Genetic and endocrine correlates of variation in human sociality

by

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B. Sc. (Psychology), University of Manitoba, 2005

Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

in the
Department of Biological Sciences
Faculty of Science

SIMON FRASER UNIVERSITY
Summer 2017

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Abstract

Hormones play evolutionarily ancient roles in social behaviour; yet the degree to which hormone systems influence human socio-emotional behaviour remains unclear. It is hypothesized that (i) hormone-associated genes linked to psychiatric conditions contribute to variation in social traits among non-clinical populations, and (ii) changes in endogenous hormone levels coordinate adaptive social behaviour with stimuli in the environment. Consistent with the first hypothesis, a vasopressin receptor polymorphism linked to autism was significantly associated with autistic-like traits in healthy individuals. Consistent with the second hypothesis, an empathy-inducing stimulus was found to mediate a trade-off in hormone levels, with oxytocin increasing and testosterone decreasing. Furthermore, a common polymorphism in the general transcription factor II-I gene, which is linked to Williams syndrome, was associated with oxytocin response to the empathy-inducing stimulus and social anxiety among healthy individuals. Together, these findings highlight the diverse ways through which hormone systems contribute to variation in human sociality.

Keywords: autism; behaviour; genetics; oxytocin; testosterone, vasopressin

Acknowledgements

Thank you to everyone who has encouraged and guided me during my time at Simon Fraser University, including friends, family, collaborators, co-authors, fellow grad students, professors, my supervisory committee, and members of FAB*Lab and HESP. Special thanks to Silven Read for teaching me everything I know about working with DNA, and to Dr. Neil Watson and Justin Jagore for accompanying me on the journey of learning to measure oxytocin. Endless gratitude to Dr. Bernard Crespi, my supervisor, for his relentless inspiration, guidance, and prodding; this thesis certainly would not exist without his support. And thank you to Peter Mayer, my partner in exploring the world and collecting its experiences, for actively encouraging all this.

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List of Acronyms

AQ	Autism spectrum quotient
ASD	Autism spectrum disorders
AVPR1a	Arginine vasopressin receptor 1a
CD38	Cluster of differentiation 38
EQ	Empathy quotient
GTF2I	General transcription factor II-I
IRI	Interpersonal reactivity index
OXTR	Oxytocin receptor
SNP	Single nucleotide polymorphism
SPQ-BR	Schizotypal personality questionnaire brief revised
SQ	Systemizing Quotient

Chapter 1.

Introduction

1.1. Evolution of variation in sociality

Through the lens of evolution, social behaviour can be viewed as a set of adaptations that increase fitness via the establishment and maintenance of social bonds with reproductive partners, family members, or unrelated individuals for such purposes as rearing offspring, acquiring resources, and deterring predation [1,2]. As an adaptation, social behaviour is expected to be subject to the homogenizing force of natural selection, which should eliminate all variants except that conferring the highest fitness advantage [3]. However, a staggering amount of variation in social traits persists within species.

While most exhaustively studied in our own species as “personality” [4], individual differences in social traits have been reported in diverse taxa. For example, non-human primates vary in levels of sociability, positive affect, and anxiety [5]; zebra finches vary in quality of parental behaviour [6]; and social spiders show stable differences in levels of aggression and boldness [7]. Among humans, three of the “big five” personality traits have been linked to reproductive success in both sexes, with higher extraversion, lower conscientiousness, and lower openness to experience in both sexes associated with having a greater number of children and grandchildren [8,9]. The existence of such variation in social traits—particularly those with direct implications to fitness—begs essential, yet unresolved, questions: why do individuals vary in social traits and how is such variation maintained?

The existence of variation in social traits—whether personality or parental care—is perhaps best accounted for by the concept of trade-offs, where a balance is achieved between two beneficial but incompatible phenotypes [10,11]. Variation in social behaviour may thus reflect different, yet equally adaptive, strategies, with individuals achieving similar overall levels of fitness, although potentially differing in the specific social traits that contribute to their fitness [4,12]. The conceptual framework of trade-offs may also be useful for understanding the proximate causes—i.e., neurobiological mechanisms—that underlie variation in social behaviour. For example, while empathy

and aggression are each adaptive in specific contexts and individuals are expected to be capable of both behaviours, the neurobiological mechanisms that would promote aggressive behaviour are likely incompatible with the neurobiological mechanisms that would promote empathetic behaviour. The goal of the studies comprising this thesis is to broadly explore what appears to be an important neurobiological mechanism mediating variation in social behaviour between individuals, between males and females, and even across life history stages: hormones.

1.2. Social hormones

Hormones—from the Greek *hormon*, meaning to set in motion or stimulate —are chemical messengers produced by endocrine glands (e.g., hypothalamus, pituitary, gonads) that act over relatively long distances in the body and brain to coordinate changes in physiology, development, and behaviour [13,14]. Although hormones were first studied for their roles in regulatory functions like metabolism and growth, more than 20 hormones are now recognized as capable of influencing social behaviour through effects at hormone receptors in the central nervous system [13]. In contrast to neurotransmitters, most hormones are not released at specific synapses; rather, hormones may be released directly within the central nervous system or be released into the bloodstream and reach the central nervous system via circulation. Hormones thus act more broadly than neurotransmitters, and a single pulse of hormone release is capable of exerting effects at distant sites in the brain and body. As many social behaviours are tied to specific stages of life history, hormones may be viewed as a mechanism for coordinating changes in physiology with changes in adaptive behaviour. For example, among female mammals, the hormone oxytocin is released when giving birth and binds to oxytocin receptors in the uterus to induce contractions, but also to oxytocin receptors in the brain to induce the maternal behaviours that will promote the survival of the impending offspring [15].

Oxytocin, along with closely-related vasopressin, are peptide hormones recognized for their broadly prosocial effects on social behaviour across vertebrate taxa [16]. In addition to its demonstrated role in inducing maternal behaviour [17], administering oxytocin to rodents has been shown to promote social recognition, affiliative huddling behaviour, and preference for a familiar partner in both males and females [18–20]. Oxytocin is also linked to prosocial behaviour in non-human primates, with higher urinary oxytocin levels

associated with greater affiliation between monogamous pairs of cotton-topped tamarins [21], and chimpanzees showing increased oxytocin levels after grooming and food-sharing [22,23]. By contrast, the prosocial effects of vasopressin appear to be largely restricted to males, with vasopressin administration promoting paternal behaviour and partner preference in monogamous vole species where males provide parental care; by contrast, in a vole species with a polygamous mating strategy and no paternal care, vasopressin administration does not alter the social behaviour of males [24,25]. Furthermore, within a monogamous vole species, individual differences in males' level of partner preference and paternal care are associated with variation in the vasopressin receptor gene, which predicts vasopressin receptor distribution in several brain areas [26]. Sex differences in the effects of oxytocin and vasopressin on social behaviour may be due to differences in hormone receptor distribution in social brain areas [27], or through interactions between peptide hormones and sex steroid hormones [16,28], which themselves are capable of influencing behaviour.

The sex steroid hormones, including estrogen, testosterone, and related forms, are produced primarily by the gonads (ovaries, testes). As such, males and females vary greatly in their average levels of steroid hormones at different life history stages, although all sex steroid hormones are typically present in each sex. The chemical structure of estrogen and testosterone allows them to permeate the blood-brain barrier and bind to estrogen or androgen receptors, respectively, in the central nervous system. The effects of steroid hormones on the behaviour of non-human animals has been studied primarily in the context of sex-specific behaviours, with males' testosterone levels increasing during breeding season [29] and paired increases in estrogen along with oxytocin necessary to induce maternal behaviour among mammals [17].

Taken together, these studies indicate that variation in hormone levels, variation in hormone associated-genes, and variation in hormone receptor distribution patterns in the brain contribute to individual differences, sex differences, and even species differences in social behaviour. The evidence that these findings can be extended to hormonal regulation of variation in human sociality is discussed in the following section.

1.3. Hormones and human sociality

Humans, with our nuanced social traits and unusually large brains, were once thought to be emancipated from simple, hormonal regulation of social behaviour [30]. The roles of hormones—particularly oxytocin and testosterone—in complex aspects of socio-emotional behaviour have, however, recently become subjects of intense interest in the fields of psychology, neuroscience, endocrinology, and psychiatry.

Administration studies, where hormone levels are temporarily altered using a hormone-containing nasal spray or gel, indicate that hormones have powerful effects on diverse aspects of human social and emotional cognition. Such studies have demonstrated that oxytocin enhances empathy, trust, and generosity; vasopressin enhances social stress response and memory of emotional stimuli; and testosterone enhances risk-taking and motivation for action, while reducing trust and empathy [reviewed in 31]. Studies of hormone levels in blood or saliva have reported a positive correlation of plasma oxytocin with attachment and sensitive parenting [32,33]; by contrast, testosterone levels correlate positively with aggression [34] and narcissism [35], and negatively with providing parental care [36–39]. Although non-invasive methods for assessing the distribution of hormone receptors in the human brain have not yet been developed, variation in hormone receptor genes may regulate transcriptional activity or predict brain region-specific gene expression [40,41]. Common polymorphisms in the oxytocin receptor (OXTR) gene have been linked to individual differences in response to stress [42] and response to betrayal [43]; polymorphisms in the arginine vasopressin receptor 1a (AVPR1a) gene have been linked to altruism [44] and pair-bonding in men, but not women [45]; and polymorphisms in both genes have been linked to prosocial behaviour [46], empathy [47,48], and risk of autism spectrum conditions [49,50]. Taken together, these lines of research are suggestive that hormones levels, hormone receptor distribution in social brain regions, and interactions between hormone levels and hormone receptors mediate variation in social traits among healthy individuals.

