and/or ToM deficits in an ASD sample?

Hypothesis 3.1. Children with ASD will show a higher rate of the 5-HTTLPR short allele, which indicates atypical serotonergic transmission, than is reported for neurotypical samples.

Hypothesis 3.2. ASD children carrying the short allele for 5-HTTLPR will present with higher ADOS2 scores than those without a short allele.

Hypothesis 3.3. ASD children carrying the short allele for 5-HTTLPR will score lower in ToM tests than those without a short allele.

Hypothesis 3.4. In children with ASD, the 5-HTTLPR short allele will be associated with poor ToM performance, and ToM performance will mediate a relationship between 5-HTTLPR genotypes and ADOS2 scores.

Method

Research Design

The Theory of Mind Study was the second of two studies in this protocol. This study had the same design as the Social Motivation Study (Chapter 3: The Social Motivation Chapter - Method, pg.52) as it had a cross-sectional relational design. This study assessed the degree of ToM deficits in ASD, the possible associations between ToM deficits and ASD-related deficits, and then assessed whether 5-HTTLPR genotypes were implicated in ASD-related deficits and/or ToM deficits.

Participants

Participants were drawn from the Social Motivation Study (Chapter 3: The Social Motivation Chapter – Method – Participants, pg.53) sample and formed the same three groups (i.e. non-verbal ASD, verbal ASD, neurotypical). The recruitment of these participants into this second study is shown in Figure 6.

The non-verbal ASD group was included in analyses for genotype-phenotype associations (i.e. associations between ADOS2 scores and 5-HTTLPR genotypes; Question 3), so only those who provided DNA were included. In the Social Motivation Study 34 non-verbal ASD participants provided DNA and were successfully genotyped for 5-HTTLPR and were included in the current study.

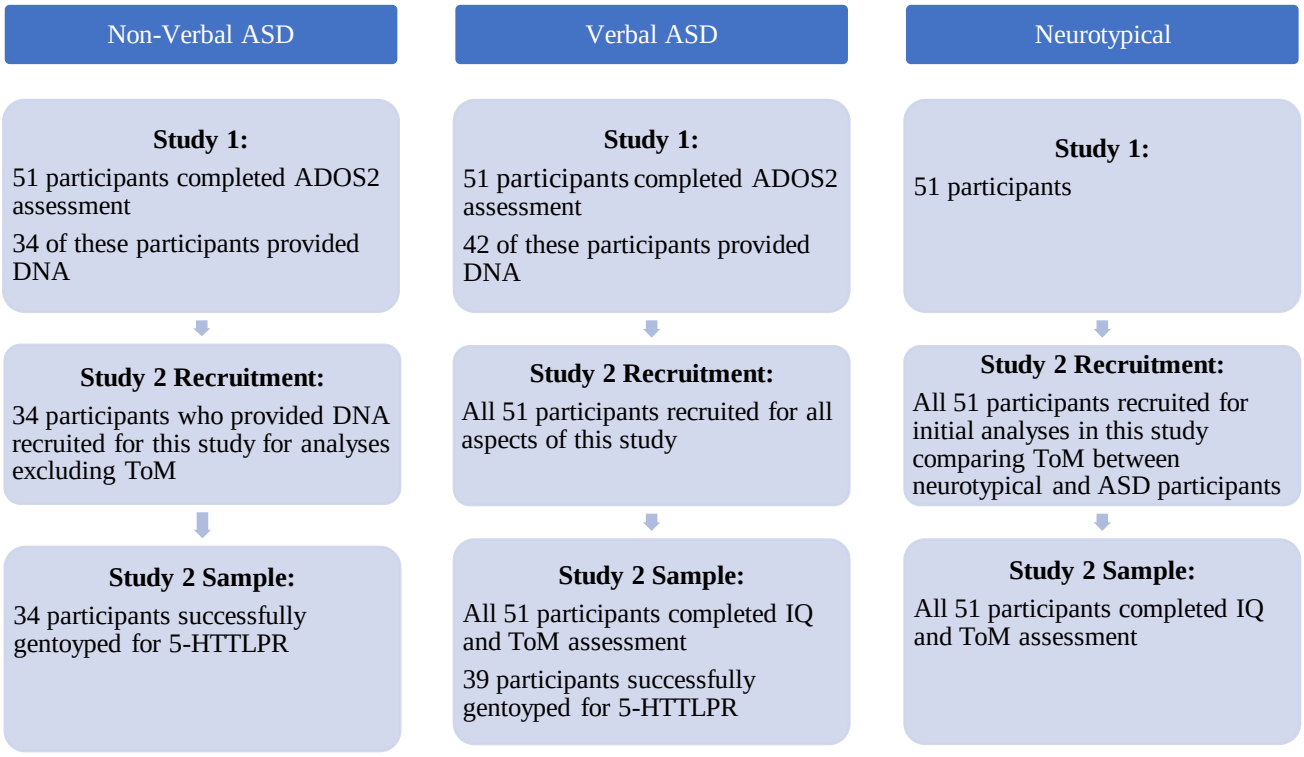

Figure 6. Samples for the Theory of Mind Study drawn from the Social Motivation Study

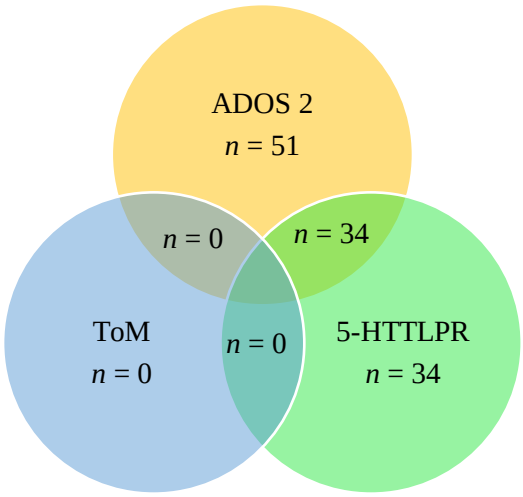

Figure 7. Non-verbal ASD group data collected per measure, and sample available for each research question

All children from the verbal ASD group completed ToM assessment. There were therefore 51 children available for Question 1 (i.e. ToM performance comparison between ASD and neurotypical samples) and for Question 2 (i.e. possible associations between ADOS2 and ToM scores). Of these children, 42 provided DNA; 39 were successfully genotyped for 5-HTTLPR. This left a sample of 39 verbal children with ASD for Question 3 (i.e. genotype-phenotype associations between ADOS2 scores and 5-HTTLPR, and ToM scores and 5-HTTLPR).

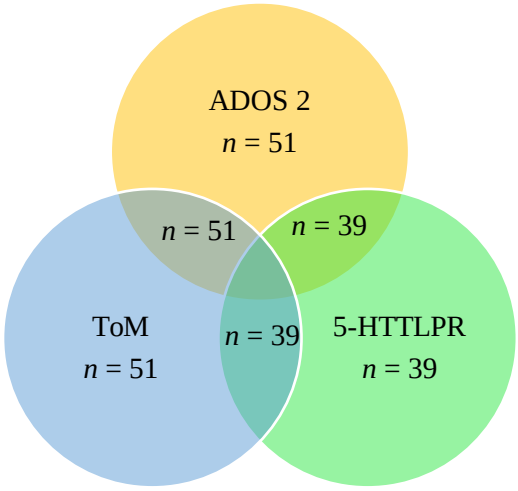

Figure 8. Verbal ASD group data collected per measure, and sample available for each research question.

All neurotypical children completed the ToM assessment and served as a control group for Question 1, resulting in a sample size of 51 participants. (For full participant information see Chapter Three: The Social Motivation Study – Results, pg.76).

Given the limitations on recruitment when working with a clinical sample, and that my exclusion criteria further limited this pool, *a prioiri* power analyses were not relied on for sample sizes. Instead, recruitment aimed to exhaust the possible recruitment pool, and to approximate the sample sizes in the literature for genotype-phenotype studies with ASD samples. The sample

sizes for both studies in this protocol were decided based on studies with a similar design to the Theory of Mind Study, as genotype-phenotype studies for OPRM1 in ASD were not found. The two genotype-phenotype studies for 5-HTTLPR in ASD research were consulted (Brune et al., 2006; Tordjman et al., 2001). These studies conducted ADI-R parent interviews and genotyping on 71 children (Tordjman et al., 2001), or ADI-R parent interviews and ADOS assessment with genotyping on 73 children (Brune et al., 2006). My full protocol included parent interviews and ADOS2 assessments, much like these two studies, and therefore aimed to recruit a minimum of 80 child-parent pairs; a total of 102 ASD child-parent pairs were recruited, and DNA was obtained from 76 children within this sample.

Procedure

The Theory of Mind Study was an extension of the Social Motivation Study, and therefore had the same initial procedure; that is, participants were recruited, screened, and ASD participants completed ADOS2 assessment as part of the first study and relevant data was included in both studies. Non-verbal participants did not need to complete any further data collection for the current study as DNA collected for the first study was sufficient for further genotyping for 5-HTTLPR. The verbal ASD participants and neurotypical participants completed IQ and ToM assessments to provide data for this protocol. Parents did not need to provide any further data.

Child Sessions. Once children were allocated to their groups, child sessions were conducted at their schools or at their homes for children with ASD who were not currently placed at schools. Non-verbal children with ASD did not need to complete further sessions as their ADOS2 data had been collected during screening. Verbal children with ASD and neurotypical children were seen three to four times to complete IQ assessment and

comprehensive ToM assessment; each session was 30-60 minutes long and duration of the session was flexible, based on the child's age and attention span to avoid fatigue.

Measures

The non-verbal ASD children only completed the ADOS2, and no other child-measures were administered. The verbal ASD and neurotypical sample completed additional assessment for IQ and ToM data, and the ADOS2 data for the verbal ASD group had been collected in the first study.

General intellectual functioning. Participants from the verbal ASD group and neurotypical group completed IQ assessments and verbal IQ (VIQ) percentile scores were used in analyses due to the known relationship between VIQ and ToM performance (Fombonne et al., 1994; Happe, 1995). These participants completed the Wechsler Abbreviated Scale of Intelligence (WASI; Weschler, 1999).

The WASI consists of four subtests that are collectively scored for an intelligence quotient (IQ) estimate. Two subtests assess VIQ, namely the *Similarities* and the *Vocabulary* subtests, and two assess performance IQ, namely the *Block Design* and the *Matrix Reasoning* subtests (Weschler, 1999). The WASI is designed for use in individuals 6-89 years old. Test-retest reliability scores range from .92-.95, and validity has been comprehensively assessed and found to be appropriate (Stano, 2004; Weschler, 1999). The WASI has been translated and adapted for some non-English populations (Abu-Hilal et al., 2011; Trentini et al., 2014), and Afrikaans translations have been used in local research (Ferrett et al., 2010). For this study I administered the WASI in English, as per the ADOS2 assessment.

Theory of Mind Assessment. Participants from the verbal ASD group and the neurotypical group completed the UCT ToM Battery (Hoogenhout & Malcolm-Smith, 2014).

This battery has four modules of increasing difficulty and allows comprehensive assessment of ToM with a developmental approach (Appendix M). This battery consists of recognised ToM tasks from the literature (Baron-Cohen et al., 1999; Peterson et al., 2012; Steele et al., 2003) that have been adapted for use in the South African population.

The first module is termed the Early Module and assesses the most rudimentary ToM skills. This module consists of the *Desire task* (understanding intentions), the *Pretend Play task*, the *Perception-Knowledge task*, the *Diverse Desires task*, and the *Diverse Beliefs task*. The Basic Module consists of the *Location-Change False Belief task*, the *Unexpected-Contents False Belief task*, the *Belief-Emotion* tasks, and the *Real-Apparent Emotion task*. The Intermediate Module consists of the *Second-Order False Belief task* and the *Strange Stories task*. The Advanced Module of this ToM battery consists of the *Lies and Jokes task* and the Children's Version of the *Faux Pas task*. This battery is appropriate for children as young as 2 years as pretend play develops in neurotypical children between 14 and 24 months (Frith & Frith, 2003), and assesses ToM development up until age 11 years, which is when neurotypical children generally develop the ability to recognise social faux pas (Baron-Cohen et al., 1999).

All tasks except the *Pretend Play task* include control and test questions, and if children fail the control questions their ToM scores are voided as they may not have understood the task. Other than the *Faux Pas task*, all tasks either used dolls or pictures to minimize linguistic and memory demands. Each module was worth 25% of the total possible score for the battery, and within each module tasks were weighted equally. Children were therefore able to achieve a total overall score which indicated their level of ToM development. Children started the battery at either the Basic or Intermediate Module, based on their age. If they achieved below 50% for this module they reversed to earlier modules, but if they achieved 50% or more for the first module

they were credited 100% for earlier modules and moved to the next module, continuing until either completing the battery or failing a module.

This raw scoring did not take age into account. Children were therefore divided into three age bands: 6-7 years, 8-10 years, and 11+ years. The neurotypical group's age bands were considered as controls, and means and standard deviations from these age bands were used in the neurotypical and ASD samples to calculate *z*-scores for each participant. These scores were then converted into percentiles to allow for direct comparisons to be made between all participants, and for consistency in analyses as VIQ percentiles were always considered alongside ToM scores. The use of percentile ToM scores allowed us to report scores that were developmentally relevant, and to directly compare the scores between the two groups.

DNA Collection and Genotyping. DNA extracted for Study One was also genotyped for 5-HTTLPR. For DNA extraction details and Direct (Sanger) sequencing details see Chapter 3: The Social Motivation Study – Methods – DNA Collection and Genotyping, pg. 70.

5-HTTLPR Genotyping. All reagents and kits were used according to the manufacturer's instructions. 5-HTTLPR was amplified with primer sequences from Klauck et al. (1997) and GoTaq G2 Taq polymerase (Promega, Madison, WI, USA). Amplicons were separated on a 2.5% agarose gel and fragments sizes were estimated by comparison to a DNA ladder (GeneRuler 100bp Plus; Thermo Fisher Scientific, Johannesburg, South Africa). Genotypes were classified as long (*L*) or short (*S*) alleles, with the latter amplicon 43 bp shorter than the reference allele. Selected genotypes were confirmed with DNA cycle sequencing. DNA was amplified as before and products were purified with 1U each of *Exo*I and FastAP (both from Thermo Fisher Scientific) according the manufacturer's protocols. DNA sequencing was performed with a BigyDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) according to the

manufacturer's instructions. Capillary electrophoresis was performed on a 3130*xl* Genetic Analyzer (Applied Biosystems) using standard run conditions. DNA sequencing data was analysed with Sequencing Analysis v5.4 software. Direct (Sanger) Sequencing used to confirm subset of genotypes for 5-HTTLPR with primer sequences from Klauck et al. (1997).

Analyses

All statistical analyses were run using IBM SPSS Statistics, Version 25 (IBM, 2017). All verbal ASD and neurotypical participants from the Social Motivation Study were included in the current study. Of the 51 non-verbal participants in that study, 34 were successfully genotyped for 5-HTTLPR and were included in Question 3 with the verbal ASD group. The verbal ASD and neurotypical groups had already been assessed for group differences in demographic variables, and were aggregate matched for age and SES (Chapter 3: The Social Motivation Study - Results Participant Characteristics, pg. 76). As the non-verbal group for the current study was a sub-sample from the previous study, this group was compared to the verbal ASD group for differences in age and SES. It was expected that the non-verbal ASD group would be younger on average, which is to be expected due to the nature of language delays in ASD, so where necessary age was included the analyses.

The Theory of Mind Study asked three main questions, with a set of hypotheses for each question. For this study ADOS2 scores were again used to indicate level of ASD-related deficits. ToM scores were calculated across a developmental battery and compared to neurotypical scores to establish age-adjusted percentile scores. These ToM percentiles were continuous with higher scores indicating better ToM ability. Finally, 5-HTTLPR genotypes were processed for a subset of the ASD participants, and the presence of at least one short allele was indicative of reduced

serotonergic transmission. All assumptions for each statistical test were assessed and the analyses only continued if assumptions were upheld, or if the test would be robust against such violations.

The first question for this study assessed whether male children with ASD had lower ToM scores than age, and SES matched male neurotypical children. This question utilised data from the verbal ASD group and the neurotypical group. First, the percentiles for ToM scores were calculated (see Chapter 4: The Theory of Mind Study – Method – Measures – Theory of Mind Assessment, pg. 151 for procedure). I then conducted an ANCOVA for ToM percentiles across groups with VIQ percentile as the covariate to assess for group differences in ToM performance. Further analyses to explore the nature of the relationship between VIQ and ToM were also conducted with an MRA for each group with age, SES, and VIQ percentile as predictors of ToM percentile score.

The second question for this study assessed for associations between ToM deficits and ASD-related deficits. These analyses were limited to the verbal ASD group. Using partial correlations that controlled for age, correlations between ToM and ADOS2 scores were assessed.

The third question looked at the possible role of 5-HTTLPR in ASD-related deficits and ToM deficits in ASD. A subsample of ASD participants provided data for genotyping, and the genotype and allelic distribution for this subsample was reported for the non-verbal ASD and verbal ASD groups for comparison to the literature, with an expectation of a higher incidence of the short allele. Data was reported for each ASD group and across ethnic groups. To assess for relationships between 5-HTTLPR and ASD-related deficits MRA was conducted for each ASD group with dummy variables for the 5-HTTLPR genotypes. In these MRAs age, SES, and genotype dummy variables were predictors of ADOS2 scores. To assess for a relationship between 5-HTTLPR and ToM, analyses were limited to the verbal ASD participants who

provided DNA samples. Here MRA was conducted with age, SES, VIQ percentile, and genotype as predictors of ToM scores. Finally, if above analyses found significant associations, MRA models would assess whether ADOS2 scores could be predicted by age, SES, genotype, VIQ and ToM, such that ToM could mediate the relationship between genotypes and ADOS2 outcomes.

This study is one of a very small body of genotype-phenotype studies for 5-HTTLPR, and the first to my knowledge to include ToM in the phenotypical assessment with an ASD sample, so the analyses are exploratory in nature. Although the high number of analyses do increase the possibility of Type 1 error, this was necessary due to the exploratory nature of this study. To allow possible associations to emerge, I set alpha to 0.05, as is the convention. I also included effect size and power analyses where appropriate as an additional estimation of the associations seen. All such findings would ideally be further explored by later confirmatory studies

Ethical considerations

This study was part of a two-study protocol, and all ethical considerations are detailed in Chapter Three: The Social Motivation Study (Ethical Considerations, pg. 72). The only additional consideration in the current protocol was that children from the verbal ASD group and the neurotypical group completed ToM and IQ assessment and could therefore have experienced fatigue. Children were given breaks or sessions were shortened whenever necessary to prevent fatigue, and children were able to end a session and/or withdraw from the study at all times. Non-verbal children with ASD received research reports detailing their ADOS2 performance as part of the Social Motivation Study (Chapter Three: The Social Motivation Study – Method – Ethical Considerations, pg. 72). Verbal children also received an ADOS2 research report, but this was supplemented with details from their WASI performance from the current study.

Neurotypical children received a research report with details from their WASI and ToM performance.