Evidence of hormonal dysfunction in psychiatric conditions further supports the role of hormone system variation in mediating human socio-behavioural diversity. In particular, hormones may be useful for explaining sex differences in the incidence rates of certain conditions. For example, a growing body of evidence supports effects from elevated prenatal testosterone and reduced postnatal oxytocin levels [51,52] in autism spectrum

conditions, which are male-biased and characterized by underdeveloped social cognition and, often, enhanced non-social cognition [53]. Dysregulation of hormone levels has also been reported in psycho-affective conditions, which are characterized, in part, by exaggerated social cognition, with some evidence of elevated oxytocin and/or reduced testosterone in schizophrenia, bipolar disorder, and depression [54–57]. Variation in multiple social hormone systems, whether levels of circulating hormones or alterations to the receptors, may thus underlie the abnormal patterns of social behaviour that characterize these conditions.

While the above-described studies have shed considerable light on specific hormones and specific behaviours or psychiatric conditions, notable gaps remain in our understanding of the broader role of hormones in social behaviour. First, what is the relationship between the role of hormone variation in the typical range of human social variation and disorders of social behaviour? Specifically, do hormonal contributions to individual differences in social behaviour grade continuously from typical to atypical functioning, i.e. from typical variation in a social trait to an extreme phenotype that would meet criteria for a clinical diagnosis? Second, how do hormone systems coordinate adaptive social behaviour with stimuli in the environment? There is a large body of evidence, as briefly described above, demonstrating that altering hormone levels can alter behaviour [31]; however, relatively few studies have tested if and how hormone levels change in response to stimuli in the environment. Of particular interest are oxytocin and testosterone, the hormones most commonly used in administration studies, which have opposing effects on aspects of human behaviour and cognition [58], e.g., oxytocin increases empathy, testosterone reduces empathy; oxytocin promotes parental behaviour, testosterone reduces parental behaviour. The joint responses of these hormones to an ecologically-valid stimulus has not previously been tested.

These questions form the basis for the two main hypotheses tested in this thesis: (i) hormone-associated genes linked to psychiatric conditions contribute to variation in social traits among non-clinical populations, and (ii) changes in endogenous hormone levels coordinate adaptive social behaviour with stimuli in the environment.

1.4. Thesis structure

To explore these over-arching questions about the roles of hormones and hormone-related genetic polymorphisms in human sociality, three studies are presented in this thesis. In Chapter 2, I use genetic data and psychometric data to test if variation in the arginine vasopressin receptor 1a (AVPR1a) gene, previously identified as an autism risk gene, is associated with variation in autistic-like traits in a non-clinical population. Chapter 3 examines the roles of oxytocin and testosterone in empathy, a complex socio-emotional behaviour with links to autism, by assessing changes in hormone levels after watching an emotional video; given the seemingly opposite effects of these hormones on empathy, as described above, endogenous oxytocin is predicted to increase while endogenous testosterone is predicted to decrease. Relationships between hormone levels and reactivity, self-reported psychological traits, and hormone-associated genetic polymorphisms are also examined. In Chapter 4, I test if a common polymorphism in the General Transcription Factor II-I (GTF2I) gene, which is associated with normal variation in sociality in healthy populations as well as the extreme social phenotype of Williams syndrome, mediates variation in social traits or oxytocin reactivity. Chapter 5 concludes the thesis, highlighting its contributions to the literature on the roles of hormones and hormone-associated genes in human sociality.

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Chapter 2.

Association testing of vasopressin receptor 1a microsatellite polymorphisms in non-clinical autism spectrum phenotypes

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Autism Research, doi: 10.1002/aur.1716

2.1. Abstract

Variation in the arginine vasopressin receptor 1a (AVPR1a) gene is associated with autism risk in clinical populations and with variation in social behaviour in non-clinical populations. However, whether a relationship exists between AVPR1a polymorphisms and non-clinical manifestations of autism spectrum phenotypes has not been established. In this study, 873 Caucasian university students were administered the Autism Quotient (AQ) questionnaire and genotyped for the RS1 and RS3 microsatellites. A significant association was found between RS3 microsatellite variation and AQ score, with the long/long RS3 genotype associated with higher AQ score. Analysis by sex revealed that the association was only significant for females. Significantly higher AQ scores were also observed for individuals with a specific RS3 allele ("target allele"), which previous researchers have associated with increased autism risk, impaired bonding, and reduced altruistic behaviour. Analyses excluding carriers of target alleles indicated that the findings were driven by their presence or absence. Examination of AQ questionnaire subscales indicated that associations with RS3 were mediated predominantly by variation in attention switching, a cognitive function commonly impaired in autism. Effects on attention may thus mediate these relationships and represent one direction for future research. The findings also indicate, for AVPR1a, the importance of testing for sex differences and effects of target alleles. No associations were observed between RS1 microsatellite variation and AQ score. Overall, this work supports the idea that autism risk genes contribute to behavioural variation in the

general population, with AVPR1a polymorphisms relating to a healthy individual's location on the autism phenotype continuum.

2.2. Background

The peptide hormone arginine vasopressin (AVP) binds to arginine vasopressin 1a receptors (AVPR1a) to modulate aspects of mammalian physiology and behaviour (Albers, 2014). Among humans, the AVPR1a gene is characterized by two highly polymorphic promoter microsatellites: RS1, a $(GATA)_{14}$ repeat sequence, and RS3, a complex $(CT)_4TT(CT)_8(GT)_{24}$ repeat sequence (Thibonnier et al., 2000). Microsatellite repeat number can affect AVPR1a expression in the brain, as suggested by the higher AVPR1a mRNA levels found in post-mortem hippocampal specimens from individuals with long RS3 alleles (Knafo et al., 2008). As such, variation in AVPR1a microsatellite repeat number may modulate its effects on social behaviour, contributing to individual differences in social behaviour as well as liability to autism.

An association between AVPR1a microsatellites and autism risk was first reported by Kim et al. (2002), who found significant transmission disequilibrium between autism and RS3 polymorphisms in 115 autism parent-offspring trios. Wassink et al. (2004) reported associations of two RS1 alleles and one RS3 allele with autism in 65 sibling-pair families. Associations between RS1 and RS3 polymorphisms and autism risk were also reported by Yang et al. (2010), while Tansey et al. (2011) and Kantojarvi et al. (2015) only found associations with RS1. Yirmiya et al. (2006) reported associations with a third AVPR1a microsatellite, the AVR intronic microsatellite, and in a haplotype analysis of all three microsatellite markers. Associations are also reported between autism risk and single nucleotide polymorphisms (SNPs) in AVPR1a (Kantojärvi et al., 2015; Tansey et al., 2011; Yang et al., 2010). Taken together, these genetic studies provide support for relationships between AVPR1a and autism risk.

The majority of genetic risk factors for autism are also present in non-clinical populations (Robinson et al., 2016). AVPR1a microsatellites are highly polymorphic in the general population, and associations have been reported between RS1 and RS3 and individual variation in social behaviour. Such studies commonly use a length classification scheme where approximately half of alleles are considered "short" and half "long". Findings include an association of short RS1 alleles with lower prosocial activity (Poulin et al.,

2012) and higher amygdala activation during a facial recognition task (Meyer-Lindenberg et al., 2009). For RS3, short alleles have been associated with lower amygdala activation and less altruistic behaviour in economic games (Knafo et al., 2008; Meyer-Lindenberg et al., 2009). These findings support the hypothesis that AVPR1a variation influences important aspects of sociality in the general population.

In several non-clinical studies, it has been recognized that RS1 and RS3 alleles of a specific length (referred to as “target alleles” by Avinun et al. (2011)) were linked with especially strong associations with the social phenotype under investigation. Thus, in a study of personality traits, the RS1 320bp target allele mediated the association with harm avoidance and novelty seeking; carriers (individuals with one or two copies) also showed significantly lower left amygdala activity relative to non-carriers (Meyer-Lindenberg et al., 2009). For RS3, the presence of a target allele (327-334bp, depending on the primers used) has been associated with less altruistic behaviour (Avinun et al., 2011), increased marital problems (Walum et al., 2008), and reduced maternal structuring and support (Avinun et al., 2012). RS3 target allele carriers also showed significantly higher amygdala activation during a face-matching task (Meyer-Lindenberg et al., 2009). Intriguingly, clinical studies reported overtransmission of the target alleles (Kim et al., 2002; Wassink et al., 2004) to individuals with autism, although the significance of these alleles was not recognized at the time. These results demonstrate the important effects of AVPR1a microsatellite target alleles on behaviour and clinical phenotypes.

To span the gap between the research examining AVPR1a and aspects of social behaviour in clinical and non-clinical populations, we tested for associations between RS1 and RS3 polymorphisms—including the target alleles—and autistic-like traits in the general population. Although autism was first identified as a distinct condition, there is evidence that autism phenotypes (e.g., poor social skill, poor communication, exceptional attention to detail) exist as a continuum in the general population with clinical autism at the extreme end (e.g., Baron-Cohen et al., 2001; Focquaert & Vanneste, 2015). Thus, the aims of this study were twofold: 1) to test for effects of AVPR1a microsatellite variation in non-clinical autism spectrum phenotypes; and 2) to evaluate the degree to which specific aspects of autism spectrum phenotypes (e.g., social, communicative, attentional) are mediated by AVPR1a variation.

2.3. Methods

2.3.1. Participants and measurements

Genetic and questionnaire data were collected from Caucasian undergraduate students at two Canadian universities, resulting in a combined sample of 873 individuals (575 females, 298 males) with a mean age of 19.4 years (SD = 2.54). Research protocols were approved by the Ethics Boards at each university and written informed consent was obtained from participants.

Participants were administered the Autism Spectrum Quotient (AQ) (Baron-Cohen et al, 2001), a questionnaire used to evaluate autistic-like traits in individuals of normal intelligence. The AQ quantifies five aspects of psychological variation that show high levels of alteration in autism: attention switching, attention to detail, communication, imagination, and social skill. The AQ is scored out of 50 (10 items per domain), with a higher score representing a more autistic-like phenotype. A recent systematic review by Ruzich et al. (2015) reported a mean AQ score of 17 for non-clinical populations with males scoring significantly higher than females; for clinical populations, mean AQ score is approximately 35, though a score of 26+ is useful for identifying individuals with autism spectrum conditions.

2.3.2. Microsatellite genotyping and statistical analyses

DNA was extracted from saliva. The RS1 microsatellite was amplified with primers 5’ AGGGACTGGTTCTACAATCTGC 3’ (forward) and 5’ ACCTCTCAAGTTATGTTGGTGG 3’ (reverse), and RS3 was amplified with primers 5' CCTGTAGAGATGTAAGTGCT 3' (forward) and 5'GTTTCTTTTTGGAAGAGACTTAGATGG 3' (reverse). Based on these primer pairs, the 315bp allele for RS1 and the 330bp allele for RS3 in this study corresponded to the target alleles identified in prior research. The microsatellite-containing fragments were genotyped by size using a LI-COR 4300 DNA Analysis System and Gene ImagIR software (Scanalytics). Consistent with methods used in previous AVPR1a microsatellite work (Knafo et al., 2008; Tansey et al., 2011), RS1 repeats 315bp or higher were termed long; those less than 315bp were termed short. For RS3, 330bp and higher were termed long and others were termed short. Each

participant was then assigned a genotype of short/short (SS), short/long (SL), or long/long (LL) for the RS1 and RS3 microsatellites.

Analysis of variance (ANOVA) was used to test the difference in mean AQ score between the SS, SL, and LL genotypes for RS1 and RS3. Two models of dominance were tested: one comparing SS and SL genotypes against LL genotype, and another comparing SS genotype against SL and LL genotypes. The effect of the presence or absence of target alleles on AQ score was also examined by ANOVA. In addition, a test for linearity was performed to examine the effect of target allele copy number (0, 1, and 2 copies) on AQ score. All statistical tests were performed using R (version 3.2.0). In cases of unplanned multiple comparisons, Bonferroni adjustments were applied: for analysis by sex (two comparisons), p was adjusted to < 0.025; for analysis of the five AQ subscales, p was adjusted to < 0.01.

2.4. Results

In the study population, nine alleles were observed for RS1 and 19 alleles for RS3 (Table 2.1). The microsatellite length frequencies were similar to previous reports for non-clinical populations (Avinun et al., 2011; Meyer-Lindenberg et al., 2009).

Significant associations were found between total AQ score and RS3 genotype, but not RS1 genotype (Table 2.2). Specifically, the RS3 long/long genotype was significantly associated with higher AQ score. The strongest association was found for the model comparing short/short and short/long individuals against long/long individuals (Table 2.2).

Table 2.1 Length classification scheme ("short" or "long") and frequency of AVPR1a RS1 and RS3 microsatellite polymorphisms in a non-clinical Caucasian population (N = 873).

RS1		
	Length (bp)	%
Short	303	0.52%
	307	14.89%
	311	38.26%
Long	315	24.91%
	319	8.76%
	323	9.91%
	327	0.97%
	331	1.43%
	335	0.34%

RS3		
	Length (bp)	%
Short	310	0.12%
	312	0.06%
	314	1.38%
	316	0.12%
	318	0.23%
	320	0.12%
	322	1.38%
	324	7.62%
	326	12.72%
	328	23.25%
Long	330	19.53%
	332	11.40%
	334	10.20%
	336	1.83%
	338	3.21%
	340	4.75%
	342	1.49%
	344	0.46%
	346	0.17%

RS1 and RS3 alleles are classified by length such that approximately half of alleles are "short" and half are "long". Based on the primers used in this study, RS1 315 and RS3 330 (in bold) correspond to the "target alleles" identified in previous work.

Table 2.2 Analysis of effects of RS1 and RS3 genotype on AQ score, including two models of dominance.

	RS1				RS3		
	Both (N = 873)	**Males (N = 298)**	**Females (N = 575)**		**Both (N = 873)**	**Males (N = 298)**	**Females (N = 575)**
SS Mean AQ ± SD (N)	17.06 ± 5.12 (252)	17.51 ±5.44 (86)	16.82 ±4.95 (166)		16.94 ±5.42 (200)	18.14 ±5.69 (70)	16.29 ±5.18 (130)
SL Mean AQ ± SD (N)	17.34 ± 5.58 (433)	18.37 ±5.97 (154)	16.77 ±5.27 (279)		16.68 ±5.20 (420)	17.37 ±5.73 (145)	16.31 ±4.87 (275)
LL Mean AQ ± SD (N)	16.59 ±5.30 (188)	17.28 ±5.56 (58)	16.28 ±5.18 (130)		17.91 ±5.60 (253)	18.65 ±5.79 (83)	17.55 ±5.49 (170)
SS + SL Mean AQ ± SD (N)	17.24 ±5.41 (685)	18.06 ±5.79 (240)	16.79 ±5.15 (445)		16.76 ±5.27 (620)	17.62 ±5.72 (215)	16.31 ±4.97 (405)
SL + LL Mean AQ ± SD (N)	17.11 ±5.50 (621)	18.07 ±5.87 (212)	16.62 ±5.24 (409)		17.14 ±5.39 (673)	17.84 ±5.77 (228)	16.79 ±5.15 (445)
SS/SL/LL F p	1.3016 (0.2726)	1.0540 (0.3499)	0.5035 (0.6047)		4.2782 (0.0142*)	1.3850 (0.2519)	3.5344 (0.0298*)
SS+SL/LL F p	2.1554 (0.1424)	0.8750 (0.3503)	1.0008 (0.3175)		8.2419 (0.0042**)	1.9201 (0.1669)	7.0798 (0.0080**)
SS/SL+LL F p	0.0201 (0.8872)	0.5784 (0.4475)	0.1831 (0.6689)		0.2175 (0.6410)	0.1506 (0.6982)	0.9248 (0.3366)

SS = short/short, SL = short/long, LL = long/long.

* denotes significance at $p < 0.05$, ** denotes significance at $p < 0.01$.

For analyses by sex, the significance cut-off is adjusted to $p < 0.025$.

Separate analysis by sex revealed that the association between higher AQ score and long/long RS3 genotype was significant for females but not males. This result indicates that the association was mediated primarily by results for females, although the lack of significant results for males may also be affected by smaller sample sizes and higher variability for this group. Subscale analysis revealed that the association was primarily due to variation among RS3 genotypes in attention switching ($p = 0.0029$ both sexes, $p = 0.0072$ females). Complete results (means, standard deviations, analyses by sex) for the five AQ subscales are provided in Supplementary Table 2.5.

Target allele analysis revealed no associations between the RS1 target allele and AQ score. For the RS3 target allele, carriers were found to exhibit significantly higher AQ scores than non-carriers (Table 2.3). Analysis by sex revealed that this association also appeared to be mediated primarily by effects among females. A significant linear trend was observed, whereby mean AQ score increased with RS3 target allele copy number (Table 2.4).

Table 2.3 Analysis of effects of RS3 target allele on total AQ score.

	Without Target Allele	**With Target Allele**			
	Mean ± SD (N)	**Mean ± SD (N)**	**Df**	**F**	**p**
Both	16.83 ±5.31 (572)	17.61 ±5.51 (301)	1, 871	4.153	0.0419*
Males	17.87 ±5.83 (198)	17.98 ±5.60 (100)	1, 296	0.023	0.88
Females	16.27 ±4.93 (374)	17.42 ±5.47 (201)	1, 573	6.573	0.0106*

* denotes significance at $p < 0.05$, ** denotes significance at $p < 0.01$.
For analyses by sex, the significance cut-off is adjusted to $p < 0.025$.

Table 2.4. Regression testing for linear effect of 0, 1, or 2 copies of RS3 target allele on AQ score.

	Df	SE	F	p
Both	871	5.382	4.344	0.0374*
Males	296	5.752	0.4047	0.5252
Females	573	5.136	5.051	0.02499*

* denotes significance at $p < 0.05$, ** denotes significance at $p < 0.01$.
For analyses by sex, the significance cut-off is adjusted to $p < 0.025$.

Given that the RS3 target allele is a long allele, the relationship between the long RS3 genotype and higher AQ score may be due primarily to target allele effects. To evaluate this hypothesis more directly, three additional ANOVA analyses were run: a) excluding homozygous RS3 target allele carriers; b) excluding LL genotype target allele carriers; and c) excluding all target allele carriers. Significance was retained in the analysis excluding 330/330 individuals ($p = 0.0357$, both sexes, Supplementary Table 2.6), but not in the other analyses ($p > 0.35$ for both tests, both sexes, Supplementary Tables 2.7 and 2.8). These analyses indicate that the relationship between AQ score and RS3 genotype was largely due to the presence, absence, and number of target alleles.

2.5. Discussion

Significant associations were observed between RS3 polymorphisms and non-clinical autism spectrum phenotypes in this study. The so-called target allele was found to mediate the relationship between long RS3 allele length and higher AQ score. This result is concordant with previous reports of relationships between the RS3 target allele and autism in clinical populations (Kim et al., 2002) and reduced prosocial behaviour in non-clinical populations (Avinun et al., 2011; Knafo et al., 2008; Walum et al., 2008). In this study, increased number of RS3 target alleles was associated with higher AQ score. Such a “dose-dependent effect” was also observed by Walum et al. (2008), whereby more copies was associated with reduced human pair-bonding. These findings highlight the importance of investigating the effects and functional basis of AVPR1a target alleles.