Results

Participant Characteristics

The participants for this study were drawn from the Social Motivation Study. The 51 male children in the verbal ASD group and the 51 male children in the neurotypical group were aggregate matched on age and SES (for detailed demographics see Chapter 3: The Social Motivation Study – Results – Participant Characteristics, pg. 76).

The non-verbal ASD group was a sub-sample from the sample in the Social Motivation Study as only those who were genotyped for 5-HTTLPR were included in the current study. As they would be included in the genotype-phenotype analyses with the verbal ASD participants who were genotyped, the two ASD groups were assessed for differences in age and SES. As in the previous study, and as expected, the non-verbal ASD group was younger (M = 8.57, SD = 3.66) than the verbal ASD group (M = 10.06, SD = 2.63). An independent t-test showed this difference was significant, $t(71) = -2.02$. $p = .047$. with a medium effect size, Cohen's $d = .468$. The groups were no longer matched on SES either, as the non-verbal ASD group had a higher average SES (M = 29385.79, SD = 13566.27) than the verbal ASD group (M = 22334.92, SD = 14889.48). Age and SES were therefore considered in Question 3 when the non-verbal ASD and verbal ASD group were both included in analyses.

Table 35.

Demographic Characteristics Across ASD Groups

Characteristics	Group		*t*	*p*	*Cohen's d*
	Non-Verbal ASD (*n*=34)	Verbal ASD (*n*=39)			
Age (years):					
$\bar{X}$ *(SD)*	8.57 (3.66)	10.06 (2.63)	-2.02	.047	.468
Range	3.33-15.83	6.08-16.92			
SES:					
$\bar{X}$ *(SD)*	29385.79 (13566.27)	22334.92 (14889.48)	2.16	.035	.495
Range	1499.50 – 45875.50	4649.50 - 45875.50			

Notes. ASD = Autism Spectrum Disorder. SES = Socio-economic status (total annual household income in Rands per year). $\bar{X}$ = Mean. *SD* = Standard deviation.

Question 1: Do Children with ASD Exhibit Lower Levels of ToM Than Neurotypical Children?

The Theory of Mind Study started by exploring whether the verbal ASD group had ToM deficits compared to a neurotypical sample, as is often reported in the literature. All 51 verbal ASD participants and the 51 neurotypical participants were included in this analysis.

Hypothesis 1.1 Children with ASD Will Have Lower ToM Scores Than Neurotypical Children. In order to test this hypothesis, I compared the overall scores for ToM between the two groups for three age bands. I then assessed whether the verbal ASD group and neurotypical group differed significantly on VIQ percentile scores. As they did, I then looked for group differences in ToM when VIQ was considered.

ToM Scoring. The participants for this study completed the *University of Cape Town Theory of Mind Battery* (Appendix M). Initially participants scored an overall percentage for the

battery, and these scores were assessed and compared between the groups (Table 36). The mean score for the neurotypical group in the age band 6-7 years (*M* = 72.05, *SD* = 15.45) showed that they were able to understand first-order false belief tasks (Basic Module) but had not consistently mastered second-order false belief tasks (Intermediate Module). The neurotypical group in the age band 8-10 years (*M* = 80.85, *SD* = 8.01) had mastered second-order false beliefs (Intermediate Module) but struggled with social *faux pas* and with distinguishing between lies and jokes (Advanced Module). The final age band, 11 years and older (*M* = 86.02, *SD* = 4.85), showed improved performance in social *faux pas* and differentiating lies and jokes (Module 4) in comparison to the previous age band, although they did not have complete mastery of these skills yet. These scores were all age-appropriate compared to the South African literature (Robberts, 2011).

Table 36.

ToM Performance (Raw Scores) across Age Bands

Age Bands	Neurotypical		ASD	
	n	ToM Percentage *M(SD)*	*n*	ToM Percentage *M(SD)*
6 – 7	9	72.05 (15.46)	11	42.06 (17.22)
8 – 10	24	80.85 (8.01)	22	61.26 (24.09)
11 +	18	86.02 (4.85)	18	63.56 (21.33)

Notes. ASD =Autism Spectrum Disorder. ToM = Theory of Mind. *M* = Mean. *SD* = Standard Deviation

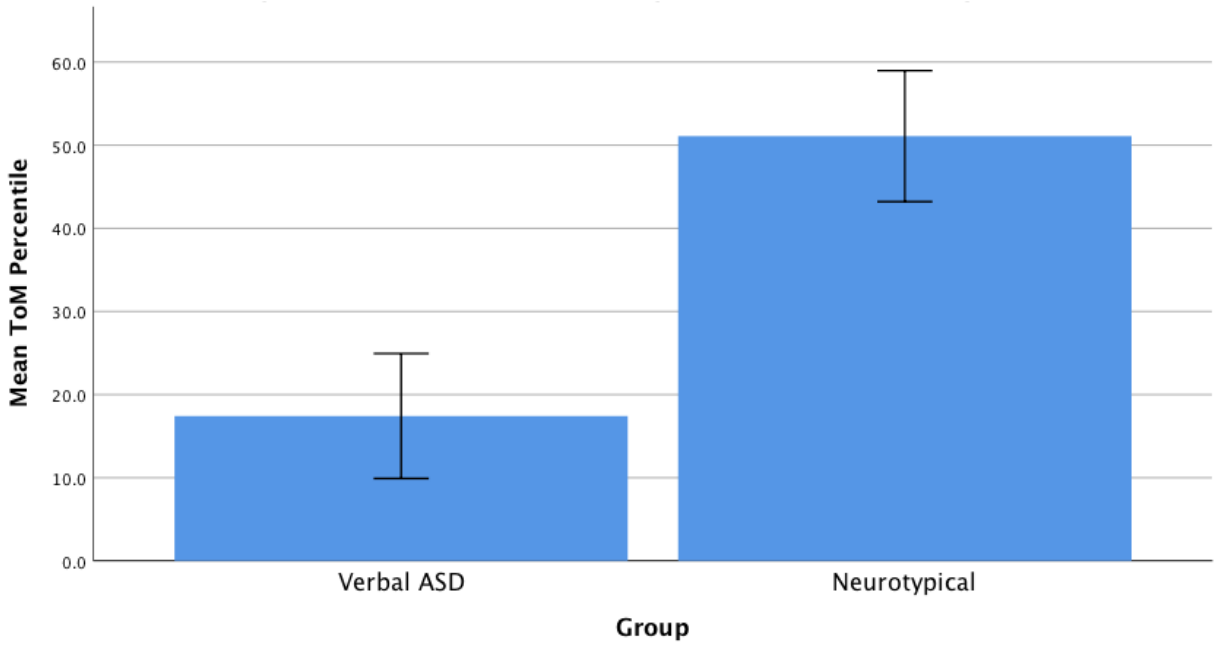

Figure 9. Mean Theory of Mind percentile per group with 95% confidence interval.

By comparison, the ASD sample showed considerably poorer performance on ToM tasks, and greater variability in scores within each age band. The ASD age band 6-7 years (M = 42.06, SD = 17.22) were able to pass basic tasks such as Pretend Play (Early Module) but struggled with first-order false belief tasks (Basic Module), placing them an entire module behind the age-matched neurotypical group. The ASD groups for ages 8-10 years (M = 61.26, SD = 24.09) and ages 11 years and older (M = 63.56, SD = 21.33) were able to complete first-order false belief tasks (Basic Module) but struggled with second-order false belief tasks (Intermediate Module), again placing them a module behind the comparable neurotypical groups.

Although the above analyses divided participants into age bands, the battery does not account for age in the overall scoring percentage, and norms have not yet been developed. To overcome this limitation, standardised z-scores were calculated for each age band (see Chapter 4:

The Theory of Mind Study – Method – Measures – Theory of Mind Assessment, pg. 151 for details). Using the means and standard deviations from the neurotypical group, whose scores did not deviate from the reported norms in the literature for neurotypical samples (Robberts, 2011), I was able to get a better comparison of the differences between the groups. To allow for easier interpretations, these *z*-scores were then converted into percentiles, and these percentiles were used in all later analyses. On average, the ASD group had lower ToM percentile scores (M = 22.59; SD = 22.86) than the neurotypical group (M = 75.31; SD = 22.8). An independent sample *t*-test showed that this difference was significant, $t(100) = -11.65$, $p < .001$, with a large effect, $r = .76$.

Table 37.

ToM Percentile Scores Across Groups

	Group		Significance Across Groups		Effect Size
	Neurotypical (n=51)	ASD (n=51)	*t*	*p*	*r*
$\bar{X}$ *(SD)*	75.31 (22.85)	22.59 (22.86)	-11.65	<.001	.73

Notes. ASD=Autism Spectrum Disorder. ToM = Theory of Mind. $\bar{X}$ = Mean. *SD* = Standard Deviation.

Verbal IQ Scores. VIQ is implicated in the ability to complete ToM tasks and was assessed in my sample so that any differences between groups in ToM would be seen over and above the impact of VIQ abilities. An independent *t*-test found that on average neurotypical participants had higher VIQ scores (M = 113.33, SD = 12.73) than the verbal ASD group (M = 84.55, SD = 14.17). This difference was significant, $t(100) = -10.79$, $p<.001$, with a large effect $r = .73$ (Table 38). Due to the groups having differences in both VIQ percentile scores and in ToM scores, the groups were assessed independently to see what role VIQ played in ToM performance, and whether it differed across the groups. For consistency, as ToM scores were

presented as percentiles, I also used the VIQ percentile scores in all analyses which included ToM scores.

Table 38.

VIQ Scores Across Groups

	Group		Significance Across Groups		Effect Size
	Neurotypical (*n*=51)	ASD (*n*=51)	*t*	*p*	*r*
$\bar{X}$ (*SD*)	113.33 (12.73)	84.55 (14.17)	-10.79	<.001	.73

Notes. ASD=Autism Spectrum Disorder. VIQ = WASI Verbal Intelligence Quotient. $\bar{X}$ = Mean. *SD*=Standard Deviation.

ToM Across Groups. To explore ToM differences across groups, I used an ANCOVA to assess whether any between-group difference in ToM was present over and above the group difference in VIQ performance. The correlation between VIQ percentile scores and ToM percentile scores for all participants was high, $r = .66$, $p < .001$. VIQ percentile score was a significant covariate, $F(1,99) = 29.06$, $p < .001$. However, group no longer had a significant association with ToM when the covariate was considered, $F(1,99) = .235$, $p = .629$, $\omega^2 = -.004$.

As the literature consistently reports ToM deficits over and above VIQ deficits in ASD, I ran separate MRAs for the neurotypical group and the verbal ASD group to establish whether the role of VIQ was consistent across the two groups. The models placed age and SES first, followed by VIQ percentile, with ToM percentile as the outcome variable.

For the neurotypical group, ToM percentile was significantly correlated with the predictor, VIQ percentile, as predicted, $r = .41$, $p < .001$ (Table 39). No issues with

multicollinearity were noted, as the only other correlations were weak and all tolerance scores and VIF scores were acceptable, confirming no multicollinearity (Field, 2013).

Table 39.

Zero-Order Correlation Matrix for Neurotypical Participants: ToM

	1	2	3	4
1. Age (Months)	-			
2. SES	-.18	-		
3. VIQ percentile	-.16	.40**	-	
4. ToM percentile	.01	.19	.41**	-

Notes. SES=Socio-Economic Status (total annual household income).

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The final model was significant, F (3,47) = 3.41, p = .025. This model had a medium effect size, f_2 = .22, and an observed power of .78. The adjusted R_2 value showed that this model explained 12.6% of the variance in ToM percentile scores, and the R^2 change value of .140 showed that this was accounted for by the VIQ percentile scores (Table 40). Table 41 shows that VIQ percentile was the only significant predicator.

Table 40.

Predictors of ToM Percentile for Neurotypical Participants: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	*R Square Change*	*F Change*	Change Statistics *df 1*	*df 2*	*Sig. F Change*
1	.197	.039	-.001	28.04	.039	0.97	2	48	.386

2	.423	.179	.126	26.20	.140	7.99	1	47	.007

Notes. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, Total Annual Family Income (SES), Age

Model 2: Constant, Total Annual Family Income (SES), Age, VIQ Percentile

Table 41.

Coefficients for Neurotypical Model 2: ToM Percentile

		Unstandardized coefficients		*Standardized coefficients*			Collinearity Statistics	
Model	Predictors	*B*	Std. Error	*β*	*t*	*p*	Tolerance	VIF
2	(Constant)	-1.94	26.20		-0.07	.941		
	Age	0.11	0.17	.09	0.64	.528	0.96	1.05
	SES	<0.01	0.00	.04	0.30	.766	0.83	1.21
	VIQ percentile	0.50	0.18	.41	2.83	.007	0.83	1.20

Notes. SES = Socio-Economic Status (total annual household income)

For the verbal ASD group, the outcome variable, ToM percentile, was significantly correlated with the predictor, VIQ percentile, as predicted, $r = .54$, $p < .001$ (Table 42). No issues with multicollinearity were noted, as the only other correlations were weak and all tolerance scores and VIF scores were acceptable, confirming no multicollinearity (Field, 2013).

Table 42.

Zero-Order Correlation Matrix for ASD Participants: ToM

	1	2	3	4
1. Age (Months)	-			

2. SES	-.18	-		
3. VIQ percentile	-.29*	.12	-	
4. ToM percentile	-.06	.08	.54**	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The overall model was significant, $F(3,47) = 3.95$, $p = .001$, with a large effect size, $f_2 = .44$, and an observed power of .98. This model explained 26.3% of the variance in ToM percentile scores, and the R^2 change value of .30 showed that this was accounted for by the VIQ percentile (Table 43). Table 44 showed that VIQ percentile was the only significant predicator.

Table 43.

Predictors of ToM Percentile for ASD Participants: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	*R Square Change*	*F Change*	Change Statistics		
							df 1	*df 2*	*Sig. F Change*
1	.086	.007	-.034	27.18	.007	0.18	2	48	.837
2	.554	.307	.263	22.94	.300	20.34	1	47	<.001

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, Total Annual Family Income (SES), Age

Model 2: Constant, Total Annual Family Income (SES), Age, VIQ Percentile

Table 44.

Coefficients for ASD Model 2: ToM

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*	*t*	*p*	Collinearity Statistics	
		B	Std. Error	*β*			Tolerance	VIF
2	(Constant)	-11.15	16.64		-0.67	.506		
	Age	0.10	0.11	.12	0.92	.364	0.89	1.12
	SES	<0.01	0.00	.03	0.20	.840	0.96	1.04
	VIQ Percentile	0.67	0.15	.57	4.51	<.001	0.91	1.10

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

As per my hypothesis, the verbal ASD group had significantly worse performances for ToM tasks than the neurotypical sample. Comparing the two regression models, we see that VIQ percentile and ToM percentile had a stronger association for the verbal ASD group than that seen

in the neurotypical group. I also note that VIQ percentile appeared to explain a greater amount of variance in ToM performance for the verbal ASD group than the neurotypical group. The role of VIQ in ToM therefore seems different in ASD compared to the neurotypical sample, and VIQ was considered in all further analyses.

Question 2: Are ToM Deficits Associated with ASD-Related Deficits?

ToM deficits are common in ASD, but the relationships between these deficits and ASD symptoms is unclear. I therefore investigated whether ToM deficits were associated with specific aspects of ASD. The above analyses also showed that VIQ can be a protective factor when considering ToM deficits in ASD, so VIQ further complicates the relationship between ToM deficits and ASD deficits.

Hypothesis 2.1 Lower ToM Scores Will Be Associated with Higher ADOS2 Scores. This analysis was limited to the 51 verbal ASD participants, and I expected higher ToM percentile scores to correlate with lower ADOS2 scores. To assess for these relationships, I initially looked at partial correlations between ToM scores and ADOS2 Scores while controlling for VIQ percentile scores. An MRA assessing the association between ToM percentile and ADOS2 scores over and above the roles of age, SES, and VIQ percentile was considered, but was rejected due to the multicollinearity issues that would arise from the correlation between VIQ percentile and ToM. Partial correlation analysis between ToM and ADOS2 scores when controlling for VIQ percentile was therefore conducted.

Where VIQ percentile was controlled for with partial correlations, ToM was inversely correlated with greater severity scores for ADOS2 Comparison score (r = -.40, p = .002) and ADOS2 Social Affect score (r = -.28, p = .024), but not with ADOS2 RRB Score (r = -.20, p =

.083). These correlations indicated a specific relationship between ToM and the ASD deficits associated with the social communication and interaction symptom domain.

Table 45.

Partial Correlation Matrix for ASD Participants: ToM when controlling for VIQ percentile

	1	2	3	4
1. ToM	-			
2. ADOS2 Comparison	-.40**	-		
3. ADOS2 SA	-.28*	.85**	-	
4. ADOS2 RRB	-.20	.31*	-.08	-

Notes. ASD = Autism Spectrum Disorder. RRB = Restricted and Repetitive Behaviours and Interests Symptom Domain.

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

Question 3: Could 5-HTTLPR Play A Role In ASD-Related Deficits and/or ToM Deficits?

As serotonin is implicated in a multitude of social functions and in ASD, I aimed to clarify whether 5-HTTLPR linked to specific ASD characteristics assessed by the ADOS2, and/or linked to ToM. I initially assessed the allelic distribution of 5-HTTLPR in the sample for the full 73 ASD participants who provided DNA (34 non-verbal; 39 verbal) and then assessed whether ADOS2 scores related to 5-HTTLPR genotypes for this sample. I then looked at the 39 verbal ASD children who provided DNA to assess for relationships between 5-HTTLPR and ToM performance.

Hypothesis 3.1 Children with ASD Will Show A Higher Rate of the 5-HTTLPR Short Allele Than Reported in Neurotypical Samples. For this aspect of the study, 73 children with ASD were genotyped for 5-HTTLPR. For the current sample, the distribution was 38.4% *L/L* genotype, 47.9% *L/S* genotype, and 13.7% *S/S* genotype (Table 46). It was noted that the

verbal ASD group tended to have higher prevalence of the short allele than the non-verbal ASD group, and particularly to have higher rates of the *S/S* genotype. Overall, the current sample showed a higher prevalence of the short allele, and specifically the *S/S* genotype, than reported in a neurotypical South African sample, where the *S/S* genotype only occurred in 5% of their sample (Esau et al., 2008).

Table 46.

5-HTTLPR Genotypes and Allelic Distribution in ASD

	Genotypes			*Allelic Distribution*	
	L/L	*L/S*	*S/S*	*L*	*S*
Total (*n*=73)	28 (38.36%)	35 (47.95%)	10 (13.70%)	.62	.38
Non-Verbal ASD (*n*=34)	15 (44.12%)	17 (50.00%)	2 (5.88%)	.69	.31
Verbal ASD (*n*=39)	13 (33.33%)	18 (46.15%)	8 (20.51%)	.56	.44

Notes. ASD = Autism Spectrum Disorder.