The effects of RS3 genotype and target alleles were significant among females but not males in this study, suggesting a sex-differential role of vasopressin in social cognition. In non-human mammals, vasopressin is known to exert sex-specific effects on social behaviour, such as enhancing social play in male juvenile rats but reducing it in females

(Bredewold et al., 2014), and reducing latency to respond to infant stimuli in female but not male marmosets (Taylor and French, 2015). Furthermore, AVPR1a receptor distribution and binding affinity have been shown to differ in the brains of male versus female rodents (Dumais & Veenema, 2015). Fewer studies have examined sex differences in the vasopressin system for humans. Among the AVPR1a studies that examined sexes separately, Knafo et al. (2008) reported no interaction between sex and RS3 length, while Walum et al. (2008) reported an association for males only. Vasopressin administration studies report sex differences in its effects on human social interactions (e.g., Thompson et al., 2006; Rilling et al., 2014). The finding of a sex difference in the current study reaffirms the importance of separate analyses for males and females in studies of vasopressin and identifies a dimension of social cognition whereby this neurohormone may play a significant role for females.

In our study, the association between RS3 genotype and AQ score was mediated by attention switching—the ability to shift attention from one task to another—more so than by other subscales. This finding may be pertinent to the over-focus of attention common in ASD (Ploog, 2010). The ability of vasopressin to modulate human attention has been recognized since the early 1980s (Beckwith et al., 1982), with one study suggesting that vasopressin affects the proportion of attention dedicated to a primary task (Jennings et al., 1986). Interestingly, a study reporting a female-specific association between AQ score and oxytocin receptor (OXTR) haplotype in a non-clinical population reported a relationship due mainly to variation in the attention switching subscale (Kawamura et al., 2011). A recent study of AVPR1a variation in chimpanzees (Hopkins et al., 2014) also reported an association with attention: male (but not female) chimpanzees with a copy of the DubB sequence (which contains the RS3 repeat element) performed better on a receptive joint attention task than did males homozygous for deletion of the DubB sequence. Taken together, research in humans and chimpanzees thus suggests that vasopressin and oxytocin may exert effects on social cognition in part through modulation of attentional focus. More direct tests of this hypothesis should provide useful insights.

Lastly, these results add to the evidence that alleles related to autism risk are associated with behavioural variation in non-clinical populations. This hypothesis is supported by Robinson et al. (2016), who reported genome-wide genetic links between autism spectrum disorders and variation in sociality and communication measures in general

populations. Our results further support this theory, indicating that AVPR1a alleles associated with autism risk contribute to a non-clinical individual's position on the autism spectrum continuum.

2.6. Conclusions

This study serves to establish continuity between the three domains of AVPR1a research: its relationship to clinical autism, its relationship to social behaviour in non-clinical populations, and its relationship to non-clinical autism phenotypes. The finding that RS3 microsatellite length is associated with AQ score supports a role for AVPR1a variation in individual differences in social cognition, and also supports effects of the RS3 target allele and sex differences. The finding that differences in AQ scores were predominantly due to attention switching contributes to the literature implicating vasopressin in aspects of attention, and highlights a potential avenue for further research.

2.7. Acknowledgements

The authors thank Julie Couture, Zoe Francis, and Nicole McEwen for data collection, Silven Read and Suhani Thakore for technical assistance, Fab*Lab and Neil Watson at SFU for helpful comments, and NSERC for support

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2.9. Supplementary analyses

Table 2.5. Association analyses of relationships between AQ subscale scores and RS3 genotype, including two models of dominance.

	attention to detail			attention switching			communication			imagination			social skill		
	Both	male	female	both	male	female	both	male	female	both	male	female	both	male	female
SS Mean ± SD	5.36 ±2.26	5.44 ± 2.36	5.42 ± 2.20	4.77 ±1.96	5.11 ± 2.14	4.58 ± 1.84	2.14 ±1.83	2.36 ±1.95	2.02 ± 1.76	2.27 ±1.67	2.63 ±1.64	2.08 ± 1.66	2.40 ±1.67	2.60 ± 1.83	2.29 ± 1.57
SL Mean ± SD	5.34 ±2.16	5.39 ± 2.24	5.32 ± 2.11	4.55 ±1.98	4.62 ± 2.01	4.52 ± 1.97	2.17 ±1.73	2.37 ±1.81	2.07 ± 1.68	2.15 ±1.57	2.46 ± 1.69	1.99 ± 1.48	2.45 ±1.60	2.54 ± 1.62	2.41 ±1.60
LL Mean ± SD	5.42 ±2.12	5.59 ± 2.10	5.34 ± 2.14	5.09 ±1.99	5.07 ± 1.84	5.11 ± 2.07	2.48 ± 2.00	2.60 ±2.04	2.42 ± 1.98	2.35 ±1.74	2.76 ± 1.84	2.15 ± 1.66	2.57 ±1.72	2.63 ± 1.69	2.54 ± 1.73
SS + SL Mean ± SD	5.35 ±2.19	5.41 ± 2.28	5.32 ± 2.14	4.62 ±1.98	4.78 ± 2.07	4.54 ± 1.92	2.16 ±1.76	2.36 ±1.85	2.06 ± 1.71	2.19 ±1.61	2.51 ± 1.67	2.02 ± 1.54	2.44 ±1.62	2.56 ± 1.69	2.37 ± 1.59
SL + LL Mean ± SD	5.37 ± 2.14	5.46 ± 2.19	5.33 ± 2.12	4.76 ±2.00	4.79 ± 1.96	4.74 ± 2.03	2.29 ±1.84	2.45 ±1.89	2.21 ± 1.81	2.23 ±1.64	2.57 ±1.75	2.05 ± 1.55	2.50 ±1.65	2.57 ±1.64	2.46 ± 1.65
SS/SL/LL F p	0.1102 0.8956	0.209 0.8115	0.0082 0.9918	5.8734 0.0029 **	2.0733 0.1276	4.9736 0.0072 **	2.7433 0.0649	0.473 0.624	2.5357 0.0801	1.1695 0.311	0.8567 0.4256	0.5152 0.5976	0.614 0.541	0.0807 0.9225	0.8251 0.4387
SS+SL/LL F p	0.2123 0.6451	0.3958 0.5297	0.0165 0.8978	10.1397 0.0015 **	1.2601 0.2625	9.8678 0.0018 **	5.4465 0.0198 *	0.948 0.331	5.0119 0.0256*	1.6472 0.1997	1.2373 0.2669	0.7802 0.3774	1.081 0.299	0.0981 0.7543	1.1858 0.2766
SS/SL+LL F p	0.0055	0.0052	0.0024	0.0058	1.4442	0.6476	1.0227	0.132	1.0489	0.1108	0.071	0.0257	0.524	0.0167	1.0416

SS = short/short, SL = short/long, LL = long/long. * denotes significance at $p < 0.05$, ** denotes significance at $p < 0.01$.
For analyses of the five subscales, the significance cut-off is adjusted to $p < 0.01$ for the total population and $p < 0.005$ for analyses by sex

Table 2.6. Analysis of relationships between AQ score and RS3 genotype excluding individuals homozygous for the target allele.

RS3			
	Both (N = 833)	**Males** (N = 284)	**Females** (N = 549)
SS Mean AQ ± SD N	16.94 ± 5.42 200	18.14 ± 5.69 70	16.29 ± 5.18 130
SL Mean AQ ± SD N	16.68 ± 5.20 420	17.37 ± 5.73 145	16.31 ± 4.87 275
LL Mean AQ ± SD N	17.85 ± 5.74 213	18.64 ± 6.00 69	17.47 ± 5.59 144
SS + SL Mean AQ ± SD N	16.76 ± 5.27 620	17.62 ± 5.72 215	16.31 ± 4.97 405
SL + LL Mean AQ ± SD N	17.07 ± 5.41 633	17.78 ± 5.84 214	16.71 ± 5.15 419
SS/SL/LL F p	3.3447 0.0357*	1.2199 0.2968	2.6990 0.0682
SS+SL/LL F p	6.3769 0.0117*	1.6047 0.2063	5.4065 0.0204*
SS/SL+LL F p	0.0890 0.7655	0.2059 0.6504	0.6464 0.4217

Individuals with the 330/330 genotype (N = 40) were excluded from analysis.
SS = short/short, SL = short/long, LL = long/long.
* denotes significance at $p < 0.05$, ** denotes significance at $p < 0.01$.
For analysis by sex, the significance cut-off is adjusted to $p < 0.025$.

Table 2.7. Analysis of relationships between AQ score and RS3 genotype excluding LL genotype carriers of the target allele.

RS3			
	Both (N = 715)	**Males** (N = 249)	**Females** (N = 466)
SS Mean AQ ± SD N	16.94 ± 5.42 200	18.14 ± 5.69 70	16.29 ± 5.18 130
SL Mean AQ ± SD N	16.68 ± 5.20 420	17.37 ± 5.73 145	16.31 ± 4.87 275
LL Mean AQ ± SD N	17.39 ± 5.44 95	18.26 ± 5.97 34	16.90 ± 5.12 61
SS + SL Mean AQ ± SD N	16.76 ± 5.274 620	17.62 ± 5.72 215	16.31 ± 4.97 405
SL + LL Mean AQ ± SD N	16.81 ± 5.25 515	17.54 ± 5.77 179	16.42 ± 4.92 336
SS/SL/LL F p	0.7408 0.4771	0.6057 0.5465	0.3777 0.6857
SS+SL/LL F p	1.1529 0.2833	0.3651 0.5462	0.7555 0.3852
SS/SL+LL F p	0.0870 0.7681	0.5498 0.4591	0.0610 0.8050

Individuals with a 330/330 or 330/long genotype (N = 158) were excluded from analysis.
SS = short/short, SL = short/long, LL = long/long.
* denotes significance at $p < 0.05$, ** denotes significance at $p < 0.01$.
For analysis by sex, the significance cut-off is adjusted to $p < 0.025$.

Table 2.8. Analysis of relationships between total AQ score and RS3 genotype, excluding all carriers of the RS3 target allele.