The distribution of 5-HTTLPR genotypes and alleles across ethnic groups in the ASD samples is shown below (Tables 47-49). As ethnicity was not a factor in recruitment, there were not equal sample sizes for each ethnic group. The current study had a similar ethnic distribution to the local ASD sample report by Arieff et al. (2010), with similar allelic frequency prevalence as in their findings. The current sample, however, had lower rates of the *S/S* genotype for each ethnic group compared to their study. When I compared the three local studies, I noted that the Caucasian ASD samples in my study and the Arieff et al. (2010) study did not show the predicted disparity in allelic frequency between these ASD samples and neurotypical sample in the Esau et al. (2008) study.

Table 47.

5-HTTLPR Genotypes and Allelic Distribution in ASD Across Ethnic Groups

	Genotypes			*Allelic Distribution*	
	L/L	*L/S*	*S/S*	*L*	*S*
Caucasian (*n*=25)	8 (32.00%)	13 (52.00%)	4 (16.00%)	.58	.42
African (n=6)	3 (50.00%)	3 (50.00%)	0 (0.00%)	.75	.25
Mixed race (*n*=38)	16 (42.11%)	17 (44.74%)	5 (13.12%)	.64	.36
Asian (*n*=2)	1 (50.00%)	1 (50.00%)	0 (0.00%)	.75	.25
Other (*n*=2)	0 (0.00%)	1 (50.00%)	1 (50.00%)	.25	.75

Notes. ASD = Autism Spectrum Disorder.

Table 48.

5-HTTLPR Genotypes and Allelic Distribution in Non-Verbal ASD across Ethnic Groups

	Genotypes			*Allelic Distribution*	
	L/L	*L/S*	*S/S*	*L*	*S*
Caucasian (n=12)	4 (33.33%)	6 (50.00%)	2 (16.67%)	.58	.42
Mixed race (n=16)	9 (56.25%)	7 (43.75%)	0 (0.00%)	.78	.22
African (n=4)	1 (25.00%)	3 (75.00%)	0 (0.00%)	.63	.38
Asian (*n*=1)	1 (100.00%)	0 (0.00%)	0 (0.00%)	1.00	.00
Other (*n*=1)	0 (0.00%)	1 (100.00%)	0 (0.00%)	.50	.50

Notes. ASD = Autism Spectrum Disorder.

Table 49.

5-HTTLPR Genotypes and Allelic Distribution in Verbal ASD across Ethnic Groups

	Genotypes			*Allelic Distribution*	
	L/L	*L/S*	*S/S*	*L*	*S*
Caucasian (*n*=13)	4 (30.77%)	7 (53.85%)	2 (15.38%)	.58	.42
Mixed race (*n*=22)	7 (31.82%)	10 (45.45%)	5 (22.73%)	.55	.45
African (*n*=2)	2 (100%)	0 (0.00%)	0 (0.00%)	1.00	.00
Asian (*n*=1)	0 (0.00%)	1 (100%)	0 (0.00%)	.50	.50
Other *(n*=1)	0 (0.00%)	0 (0.00%)	1 (100%)	.00	1.00

Notes. ASD = Autism Spectrum Disorder.

Hypothesis 3.2 Children with ASD Carrying the 5-HTTLPR Short Allele Will Have Higher ADOS2 Scores Than Those Without This Allele. As ASD is likely to arise from multiple genetic contributions, I aimed to see whether 5-HTTLPR linked to particular aspects of ASD. I therefore assessed ADOS2 scores across the genotypes for the ASD participants who had provided DNA samples and I had predicted that the short allele would relate to higher deficits. As the Social Motivation Study (Chapter 3: The Social Motivation Study – Results – Question 2, Hyp. 2.2, pg. 83) found significant group differences for ADOS2 scores between the non-verbal ASD group and the verbal ASD group, I conducted separate analyses for these two groups.

ADOS2 Comparison Scores Across 5-HTTLPR Genotypes. I conducted a hierarchical regression model to assess for a relationship between 5-HTTLPR and ADOS2 Comparison scores. I entered age and SES, followed by the dummy variables for 5-HTTLPR, with ADOS2 Comparison score as the outcome variable. The dummy variables for 5-HTTLR were coded as follows: Dummy1 assigned a value of zero to all genotypes with a short allele (i.e. *S/S* and *L/S* genotypes); Dummy2 assigned a zero to all genotypes without a long allele (i.e. *S/S* genotype

only). This analysis was conducted for the non-verbal ASD group and for the verbal ASD group separately.

For the non-verbal ASD group, 34 participants had the required data. Assessing ADOS2 Comparison scores across genotypes showed that the *L/L* genotype had the lowest deficit score (M = 5.87, SD = 1.73), followed by the *L/S* genotype (M = 6.29, SD = 0.85), and the *S/S* genotype (M = 8.50, SD = 0.71) had the highest deficit score. Although this followed the hypothesised pattern of scoring, as the *S/S* genotype only had 2 participants, the relevance of the mean scores is limited.

No issues for multicollinearity were noted in the correlation matrix or when assessing tolerance and VIF scores (Field, 2013). Table 50 shows the zero-order correlations for this model, and I noted only one significant correlation to the outcome variable, namely the second 5-HTTLPR dummy variable to ADOS2 Comparison score, r = -.41, p = .009. This dummy variable compared the *S/S* genotype to the genotypes that carried at least one long allele and the correlation indicated that children carrying a long allele tended to show lower deficits scores than those only carrying short alleles, as hypothesised.

Table 50.

Zero-Order Correlation Matrix for Non-Verbal ASD Participants: ADOS2 Comparison Score

	1	2	3	4	5
1. Age (Months)	-				
2. SES	.03	-			
3. S-carriers v L/L [a]	.07	-.10	-		
4. SS v L-carriers [a]	.19	-.15	.22	-	
5. ADOS2 Comparison score	.14	-.12	-.24	-.41**	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income). [a] 5-HTTLPR dummy variables

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The first model included SES and age, and was not significant, F (2,31) = 0.56, p = .576. The second model included the 5-HTTLPR dummy variables and was significant, F (4,29) = 2.75, p = .042, with a large effect size, f^2 =.39, and an observed power of .78. The adjusted R^2 valued showed that this second model explained 18.3% of the variance in ADOS2 Comparison score, and the R^2 change value of .247 showed that this was accounted for by the 5-HTTLPR dummy variables (Table 51). Table 52 shows that the second 5-HTTLPR dummy variable was the only significant predicator, indicating that the *S/S* genotype had higher deficits than those carrying a long allele. Although this supported my hypothesis, it contradicted the reported dominant effect for the short allele.

Table 51.

Predictors of ADOS2 Comparison Score for Non-Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	Change Statistics				
					R Square Change	*F Change*	*df 1*	*df 2*	*Sig. F Change*
1	.187	.035	-.027	1.44	.035	0.56	2	31	.576
2	.531	.282	.183	1.28	.247	4.99	2	29	.014

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, 5-HTTLPR Dummy1, 5-HTTLPR Dummy2

Table 52.

Coefficients for Model 2: ADOS2 Comparison Score for Non-Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*	*t*	*p*	Collinearity Statistics	
		B	Std. Error	*β*			Tolerance	VIF
2	(Constant)	8.81	1.15		7.70	<.001		
	Age (months)	0.01	0.01	.241	1.50	.145	0.96	1.04
	SES	<-0.01	0.00	-.217	-1.36	.185	0.97	1.03
	S-carriers v L/L*	-0.49	0.46	-.174	-1.08	.291	0.95	1.06
	SS v L-carriers*	-2.65	0.98	-.447	-2.69	.012	0.90	1.11

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

* 5-HTTLPR genotype dummy variables

For the verbal ASD group, 39 participants had the required data. Assessing ADOS2 Comparison scores across genotypes showed that the *L/S* genotype (*M* = 6.06, *SD* = 1.96) had the highest deficit score, followed by the *S/S* genotype (*M* = 5.38, *SD* = 2.00), with the *L/L* genotype having the lowest deficit score (*M* = 5.08, *SD* = 2.15). Although this supported my hypothesis

that short allele carriers would have higher ASD-related deficits, for the *L/S* genotype to have the highest deficit score I would need the short allele for 5-HTTLPR to have a dominant effect, yet this scoring pattern does not clearly indicate that the *L/S* genotype and *S/S* genotype have similar scores.

No issues for multicollinearity were noted in the correlation matrix or when assessing tolerance and VIF scores (Field, 2013). Table 53 shows the zero-order correlations for this model, and I noted only one significant correlation to the outcome variable, namely age to ADOS2 Comparison score, $r = .31$, $p = .028$. Neither of the 5-HTTLPR dummy variables correlated to the outcome variable.

Table 53.

Zero-Order Correlation Matrix for verbal ASD Participants: ADOS2 Comparison Score

	1	2	3	4	5
1. Age (Months)	-				
2. SES	-.28*	-			
3. S-carriers v L/L [a]	-.01	.06	-		
4. SS v L-carriers [a]	.16	.26	.36*	-	
5. ADOS2 Comparison score	.31*	-.20	-.18	.05	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income). [a] 5-HTTLPR dummy variables

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed). **. Correlation is significant at the .01 level (1-tailed).

The first model included SES and age, and was not significant, $F(2,36) = 2.24$, $p = .121$. The second model included the 5-HTTLPR dummy variables and was also not significant, *F*

(4,34) = 2.75, p = .215, f_2 =.18. This analysis therefore did not support the hypothesis that short allele carriers would have higher ADOS2 Comparison scores compared to non-carriers.

Table 54.

Predictors of ADOS2 Comparison Score for Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	*R Square Change*	*F Change*	Change Statistics *df 1*	*df 2*	*Sig. F Change*
1	.333	.111	.061	1.99	.111	2.24	2	36	.121
2	.391	.153	.053	1.99	.042	0.84	2	34	.440

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, 5-HTTLPR Dummy1, 5-HTTLPR Dummy2

Table 55.

Coefficients for Model 2: ADOS2 Comparison Score for Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*			Collinearity Statistics	
		B	Std. Error	*β*	*t*	*p*	Tolerance	VIF
2	(Constant)	3.98	1.57		2.54	.016		
	Age (months)	0.02	0.01	.242	1.12	.165	0.86	1.17
	SES	<-0.01	0.00	-.157	-0.90	.373	0.83	1.21
	S-carriers v L/L*	-0.92	0.73	-.215	-1.27	.215	0.86	1.16
	SS v L-carriers*	0.66	0.91	.132	0.73	.472	0.76	1.32

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

* 5-HTTLPR genotype dummy variables

ADOS2 Social Affect Scores Across 5-HTTLPR Genotypes. I conducted similar hierarchical regression models to assess for a relationship between 5-HTTLPR and ADOS2

Social Affect scores for each ASD group. I entered age and SES, followed by the dummy variables for 5-HTTLPR, with ADOS2 Social Affect score as the outcome variable.

For the non-verbal ASD group, 34 participants had the required data. Assessing ADOS2 Social Affect scores across genotypes showed that the *S/S* genotype (M = 15.50, SD = 2.12) had the highest deficit score, followed by the L/S genotype (M = 13.12, SD = 3.95), with the *L/L* genotype having the lowest deficit score (M = 12.80, SD = 4.09). Although this followed the hypothesised pattern of scoring, as the *S/S* genotype only had 2 participants, the relevance of the mean scores is limited, and the pattern does not clearly indicate a dominant effect for the short allele as predicted.

No issues for multicollinearity were noted in the correlation matrix or when assessing tolerance and VIF scores (Field, 2013). Table 56 shows the zero-order correlations for this model, and none of the predictor variables correlated with the outcome variable.

Table 56.

Zero-Order Correlation Matrix for Non-Verbal ASD Participants: ADOS2 Social Affect Score

	1	2	3	4	5
1. Age (Months)	-				
2. SES	.03	-			
3. S-carriers v L/L [a]	.07	-.10	-		
4. SS v L-carriers [a]	.19	-.15	.22	-	
5. ADOS2 Social Affect score	.18	-.09	-.07	-.16	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income). [a] 5-HTTLPR dummy variables

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The first model included SES and age, and was not significant, $F(2,31) = 0.64$, $p = .534$. The second model included the 5-HTTLPR dummy variables and was also not significant, $F(4,29) = 0.69$, $p = .605$, $f^2 = .10$. The hypothesised role for 5-HTTLPR in ADOS2 Social Affect scores was therefore not supported for the non-verbal ASD group.

Table 57.

Predictors of ADOS2 Social Affect Score for Non-Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	Change Statistics				
					R Square Change	*F Change*	*df 1*	*df 2*	*Sig. F Change*
1	.199	.040	-.022	3.94	.040	0.64	2	31	.534
2	.295	.087	-.039	3.97	.047	0.75	2	29	.482

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).
Model 1: Constant, SES, Age
Model 2: Constant, SES, Age, 5-HTTLPR Dummy1, 5-HTTLPR Dummy2

Table 58.

Coefficients for Model 2: ADOS2 Social Affect Score for Non-Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*	*t*	*p*	Collinearity Statistics	
		B	Std. Error	*β*			Tolerance	VIF
2	(Constant)	15.52	3.56		4.36	<.001		
	Age (months)	0.02	0.02	.224	1.24	.226	0.96	1.04
	SES	<-0.01	0.00	-.130	-0.72	.476	0.97	1.03
	S-carriers v L/L*	-0.43	1.41	-0.06	-0.31	.761	0.95	1.06
	SS v L-carriers*	-3.36	3.05	-0.21	-1.10	.281	0.90	1.11

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income). * 5-HTTLPR genotype dummy variables

For the verbal ASD group, 39 participants had the required data. Assessing ADOS2 Social Affect scores across genotypes showed that the *L/S* genotype (*M* = 8.11, *SD* = 3.20) had the highest deficit score, followed by the *L/L* genotype (*M* = 6.92, *SD* = 4.13), with the *S/S* genotype (*M* = 6.75, *SD* = 2.55) showing the lowest deficit score. This was contrary to my hypothesised expectation that the short allele would be linked to greater ASD-related deficits, and once again did not support a dominant role for the short allele.

No issues for multicollinearity were noted in the correlation matrix or when assessing tolerance and VIF scores (Field, 2013). Table 59 shows the zero-order correlations for this model, and I noted the only one significant correlation to the outcome variable was age, *r* = .36, *p* =.013. Neither of the 5-HTTLPR dummy variables correlated to the outcome variable.

Table 59.

Zero-Order Correlation Matrix for Verbal ASD Participants: ADOS2 Social Affect Score

	1	2	3	4	5
1. Age (Months)	-				
2. SES	-.28*	-			
3. S-carriers v L/L [a]	-.01	.06	-		
4. SS v L-carriers [a]	.16	.26	.36*	-	
5. ADOS2 Social Affect score	.36*	-.15	-.11	.10	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income). [a] 5-HTTLPR dummy variables

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The first model included SES and age and was not significant, F (2,36) = 2.69, p = .082. The second model included the 5-HTTLPR dummy variables and was also not significant, F (4,34) = 1.54, p = .214, f_2 =.18. This analysis therefore did not support the hypothesis that short allele carriers would have higher ADOS2 Social Affect scores compared to non-carriers.

Table 60.

Predictors of ADOS2 Social Affect Score for Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	*R Square Change*	*F Change*	Change Statistics *df 1*	*df 2*	*Sig. F Change*
1	.360	.130	.082	3.26	.130	2.69	2	36	.082
2	.391	.153	.053	3.31	.023	0.46	2	34	.633

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, 5-HTTLPR Dummy1, 5-HTTLPR Dummy2

Table 61.

Coefficients for Model 2: ADOS2 Social Affect Score for Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*			Collinearity Statistics	
		B	Std. Error	*β*	*t*	*p*	Tolerance	VIF
2	(Constant)	3.43	2.61		1.32	.197		
	Age (months)	0.03	0.02	.307	1.80	.081	0.86	1.17
	SES	<-0.01	0.00	-.093	-0.54	.595	0.83	1.21
	S-carriers v L/L*	-1.04	1.21	-.15	-0.86	.396	0.86	1.16
	SS v L-carriers*	1.08	1.51	.130	0.72	.478	0.76	1.32

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

* 5-HTTLPR genotype dummy variables

ADOS2 RRB Scores Across 5-HTTLPR Genotypes. I conducted hierarchical regression models to assess for a relationship between 5-HTTLPR and ADOS2 RRB scores for each ASD group. I entered age and SES, followed by the dummy variables for 5-HTTLPR, with ADOS2 RRB score as the outcome variable.

The non-verbal ASD group had 34 participants with the required data. Assessing ADOS2 RRB scores across genotypes showed that the *S/S* genotype (M = 6.00, SD = 1.41) had the highest deficit score, followed by the L/S genotype (M = 3.47, SD = 2.15), with the *L/L* genotype having the lowest deficit score (M = 3.00, SD = 1.96). Although this followed the hypothesised pattern of scoring, the *S/S* genotype only had 2 participants so the relevance of the mean scores is limited. Further, the scoring pattern did not support a dominant role for the short allele.

No issues for multicollinearity were noted in the correlation matrix or when assessing tolerance and VIF scores (Field, 2013). Table 62 shows the zero-order correlations for this model, and I noted only one significant correlation to the outcome variable, namely 5-HTTLPR Dummy2 to ADOS2 RRB score, r = -.31, p =.036. This dummy variable compared the *S/S* genotype to the genotypes that carried at least one long allele and the correlation indicated that children carrying a long allele tended to show lower deficits scores than those only carrying short alleles, as hypothesised. However, this supports a dominant role for the long allele and not the short allele.

Table 62.

Zero-Order Correlation Matrix for Non-verbal ASD Participants: ADOS2 RRB Score

	1	2	3	4	5
1. Age (Months)	-				
2. SES	.03	-			
3. S-carriers v L/L [a]	.07	-.10	-		
4. SS v L-carriers [a]	.19	-.15	.22	-	
5. ADOS2 RRB score	.17	-.28	-.18	-.31*	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income). [a] 5-HTTLPR dummy variables

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed

The first model included SES and age and was not significant, F (2,31) = 1.88, p = .169. The second model included the 5-HTTLPR dummy variables and was significant, F (4,29) = 2.96, p = .036, with a large effect size, f^2 =.41, and an observed power of .79. The adjusted R^2 value showed that the second model explained 19.2% of the variance in ADOS2 RRB score and the R^2 change value of .182 showed that this was accounted for by the 5-HTTLPR dummy variables (Table 63). Table 64 showed that SES was a significant predictor. The second 5-HTTLPR dummy variable was also a significant predicator, indicating that the *S/S* genotype had higher deficits than those carrying a long allele. Although this supported the hypothesis, it again contradicted the expected dominant role for the short allele.

Table 63.

Predictors of ADOS2 RRB Score for Non-Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	*R Square Change*	*F Change*	Change Statistics *df 1*	*df 2*	*Sig. F Change*
1	.329	.108	.051	2.05	.108	1.88	2	31	.169
2	.538	.290	.192	1.89	.182	3.71	2	29	.037

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, 5-HTTLPR Dummy1, 5-HTTLPR Dummy2

Table 64.