RS3			
	Both (N = 572)	**Males** (N = 198)	**Females** (N =374)
SS Mean AQ ± SD N	16.94 ± 5.42 200	18.14 ± 5.69 70	16.29 ± 5.18 130
SL Mean AQ ± SD N	16.55 ± 5.19 277	17.53 ± 5.93 94	16.05 ± 4.70 183
LL Mean AQ ± SD N	17.39 ± 5.44 95	18.26 ± 5.97 34	16.90 ± 5.12 61
SS + SL Mean AQ ± SD N	16.71 ± 5.29 477	17.79 ± 5.82 163	16.15 ± 4.90 313
SL + LL Mean AQ ± SD N	16.77 ± 5.26 372	17.73 ± 5.93 128	16.26 ± 4.81 244
SS/SL/LL F p	0.9475 0.3883	0.3099 0.7338	0.6831 0.5057
SS+SL/LL F p	1.2776 0.2588	0.1836 0.6688	1.1846 0.2771
SS/SL+LL F p	0.1391 0.7093	0.2295 0.6324	0.0031 0.9554

Individuals with the 330/330, 330/long, or 330/short genotype (N = 301) were excluded from analysis.
SS = short/short, SL = short/long, LL = long/long.
* denotes significance at $p < 0.05$, ** denotes significance at $p < 0.01$.
For analysis by sex, the significance cut-off is adjusted to $p < 0.025$

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Chapter 3.

Endogenous oxytocin and testosterone response to experimental empathy induction

3.1. Abstract

The neurohormones oxytocin and testosterone are evolutionarily ancient regulators of sociality, serving to coordinate adaptive social behaviour with stimuli in the environment. Administration of oxytocin and testosterone has been shown to increase and reduce empathy, respectively; yet how these hormones levels change in response to naturalistic empathy-inducing stimuli has seldom been tested. In our study, healthy adults watched an emotional, empathetic video, with salivary oxytocin and testosterone measured before and after. Overall, on average, there were significant increases in oxytocin ($p<0.01$) and decreases in testosterone ($p<0.001$). Moreover, these changes in hormone levels tended to occur together, as supported by a chi square test ($p < <0.001$) and a circular statistics test of directionality ($p <0.05$). Research participants also completed questionnaires to assess individual variation in relevant socio-cognitive traits (Autism Spectrum Quotient, Schizotypal Personality Questionnaire, Interpersonal Reactivity Index, and Empathizing and Systemizing Quotients) and provided DNA samples for genotyping of hormone-associated genes. The results showed a nominally significant positive correlation between pre- to post-video oxytocin change and the Perspective Taking subscale of the Interpersonal Reactivity Index ($r = 0.18$, $p < 0.05$) for the overall population, as well as a positive correlation between baseline oxytocin-testosterone balance and Empathizing-Systemizing balance ($r = 0.16$, $p < 0.05$), when adjusted to account for sex differences in these variables. Variants in the oxytocin receptor (OXTR) and cluster of differentiation 38 (CD38) genes previously linked to plasma oxytocin were not associated with baseline salivary oxytocin in this study. The genetic analyses do, however, provide preliminary evidence of an association of CD38 SNP rs3796863 with baseline testosterone and systemizing in males, and some further support of associations of OXTR variants with empathy. Taken together, these results offer support for the diametric hypothesis of the roles of oxytocin and testosterone in empathizing.

3.2. Introduction

The neuropeptide hormone oxytocin and the steroid hormone testosterone are evolutionarily ancient mediators of social behaviour, playing roles in pair-bonding, parenting, affiliation, aggression, and anxiety across mammal species [1]. Inputs from the environment, such as social interactions, have been shown to mediate changes in oxytocin and testosterone levels in rodents [2,3], and experimental manipulation of these hormones can influence social behaviour [4,5]. Variation in levels of social hormones may thus serve as a mechanism for "fine tuning" adaptive social behaviour in the ever-changing environments that social species inhabit.

The extent to which oxytocin and testosterone mediate complex aspects of human socio-emotional behaviour have become subjects of intense interest. Administration studies, where hormone levels are temporarily altered using a nasal spray, have shown that oxytocin increases empathy [6–8], compassion [9], cooperation [10,11], trust [12,13], and positive perceptions of one's own personality [14], and promotes positive social interactions between spouses and between parents and children [15,16]. By contrast, testosterone administration decreases empathy, generosity, and trust [17–20], and increases risk-taking behaviour [21]. Oxytocin levels in blood or saliva correlate positively with bonding, attachment, and sensitive parenting [22–24]; testosterone levels correlate positively with aggression and narcissism [25,26], and negatively with parental effort [27,28]. Variation in oxytocin and testosterone levels has also been reported in psychiatric conditions, with evidence of elevated testosterone or reduced oxytocin levels in autism spectrum conditions [29,30], and elevated oxytocin or reduced testosterone in schizophrenia, bipolar disorder, and depression [31–34], although these patterns remain inconsistent across studies [reviewed in 35]. Dysregulation of levels of social hormones may relate to the alterations in social behaviour that characterize these conditions. Taken together, findings from experimental, correlational, and clinical research suggest a broadly prosocial role for oxytocin and an asocial or antisocial role for testosterone.

Crespi [35] proposed an integrative model whereby oxytocin activates neural regions that promote mentalizing—described as "engaging in social cognition and making sense of each other, and ourselves, in terms of subjective empathic and cognitive states" — while testosterone favours "self-oriented, ego-centric, and non-social attention and information processing". Patterns of endogenous oxytocin and testosterone release are

thus expected to adaptively shift cognition between social and non-social domains dependent upon immediate environmental costs and benefits. As prosocial and antisocial cognition are incompatible, Crespi's diametric-effects model predicts a pattern where increased oxytocin is paired with decreased testosterone, and vice versa. However, the ability to evaluate any trade-off between oxytocin and testosterone in the hormones and behaviour literature is limited, as experimental studies suitable for testing this hypothesis have assessed oxytocin or testosterone separately, rather than assessing both hormones within individuals.

The primary goal of the current study was thus to test for joint, opposite changes in oxytocin and testosterone in response to a naturalistic, ecologically-valid experimental stimulus. This methodology contrasts with administration studies where hormone levels are inflated independent of social context. To date, only a handful of studies have examined oxytocin response to "real life" conditions: de Jong et al. [36] reported significant oxytocin increases after exercise, sexual self-stimulation, and social stress, but not after breast-feeding (the authors suspect pulses of oxytocin release may have been missed due to a lag in saliva sampling). Brondino et al. [37] found that engaging in gossip, but not emotional non-gossip or neutral conversation, increased oxytocin in college-age females. Feldman et al. [38] reported that oxytocin levels in mothers and fathers increase after 15 minutes of play with their infants, dependent on the level of affectionate or stimulatory contact. Barraza & Zak [39] showed that plasma oxytocin levels increased after watching an empathy-inducing video about a young child with terminal cancer. Testosterone response has been well studied in the context of competition, with winners often, but not always, showing a testosterone increase mediated by psychological factors and stress [40–43]. In addition, males in committed relationships have been found to have lower basal testosterone levels [44,45], and basal testosterone decreases in both males and females with the onset of parenthood [46,47].

Although joint responses of oxytocin and testosterone were also untested in these studies, they provide further support for Crespi's [35] model, with oxytocin increasing in contexts demanding social cognition—regardless if the social context may be perceived as malicious or stressful—and testosterone increasing with experiences of individual success and decreasing with the anticipation of parental effort. Interestingly, Jaeggi et al. [48] reported unexpected, concurrent increases in both oxytocin in testosterone in

Tsimane' men returning home after hunting, although the stimuli that prompted the changes in hormone levels were unclear.

In the current study, we used the emotional video validated by Barraza & Zak [39] to induce feelings of empathy in a large, mixed-sex population of healthy young adults. Saliva samples were collected before and after watching the video to assess changes in salivary oxytocin and testosterone levels. In addition, research participants reported their emotional response to the video and completed questionnaires assessing variation in relevant socio-cognitive traits, namely the Autism Spectrum Quotient (AQ), Schizotypal Personality Questionnaire Brief-Revised (SPQ-BR), Interpersonal Reactivity Index (IRI), and the Empathy Quotient (EQ) and Systemizing Quotient (SQ). Lastly, research participants provided DNA samples for genotyping of hormone-related polymorphisms. These data were used to explore if psychological or genetic factors mediated hormonal response to the empathy-inducing video, and if individual variation in oxytocin, testosterone, or hormone-associated genes were related to self-reported psychological traits.

3.2.1. Predictions

The empathetic video was predicted to induce an increase in oxytocin levels and a decrease in testosterone levels relative to baseline. Moreover, the oxytocin increase and testosterone decrease were expected to occur together, a prediction from Crespi's diametric-effects model [35]. Individuals reporting a more empathetic response to the video and/or scoring higher on questionnaire measures of empathy (i.e., IRI, EQ) were predicted to show greater increases in oxytocin.

Informed by previous findings of hormone level alterations in psychiatric conditions, we predicted that variation in baseline oxytocin, testosterone, or oxytocin relative to testosterone may predispose individuals towards certain social traits. Specifically, basal oxytocin was predicted to correlate positively with EQ and SPQ-BR—particularly the Cognitive-Perceptual subscale of the SPQ-BR, which represents a measure of positive schizotypy [49,50]. In contrast, basal testosterone was predicted to correlate positively with SQ and AQ scores; a positive correlation has been reported between salivary testosterone and the Japanese version of the AQ, although the relationship disappeared when sex differences were accounted for [51].

Lastly, with regard to hormone-related genes, we predicted that single nucleotide polymorphisms (SNPs) in the oxytocin receptor gene (OXTR, rs2254298 and rs1042778) and cluster of differentiation 38 gene (CD38, rs3796863) previously associated with variation in plasma oxytocin [22] would also be associated with variation in salivary oxytocin. In addition, we predicted that OXTR SNPs (rs53576, rs1042778, rs7632287, rs2254298, rs237887) previously associated with empathy and prosocial behaviour [52–54] would be associated with a greater oxytocin increase, a more empathetic response to the video, and higher IRI and EQ scores.