Coefficients for Model 2: ADOS2 RRB Score for Non-Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*	*t*	*p*	Collinearity Statistics	
		B	Std. Error	*β*			Tolerance	VIF
2	(Constant)	7.20	1.69		4.25	<.001		
	Age (months)	0.01	0.01	.266	1.66	.107	0.96	1.04
	SES	<-0.01	0.00	-.358	-2.25	.032	0.97	1.03
	S-carriers v L/L*	-0.61	0.67	-.145	-0.90	.374	0.95	1.06
	SS v L-carriers*	-3.40	1.45	-.385	-2.34	.027	0.90	1.11

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

* 5-HTTLPR genotype dummy variables

For the verbal ASD group, 39 participants had the required data. Assessing ADOS2 RRB scores across genotypes showed that the *S/S* genotype (*M* = 2.38, *SD* = 1.41) had the highest deficit score, followed by the *L/L* genotype (*M* = 1.92, *SD* = 1.66), and the *L/S* genotype (*M* =

1.83, *SD* = 1.69) with the lowest deficit score. This scoring pattern indicated that there was not a relationship between 5-HTTLPR genotypes and ADOS2 RRB scores.

There were no indications of multicollinearity in the correlation matrix or when assessing tolerance and VIF scores (Field, 2013). Table 65 shows the zero-order correlations for this model, and there were no significant correlations between the predictors and the outcome variable. It was therefore unsurprising that the regression models were not significant, $F(4,34) = 0.25$, $p = .907$, $f^2 = .03$. Analysis therefore did not support the hypothesised relationship between 5-HTTLPR and ADOS2 RRB scores.

Table 65.

Zero-Order Correlation Matrix for Verbal ASD Participants: ADOS2 RRB Score

	1	2	3	4	5
1. Age (Months)	-				
2. SES	-.28*	-			
3. S-carriers v L/L [a]	-.01	.06	-		
4. SS v L-carriers [a]	.16	.26	.36*	-	
5. ADOS2 RRB score	-.03	-.13	-.02	-.13	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income). [a] 5-HTTLPR dummy variables

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

Table 66.

Predictors of ADOS2 RRB Score for Verbal ASD: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	*R Square Change*	*F Change*	Change Statistics *df 1*	*df 2*	*Sig. F Change*
1	.146	.021	-.033	1.62	.021	0.39	2	36	.680
2	.170	.029	-.086	1.66	.008	0.13	2	34	.877

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

Model 1: Constant, SES, Age

Model 2: Constant, SES, Age, 5-HTTLPR Dummy1, 5-HTTLPR Dummy2

Table 67.

Coefficients for Model 2: ADOS2 RRB Score for Verbal ASD

Model	Predictors	Unstandardized coefficients		*Standardized coefficients*	*t*	*p*	Collinearity Statistics	
		B	Std. Error	*β*			Tolerance	VIF
2	(Constant)	2.83	1.31		2.16	.038		
	Age (months)	<-0.01	0.01	-.045	-0.25	.805	0.86	1.17
	SES	<-0.01	0.00	-.118	-0.63	.532	0.83	1.21
	S-carriers v L/L*	.063	0.61	.019	0.10	.918	0.86	1.16
	SS v L-carriers*	-.384	.759	-.098	-0.51	.616	0.76	1.32

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

* 5-HTTLPR genotype dummy variables

Summary of Findings for Hypothesis 3.2. I had hypothesised that children with ASD who carried a short allele for 5-HTTLPR would present with greater ASD-related deficits than those who only carried the long allele. I found two significant relationships for the non-verbal ASD group, and these indicated that carriers of the *S/S* genotype scored higher for ADOS2

Comparison scores, indicating ASD severity, and ADOS2 RRB scores than did carriers of a long allele. This supported the hypothesis but did not support the reported dominant effect for the 5-HTTLPR short allele. There were no significant findings for the verbal ASD group, indicating that 5-HTTLPR did not play a role in ASD-related deficits in this group.

Hypothesis 3.3 Children with ASD Carrying the 5-HTTLPR Short Allele Will Have Lower ToM Scores Than Those Without This Allele. I hypothesised that short allele carriers for 5-HTTLPR would have lower ToM scores than those who did not carry short alleles. To assess the relationship between 5-HTTLPR and ToM I conducted hierarchical regression with the 39 verbal ASD participants who provided DNA. I placed age and SES first, followed by VIQ percentile, followed by genotype dummy variables, with ToM percentile score as the outcome variable.

Assessing ToM scores across genotypes showed that the *L/L* genotype (M = 30.04, SD = 32.02) tended to score higher than the other two genotypes, followed by the *S/S* genotype (M = 22.90 SD = 31.28), with the *L/S* genotype scoring the lowest (M = 12.30, SD = 24.49). This suggested that having one or more short alleles would undermine ToM performance, as predicted, indicating a dominant effect for the short allele for 5-HTTLPR, as found in the literature (Arieff et al., 2010; Tordjman et al., 2001). Due to the strong relationship between VIQ and ToM, VIQ percentile scores were also noted for the genotypes. Here no clear pattern emerged: the *S/S* genotype had the least deficits (M = 33.50, SD = 35.73), followed by very similar scores for the *L/L* genotype (M = 20.42, SD = 16.83) and *L/S* genotype (M = 20.85, SD = 22.33). This suggested a dominant effect for the long allele on VIQ percentile scores, which contrasted with the pattern for ToM. However, for both ToM percentile and VIQ percentile

scores the variability for each genotype represented in standard deviation scores indicated that genotypes did not represent homogenous groups for either of these abilities.

No issues for multicollinearity were noted in the correlation matrix (Table 68) or when assessing tolerance and VIF scores (Field, 2013). ToM correlated with VIQ, r =.54, p <.001, but not with either of the 5-HTTLPR genotype dummy variables.

Table 68.

Zero-Order Correlation Matrix for ToM

	1	2	3	4	5	6
1. Age (Months)	-					
2. SES	-.28*	-				
3. VIQ	-.31*	.20	-			
4. Genotype: SS v L-carriers	-.01	.06	-.07	-		
5. Genotype: LL v S-carriers	.16	.26	-.23	.36*	.	
6. ToM	-.04	.16	.54**	-.24	-.05	-

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).

*. Correlation is significant at the .05 level (1-tailed).

**. Correlation is significant at the .01 level (1-tailed).

The first regression model had only age and SES as predictors and was not significant, $F(2,36) = 0.49$, p =.615. The second model added VIQ percentile and was significant, F (3,35)= 5.59, p = .003, and the adjusted R_2 value showed that the model explained 26.6% of the variance in ToM, and the R^2 change value of .297 showed that this was accounted for by VIQ percentile (Table 69). When 5-HTTLPR genotype was added to the model, the overall model remained

significant, $F(5,33) = 4.52$, $p = .003$, with a large effect size, $f^2 = .68$, and an observed power of .97. The adjusted R^2 value for the third model explained 31.6% of the variance in ToM, although the R^2 change value was only .082 and the F change was not significant, indicating that the 5-HTTLPR genotypes did not add value to the model beyond that already added by VIQ percentile scores.

Table 69.

Predictors of ToM Percentile: Model Summary

Model	*R*	*R Square*	*Adjusted R Square*	Std. Error of the Estimate	*R Square Change*	*F Change*	Change Statistics *df 1*	*df 2*	*Sig. F Change*
1	.163	.027	-.027	29.32	.027	0.49	2	36	.615
2	.569	.324	.266	24.78	.297	15.38	1	35	<.001
3	.637	.406	.316	23.91	.082	2.29	2	33	.117

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income).
Model 1: Constant, Total Annual Family Income (SES), Age
Model 2: Constant, Total Annual Family Income (SES), Age, VIQ percentile
Model 3: Constant, Total Annual Family Income (SES), Age, VIQ percentile, 5-HTTLPR (Dummy Variables)

Table 70 shows that VIQ percentile was the only significant predicator in Model 2. For Model 3 VIQ percentile and the first dummy variable for 5-HTTLPR were significant predictors of ToM percentile. The dummy variable that was significant was the coding for a change from carrying a short allele to being long-allele homogenous (i.e. it indicates the difference between *S/S* and *L/S* as one group compared to the *L/L* genotype group). This positive relationship supports a dominant role for the short allele, and indicates, as was suggested by the descriptive statistics, that having one or more short alleles for 5-HTTLPR was associated with poor ToM

performance when compared to long allele carriers, as predicted. However, the *F*-change statistic was not significant when the genotypes were added to the model, and it is noted that genotypes did not show consistent scoring in either ToM or VIQ.

Table 70.

Coefficients for Model 3: ToM Percentile

		Unstandardized coefficients		*Standardized coefficients*			Collinearity Statistics	
Model	Predictors	*B*	Std. Error	*β*	*t*	*p*	Tolerance	VIF
3	(Constant)	-24.26	20.61		-1.18	.248		
	Age	0.18	0.14	.194	1.31	.201	0.82	1.23
	SES	0.00	0.00	.102	0.68	.503	0.79	1.26
	VIQ Percentile	0.73	0.18	.588	4.01	<.001	0.84	1.19
	S-carriers v LL*	18.58	8.75	.307	2.12	.041	0.86	1.16
	SS v L-carriers*	-5.66	11.18	-.080	-0.51	.616	0.72	1.39

Notes. ASD = Autism Spectrum Disorder. SES = Socio-Economic Status (total annual household income). *5-HTTLPR genotype dummy variables

Hypothesis 3.4 In children with ASD, the 5-HTTLPR Short Allele Will Be Associated with Poor ToM Performance, and ToM Performance Will Mediate A Relationship Between 5-HTTLPR Genotypes and ADOS2 Scores. I had predicted that the 5-HTTLPR genotype would be associated with both ToM performance and ADOS2 scores. However, hypothesis 3.2 found no association between 5-HTTLPR genotypes and ADOS2 scores for the verbal ASD group. Analyses to assess for ToM having a mediation effect between genotypes and ADOS2 scores was therefore not conducted.

Summary of Findings

The Theory of Mind Study found that the verbal ASD group performed significantly worse on ToM tasks and had lower VIQ percentile scores than the neurotypical sample. I found a stronger association between VIQ percentile and ToM performance for the ASD group than for the neurotypical group. When I assessed the role of ToM in ASD while controlling for VIQ percentile I found that ToM deficits linked to greater deficits in ADOS2 Comparison scores and ADOS2 Social Affect scores.

I then considered the role of 5-HTTLPR and had anticipated that carriers of the short allele for this gene would have greater deficits in ToM and for ASD-related deficits measured by the ADOS2. I found a higher incidence of the *S/S* genotype in the current sample compared to the literature for neurotypical South Africans (Esau et al., 2008). For the non-verbal ASD group I found that children with the *S/S* genotype tended to have higher scores for ADOS2 Comparison scores and ADOS2 RRB scores compared to carriers of the long allele. This supported the hypothesis but did not support the dominant role for the short allele as reported in the literature. There were no significant relationships between 5-HTTLPR and ADOS2 scores for the verbal ASD group. However, there was a possible relationship between 5-HTTLPR and ToM performance for the verbal group, with carriers of the short allele performing more poorly on ToM tasks than carriers of the *L/L* genotype as predicted, although the high variability in ToM and VIQ performance when the sample was divided by genotype indicates that this is unlikely.

Discussion

The Theory of Mind Study aimed to further our knowledge regarding the role of ToM deficits in ASD, and to explore the possible role of 5-HTTLPR genotypes in ToM deficits and in the ASD phenotype. Although these deficits have been widely recognised and theories for the

role of ToM deficits have been developed, the mechanisms underlying these deficits are not fully understood. My results were consistent with the literature showing that children with ASD have ToM deficits, and that these deficits correlated with overall ASD severity and with greater impairment in the social communication and interaction symptom domain. VIQ played a significant role in ToM performance for neurotypical children and children with ASD but appeared to play a larger role in performance for the children with ASD, suggesting a different relationship between ToM and VIQ for children with ASD. My study was able to establish the ASD participants' developmental stage for ToM, a novel finding, and showed that their ToM skills were consistently behind those of neurotypical children over the early and middle school years.

Finally, 5-HTTLPR did have an atypical allelic distribution in my ASD sample when compared to reported distributions for neurotypical samples, supporting a role for this polymorphism and the serotonergic system in ASD. Although my sample sizes were small, I found that 5-HTTLPR was associated with overall ASD severity and the restricted and repetitive behaviours and interests symptom domain for the non-verbal ASD group, but no associations between 5-HTTLPR and ASD-related deficits were found for the verbal ASD group. The verbal ASD group also failed to show the predicted relationships between the 5-HTTLPR short allele and poor ToM performance. The 5-HTTLPR genotypes added some value to the models predicting ToM performance in this group, but the contribution was not significant and the relationship between ToM, VIQ and 5-HTTLPR was unclear. This study supported a core role for ToM in ASD presentations and showed that 5-HTTLPR and the serotonergic system is implicated in specific ASD-related impairment in non-verbal ASD. The possible role of 5-HTTLPR in verbal children with ASD did not clearly emerge in this study. As with the previous

study in this protocol, this suggests that verbal abilities play a significant role in ASD presentations.

This study was limited by a small sample size but benefited from the comprehensive ToM assessment that went beyond typical false belief testing. This is also one of very few studies internationally to assess for genotype-phenotype relationships for 5-HTTLPR in ASD, and, to my knowledge, the first to do so in a South African sample. My findings are discussed in greater detail below.

Theory of Mind Assessment. One of the strengths of my study was the use of a comprehensive ToM battery that went beyond false-belief testing and assessed ToM along its developmental trajectory. The assessment included pretend play tasks, which assess behaviours considered as pre-cursors for later ToM development, and then increased in complexity until the final module assessed the child's ability to understand social *faux pas* and to differentiate lies from jokes using sarcasm. Using this developmental approach, I was able to directly compare children with ASD's performance to neurotypical children's performance on the same tasks. In this way I was able to overcome the limitations of binary pass-fail ToM assessments of false belief tasks (Liddle & Nettle, 2006; Peterson et al., 2012), and to establish a ToM developmental stage for participants.

This study also strove to overcome some important confounding variables such as culture, cognitive and language difficulties, and age. The comparison to a matched group of neurotypical children reduced the effects of cultural factors possibly confounding the assessment (Shahaeian et al., 2014). The tasks were also presented in such a way that typical concerns regarding cognitive and language functions were minimized: earlier tasks included props or comic-like stories so that children could point to answers if they did not wish to speak, and the

instructions and story details were simplistic so that language difficulties did not undermine understanding; story tasks included pictures that were left in view to limit the load on working memory; and tasks were short and again the use of visual stimuli prevented memory difficulties from confounding the results. I also screened all verbal children prior to ToM testing to ensure they could follow at least two-stage instructions and ensured the instructions did not exceed this. Further, VIQ was assessed and included in all analyses for ToM performance. Finally, age was included in the calculation of ToM percentile scores and included in analyses.

In terms of ecological validity, my tests were conducted in a one-on-one assessment format, and although the tasks were game or story-based, they did still face the limitations of being more simplistic than real-world scenarios relying on ToM skills (Hutchins et al., 2012; Scheeren et al., 2013; Senju, 2012). When this concern is raised in the literature, however, the concern is that ToM tasks overestimate children with ASD's true capabilities. If this is the case, then it only serves to reinforce my finding that children with ASD had significant ToM deficits compared to matched neurotypical children who completed the same tasks.

Theory of Mind deficits in ASD. My study found that children with ASD tended to struggle with ToM tasks a module behind the matched neurotypical children, indicating a significant delay in ToM development. My results support the known correlation between VIQ and ToM in both ASD and neurotypical samples but found that this association may differ between the groups as VIQ appeared to play a larger role in ToM performance in the current ASD sample (see Chapter 4: The Theory of Mind Study - Results – Question1, Hyp. 1.1, pg.158).

I compared the performance of my ASD sample with a matched neurotypical sample on the same ToM battery and found that the children with ASD were at least one module behind

neurotypical children of the same age. While the ages at which children progressively develop ToM can differ across countries (Liu et al. 2008), the children in my study had similar demographics so their differences in performance are more likely associated with the presence (or absence) of ASD.

For example, the neurotypical children in the 6-7 year age band in my study could complete the Basic Module (tasks generally achieved at 3-5 years old) and were developing the ability to complete the Intermediate Module (tasks generally achieved at 5-7 years old). This indicated that the neurotypical children's ToM was in keeping with the generally reported developmental stage for their age in the literature. By contrast, the children with ASD in this age band could only complete tasks of ToM precursors (Early Module), but they struggled with tasks in the Basic Module. This suggests that these children with ASD had only developed the ToM skills neurotypical children generally master by age 5 years.

A similar pattern was seen for the older samples (i.e. the 8-10 year age band and 11+ year age band). The neurotypical samples for these two age bands performed as the literature predicted and were able to complete the Intermediate Module (tasks generally achieved 5-7 years old) and were developing the ability to complete the Advanced Module (tasks generally achieved 9-11 years old). The ASD samples in these age bands were able to complete the Basic Module but struggled with the Intermediate Module. These two ASD age bands showed very similar performance, indicating little developmental difference.

It was also noted that there was less variance in performance for the neurotypical sample as they got older, whereas the ASD group showed greater variance in scoring in the older age bands. This could suggest that while neurotypical children are developing their skills and ultimately "catch up" with each other, the delays seen in ASD are variable and as children age

and the expectations regarding their abilities are raised, their deficits become more apparent. This finding of variable degrees of delay is in keeping with Hoogenhout and Malcolm-Smith (2014) finding of both plateaued and delayed ToM development in a local ASD sample. However, they found that the difference was associated with IQ, as plateaued development was seen in the children classified as having low functioning ASD vs. delayed development in those with high functioning ASD. Other studies are mixed, as some also noted ToM development as delayed rather than having plateaued in higher functioning ASD samples (Paynter & Peterson, 2010) while others found their performance was comparable to neurotypical peers (Scheeren et al., 2013). As I did not classify level of functioning in the current sample, I cannot directly compare my results with this literature. However, given the variability in ToM performance in the later age bands, I think that the older children with a more severe deficit in ToM performance could perhaps indicate plateaued development, while the others may represent delayed development. I have established the developmental degree to which participants with ASD were delayed compared to their neurotypical peers in regard to ToM development. If future research utilised the same approach and conducted further developmental tracking, we could see points where, and to what degree, ToM development would stop, accelerate, or "catch up" for these children.

ToM performance in ASD is variable, as some children have greater deficits than others, so age norms are not available (Hoogenhout & Malcolm-Smith, 2014). It has been reported that children with ASD require a verbal mental age of 11 years and older in order to pass first-order false belief tasks (Happe, 1995). The majority of the current ASD sample showed VIQ deficits, indicating lower verbal mental age than chronological age, but some of these children were able to complete first-order false belief tasks, which is not in keeping with Happe's (1995) findings. The verbal ASD sample showed reduced VIQ compared to the neurotypical group, but their

mean score was 85. This score is not considered a great deficit in the South African context as tests were normed and developed for Western cultures and tend to under-estimate true IQ in local children (Lynn & Meisenberg, 2010; Wicherts et al., 2010).

The role of VIQ in ToM performance was particularly important in this study. VIQ was a stronger predictor of ToM performance than either age or SES for both samples. The neurotypical sample had a mean VIQ in the high average range, and the ASD sample had a mean VIQ in the low average range. It is noted that I only included children who were able to pass two-stage commands and were verbally fluent, so children with more severe language deficits were excluded from this aspect of the study. VIQ did not fully account for the ToM deficits seen in the current ASD sample, however, as the level of deficits in ToM appeared more severe than their slightly reduced VIQ abilities.