3.3. Material & methods

3.3.1. Subjects

176 research participants were recruited from Simon Fraser University via the Department of Psychology undergraduate research pool and posters, receiving either course credit or $10 CAD for their participation and time. Participants self-reported being healthy, avoiding food and drink for a minimum of 1 hour before participation, and not taking drugs that may interfere with hormone levels (excluding contraceptive use by females).

The mean age of the research participants was 20.4 years with a standard deviation of 2.2 years. Participants self-reported diverse ethnic backgrounds: 42% were East Asian, 25% Caucasian, 22% South Asian, and 11% other or multiple races. No participants reported having children and no female participants reported being pregnant. Data was collected on use of hormonal contraception and stage of menstrual cycle in female participants. Hormone levels were not significantly associated with ethnicity, stage of menstrual cycle, or use of hormonal contraception, and these variables were thus excluded from further analyses ($p > 0.18$ for all tests).

3.3.2. Experimental design and empathy induction

Experiments took place between 1:00pm and 5:00pm and were conducted by the same researcher. After written informed consent was obtained, participants provided a saliva sample for baseline hormone level measurement and completed a brief demographics questionnaire (provided in 3.7.1). Next, participants watched a two-minute video

intended to elicit empathy; watching a video is an established method for inducing emotional states [55]. The video is about a young child with terminal brain cancer and is narrated by the child's father, and was previously validated by [39].

Immediately after watching the empathy-inducing video, participants completed the post-video questionnaire (provided in 3.7.2) regarding the video's content and their emotional response and provided a second saliva sample. Collection of a third saliva sample began exactly 20 minutes after watching the video. Due to the volume of saliva required for assaying multiple hormones, participants took upwards of 10 minutes to provide a sufficient sample. During this time, participants completed the questionnaires described in Section 2.3. Lastly, after all saliva samples were collected, participants provided a mouthwash sample for DNA extraction. All protocols were approved by the Office of Research Ethics, Simon Fraser University (study number 2015s0228).

3.3.3. Psychometric data

To explore relationships between psychological factors and hormones, research participants completed the following questionnaires:

Post-video emotional response

Immediately after watching the emotional video, participants completed an ad hoc questionnaire about the content of the video and their emotional response. The emotional response ratings were modeled on the video-rating questionnaire used by [39]. Participants were instructed to indicate, on a scale from 1-5, how strongly they experienced 12 emotions. Overall "Empathy Response" was determined by summing the ratings of 6 emotions (sympathetic, warm, compassionate, tender, soft-hearted, and moved), and 'Distress Response" was determined by summing the ratings of the other 6 emotions (anxious, annoyed, sad, distressed, frightened, and disturbed). Scores thus have a possible range of 6-60, with higher scores indicating greater degree of response. This questionnaire is provided in 3.6.2.

Autism spectrum quotient

The autism spectrum quotient (AQ) quantifies non-clinical autism phenotypes in populations of normal intelligence [56]. The AQ comprises 50 questions that assess autistic-like traits across five domains: imagination, social skill, communication, attention

switching, and attention to detail. The first four domains show relative impairment in autism spectrum conditions, while the fifth shows relative enhancement. Responses are in forced choice format: endorsing an autistic-like trait is scored as 1, otherwise items are scored 0. Total AQ scores thus range from 0-50, with domain subscale scores ranging from 0-10, with higher scores indicating a more autistic phenotype. A recent review reported an average AQ score of 17 in non-clinical populations, with males scoring significantly higher than females [57]. This questionnaire is provided in 3.7.3.

Schizotypal personality questionnaire

The schizotypal personality questionnaire brief-revised (SPQ-BR) [49,50] is a short, sensitive tool for assessing dimensions of schizotypy in non-clinical populations. The SPQ-BR comprises 32 items that cluster to three higher-order factors: cognitive-perceptual schizotypy, interpersonal schizotypy, and disorganized schizotypy. Responses are on a Likert scale that ranges from “strongly disagree” to “strongly agree”. Higher scores are given for greater agreement with schizotypal traits. A higher SPQ-BR-BR score thus indicates higher schizotypy. This questionnaire is provided in 3.7.4.

Interpersonal reactivity index

The interpersonal reactivity index (IRI) [58] is used to assess four distinct components of empathy: empathic concern, perspective taking, personal distress, and fantasy scale. The 28 items of the IRI are scored 0-4, with a higher score indicating greater empathy. Total IRI score thus ranges 0-112 with subscale scores ranging 0-28. The IRI has been validated cross-culturally [59,60] and shows significantly higher mean scores for females [58]. This questionnaire is provided in 3.7.5.

Empathy quotient and systemizing quotient

The short-form empathy quotient (EQ) and systemizing quotient (SQ) are used to assess social (empathizing) and non-social (systemizing) aspects of cognition [61,62]. The EQ comprises 22 items and the SQ comprises 25 items, both with a forced-choice response format. Items are scored 2 if the empathizing/systemizing item is strongly endorsed, 1 if the item is slightly endorsed, and 0 is the item is not endorsed. Possible EQ scores thus range 0-44 and SQ scores from 0-50. “Balance” between EQ and SQ was calculated, as per Wakabayashi et al. [61], by standardizing the scores and then subtracting the normalized EQ score from the normalized SQ score: a positive value thus indicates

higher systemizing relative to empathizing, and a negative value indicates higher empathizing relative to systemizing. Scores within one standard deviation of the mean represent a “balanced” brain type; scores 1-2 standard deviations above the mean represent a “systemizing” brain type; scores 1-2 standard deviations below the mean represent an “empathizing” brain type; and scores more than 2 standard deviations above or below the mean represent “extreme systemizing” and “extreme empathizing” brain types, respectively. Higher mean systemizing scores, and systemizing-biased brain types, are more common among males and individuals with autism spectrum conditions [29,63,64]. This questionnaire is provided in 3.7.6.

3.3.4. Saliva collection and hormone analysis

Collection of saliva samples took place between 1:00pm and 5:00pm to minimize any effects of diurnal variation, and participants were instructed to refrain from eating or drinking anything except water for a minimum of one hour. Prior studies testing stimulus-induced hormone change allow a 20-minute delay for endogenous testosterone changes to be manifested in saliva; however, for oxytocin, the optimal sampling time is not clear. Thus, three saliva samples were collected per participant: a baseline sample (referred to as timepoint “A”), an immediate post-empathy induction sample (“B”), and a 20-minute post-empathy induction sample (“C”). Saliva was expressed directly into pre-chilled polypropylene 15 ml tubes and kept on ice until a sufficient volume (> 2 mL) was collected, then immediately frozen at -20°C. Prior to assays, samples were thawed at 4°C and centrifuged at 4°C at 1600 x g for 15 minutes. All samples were run in duplicate and samples from the same individual were always analyzed together on the same plate.

Oxytocin was assayed on the first freeze-thaw cycle using Enzo Life Sciences enzyme-linked immunosorbent assay kit ADI-901-153A, which has a sensitivity range of 15.6 – 1,000 pg/ml. A pilot study found that concentrating saliva samples twofold resulted oxytocin measurements sufficiently above the minimum detection limit. Consistent with protocols described in the literature, samples were concentrated by freeze-drying, reconstituted, incubated as per the Enzo manual, and read at 405 nm. Oxytocin concentrations were then calculated from the standard curve. The intra- and inter-assay coefficients of variability were < 10% and < 18%, respectively, for n = 17 plates, which are below the ranges of 12.6–13.3% and 11.9–20.9%, respectively, reported in the

assay manual. The validity of oxytocin measured in extracted versus unextracted body fluid, as well as the relationship between peripheral oxytocin and central oxytocin, has been questioned [65]. However, the ELISA kit used in this study has undergone rigorous testing since the publication of such critiques and is highly specific to oxytocin (i.e., does not detect vasopressin) [66]. Furthermore, any methodological issue should affect samples in a similar manner, and is not expected to positively bias the results toward significant findings.

Salivary testosterone was quantified using Salimetrics enzyme-linked immunosorbent assay kit 1-2312-5. This assay kit is designed specifically for use with saliva, and thus no concentration was necessary. After centrifugation, 20µl of saliva supernatant was incubated as per kit instructions and read at 450 nm. Testosterone concentrations were then calculated from the standard curve. The intra-assay coefficient of variability was < 5%, and the inter-assay coefficients of variability were < 10% and < 15% for the high and low controls, respectively, for n = 10 plates.

Two individuals were unable to provide sufficient saliva for the quantification of both oxytocin and testosterone. Due to the lower volume of saliva required for the testosterone assay, samples from these individuals were analyzed for testosterone only. One individual was excluded from all analyses due to atypical results on genetic analyses and extremely high testosterone levels (> 3 SD above sex-specific mean).

3.3.5. Genetic analyses

DNA was extracted from saliva. SNPs in OXTR (rs53576, rs1042778, rs7632287, rs2254298, rs237887) and CD38 (rs3796863) were quantified using TaqMan® SNP Genotyping Assays and a Roche LightCycler® 96 Real-Time PCR machine, and fluorescence data were analyzed under Endpoint Genotyping with LightCycler® 96 software, version 1.1.0.1320. Genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium ($p > 0.05$ for all chi square tests).

3.3.6. Statistical analyses

Statistical tests were performed in SPSS or R version 3.3.1 (2016-06-21). Circular statistics tests were performed using the ‘circular’ package (version 0.4-7) for R. T-tests

of means were used for comparisons between two groups; analysis of variance (ANOVA) was used for tests involving more than two groups. All tests were two-tailed. Results were considered statistically significant if $p < 0.05$, unless otherwise specified.

Percentage changes in hormone levels were calculated as (final – initial)/initial; a positive value thus indicates an increase from the baseline hormone level to the post-video hormone level, and a negative value indicates a decrease from the baseline hormone level to the post-video hormone level. The balance between baseline testosterone and oxytocin (Baseline T:OT) was computed using the normalized baseline hormone values (using sex-specific mean and SD, to account for significant sex difference in baseline testosterone). Normalized baseline oxytocin was then subtracted from normalized baseline testosterone: a positive value thus indicates higher testosterone relative to oxytocin, and a negative value indicates higher oxytocin relative to testosterone.

3.4. Results

3.4.1. Salivary oxytocin and testosterone

Means and standard deviations for salivary hormone levels in the overall population, females only, and males only are presented in Table 3.1. The typical sex difference in testosterone levels was present at timepoints A and C (t-tests, $p < 0.0001$), with mean salivary testosterone levels nearly twofold higher for males than females. A statistically significant sex difference in salivary oxytocin levels was not observed at any of the three timepoints (t-tests, $p > 0.15$), which is consistent with studies of salivary oxytocin levels in comparable populations [e.g., 67].

Table 3.1 Means and standard deviations for salivary hormone levels.

		N	Mean (pg/ml)	Std. Deviation	Sex difference (p value)
Testosterone Time A	Both sexes	171	117.4	53.3	< 0.001
	Females	93	82.5	28.2	
	Males	78	159.1	45.6	
Testosterone Time C	Both sexes	171	111.7	53.7	< 0.001
	Females	93	76.7	26.0	
	Males	78	153.5	48.1	
Oxytocin Time A	Both sexes	168	104.5	65.8	0.17
	Females	93	97.8	57.6	
	Males	75	112.7	74.3	
Oxytocin Time B	Both sexes	167	115.5	84.4	0.78
	Females	93	113.9	79.3	
	Males	74	117.6	90.9	
Oxytocin Time C	Both sexes	168	116.5	84.7	0.27
	Females	93	109.9	72.8	
	Males	75	124.6	97.3	
Baseline T:OT[1]	Both sexes	168	-0.007	1.1	0.94
	Females	93	-0.009	1.1	
	Males	75	-0.005	1.2	

[1] Baseline T:OT was computed using the normalized hormone values (using sex-specific mean and SD), with the normalized baseline oxytocin subtracted from normalized baseline testosterone.

3.4.2. Pre- to post-empathy change in salivary oxytocin and testosterone

Paired t-tests were used to assess changes in salivary hormone levels in response to the empathy-inducing video. For the overall population, oxytocin increased significantly from baseline (timepoint A) to both post-empathy induction samples (timepoints B and C, $p < 0.01$ both tests). The change in salivary oxytocin from timepoint B to C was not significant ($p > 0.75$). These analyses were also performed for males and females separately. Tests of female participants only show the same pattern of pre- to post-empathy salivary oxytocin increase at both timepoints. For tests of male participants only, although there was an overall increase in salivary oxytocin from baseline (A) to timepoints B and C, only the increase from timepoint A to C reached statistical significance. Furthermore, the increases in salivary oxytocin from baseline to timepoint B and baseline to timepoint C were, on average, higher for females than males (A-B: 21.0% for females, 7.3% males; A – C: 17.7% females, 10.9% males); however, the mean difference did not reach the level of statistical significance in either case ($p > 0.06$,

both tests). The complete results of the tests for the overall population, females only, and males only are presented in Tables 3.2 and 3.3.

The results of paired t-tests of change in salivary testosterone are also shown in Table 3.2. For the overall population, there was a significant decrease in salivary testosterone from baseline (A) to the post-empathy induction sample (C) ($p < 0.001$). The significant decrease in salivary testosterone was also observed in the test of females only ($p < 0.001$), but did not meet the level of statistical significance in the test of males only ($p = 0.08$). Although the absolute mean change in salivary testosterone (measured in pg/ml) is similar for males and females, due to males' nearly twofold higher testosterone levels, this result translates to a lower percentage change for males than for females. However, the mean difference in salivary testosterone decrease between males and females did not reach the level of statistical significance ($p = 0.30$, Table 3.3).

Table 3.2 Paired t-test results for comparisons of salivary hormone levels at different timepoints pre- and post-empathy induction.

		Paired Differences					t	p
		Mean	Std. Dev.	Std. Error Mean	95% Confidence Interval of the Difference			
					Lower	Upper		
Testosterone, Time A - C	Both sexes	5.69	21.77	1.66	2.41	8.98	3.42	0.0009 **
	Females	5.81	15.28	1.58	2.66	8.96	3.67	0.0004 **
	Males	5.55	27.69	3.14	-0.69	11.79	1.77	0.081
Oxytocin, Time A - B	Both sexes	-10.95	49.82	3.86	-18.57	-3.34	-2.84	0.005**
	Females	-16.06	51.84	5.38	-26.74	-5.38	-2.99	0.004**
	males	-4.53	46.72	5.43	-15.36	6.29	-0.83	0.41
Oxytocin, Time A - C	Both sexes	-12.04	47.88	3.69	-19.33	-4.75	-3.26	0.001**
	Females	-12.04	47.10	4.88	-21.74	-2.34	-2.46	0.016*
	Males	-12.05	49.15	5.67	-23.35	-0.74	-2.12	0.037*
Oxytocin, Time B – C	Both sexes	-1.10	51.13	3.96	-8.92	6.71	-0.28	0.78
	Females	4.02	49.24	5.11	-6.12	14.16	0.79	0.43
	Males	-7.55	53.05	6.17	-19.84	4.74	-1.22	0.23

* $p < 0.05$, ** $p < 0.001$

Table 3.3 Pre- to post-empathy induction percent change in salivary oxytocin and testosterone, and t-tests of sex differences in mean change.

		N	Mean % Change	SD	Sex difference (p-value)
Testosterone % Change, A – C	Both sexes	171	-4.14%	16.86%	0.30
	Females	93	-5.37%	16.80%	
	Males	78	-2.67%	16.93%	
Oxytocin % Change, A – B	Both sexes	167	14.94%	46.24%	0.06
	Females	93	21.00%	48.60%	
	Males	74	7.33%	42.19%	
Oxytocin % Change, A – C	Both sexes	168	14.65%	42.89%	0.31
	Females	93	17.65%	46.70%	
	Males	75	10.93%	37.61%	

3.4.3. Relationship between oxytocin and testosterone change

Depending on the direction of change (increase or decrease) for oxytocin and testosterone, each research participant was assigned to one of four categories: (I) oxytocin increase, testosterone increase; (II) oxytocin increase, testosterone decrease; (III) oxytocin decrease, testosterone decrease; (IV) oxytocin decrease, testosterone increase. Figure 3.1 shows the research participants' paired hormone level changes separated into the four categories.

A chi square test was then performed as a test of variation among quadrants: in the absence of a relationship between change in testosterone and change in oxytocin, the number of observations in each category should be approximately equal. The chi square test does not directly assess the prediction of a joint increase in oxytocin and decrease in testosterone, and thus represents only a preliminary test of the relationship between the two variables. The results of the chi square tests are presented in Table 3.4. The results support the hypothesis that, for the overall population, hormonal responses to the empathy-inducing video were not equally distributed across the four quadrants ($p < 0.001$). As shown in the table, the largest proportion of individuals was in quadrant II (oxytocin increase, testosterone decrease) and the smallest proportion of individuals was in quadrant IV (oxytocin decrease, testosterone increase). When the chi square tests were performed separately for each sex, the results were statistically significant for females only ($p < 0.001$), but not for males only ($p = 0.10$).

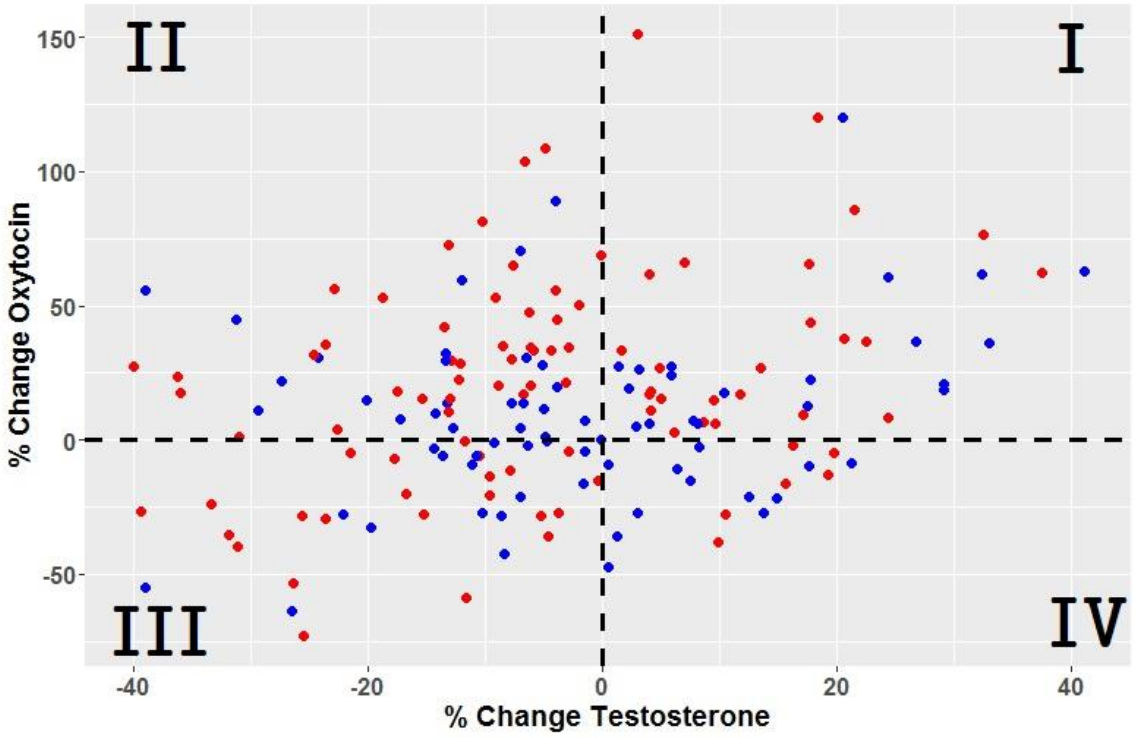

Figure 3.1 Scatterplot of pre- to post-empathy induction percent change in oxytocin and testosterone for each research participant (N = 167).