Although VIQ did not fully explain the ToM deficits in the current ASD sample, it did play a significant role in ToM performance. The ASD group showed a different correlation pattern between VIQ and ToM than the neurotypical sample, perhaps suggesting a greater role for VIQ in ToM performance in the ASD group compared to the neurotypical group. The ASD sample also had a larger standardised *beta* value for VIQ in a regression model predicting ToM performance, and the model had a larger effect size for the ASD group, confirming VIQ had a greater association with ToM for this group. Happe (1995) argued that children with ASD may be more reliant on language skills during ToM testing as they use language structure and knowledge to help solve ToM problems in the absence of fully developed ToM abilities. In the current sample, the association between VIQ and ToM in the ASD groups supports the notion of VIQ as a protective factor in ToM development.

Theory of Mind deficits and ASD-Related Deficits

I found significant relationships between ToM deficits and overall ASD severity, as well as ToM deficits and deficits in social functioning as assessed by the ADOS2 Social Affect scoring domain which focuses on symptoms in the social communication and interaction domain. This is in keeping with the literature that supports a role for ToM in ASD deficits, and specifically in social deficits in this disorder. The association between ToM and ADOS2 Comparison scores was stronger than the association between ToM and ADOS2 Social Affect score, and was not significant for ADOS2 RRB scores (i.e. symptoms in the restricted and repetitive behaviours and interests domain); this pattern indicates that ToM is directly linked to social deficits in ASD rather than those in the restricted and repetitive behaviours and interests symptom domain, but that the relationship to overall ASD severity went beyond this link to social deficits (see Chapter 4: The Theory of Mind Study – Results – Question 2, pg. 166).

I found an association between ToM ability and reduced overall ASD severity as indicated by the ADOS2 Comparison score when VIQ was controlled for. I used the ADOS2 to rate ASD-related deficits, which is a gold-standard for ASD diagnostics, and adds support to the existing literature using other measures. Lerner et al. (2011) found associations between ToM deficits and overall scores of ASD-related deficits measured by the Social Communication Questionnaire, Social Skills Rating System – Parent, and Social Responsiveness Scale (SRS). The relationship between ASD-severity and ToM ability has therefore been found across several measures.

The association in the current sample between ToM ability and lower scores on the ADOS2 Social Affect domain, and the absence of a relationship between ToM and ADOS2 RRB scores, indicates that ToM specifically has a greater role in social deficits in ASD than in restricted and repetitive behaviours and interests. This specific association has been found

previously in the literature. Fombonne et al. (1994) found an association between better ToM skills and reduced maladaptive behaviours as assessed by the VABS. Frith et al. (1994) expanded the VABS to differentiate between social behaviours that required ToM abilities and those that did not and found that children who could pass false belief tasks had better communicative abilities; they also found that only the children who passed these tasks displayed the ability to have insightful interactions.

While the current study supports the literature above, it does not support the findings of Joseph and Tager-Flusberg (2004), who found that ToM ability did not explain the variance of ASD-related deficits in either the social domain or the symptom domain of restricted and repetitive behaviours and interests. Our studies had considerable similarities: we both used the ADOS/ADOS2 to rate ASD-related deficits; my ToM battery included all three of the false belief tasks they used, although my battery was more comprehensive; and both study samples had VIQs in the low average range. Both study samples were also predominantly male children: my study sample constituted 51 children who were all male, and their sample had 31 participants, only four of which were female. Our studies were therefore highly comparable, and yet I found contrasting results. In their study they initially found a correlation between ToM and ADOS scores, but this was not significant when language ability was controlled for. The similar VIQs across the studies indicate that language capability does not explain the different results. One possible factor that might explain the differences in our findings then is that my study had a slightly older sample, with a mean age of 10 years 3 months while theirs was 8 years 9 months. I did however control for age in my analyses, which would limit the effect of this difference. It is therefore more likely that the association between ToM and ASD-related deficits emerged in my study because my ToM battery encompassed a wider set of tasks, I controlled for age in the ToM

scoring, and because my sample's wider age range allowed a better overview of ToM development in childhood.

This study therefore suggests that ToM capability in ASD is related to better outcomes in both overall severity as well as in the social domain specifically. ToM deficits are consistently noted in ASD samples. Targeting ToM deficits in ASD for intervention could therefore lead to improvements in social deficits in ASD, as well as other areas resulting in a decreased overall ASD severity.

A Possible Role for 5-HTTLPR in ASD-Related Deficits and ToM Deficits

My ASD sample showed a higher incidence of the short allele for 5-HTTLR than reported in the literature, supporting the hypothesis that this polymorphism may be, at least in part, responsible for atypical serotonergic transmission in ASD. This polymorphism is implicated in overall ASD severity and level of deficit in the ADOS2 RRB symptom domain for the current non-verbal ASD sample, although not for the verbal ASD sample. The 5-HTTLPR genotypes were also not clearly associated with ToM performance in the verbal ASD sample. This difference between ASD groups was not anticipated and was limited by the small sample sizes. A future study might examine whether these group differences could be replicated, ideally with equal group sizes and a more comprehensive assessment of language ability beyond my categorical grouping. This novel finding has considerable implications for our current application of research findings to non-verbal children with ASD, who are generally under-represented in the literature, as our findings for verbal children with ASD may not generalise to this group.

Allelic Distribution of 5-HTTLPR in ASD. My study found a similar 5-HTTLPR allelic distribution for this cohort of male children with ASD to what was noted in a previous local study with a different ASD sample (Arieff et al., 2010), and a higher rate of the short allele than

the reported rate for neurotypical South Africans (Esau et al., 2008). In my sample of 73 male children with ASD I found an incidence of .38 of the short allele, with 13.70% of the total ASD sample carrying the *S/S* genotype. When I divided the sample according to verbal ability, the non-verbal ASD group had an incidence rate of .31 for the short allele and 5.88% of this sample carried the *S/S* genotype, while the verbal ASD group had an incidence rate of .44 for the short allele and 20.51% of this sample carried the *S/S* genotype.

Previous research showed local ASD samples had an incidence rate for the short allele of .39 (Arieff et al., 2010) and .46 (Hamilton, 2014), and in these samples the *S/S* genotype was present in 25-30% of cases. The current full ASD sample therefore showed a similar incidence rate for the short allele for 5-HTTLPR, but a lower incidence of the *S/S* genotype. The non-verbal ASD group showed the lowest incidence rate of the short allele, while the verbal ASD group had an incidence of the short allele and the *S/S* genotype similar to those previously reported for ASD samples locally.

The ethnic breakdown of my study can be compared to that of the Arieff et al. (2010) local study with an ASD sample. Our studies had similar rates of Caucasian participants and these subsamples showed similar prevalence of the short allele for 5-HTTLPR: my study was 34% Caucasian with an allelic rate of 0.42 for the short allele compared to their sample at 37% Caucasian with an allelic rate of 0.49. I also had similar rates for the mixed race participants: my study constituted 52% mixed race participants with an allelic rate of 0.36 for the short allele compared to their sample at 44% mixed race participants with an allelic rate 0.33. Our samples differed in terms of African participants, however, as my sample only had 8% African participants compared to their 19%. This could explain, at least in part, our lower prevalence for the short allele, as my African participants had an allelic frequency of 0.25 for the short allele

compared to the Arieff et al. (2010) prevalence of 0.36, suggesting differences may have partly been driven by my sample underrepresenting participants of African descent. However, my sample was male, while theirs was mixed sex.

These values can therefore be seen as a baseline for the local male ASD population. When compared to a study with a neurotypical South African sample (Esau et al., 2008), I note that my ASD sample had a far higher prevalence of the short allele for the African (0.25) and mixed race / "mixed" (0.36) samples compared to the rates in their study (0.16 and 0.14 respectively). The Caucasian samples, however, showed a far slighter disparity: neurotypical rate of 0.39 (Esau et al., 2008), compared to my ASD sample at 0.42 and the previous rate of 0.49 samples (Arieff et al., 2010). Previous international studies with Caucasian samples have found similarly high rates of the short allele (for a review see Esau et al. (2008)). It therefore appears that the allelic rate of the short allele may not differ between neurotypical and ASD Caucasian samples.

The picture is more mixed when we look at the *S/S* genotype specifically, showing no clear pattern across ethnicities in local samples. For African samples, I had no incidence of this genotype, another local ASD sample found this genotype in 33% of their African participants (Arieff et al., 2010), and a local neurotypical sample had no incidence of this genotype (Esau et al., 2008). For the mixed race samples, I had a prevalence of 13.16% for this genotype, the other local ASD sample found this genotype in 20.83% of their mixed race participants (Arieff et al., 2010), and the local neurotypical sample had an incidence of 2.81% for this genotype (Esau et al., 2008). The rates for the Caucasian subsamples were similarly mixed, at 16% for my sample, 40% for the other ASD sample (Arieff et al., 2010), and 11% for the neurotypical sample (Esau et al., 2008).

It therefore appears that the allelic rate for the short allele for 5-HTTLPR is higher in ASD samples for African and mixed race participants compared to neurotypical samples, but not for Caucasian samples. This indicates that if 5-HTTLPR is implicated in male children with ASD, this may be limited to ethnicities other than Caucasians. The incidence of the *S/S* genotype does not appear to show a pattern across ethnicities and between ASD and neurotypical samples. However, as ethnicity was not considered in recruitment, I did not have adequate or balanced representation for each ethnic group, so these results must be interpreted with caution. Ideally later studies would consider better representation of ethnic groups, especially when looking for genotype-phenotype relationships.

The Role of 5-HTTLPR in ASD-Related Deficits. When I assessed ASD-related deficits as rated by the ADOS2 assessment across 5-HTTLPR genotypes, I only found relationships for the non-verbal sample. For the non-verbal ASD group I found that the *S/S* genotype was associated with greater deficits in ADOS2 Comparison scores, indicating overall ASD severity, and with greater deficits in ADOS2 RRB scores, indicating impairment in the restricted and repetitive behaviours domain. Genotype-phenotype studies for 5-HTTLPR and ASD are very limited, and to my knowledge only two studies have been published. These studies, however, disagreed on the role of the short allele in the ASD phenotype: Brune et al. (2006) were unable to replicate Tordjman et al. (2001) and Brune et al. (2006) studies Tordjman et al. (2001) finding of an association between the short allele and ASD severity. My study found strong evidence for this association, and also suggests a different role for 5-HTTLPR in non-verbal ASD vs. verbal ASD, at least for male children, which was also suggested by Brune et al. (2006).

Associations Between 5-HTTLPR and ASD-Related Deficits. My study found that 5-HTTLPR was only related to deficits in the non-verbal participants, and these associations were between the *S/S* genotype and ADOS2 Comparison scores and between the *S/S* genotype and ADOS2 RRB scores. This indicates that while 5-HTTLPR is linked to language in some way (as associations were not noted in the verbal ASD group), the actual role of this polymorphism appears to be implicated in overall ASD severity and in the restricted and repetitive behaviours and interest domain rather than the social interaction and communication domain in this study. These associations also indicate a dominant effect for the long allele, as it was the *S/S* genotype that was associated with deficits rather than the short allele specifically.

One of the strengths of my study design was the use of MRA to assess the role of the 5-HTTLPR genotypes. The Tordjman et al. (2001) and Brune et al. (2006) studies both merged the two genotypes with short alleles (i.e. *S/S* genotype and *L/S* genotype) into a reduced transmission group for comparison to the *L/L* genotype group, which would only reveal group differences if the short allele had a dominant effect. I used dummy coding for the genotypes in the MRA models so I could assess for a dominant effect for either allele. I found that the *S/S* genotype, rather than the short allele, was associated with deficits in the non-verbal ASD group. This suggests that it could be possible that the merging of groups used in the Tordjman et al. (2001) and Brune et al. (2006) studies may have masked genotype-phenotype relationships, although their data did justify this grouping as it suggested a dominant effect for the short allele. My study suggests that non-verbal children who are homogenous for the short allele are likely to have greater deficits than those who carry even a single long allele.

Association Between 5-HTTLPR and Non-Verbal Aspects of ASD. Another strength of my study was the division of the ASD sample according to verbal ability, as this revealed that

ASD-related deficits were only associated with 5-HTTLPR in the non-verbal ASD group. Although it is not clearly stated, it appears that both the previous studies recruited children with ASD regardless of language ability, and therefore were likely to have included a mix of non-verbal and verbal children. The proportion of non-verbal children included in their studies, as well as the genotype-phenotype analyses per group, was not reported, making direct comparisons less useful.

It is of interest, however, that although the Brune et al. (2006) group did not find an association between 5-HTTLPR and overall ASD severity, they did find a specific association between the short allele and non-verbal aspects of social interaction, specifically for impairment in the "failure to use non-verbal communication to regulate social interaction" domain of the ADI-R. In this analysis, a comparison between their study and mine is again limited as they merged the *L/S* genotype and *S/S* genotype groups, while I found specific associations only for the *S/S* genotype group, and only for the non-verbal ASD sample. However, both studies do support a role for the short allele in non-verbal aspects of ASD. While the Brune et al. (2006) study suggested a role in general for non-verbal aspects of ASD, my study clarifies and expands on this notion by suggesting a role for the short allele for individuals with ASD who are non-verbal, and that this association is for the restricted and repetitive behaviours and interests domain.

I did not find any associations between the *L/L* genotypes and ASD-related deficits. In contrast, the Brune et al. (2006) study found associations for the *L/L* genotype and "stereotyped and repetitive motor mannerism" on the ADI-R, and for the ADOS scores for "failure to direct facial expressions" and unusual sensory interests. This is in contrast to my study, as these scores would fall under the ADOS2 RRB score, which was associated with the *S/S* genotype in my

sample. I did not assess specific ADOS2 questions across genotypes, but rather looked at the overall scores (to limit type one error), so I cannot say which specific ADOS2 scores would be associated with 5-HTTLPR. However, based on the associations I found to the *S/S* genotype, and the absence of specific relationships to the *L/L* genotype, I do not believe I would have replicated their findings if I did assess specific ADOS2 questions rather than overall scores.

My study therefore adds to the existing body of genotype-phenotype studies for ASD and 5-HTTLPR. It indicates a potential role for this genotype in non-verbal ASD, and that this role is seen in an association between the *S/S* genotype and overall ASD severity as well as in the restricted and repetitive behaviours and interests domain. Previous studies assumed a dominant role for the short allele and grouped all short allele carriers into one group (i.e. *S/S* and *L/S* genotypes were merged), whereas my study allowed for either allele to be dominant, and the importance of the *S/S* genotype, rather than the short allele, emerged. It is noted, however, that the *S/S* genotype did not appear to be more prevalent in the ASD group compared to reported rates for neurotypical samples. My results therefore suggest that although the *S/S* genotype may not be more prevalent in ASD samples, when it is present it has implications for the ASD phenotype that emerges.

A further study should be conducted that does not assume a dominant role for either allele and that assesses for a qualitative difference in ASD based on language ability in order to clarify this. If my findings are replicated, it indicates that the serotonergic system should be targeted when a patient is non-verbal, and when they present with deficits in the restricted and repetitive behaviours and interests domain.

The Role of 5-HTTLPR in ToM. In my study, male verbal children with ASD who carried at least one short allele presented with lower ToM scores compared to those without short

alleles, as expected. However, each genotype showed high variability in ToM scores so the exact nature of this association needs to be further explored to consider other mediating variables. In regression analyses, the 5-HTTLPR genotypes were not significant predictors over and above VIQ despite increasing the strength of the model. It would appear then that 5-HTTLPR is not directly related to ToM ability in ASD, and if there is a relationship, there are further variables involved in this association that I did not identify. As the previous analyses showed no relationship between ADOS2 scores and 5-HTTLPR for the verbal ASD group, this further supports the hypothesis that in boys, if 5-HTTLPR plays a role for verbal children with ASD, it is a different role compared to that in non-verbal children with ASD.

VIQ was strongly associated with ToM in my sample and was the only significant predictor in my MRA model for ToM which included age, SES, and 5-HTTLPR genotypes as predictors. Including the genotypes in the model strengthened it, although the exact role of these genotypes was not clearly evident. Neither ToM nor VIQ correlated with 5-HTTLPR genotypes, and they showed different patterns across the genotypes: VIQ was highest for the *S/S* genotype group while the *L/S* genotype and *L/L* genotype showed similar scores; ToM scores were highest for the *L/L* genotype group, with the S/S genotype group scoring approximately midway between the *L/L* genotype group and *L/S* genotype. Therefore, although the children carrying short alleles performed poorly on ToM tasks, those with only one short allele performed worse than those with two short alleles, and there was no clear dominant effect for either allele for ToM scores in this study.

Previous studies found associations between poor serotonin binding and ToM skills in ASD samples (Murphy et al., 2006; Nakamura et al., 2010). My results did not support the hypothesis that 5-HTTLPR plays a role in the relationship between serotonin and ToM

performance, but perhaps other aspects of the system do – such as the 5-HT2A receptor researched by Murphy et al. (2006). It is also worth noting that while my study recruited children with ASD, these two previous studies recruited adult males, and one must be cautious when comparing a developing brain to that of an adult. Further, Murphy et al. (2006) had a small sample of only 8 men, and they assessed ToM indirectly by analysing ADI-R scores for reciprocal social interactions and stating that these skills were reliant on ToM abilities; the actual association to ToM is therefore less clear. The association between serotonin and ToM in the Nakamura et al. (2010) study was to performance on *faux pas* tasks. My sample was younger and were developmentally further behind in their ToM development than their neurotypical peers, so they were unable to complete social *faux pas* tasks. It is perhaps possible then that serotonin's relationship to ToM changes between childhood and adulthood, and is then evident on more advanced ToM tasks. A longitudinal study or cohort which included children and adults with ASD is required to clarify if 5-HTTLPR's possible role in ToM changes across development, or whether 5-HTTLPR is not included in the aspects of the serotonergic system that are implicated in ToM processing.

Summary of the Theory of Mind Study

The Theory of Mind Study showed that ToM was associated with ASD-related deficits in a male verbal ASD sample, and that the relationship between VIQ and ToM in ASD is different to that in neurotypical samples, potentially indicating that VIQ provides alternate means to complete ToM tasks for children with ASD. I found that the short allele for 5-HTTLPR had a higher prevalence in my ASD sample compared to reported rates for neurotypical samples. There was not an obviously higher rate of the *S/S* genotype specifically, but the *S/S* genotype did still appear to influence ASD phenotypes. The *S/S* genotype had an association with overall ASD

severity and with deficits in the restricted and repetitive behaviours and interest domain for non-verbal children with ASD, but not for verbal children with ASD. The role of 5-HTTLPR in verbal children with ASD was not elucidated in this study, and although an association between 5-HTTLPR, VIQ, and ToM did not emerge in my sample, the contribution 5-HTTLPR made to the MRA model predicting ToM performance suggests it may have a role that did not emerge due to unconsidered variables. ToM and serotonin are therefore both important aspects of ASD that should be considered during diagnostics and treatments. Targeting ToM deficits for intervention could result in decreasing other ASD-related deficits, and regulating serotonergic transmission in non-verbal children with ASD could reduce the severity of their disorder.

Chapter 5: General Discussion

The aim of this protocol was to assess for genotype-phenotype relationships in ASD, and to illustrate an approach that merged existing knowledge from psychological and medical / genetic research. Genome wide association studies are becoming the widely preferred approach in ASD genetic research and are revealing interesting findings regarding the genetic underpinning of ASD. These types of studies allow for comprehensive assessment of the human genome that reveal novel targets not yet identified by other investigations, and can identify linkages and interactions between genes that candidate genes cannot. However, I believe that these kinds of studies can be coupled with hypothesis-driven candidate gene approaches such as mine, as this may allow existing psychological knowledge regarding ASD to be paired with our expanding genetic understanding of this disorder. I propose that the historical contribution from psychological research, and how this has aided our understanding of how ASD unfolds across development, can continue to provide valuable insight into the underlying genetic mechanisms for ASD if we also pair strong psychological theories with selected ASD candidate genes in research. Using this approach, I focused on the role of reduced social motivation and ToM deficits in the overall ASD phenotype, and explored whether two ASD candidate genes, OPRM1 and 5-HTTLPR, were implicated in the ASD phenotype and in impairment in these two areas respectively.

From the time ASD was first recognised as a unique disorder, we have relied on psychological theories to deepen our understanding of this complex disorder. Recently there has been a surge in genetic studies in ASD, where researchers hope to identify key genes that underly the core phenotypic traits in ASD in an endeavour to elucidate the neurobiological mechanisms contributing to this disorder. Although there are studies which aim to expose the genetic basis for

specific ASD phenotypes and clinical variance, these studies have tended to do so without including psychological theory in their approaches. Similarly, psychological theories for ASD often implicate genetic contributors, but either do not empirically assess the genetic aspects of their claims or limit their research to animal studies. This separation of fields has resulted in a somewhat fragmented understanding of the causes and development of ASD. This greatly limits our understanding of the neurobiological basis of this disorder, which may hamper the efficacy of medical and psychological interventions. Drawing on the contributions from psychological and genetic research, and furthering research by combining these fields, we may be better positioned to identify contributing factors to ASD, and their role in the development of different phenotypes. Understanding the neurobiological basis of the core deficits within the context of psychological theory may provide the opportunity to design targeted biological and psychological interventions within developmentally appropriate windows.

The findings of each study have been presented in detail in the empirical chapters, but are briefly discussed below, with a focus on their overall implications. This is followed by a discussion of methodological considerations in this protocol and for future research.

The Social Motivation Study

The Social Motivation Study is unique in how it examined the three main tenet's of Panksepp's (1979) Social Motivation Theory for its role in ASD directly in a sample of male children with ASD. I found significantly decreased levels of social motivation in the ASD sample compared to the neurotypical sample and found associations between reduced social motivation and deficits in the social communication and interaction domain for the non-verbal ASD group. The absence of this relationship in the verbal ASD group suggests a further relationship between language acquisition or development and social motivation. Panksepp (1979) proposed that the

relationship between reduced social motivation and social impairment in ASD could extend to atypical language development, but this hypothesis remained relatively unexplored. The Social Motivation Study presented here supported this theory and led me to propose that Panksepp's (1979) theory could possibly be expanded to include a threshold effect for the impact of reduced social motivation on social development in male children with ASD. I propose that male children with more severely reduced social motivation experience, more severe deficits in social competence and that this may prevent or undermine language development, while those with less impairment to their social motivation are able to develop language more easily. Further, I propose that once language had developed, these children have a relative advantage, as other social and cognitive domains are then better able to develop - this then reduces the long term impact of their early deficits in social motivation.

Language is developed socially, with social interest and social interaction being strongly tied to language development (Kuhl, 2010). A literature review on non-verbal children with ASD by Tager-Flusberg and Kasari (2013) noted that these children tended to have severely reduced social motivation. Kintwall et al. (2014) found that reduced social motivation was associated with greater language deficits. This literature suggests that the difference in social motivation scores between the two ASD samples in the current study are, at least in part, related to their language abilities as suspected. As expressive language is one of the strongest predictors of ASD outcome (Szatmari et al., 2003), this relationship between reduced social motivation and poor language abilities remains important when considering ASD interventions. And, as there are associations between verbal communication skills and cognitive abilities (Kjellmer et al., 2012), this could further support the hypothesis that if social motivation is reduced but not sufficiently

so to meet a proposed threshold (i.e. the child has less severely reduced social motivation that does not prevent language development), then other cognitive domains are better able to develop.

This study also supported a role for altered mu-opioid processes in ASD and reported that children with ASD in this study had an unprecedentedly high prevalence of the OPRM1 *G* allele. This meant that statistical exploration of the possible link between this allele and reduced social motivation was not possible. However, I note that all of the non-verbal ASD participants carried at least one of the *G* alleles, and the two non-carriers in the verbal ASD group lacked significant reductions in social motivation (Chapter 3: The Social Motivation Study - Results – Question 3, Hyp. 3.3, pg. 102).

The suggested threshold effect for the role of reduced social motivation in ASD has important implications for the management of ASD. If early targeting of reduced social motivation could prevent this low social interest from meeting the proposed threshold, this could have a lasting impact on a child's development. If lowered social motivation can be targeted and levels increased, perhaps language may be better able to develop and remaining social motivation difficulties have less of an impact on a child's psychosocial development. Elevated mu-opioids levels are easily treated with medications such as naltrexone, and research already exists that shows that naltrexone administration has a possible ameliorating effect on ASD symptoms (Bouvard et al., 1995; Herman et al., 1987). Interventions also exist that specifically target social interest in ASD, such as the Early Start Denver Model (ESDM) which teaches parents to use the child's non-social interests as a way to engage socially (Dawson et al., 2010; Estes et al., 2015). Perhaps then, toddlers with suspected ASD could be assessed for mu-opioid levels or OPRM1 genotypes, and psychological interventions to increase their social motivation could be coupled with appropriate medications. The hope would be that this combined approach

could diminish the long-term effects of reduced social motivation and decrease the severity of symptoms in the social communication and interaction domain. This, however, is speculative at this point, but it is an area of research that may be promising and replication studies regarding the proposed threshold effect are required.

Limitations and Future Directions

The Social Motivation Study was exploratory in nature, and the findings here should be confirmed in replication studies that also address the limitations this study faced. Limitations specific to this study are discussed here, while overarching limitations for the entire protocol are discussed toward the end of this chapter.

I assessed social motivation indirectly through parent interviews. I used attachment behaviours as a proxy for level of social motivation and used a measure that looked at a child's response to feelings about social interactions and separations. Measuring attachment beyond infancy can be controversial as social behaviours become far more complex in childhood and adolescence when compared to those of early infancy (Dwyer, 2005). However, as I was not assessing true attachment, but rather indicators of social interest and pleasure, this debate is of lesser concern in the context of this particular study. The attachment measure in this study assessed whether children sought out social interaction, as well as how they responded to others initiating this contact. The responses that indicated secure attachment were those that showed a child sought interaction, enjoyed this engagement, and responded well when others initiated it. These are suggested indicators of positive social motivation, just as low scores would indicate poor social motivation. Further, as these responses were based on the general pattern of the child's behaviour over time, I believe they can give a robust indication of the child's general level of social motivation.

The use of the ASCQ as the attachment measure also had the advantage of being developmentally appropriate across the lifespan. This measure relied on parent report, which is always open to some bias, but avoided the limitations of a strange situation test (i.e. this test is not appropriate for older children and novel situations can be anxiety provoking for children with ASD). Ideally a measure of social motivation should be developed that can be administered in human samples that assesses the level of social interest behaviourally, emotionally, and cognitively. To my knowledge, two such measures have recently been developed with ASD samples (Elias & White, 2019; Schapp, 2016). These were only published after the current study had already been conceptualised and data collection had commenced, but their release indicates a growing interest in assessing social motivation in ASD samples. However, considerations will still need to be made to ensure that non-verbal children are not unfairly graded on such measures, and a measure that can be utilised without reliance on self-report is needed.

I included children with a range of symptom severities, including those with little to no language. The inclusion of these children showed the importance of language ability when considering phenotypic research, as associations between genotypes and variables of interest differed between the non-verbal ASD and the verbal ASD groups. However, in the context of the Social Motivation Theory Study, the evidence for a relationship between language acquisition and social motivation was limited by my binary categorisation of language ability into verbal (i.e. fluent expressive speech) and non-verbal.

Due to methodological considerations of the second study, which required fluent language for ToM assessment, I grouped children based on whether they were fluent or not. Although a standard definition of "non-verbal" does not exist in the literature (Tager-Flusberg & Kasari, 2013), research tends to include fully verbal children at the cost of "the rest". This results

in a pragmatic approach in research where participants are either fully verbal and able to complete most research measures, or are not fluent and fall into the non-verbal categorisation. I followed the same categorisation to ensure non-verbal children were included where possible. However, due to the low inclusion rates of non-verbal ASD children in existing research, I had no reason to anticipate different genotype-phenotype relationships between the groups, and therefore did not include a better, more nuanced, assessment of language ability. This binary characterisation of language ability as either "non-verbal" or "verbal" in the current study limits our understanding of the extent of the relationship between language and social motivation, and later studies should consider more comprehensive language assessments. Where possible a continuous measure of language ability alongside a continuous measure of social motivation should be utilised. Such a study would reveal what level, if any, of social motivation is critical for language development, and the degree to which the two are related.

While a large focus of this study was the genetic role of the OPRM1 genotypes in the ASD phenotype, unanticipated findings did not allow me to conduct the statistical analyses originally proposed. The exceptionally low rate of the *A* allele meant that statistical analyses to investigate possible associations between the OPRM1 *G* allele and phenotypic traits could not be conducted on the current sample. I had anticipated a higher rate of the *G* allele in my ASD samples compared to those reported for neurotypical samples, but as the highest previously reported prevalence of this allele for neurotypical samples was .35 (A. C. Chen et al., 2013; Way et al., 2009; Wei et al., 2017), I could not have foreseen that only two of my participants would not carry the *G* allele. The high rate of the *G* allele was a novel finding that needs to be replicated in other ASD samples.

The unprecedentedly high rate of the OPRM1 *G* allele requires that future studies have larger and more diverse samples sizes to get a higher representation of non-carriers of this allele. However, studies should include non-ASD samples to avoid this underrepresentation of the *A* allele. For these studies, I would suggest merging children with ASD and without ASD into a single group and measuring ASD-related traits / tendencies and social motivation on a continuous scale. As Panksepp (1979) proposed these relationships exist in all humans, and that children with ASD represented the extreme in regard to social motivation difficulties, such a study could allow for an overall picture of how social motivation deficits may be in part underpinned by alteration in OPRM1 alleles and genotypes. The inclusion of mixed samples will also allow a direct comparison of OPRM1 rates between ASD samples and neurotypical samples with similar genetic heritage.

The Theory of Mind Study

The Theory of Mind Study explored the phenotypical role of ToM deficits in male children with ASD, and the possible role of the 5-HTTLPR genotypes in ToM deficits and the ASD phenotype. I found that male children with ASD were typically at least one developmental stage behind neurotypical children in their ToM development, and that VIQ may play a larger role in ToM performance for the children with ASD. As expected, ToM ability was associated with symptoms in the social communication and interaction domain over and above the influence of VIQ, age, and SES. The role of 5-HTTLPR in ASD was not what I hypothesised: I found an association between the 5-HTTLPR short allele and symptoms associated with the restricted and repetitive behaviours and interests symptom domain rather than the social communication and interaction domain, and only found this association for the non-verbal ASD group. No associations between 5-HTTLPR and ToM emerged, nor between 5-HTTLPR and ASD

symptoms in the verbal ASD group. This genotype is therefore unlikely to play a major role in the ToM Theory for ASD, although it may be implicated in other aspects of this disorder.

The link between ToM and social competence suggests that targeting ToM deficits for intervention could have long lasting positive effects on social deficits in ASD. The role of VIQ must also be kept in mind and interventions should consider this domain as well, as strengthening VIQ skills may further assist in acquiring ToM capabilities. As mentioned previously, my study was limited to male children, and language and social development may be different in female children (Kuhl, 2010).

By contrast, 5-HTTLPR may play in a role in the second symptom domain, namely symptoms under the restricted and repetitive behaviours and interest domain. Previous studies have had mixed findings for the use of SSRIs for treatment of symptoms in this domain: some found improvements in this symptom domain (Hollander et al., 2005; Namerow et al., 2003), while others did not (King et al., 2009). An alternative approach to treatments might include a targeted strategy based on genotyping as well as phenotyping, and an association between 5-HTTLPR genotypes and treatment responses could emerge. Given the diverse presentations associated with ASD, being able to identify which children would most benefit from each possible treatment could be beneficial.

Limitations and Future Directions

The Theory of Mind Study used a comprehensive ToM assessment, but this meant the exclusion of the non-verbal participants from the ToM assessment. Although it would have been possible to conduct non-verbal ToM tasks, many of these tests are considered simplistic and do not assess higher order levels of ToM, which would create a ceiling effect in testing. Ideally more comprehensive non-verbal ToM tasks should be developed, and the phenotypic role of ToM

deficits should be explored in non-verbal children with ASD as well. As this protocol frequently found differences between the non-verbal ASD group and the verbal ASD group, it would be useful to explore whether ToM deficits played the same role in both groups as this will impact intervention outcomes. However, given the intertwined nature of language and other cognitive domains in development (Mody et al., 2013), and the relationship between language and ToM (Happe, 1995), it is likely that the relationship between ToM and biological processes may be different in non-verbal ASD samples compared to verbal ASD samples.

I therefore opted to limit this aspect of the study to verbal participants and conduct comprehensive ToM testing. I utilised a ToM battery that expanded the typical analyses of first- or second-order false belief assessment, and included assessment of ToM capabilities from basic pretend play through to more complex ToM tasks such as understanding social *faux pas*. This decision prevented a ceiling effect on ToM testing for the neurotypical group. It also allowed us to provide an assessment of ToM capabilities in the verbal ASD group that indicated their ToM developmental stage by having them complete tasks of increasing complexity and then directly comparing their performance to the matched neurotypical group's performance. However, as discussed above, I considered children to be verbal if they were able to follow two-stage commands. While this was sufficient for following the instructions of the ToM tasks, it is possible that for the higher order ToM tasks in the *Advanced* module, the performance of some of these children may have been impacted on by language-related difficulties. I do not think this was a large risk to the study, however, for two reasons: first, the majority of the ASD participants did not reach the *Advanced* level of the battery as they struggled with more basic ToM tasks; and, second, all tasks included control questions to assess comprehension of the material.

Nonetheless, a greater focus on assessing language more comprehensively in future studies would allow us to appreciate the influence of language skills on ToM in a more nuanced fashion.

A further limitation of this study was that I assessed only one aspect of the serotonin system. While there is support for the role of serotonin in ASD and in ToM development, I did not find strong support for 5-HTTLPR specifically in my sample. While negative findings do help research, it would be more productive if one was able to assess the serotonergic system more comprehensively. A previous study found that the 5-HT1A receptor was implicated in ToM performance in schizophrenic patient (Bosia et al., 2011), and perhaps future studies could assess 5-HT1A's role in ToM in ASD as well.

Overall Considerations From This Study

I aimed to integrate existing knowledge from psychological theories and genetic research, as I hypothesised that this approach could generate more comprehensive and nuanced knowledge regarding the neurobiological bases of core deficits in ASD within the context of our psychological understanding of this disorder. Ultimately, this knowledge may facilitate the development of better interventions for children with ASD. The Social Motivation Theory for ASD and the ToM Theory for ASD were selected as both have considerable support from psychological research and deficits in these areas are already the focus of some ASD interventions. Similarly, OPRM1 and 5-HTTLPR were selected because they are implicated in ASD and because medications already exist that can target the effects of these genes – that is, medications exist to reduce mu-opioids binding, or that target 5-HTTLPR directly if serotonergic transmission is atypical. The diverse nature of ASD presentations can make it a challenging disorder to research and there is an overwhelming amount of research from various fields. This research lacks integration and needs to be better synthesised for meaningful explanations about

the causes and development of this disorder so that treatments and interventions can be properly informed.

Several further considerations could be made in future genotype-phenotype studies. Perhaps most importantly, the current research was conducted directly with an ASD sample with a matched neurotypical group for comparison. Psychological theories about ASD that include biological aspects should ensure an element of assessment with human ASD samples. For both psychological theories here, a great deal has been learnt from animal models (e.g. Cinque et al., 2012; Moles et al., 2004), as well as from inferences from other disorders with shared traits or deficits (e.g. McDonald et al., 2013). To ensure the theories are valid, however, they do need to be assessed within human ASD samples. The complexities of human nature and the interplay between demographic, cognitive, and societal influences can only be considered when working with a human sample, and the variability of possible ASD presentations requires that these studies include participants with ASD.

Human studies are complex, however, as there is not a single ASD phenotype. I used the ADOS2 to assess ASD-related deficits, focusing on the severity of the overall disorder, as well as severity of symptoms in the two main ASD symptom domains (i.e. social communication and interaction, and restricted and repetitive behaviours and interests). My studies were exploratory and among the first to explore these genotype-phenotype relationships in ASD. As the ADOS2 is used in diagnostics, it allows an examination of the symptoms and behaviours that clinicians tend to focus on. Although this gave the research clinical validity, it only allowed for a broad examination of the ASD phenotype. As an exploratory study this can set the foundation for later studies to conduct more targeted investigations. For example, where the ADOS2 SA scores showed associations, future studies can identify more specific symptoms in their phenotyping. In

this way, we may learn more about the biological underpinnings of specific aspects ASD. Ultimately, this may help us understand the various presentations seen in ASD.

This protocol consistently found that non-verbal children with ASD showed different associations between variables of interest compared to the verbal children with ASD. Language is therefore a critical aspect of the ASD phenotype and needs to be considered in future research. My study only looked at whether language had been acquired or not, but it would have been useful to understand the role language plays in the ASD phenotype in more detail; future studies could consider the role of language delay and could aim to characterise language deficits in a more detailed manner. This protocol showed that children who acquired language had a significant advantage in their social competence compared to those who did not, and that understanding the mechanisms that prevent language acquisition as well as the influence of language deficits on the development of the ASD phenotype is needed.

Another limitation with regard to language, is how generalisable my findings may be for South African children with ASD. I limited participants to children who were fluent in English, or non-verbal children who were from English speaking homes or schools. South Africa is a very diverse country, with English being only one of our 11 national languages, and it is not the home language of the majority of residents. This limitation was due to the use of the ADOS2, but efforts are being made locally to translate and validate the ADOS2, and other measures, that will hopefully improve the potential for more representative research from South Africa.

The exclusion of females from my study allowed for clearer phenotyping but at a cost to generalisability. ASD is predominantly diagnosed in males, with a ratio of 4:1 to females (American Psychiatric Association, 2013). Further, there is some evidence that the female ASD phenotype may differ from males with the same diagnosis (River & Matson, 2011). This is

particularly relevant when studying social deficits, as female children with ASD may have a social advantage over male children with ASD. In general, female children tend to have an advantage in language development (Adani & Cepanec, 2019). Sedgewick et al. (2016) also found that female children with ASD display social motivation levels and qualities of friendships similar to those of female neurotypical children, and with considerably less deficits and difficulties in this area than male children with ASD. Female children with ASD also appear to have a better understanding of what is required in social relationships, and derive pleasure from social relationships (Cook et al., 2017; Vine Foggo & Webster, 2017). As female children with ASD may therefore show higher levels of social motivation than male children with ASD, as well as better social abilities, I did not include them in either of my studies. I did not think it would be possible to statistically control for sex in my studies, as I was unlikely to recruit sufficient females with ASD due to there being a much higher male prevalence of the disorder. Future studies with larger samples should aim to include females but must remain mindful of the possible phenotypic differences across sex in ASD.

When considering the role of different genes in the ASD presentations, I relied on reported rates of the alleles as I could not afford DNA analyses for the neurotypical sample. I am confident that the findings are valid: for OPRM1, this high rate of the *G* allele has not been reported in any samples that I am aware of, in and outside of ASD research; and for 5-HTTLPR our allelic distribution was very similar to that of another local ASD sample (Arieff et al., 2010) which had been compared to a neurotypical sample (Esau et al., 2008) from the same researchers. However, it would still be best if researchers included DNA analyses for matched controls as this allows more direct comparison for allelic distributions differences between samples. Further, it will be useful to understand whether implicated genes play a unique role in

ASD, or whether the associations between the genes and ASD-related symptoms are generally present in neurotypical populations as well albeit to a non-clinical degree.

Concluding Remarks

Combining the knowledge obtained through psychological and genetic research may enable progress in our understanding of underlying biological mechanisms for ASD, as well as their roles in ASD phenotypes across development. ASD is a prevalent and complex disorder and has therefore attracted the attention of researchers in various fields. Numerous psychological theories regarding the aetiology and development of ASD have been developed and some are well supported, while genetic studies have implicated hundreds of possible candidate genes. In the current study, I argue that there is value in combining both these fields and have done so with two psychological theories and two candidate genes.

I chose to focus on deficits that are noted early in ASD as their presence early in development suggests they would have a critical role in later social development, and I therefore explored the Social Motivation Theory for ASD and the ToM Theory for ASD. Both theories were supported in my study, and this suggests that reduced social motivation and ToM deficits are areas that should be targeted early in development. While psychological intervention may not prevent the development of ASD, it has the potential to limit the severity of symptoms and their effects across the lifespan.

I chose to focus on genes that, if found to be implicated, could be targeted with existing medications. Ideally children with ASD would be diagnosed early in life and based on the level of reduced social motivation or ToM deficits, they could be treated with medication as well as psychological intervention. The hope would be that this would give these children the best possible chance of reaching their full potential.

While my protocol included two genotype-phenotype studies, it was exploratory in nature and I faced marked limitations in time and funding. The study ideas I have suggested for future research will require multidisciplinary teams to ensure more in-depth genetic assessment, comprehensive psychological phenotyping, and clinical input regarding the role of medications. These studies are likely to be expensive and those looking at interventions or medical use will need to be longitudinal to some extent. I hope that my study has laid the foundation for such studies, as they will greatly improve our current understanding of ASD.

Understanding ASD, revealing the neurobiological factors that contribute to its emergence, and being able to predict how the disorder will present across development will help with improving diagnostics and treatments, and ultimately could help thousands of children and their families. The current protocol has contributed by demonstrating a methodology for unifying existing research in a meaningful way, by furthering our knowledge of two key areas of deficits in ASD that are not included in the diagnostic criteria, and by identifying some important future avenues of research in this field.

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Appendix A

DSM5 ASD Diagnostic Criteria for Autism Spectrum Disorder

Diagnostic Criteria299.00 (F84.0)

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history (examples are illustrative, not exhaustive; see text):
    1. Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interests, emotions, or affect; to failure to initiate or respond to social interactions.
    2. Deficits in nonverbal communicative behaviors used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of gestures; to a total lack of facial expressions and nonverbal communication.
    3. Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behavior to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers.

Specify current severity:

- **Severity is based on social communication impairments and restricted, repetitive patterns of behavior**.

B. Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following, currently or by history (examples are illustrative, not exhaustive; see text):
    1. Stereotyped or repetitive motor movements, use of objects, or speech (e.g., simple motor stereotypies, lining up toys or flipping objects, echolalia, idiosyncratic phrases).
    2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior (e.g., extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take same route or eat same food every day).
    3. Highly restricted, fixated interests that are abnormal in intensity or focus (e.g., strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interests).
    4. Hyper- or hyporeactivity to sensory input or unusual interest in sensory aspects of the environment (e.g., apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement).

Specify current severity:

- **Severity is based on social communication impairments and restricted, repetitive patterns of behavior**.

C. Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life).
D. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.
E. These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay. Intellectual disability and autism spectrum disorder frequently co-occur; to make comorbid diagnoses of autism spectrum disorder and intellectual disability, social communication should be below that expected for general developmental level.

Note: Individuals with a well-established DSM-IV diagnosis of autistic disorder, Asperger's disorder, or pervasive developmental disorder not otherwise specified should be given the diagnosis of autism spectrum disorder. Individuals who have marked deficits in social communication, but whose symptoms do not otherwise meet criteria for autism spectrum disorder, should be evaluated for social (pragmatic) communication disorder.

Specify if:

- **With or without accompanying intellectual impairment**
- **With or without accompanying language impairment**
- **Associated with a known medical or genetic condition or environmental factor** (**Coding note:** Use additional code to identify the associated medical or genetic condition.)
- **Associated with another neurodevelopmental, mental, or behavioral disorder** (**Coding note:** Use additional code[s] to identify the associated neurodevelopmental, mental, or behavioral disorder[s].)
- **With catatonia** (refer to the criteria for catatonia associated with another mental disorder, pp. 119–120, for definition) (**Coding note:** Use additional code 293.89 [F06.1] catatonia associated with autism spectrum disorder to indicate the presence of the comorbid catatonia.)

Appendix B

Study Information Sheets: ASD Participants

UCT Autism Research

Brief Overview of Psychology Doctoral Study

The Biological Bases of Social Deficits: The possible roles of two candidate genes in social motivation and social ability in Autism Spectrum Disorder

Dear Parents

You and your child are invited to participate in my study! I am a PhD Psychology student with a history in in Neuropsychology (MA Clinical Neuropsychology), and am a member of the University of Cape Town Autism Research Group (uctautism.com). I am investigating the social difficulties in Autism Spectrum Disorders (ASD). I am interested in general social ability, and specifically in social motivation and Theory of Mind. Theory of Mind refers to the ability to understand other people's thoughts, beliefs, and emotions, and to understand that these are different from one's own. For example, the ability to understand jokes and the ability to understand that when you know something, everyone else doesn't automatically know it too, are forms of Theory of Mind. I am interested in two candidate genes as one may be involved in whether children look for social interaction (the mu-opioid receptor, OPRM1), and the other may be involved in how well children understand social interaction and other people's behaviours (the serotonin transporter promoter length polymorphism, 5-HTTLPR).

Who can participate?
In order to participate, your son must be between 4-16 years old and must understand English. Children with limited language ability can participate, and even non-verbal children can participate, as long as their home language or the language their teachers use with them is English. Children can participate either if they have or are suspected to have Autism Spectrum Disorder. You as the parent must also be fluent in either English or Afrikaans as I will need to interview you about your son.

Must my child and I participate?
No, not at all – this study is completely optional. There are no negative consequences if you choose not to participate. Also, if you decide to participate and then change your mind, you can just let me know that you are withdrawing and you don't even need to provide a reason. If this happens, you and your son will not be penalised in any way.

What will happen if we take part?
If you decide to participate in the study, I will ask you to sign a consent form and complete a demographics form. The demographic forms asks about your son's medical history and your family income and education. I understand that this is personal information, so as soon as I receive it I will remove your name and record the information under a confidential participation number. This information will not be shared with anyone else. We need this personal information for two reasons: first, we need the medical information to establish whether anything else could explain the relationships we are exploring, in which case we may not be able to include your son in the study (for example, if your child experienced a severe head injury, we cannot conclude that his social difficulties are due to ASD and the genes we are exploring); and second, we need the financial information to make sure that this research recruits children from all backgrounds and is therefore representative of the South African population.

Myself or someone in my team will then call you to arrange a time to interview you. The interview will consist of two parts, each 30 minutes, and can be done telephonically or we can meet and conduct the interview in person.

I will then meet with your son at his school. At the start of every session I will ask your son if he is willing to play the games with me that day, and if he isn't then we won't have a session. I will meet each child for 1-4 sessions, where we will complete several tasks all designed to measure different aspects of social and cognitive ability. All the tasks are designed to appear as games for the children, so they are all toy or story based.

Included in these sessions is the ADOS2 assessment. The ADOS2 (Autism Diagnostic Observation Schedule, Second Edition) is the international gold-standard ASD diagnostic tool. The information from an ADOS2 assessment is very valuable to your school and any doctors or psychologists involved in your child's treatment. Unfortunately there are usually long waiting lists to get an ADOS2 assessment and having it done privately can be quite expensive. However, if your child completes this assessment as part of this study I can share this information with the appropriate professionals.

Later in my PhD I will contact some families again to arrange to use a non-invasive cheek swab to collect a DNA sample from your child, but only if you are comfortable with this. This is done to see which expression of the candidate genes I'm researching your son has. I will do this by gently rubbing a cotton swab on the inside of his cheek. This swab is similar to an earbud and will not hurt your son or pose any risk to him. To make sure your son is comfortable, I will first let him play with a cotton bud and get used to putting it in his mouth. He can then imitate me showing him how to rub the inside of the cheek. I will only collect the sample once your son is comfortable.

What will happen to the information I give you and the information from seeing my son?
All information is recorded under a confidential participant number, and your privacy will be maintained at all times. I will not share this information with others, and if any data is shared it

will be the kind of information that does not reveal who you are (for example, when I send the lab samples I may give them the age and sex of you son, but not his name, school, or anything else). Therefore, your name, income information, son's medical information, and all other information will not be shared with anyone. All information will be securely stored so that no one else can access it, and the data is coded so that your name and your son's name are removed. Any DNA that is unused will be destroyed.

What will happen with the results of this study?
At the end of this study I will provide you with a personalised report explaining what I learnt about your son. You can keep this report, and you can choose to share it with schools or any clinical professional involved in your son's care (for example, psychologists, GPs, speech therapists, etc). I am also always available to discuss anything about the research and to answer any questions.

If I publish my findings from this study, you and your son will never be identified personally. I will be delighted to share the results with you as soon as they are available.

Who has approved this study?
This study has received ethical approval from the Western Cape Education Department, the UCT Psychology Department Ethics Board, and the UCT Faculty of Science Ethics Board.

Who is responsible for this study?
I am the Doctoral Candidate who is conducting the study, and can be contacted at any time with any questions. My supervisor, Dr Susan Malcolm-Smith, is a senior lecturer and Neuropsychologist at UCT can also be contacted if you have any queries or complaints that you would rather address to her. Or, alternatively you can address these issues to Rosaline Adams, the administrative assistant for the Psychology Department Ethics board. All contact details are included at the end of this letter.

How to participate?
Thank you for considering participating in my study! In order to join the study, please sign the attached consent form, complete the demographic form and return these forms to your school. Please feel free to call me with any questions or for help submitting these forms.

I look forward to hearing from you!

Katie Hamilton
PhD Psychology Candidate
Department of Psychology, UCT
082 463 8335
kate@hamilton.co.za

Dr Susan Malcolm-Smith
Senior Lecturer
Department of Psychology, UCT
021-650-4605
susan.malcolm-smith@uct.ac.za

Rosalind Adams
Admin. Assistant: Ethics Committee
Department of Psychology, UCT
021-650-4104
rosalind.adams@uct.ac.za

Appendix C

Demographic Questionnaire

DEMOGRAPHIC QUESTIONNAIRE

Your name: _____ Date: _____

Child's Name: _____ School: _____

Age: _____ Date of Birth: _____

Please note that you may skip any questions that you are not comfortable answering.

1. Sex (circle one): *Male* *Female*
2. Ethnicity: *White* *Black* *Indian* *Coloured*
   Asian *Other* *If other please specify:* _____
3. Home Language: _____
4. Handedness (circle one): *Left* *Right* *Ambidextrous*
5. Number of siblings: _____
6. Number of **older** siblings: _____
7. Who is the child's primary caregiver? _____
8. What is your relationship to the child (e.g. mother, father, etc)? _____
9. Has your child ever been diagnosed with Autism Spectrum Disorder (ASD)? YES NO
   Please indicate any other diagnoses or information related to your child's ASD: _____
   _____
10. Has your child ever been diagnosed with a disruptive, impulse-control, or conduct disorder, such as conduct disorder or oppositional defiant disorder (ODD)? YES NO
    If yes, please specify: _____
    _____
11. Has your child ever had a communication disorder? (For example: Having problems with understanding or producing speech, slow vocabulary development, difficulties recalling words or problems with producing sentences appropriate for his/her age.) YES NO
    If yes, please specify: _____
    _____
12. Has your child ever experienced learning difficulties such as dyslexia or attention-deficit / hyperactivity disorder (ADD/ ADHD)? YES NO
    If yes, please specify: _____
    _____
13. Has your child ever experienced a head injury? (e.g., being hit on the head and losing consciousness as a result) YES NO
    If yes, please give details: _____
    _____

14. Has your child ever experienced any of the following medical conditions:

a. Neurological problems (e.g., epilepsy, meningitis, cerebral palsy, encephalitis, Tourette's syndrome, brain tumour, other) YES NO

If yes, please specify: _____

_____

_____

b. Depression YES NO

If yes, please specify: _____

_____

_____

c. Memory problems YES NO

If yes, please specify: _____

_____

_____

d. Problems with their vision: YES NO

If yes, please specify: _____

_____

_____

e. Problems with their hearing (e.g. difficulty hearing, hearing aids, grommets): YES NO

If yes, please specify (please include details on how this affected their language development): _____

_____

_____

f. Is he/she currently taking any prescription medication? YES NO

If yes, what medication(s)? _____

_____

_____

Parent / Guardian Information

Please indicate here if child is adopted): _____

Please note that information on the primary caregiver is required. If the primary caregiver is not the biological or adoptive mother or father, please place their information under "Guardian".

What is the total monthly income of your household? (Tick the appropriate block):

[NOTE: This should be total household income, not personal income.]

0 – R2999		R3000 – R6299		R6300 – R 10 499		R10 500 – R 14599	
R14 600 – R18 799		R18 800 – R22 999		R23 000 – R26 999		R27 000 – R31 299	
R31 300 – R35 499		R35 500 - R39 499		R39 500 – R43 750		more than R43 750:	
What is the estimated value of your total monthly household income: R							

Highest level of education completed for … (please circle number):	Mother	Father	Guardian
1) 0 years (Never went to school)	1	1	1
2) Grade 1	2	2	2
3) Grade 2	3	3	3
4) Grade 3 / Standard 1	4	4	4
5) Grade 4 / Standard 2	5	5	5
6) Grade 5 / Standard 3	6	6	6
7) Grade 6 / Standard 4	7	7	7
8) Grade 7 / Standard 5 [Completed primary school]	8	8	8
9) Grade 8 / Standard 6	9	9	9
10) Grade 9 / Standard 7	10	10	10
11) Grade 10 / Standard 8	11	11	11
12) Grade 11 / Standard 9	12	12	12
13) Grade 12 / Standard 10 [Matric; Completed high school]	13	13	13
14) Tertiary education: Higher education certificate	14	14	14
15) Tertiary education: Diploma received	15	15	15
16) Tertiary education: Bachelor's degree received	16	16	16
17) Tertiary education: Post graduate degree received	17	17	17
18) Don't know	18	18	18

Parental employment: (Please circle appropriate number)	Mother	Father	Guardian
1. Higher executives, owners of large businesses, major professionals (e.g. doctors, lawyers)	1	1	1
2. Business managers of medium sized businesses, professions like nurses, opticians, pharmacists, social workers, teachers, accountants	2 3	2 3	2 3
3. Administrative personnel, managers, owners / sole proprietors of small businesses (decorator, actor, reporter, travel agent)	4	4	4
4. Clerical and sales, technicians, (e.g. bank teller, bookkeeper, clerk, draftsperson, timekeeper, secretary)	5	5	5
5. Skilled manual – usually having had training (e.g. baker, barber, chef, electrician, fireman, machinist, mechanic, welder, police, plumber, electrician)	6	6	6
6. Semi-skilled (e.g. hospital aide, painter, bartender, bus driver, cook, garage guard, checker, waiter, machine operator)	7 8	7 8	7 8
7. Unskilled (e.g. attendant, janitor, construction helper, unspecified labour, porter)	9	9	9
8. Homemaker			
9. Student, disabled, no occupation			

Which of the following items, in working order, does your household have?	Yes	No
1. A refrigerator or freezer	Yes	No
2. A vacuum cleaner or polisher	Yes	No
3. A television	Yes	No
4. A hi-fi or music center (radio excluded)	Yes	No
5. A microwave oven	Yes	No
6. A washing machine	Yes	No
7. A video cassette recorder or dvd player	Yes	No

Which of the following do you have in your home?	Yes	No
1. Running water	Yes	No
2. A domestic servant	Yes	No
3. At least one car	Yes	No
4. A flush toilet	Yes	No
5. A built-in kitchen sink	Yes	No
6. An electric stove or hotplate	Yes	No
7. A working telephone / cellular phone	Yes	No

Do you personally do any of the following?		
1. Shop at supermarkets	Yes	No
2. Use financial services such as a bank account, ATM card or credit card	Yes	No
3. Have an account or credit card at a retail store	Yes	No

Thank you for your participation!

Appendix D

Consent Form: ASD Participants

UCT Autism Research 

The study has been explained to me, and my questions have been answered. I understand that participation in this study is voluntary, and that I may withdraw my child at any point. I understand that my child will not be identified except by an initial, and that this anonymity will be maintained throughout the study and when the research is published.

I consent to participate and to allow my child to **participate** in this study.

Child's name: _____

Signature of parent /guardian: _____

Date: _____

(Optional)I hereby give consent for **DNA** samples to be collected from my child using cheek swabs. I understand that this DNA will only be used for research purposes, and will be destroyed after analysis for this project.

Signature of parent /guardian: _____

Date: _____

I hereby give consent for **ADOS2** administration to be video recorded. I understand that this video will only be used for research purposes. I give consent for this video to be stored at the Department Psychology, UCT, and to be used in later research.

Signature of parent /guardian: _____

Date: _____

Please indicate below if you would like to be notified of future research conducted by our research group:
Yes, I _____ (initial) would like to be added to your research participation pool and be notified of research projects in which I or my child might participate in the future.

Phone number: _____

Cell phone number: _____

E-mail address: _____

(Parent/guardian) _____ has been informed of the purpose, procedures, and any possible risks or this study. He / she has been given time to ask any questions, and these questions have been answered to the best of my ability. He / she understands that participation is voluntary.

Researcher: _____

Signature & Date: _____

Psychology Department
University of Cape Town
UCTautism.com

Katie Hamilton
kate@hamilton.co.za
Neuropsychology, UCT

Appendix E

Study Information Sheets: Neurotypical Participants

Brief Overview of Psychology Doctoral Study
The Biological Bases of Social Deficits: Exploring social functioning in Autism Spectrum Disorder and Neurotypical Children

Dear Parents

You and your child are invited to participate in my study! I am a PhD Psychology student with a history in in Neuropsychology (MA Clinical Neuropsychology, 2014), and am a member of the University of Cape Town Autism Research Group (uctautism.com). I am investigating the social difficulties in Autism Spectrum Disorders (ASD) and I am inviting children with ASD and without ASD to participate. I am interested in general social ability, and specifically in social motivation and Theory of Mind. Theory of Mind refers to the ability to understand other people's thoughts, beliefs, and emotions, and to understand that these are different from one's own. For example, the ability to understand jokes and the ability to understand that when you know something, everyone else doesn't automatically know it too, are forms of Theory of Mind. I am interested in the functioning of two candidate genes in the ASD group. In order to conduct my study, I have recruited children with ASD and I am now inviting children who do not have ASD to participate. This will enable me to make comparisons and improve our understanding of how children with ASD may differ from other children.

Who can participate?
In order to participate, your son must be between 4-16 years old and must understand English. Children can participate as long as they do not have a diagnosed Autism Spectrum Disorder and their home language or the language their teachers use with them is English. You as the parent must also be fluent in either English or Afrikaans as I will need to interview you about your son.

Must my child and I participate?
No, not at all – this study is completely optional. There are no negative consequences if you choose not to participate. Also, if you decide to participate and then change your mind, you can just let me know that you are withdrawing and you don't even need to provide a reason. If this happens, you and your son will not be penalised in any way.

What will happen if we take part?
If you decide to participate in the study, I will ask you to sign a consent form and complete a demographics form. The demographic forms asks about your son's medical history and your family

income and education. I understand that this is personal information, so as soon as I receive it I will remove your name and record the information under a confidential participation number. This information will not be shared with anyone else. We need this personal information for two reasons: first, we need the medical information to establish whether anything else could explain the relationships we are exploring, in which case we may not be able to include your son in the study, and second, we need the financial information to make sure that this research recruits children from all backgrounds and is therefore representative of the South African population.

Myself or someone in my team will then call you to arrange a time to interview you. The interview will consist of two parts, each 30-60 minutes, and can be done telephonically or we can meet and conduct the interview in person.

I will then meet with your son at his school. At the start of every session I will ask your son if he is willing to play the games with me that day, and if he isn't then we won't have a session. I will meet each child for 2 sessions of approximately 40 minutes, where we will complete several tasks all designed to measure different aspects of social and cognitive ability. All the tasks are designed to appear as games for the children, so they are all toy or story based.

What will happen to the information I give you and the information from seeing my son?
All information is recorded under a confidential participant number, and your privacy will be maintained at all times. I will not share this information with others, and if any data is shared it will be the kind of information that does not reveal who you are. Therefore, your name, income information, son's medical information, and all other information will not be shared with anyone. All information will be securely stored so that no one else can access it, and the data is coded so that your name and your son's name are removed.

What will happen with the results of this study?
At the end of this study I will provide you with a personalised report explaining what I learnt about your son. You can keep this report, and you can choose to share it with schools or any clinical professional involved in your son's care (for example, psychologists, GPs, speech therapists, etc). I am also always available to discuss anything about the research and to answer any questions.

If I publish my findings from this study, you and your son will never be identified personally. I will be delighted to share the results with you as soon as they are available.

Who has approved this study?
This study has received ethical approval from the Western Cape Education Department, the UCT Psychology Department Ethics Board, and the UCT Faculty of Science Ethics Board.

Who is responsible for this study?

I am the Doctoral Candidate who is conducting the study, and can be contacted at any time with any questions. My supervisor, Dr Susan Malcolm-Smith, is a senior lecturer and Neuropsychologist at UCT can also be contacted if you have any queries or complaints that you would rather address to her. Or, alternatively you can address these issues to Rosalind Adams, the administrative assistant for the Psychology Department Ethics board. All contact details are included at the end of this letter.

How to participate?

Thank you for considering participating in my study! In order to join the study, please sign the attached consent form, complete the demographic form and return these forms to your school. Please feel free to call me with any questions or for help submitting these forms.

I look forward to hearing from you!

Katie Hamilton
PhD Psychology Candidate
Department of Psychology, UCT
082 463 8335
kate@hamilton.co.za

Dr Susan Malcolm-Smith
Senior Lecturer
Department of Psychology, UCT
021-650-4605
susan.malcolm-smith@uct.ac.za

Rosalind Adams
Admin. Assistant: Ethics Committee
Department of Psychology, UCT
021-650-4104
rosalind.adams@uct.ac.za

Appendix F

Consent Form: Neurotypical Participants

The study has been explained to me, and my questions have been answered. I understand that participation in this study is voluntary, and that I may withdraw my child at any point. I understand that my child will not be identified except by an initial, and that this anonymity will be maintained throughout the study and when the research is published.

I consent to participate and to allow my child to **participate** in this study.

Child's name: _____

Signature of parent /guardian: _____

Date: _____

Please indicate below if you would like to be notified of future research conducted by our research group:
Yes, I _____ (initial) would like to be added to your research participation pool and be notified of research projects in which I or my child might participate in the future.

Phone number: _____

Cell phone number: _____

E-mail address: _____

(Parent/guardian) _____ has been informed of the purpose, procedures, and any possible risks or this study. He / she has been given time to ask any questions, and these questions have been answered to the best of my ability. He / she understands that participation is voluntary.

Researcher: _____

Signature & Date: _____

Psychology Department
University of Cape Town
UCTautism.com

Katie Hamilton
kate@hamilton.co.za
Neuropsychology, UCT

Appendix G

ADOS2 Research Reliability Certification

Certificate of Training

Awarded to

Kate Hamilton

for successfully completing a course in administration and coding of the Autism Diagnostic Observation Schedule (ADOS-2) to the level of

Research Reliability

This is to certify that the above named has demonstrated research reliability in use of the ADOS-2 having attended a course of study from

13th October to 17th October 2014

and having completed post-course assignments to the required standard.

Course Leader

beginningwithA
Autism Consultancy and Training

Appendix H

Attachment Style Classification Questionnaire

For the following questions, I would like you to think about how your son is around friends, or around other children. For each question, I would like to know whether the statement is true or not for your son. If you are not sure, that is also fine.

	Not true	Unsure	True
1. My son makes friends with other children easily. True or not true?*	0	1	2
2. My son doesn't feel comfortable trying to make friends.	0	1	2
3. It is easy for my son to depend on others, if they're good friends of his. *	0	1	2
4. Sometimes others get too friendly and too close to my son.	0	1	2
5. Sometimes my son is afraid that other kids won't want to be with him.	0	1	2
6. My son would like to be really close to some children and always be with them.	0	1	2
7. It's all right with my son if good friends trust and depend on him.*	0	1	2
8. It's hard for my son to trust others completely.	0	1	2
9. My son sometimes feels that others don't want to be good friends with him as much as he does with them.	0	1	2
10. My son usually believes that others who are close to him will not leave him.*	0	1	2
11. My son is sometimes afraid that no one really loves him.	0	1	2
12. My son finds it uncomfortable and gets annoyed when someone tries to get too close to him.	0	1	2
13. It's hard for my child to really trust others, even if they're good friends of his/hers.	0	1	2
14. Children sometimes avoid my child when he wants to get too close and be a good friend of theirs.	0	1	2
15. Usually when anyone tries to get too close to my child, it does not bother him.*	0	1	2

** Items indicating Secure Attachment*

Appendix I

UCT Psychology Department Ethics Board Clearance

UNIVERSITY OF CAPE TOWN

Department of Psychology

University of Cape Town Rondebosch 7701 South Africa
Telephone (021) 650 3414
Fax No. (021) 650 4104

13 April 2015

Mr K. Hamilton
Department of Psychology
University of Cape Town
Rondebosch

Dear Ms Hamilton,

This is to confirm that ethical clearance has been given by an Ethics Review Committee of the Faculty of Humanities for your study, *The Biological Bases of Social Deficits*. The reference number is PSY2014-024.

I wish you all the best for your study.

Yours sincerely,

Johann Louw PhD
Professor
Chair: Ethics Review Committee

Appendix J

UCT Faculty of Science Research Ethics Committee Clearance

Faculty of Science
University of Cape Town
RONDEBOSCH 7701
South Africa

E-mail: richard.hill@uct.ac.za
Telephone: + 27 21 650 2786
Fax: + 27 21 650 3456

7 October 2014

Dr Colleen O'Ryan
Department of Molecular and Cell Biology

Dear Dr O'Ryan

GENOTYPE: PHENOTYPE ASSOCIATIONS IN AUTISM SPECTRUM DISORDER

I am pleased to inform you that the Faculty of Science Research Ethics Committee has approved the above-named application for research ethics clearance, subject to the conditions listed below. You are required to:

- implement the measures described in your application to ensure that the process of your research is ethically sound; and
- uphold ethical principles throughout all stages of the research, responding appropriately to unanticipated issues: please contact me if you need advice on ethical issues that arise.

Your approval code is: FSREC 076– 2014

I wish you success in your research.

Yours sincerely

RC Hill

Dr Richard Hill
Chair: Faculty of Science Research Ethics Committee

Appendix K

UCT Faculty of Health Sciences Human Research Ethics Committee Clearance

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

20 October 2017

HREC REF: 346/2017

Dr S Malcolm-Smith
Psychology Department
Upper Campus
UCT

Dear Dr Malcolm-Smith

PROJECT TITLE: THE BIOLOGICAL BASES OF SOCIAL DEFICITS: THE POSSIBLE ROLES OF THE MU-OPIOID RECEPTOR AND THE SEROTONIN TRANSPORTER PROMOTER LENGTH POLYMORPHISM IN SOCIAL MOTIVATION AND THEORY OF MIND IN AN AUTISM SPECTRUM DISORDER SAMPLE-PhD-candidate-K Hamilton

Thank you for your response letter dated 13 October 2017, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study, including the following documentation: -

1. PI Generated Synopsis Instruction version date 23 July 2014
2. Research Protocol version date 16 April 2012
3. Information Sheet and Parent Consent Form
4. Child Assent Form
5. Parent Questionnaire: ASCQ
6. Ethical Approval: Psychology Ethics Board, UCT
7. Ethics Approval: Science Faculty Ethics Board, UCT
8. Permission to approach schools: Western Cape Education Department
9. Budget Summary
10. Form A: Version 19, dated February 2015
11. Research Protocol for Phase Two: DNA Component of Study
12. Consent Form for Phase Two: DNA Component of Study
13. Assent form for Phase Two: DNA Component of Study

Approval is granted for one year until the 30 October 2018.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Katie Hamilton will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC 346/2017

Appendix L

Western Cape Government Education Department Permission to Conduct Research

Western Cape Government
Education

Directorate: Research

Audrey.wyngaard@westerncape.gov.za
tel: +27 021 467 9272
Fax: 0865902282
Private Bag x9114, Cape Town, 8000
wced.wcape.gov.za

REFERENCE: 20150422-46598
ENQUIRIES: Dr A T Wyngaard

Ms Kate Hamilton
PO Box 1694
Milnerton
7435

Dear Ms Kate Hamilton

RESEARCH PROPOSAL: THE BIOLOGICAL BASES OF SOCIAL DEFICITS: THE POSSIBLE ROLES OF THE MU-OPIOID RECEPTOR (OPRM1) AND THE SEROTONIN TRANSPORTER PROMOTER LENGTH POLYMORPHISM (5-HTTLPR) IN SOCIAL MOTIVATION AND THEORY OF MIND IN AN AUTISM SPECTRUM DISORDER (ASD) SAMPLE

Your application to conduct the above-mentioned research in schools in the Western Cape has been approved subject to the following conditions:

1. Principals, educators and learners are under no obligation to assist you in your investigation.
2. Principals, educators, learners and schools should not be identifiable in any way from the results of the investigation.
3. You make all the arrangements concerning your investigation.
4. Educators' programmes are not to be interrupted.
5. The Study is to be conducted from **24 April 2015 till 30 September 2017**
6. No research can be conducted during the fourth term as schools are preparing and finalizing syllabi for examinations (October to December).
7. Should you wish to extend the period of your survey, please contact Dr A.T Wyngaard at the contact numbers above quoting the reference number?
8. A photocopy of this letter is submitted to the principal where the intended research is to be conducted.
9. Your research will be limited to the list of schools as forwarded to the Western Cape Education Department.
10. A brief summary of the content, findings and recommendations is provided to the Director: Research Services.
11. The Department receives a copy of the completed report/dissertation/thesis addressed to:

    The Director: Research Services
    Western Cape Education Department
    Private Bag X9114
    CAPE TOWN
    8000

We wish you success in your research.

Kind regards.
Signed: Dr Audrey T Wyngaard
Directorate: Research
DATE: 22 April 2015

Appendix M

University of Cape Town Theory of Mind Battery: Task Descriptions

EARLY MODULE

TASK	ASSESSES	EXAMPLE
Desire	Ability to predict behaviour based on a character's stated desire in a simple comic	*Comic explaining Sam wants to find his cellular phone. He thinks it is either in the cupboard under the bed. He looks in the cupboard but finds a different item. Check child knows what Sam was looking for. Ask child,* "What will Sam do now – will be go to school or will be look under the bed?"
Pretend Play	Ability to use a doll appropriately (i.e. as the agent of action) in a structured pretend situation	*Use doll and props.* "It is time to water the plants. Watch what Sam does. Look, Sam is getting the watering can." *Examiner makes doll take watering can.* "Show me what Sam does next." *Hand items to child, assess if child uses doll as agent.*
Perception-Knowledge	Ability to understand that a character obtains knowledge from visual access to a box the child cannot see inside of	*Using two dolls and props; act out while dictating.* "Tina looks in the box. Sam pushes the box. Does Tina know what is in the box? Does Sam know what is in the box?"
Diverse Desires	Ability to understand that a character has desires different to one's own, and that this will guide their choices.	*Use dolls and images of food items.* "It is snack time, and Sam wants a snack to eat. There are carrots and chips. Which would you like best?" *Allow child to respond.* "That's a good choice, but Sam really likes [alternate food to child's choice]. Which snack will Sam choose, carrots or chips?"
Diverse Beliefs	Ability to understand a character can have different thoughts to their own, and that this will guide their actions.	*Use doll and images of locations.* "Tina wants to find her cat. Her cat might be hiding in the bushes or it might be hiding in the garage. Where do you think the cat is?" *Allow child to respond.* "That's a good idea, but Tina thinks her cat is in [alternate location to child's choice]. So where will Tina look, the garage or the bushes?"

BASIC MODULE

TASK	ASSESSES	EXAMPLE
Location Change False Belief	First-order false belief; Ability to understand that a character's false belief about an item's location would guide their behaviour when looking for the item	*Illustrated picture story shows Emma eating chocolate in the kitchen, then playing it in a drawer before leaving. While away, her mother moves it to the fridge. Emma comes back into the kitchen later. Ask child where Emma would look first for her chocolate.*
Unexpected Contents False Belief	First-order false belief; Ability to understand that a character's false belief about a box's content would guide the expectation of its contents	*Place crayons inside a Smarties (chocolate) box without the child seeing. Ask the child what they think is inside the box. Show them the items and ask, then close the box again. Ask child what is in the box [reality]. Ask child what they had thought was in the box [representational change]. Ask if their mother would know what was in the box if she entered the room now and saw the closed box [ignorance]. Ask child what their mother would think is in the box [false belief].*

Belief-Emotion	First-order false beliefs; Ability to understand how a character's false beliefs will guide their expectations and emotions, and then how their emotions will change when these expectations are not met by reality.	*Use dolls and props. Show child a closed cookie box containing a spoon, without letting them touch the box. Ask child what they think is in the box. State the doll's favourite snack is cookies. Remove dolls so it does not have visual access and show the child the real contents of the box. Bring doll back and give doll the box. Ask the child how the doll would feel on receiving the box. Open the box, and ask child how doll would feel now.*
Real-Apparent Emotion	Ability to differentiate how a character's true feelings may differ from their expressed emotion state	*Story task with pictures. Tell a story about a child who is joking around with his friends. A friend tells a mean joke about the main character, Matt. Tell child that Matt did not find the joke funny, but did not want the other children to know how he felt so he tried to hide how he felt. Ask child control questions, ask child how Matt really felt when the joke was told [emoticon faces of happy, sad, okay provided]. Ask child how he tried to look on his face when the joke was told.*

INTERMEDIATE MODULE

TASK	ASSESSES	EXAMPLE
Second Order False Belief	Second order false beliefs; Knowledge of a character's beliefs about another character's beliefs.	*Story with pictures. Ted wants to surprise his mother with breakfast in bed for Mother's Day. He starts to cook the meal, then goes to his mother's room. She is in bed and states she wants breakfast in bed. Ted wants it to be a surprise, so states that he actually made her a card. Ted returns to kitchen and does not notice his mother observe him doing so. Later, his father enters the kitchen and asks Ted: "Does Mom know what you're making her for Mother's Day? [child answers] What does mom think you are making her?" [child answers].*
Strange Stories	Ability to identify situations where language was used in lies, white lies, jokes, pretend/play, double-bluff, persuasion, figures of speech, appearance vs reality, irony, or contrary emotions, or to indicate situations where characters had forgotten or misunderstood.	*Story task. Philip and Ian see Mrs Thompson coming out of the hairdresser's one day. She looks a bit funny because they cut her hair much too short. Phlilip says to Ian, "She must have been in a fight with a lawnmower!". ASD child if what Phillip says is true. Ask child why Phillip said it.*

ADVANCED MODULE

TASK	ASSESSES	EXAMPLE
Lie-Joke	Ability to identify whether something that was untrue was a lie or a joke based on a character's knowledge (e.g. Patricia) of another character's knowledge (e.g. Mom).	*Story task. Patricia's mom based muffin and told Patricia not to eat any. Mom then leaves, and a friend comes over to play, Tina. When Tina leaves the room, Patricia eats a muffin, but Mom has returned and sees her through the window. Patricia sees her mom looking angrily at her as she eats the muffin, but continues to do so. Tina returns and asks if Mom knows Patricia is eating the muffin [child responds]. Mom enters the kitchen and asks Patricia if she ate a muffin and Patricia denies it. Ask child,* "When Patricia said that, did she think her

		mom would believe her? [*child responds*] Was she lying to avoid getting caught or joking to cover her embarrassment?"[*child responds*]
Faux Pas	Ability to identity if a character makes a social *faux pas* - that is, how socially adept the child is at identify when an action / statement offends someone	*Story task. Kim helped mom bake an apple pie for her uncle and presents it to him. He responds, "That looks lovely. I love pies, except for apple, of course". Ask child what kind of pie it was. Ask child if the uncle knew what kind of pie it was. Ask child if anyone said something they shouldn't have.*