Oxytocin change is the average of the percentage change from baseline to time points B and C. Red dots indicate females, blue dots indicate males.

Table 3.4 Chi square test assessing relationship between pre- to post-empathy induction paired change in oxytocin and testosterone.

		OT increase, T increase	OT increase, T decrease	OT decrease, T decrease	OT decrease, T increase	X^2	P
Both sexes n = 167	**Observed n (%)**	44 (26%)	65 (39%)	40 (24%)	18 (11%)		
	Expected n (%)	41.75 (25%)	41.75 (25%)	41.75 (25%)	41.75 (25%)		
	$(O-E)^2/E$	0.12	12.95	0.07	13.51	26.7	< 0.0001
Females n = 92	**Observed n (%)**	25 (27%)	38 (41%)	23 (25%)	6 (7%)		
	Expected n (%)	23 (25%)	23 (25%)	23 (25%)	23 (25%)		
	$(O-E)^2/E$	0.17	9.78	12.57	0	22.5	< 0.0001
Males n = 75	**Observed n (%)**	19 (25%)	27 (36%)	17 (23%)	12 (16%)		
	Expected n (%)	18.75 (25%)	18.75 (25%)	18.75 (25%)	18.75 (25%)		
	$(O-E)^2/E$	0.00	3.63	0.16	2.43	6.2	0.10

df = 3 for all tests. Oxytocin change used in the tests is the average of the percentage change at time points B and C. The results are the same (significant for overall population and females only) if percentage change at each individual time point (A to B, or A to C) is used.

As a direct test of the joint change in salivary oxytocin and testosterone, circular statistical tests were employed. To run circular statistical tests, the percentage changes in salivary oxytocin and testosterone were transformed to vectors originating from the origin (0,0) (as shown in Supplementary 3.7.7). The angle at which the vector radiated from the origin (0,0) was then converted to radians for circular statistical tests. Given the variability in hormonal responses in our population, Rao's Spacing Test of Uniformity was used to test for the presence or absence of underlying directionality. This circular statistics test is based on the idea that, in the absence of underlying directionality, observations should be evenly spaced; clustered observations or unusually large spaces between observations thus constitute evidence for directionality [68].

Plots of the circular data are presented in Figure 3.2 for the overall population, females only, and males only. Each black arrow originating from the centre of the circle (0,0) represents one individual, and the red arrow indicates the mean direction of joint hormone change. The quadrants of the circle correspond with the quadrants in Figure 3.1 used for the chi square test. For the overall population, Rao's Spacing Test rejects the null hypothesis of uniformity (test statistic = 147.6, $p < 0.05$), supporting the presence of directionality in the dataset. The mean direction is 2.03 radians, which falls in quadrant II, i.e., oxytocin increase, testosterone decrease. Rao's Spacing Test also indicates significant directionality (test statistic = 159.7, $p < 0.001$) in the hormone level changes for females only, with a mean direction of 1.93 radians (Quadrant II). For males, the mean direction of hormone level change is also in Quadrant II (2.21 radians), although the test of directionality is not statistically significant (test statistic = 144.1, $p < 0.15$).

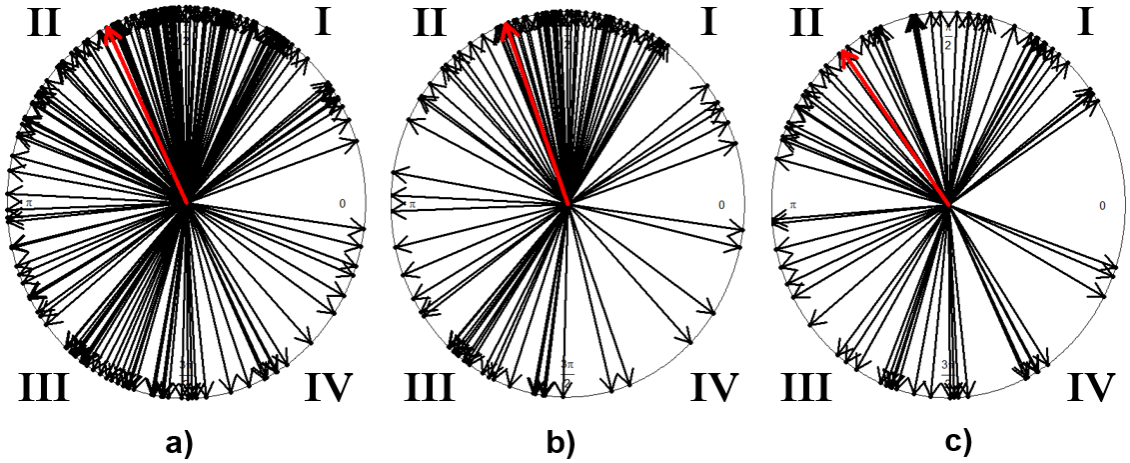

Figure 3.2 Circular plots of angles derived from joint percent change in salivary oxytocin and testosterone.

Plot a) is for the overall population, b) is females only, and c) is males only.
Each black arrow indicates the joint change in oxytocin and testosterone for one individual.
The red arrow indicates the mean change.
Quadrant I indicates increased oxytocin, increased testosterone; Quadrant II indicates increased oxytocin, decreased testosterone; Quadrant III indicates decreased oxytocin, decreased testosterone; and Quadrant 4 indicates deceased oxytocin, increased testosterone.

3.4.4. Psychological questionnaire scores

Descriptive statistics for the psychological questionnaires used in the study, as well as their subscales, are presented in Table 3.5. As the means for several questionnaires appeared to vary between males and females, t-tests for equality of means were performed. Females, on average, scored higher on the Interpersonal Reactivity Index and its subscales and males, on average, scored higher on the Autism Spectrum Quotient, Schizotypal Personality Questionnaire, and Systemizing Quotient. The sex difference in the Empathy Quotient did not quite reach the pre-determined level of statistical significant ($p = 0.055$). However, when Empathizing and Systemizing scores are normalized using the population means and considered together, males, on average, show greater Systemizing relative to Empathizing ($p < 0.01$).

To assess relationships between psychological questionnaires, correlations were calculated and are presented in Table 3.6. The two measures of empathy used in this study, IRI and EQ, were significantly and positively correlated with each other ($r = 0.33$, overall population, $p < 0.01$). AQ score was significantly and negatively correlated with IRI and EQ ($r = -0.22$, -0.41, respectively, for the overall population; both tests $p < 0.001$). Although AQ score showed no relationship with SQ score in our study population, Systemizing relative to Empathizing (SQ:EQ) was positively correlated with AQ score ($r = 0.26$, $p < 0.01$)

Table 3.5 Mean and standard deviation of questionnaire scores for the overall population, females only, and males only.

	Mean (SD)			Sex difference (p value)
	Both sexes	**Females**	**Males**	
Autism Spectrum Quotient	18.35 (5.72)	17.26 (5.41)	19.62 (5.84)	0.008**
- attention switching	5.34 (1.94)	5.13 (1.89)	5.58 (1.98)	0.137
- attention to detail	5.45 (2.12)	5.38 (2.39)	5.53 (1.75)	0.658
- communication	2.51 (1.84)	2.22 (1.78)	2.85 (1.93)	0.027*
- imagination	2.45 (1.57)	2.20 (1.49)	2.76 (1.62)	0.020*
- social skill	2.62 (2.26)	2.44 (2.17)	2.83 (2.35)	0.259
Schizotypal Personality-BR	89.50 (16.18)	86.16 (15.57)	93.18 (16.14)	0.005**
- constricted affect	15.7 (4.89)	14.01 (4.57)	17.64 (4.53)	0.000**
- eccentric behaviour	11.38 (3.43)	10.94 (3.55)	11.87 (3.24)	0.081
- ideas of reference	18.66 (4.31)	18.07 (4.07)	19.36 (4.5)	0.051
- magical thinking	9.06 (3.72)	9.54 (3.77)	8.47 (3.59)	0.060
- odd speech	12.8 (2.84)	12.67 (2.66)	12.95 (3.05)	0.531
- social anxiety	12.00 (4.07)	11.66 (3.98)	12.4 (4.18)	0.241
- unusual perceptual experiences	10.15 (2.98)	9.77 (2.90)	10.6 (3.03)	0.070
- cognitive-perceptual	37.77 (8.33)	37.23 (8.13)	38.39 (8.57)	0.373
- disorganized	24.2 (5.07)	23.66 (5.04)	24.82 (5.05)	0.141
- interpersonal	27.66 (7.81)	25.58 (7.57)	30.04 (7.43)	0.000**
Interpersonal Reactivity Index	69.28 (13.41)	72.86 (13.44)	64.93 (12.09)	0.000**
- empathic concern	19.47 (5.25)	21.00 (4.92(	17.64 (5.07)	0.000**
- fantasy scale	18.49 (5.22)	19.31 (5.18)	17.49 (5.11)	0.023*
- personal distress	13.06 (4.57)	13.71 (4.62)	12.29 (4.42)	0.044*
- perspective taking	18.40 (4.38)	19.05 (4.45)	17.61 (4.17	0.032*
Empathy Quotient	24.64 (7.19)	25.60 (7.53)	23.45 (6.59)	0.055
Systemizing Quotient	18.48 (9.33)	14.67 (7.88)	22.97 (8.94)	0.000**
SQ:EQ [1] (overall)	-0.044 (1.27)	-0.59 (1.15)	0.63 (1.07)	0.000**

[1] for this test of sex difference only, SQ:EQ was calculated using the overall population mean and SD for EQ and SQ

Table 3.6 Correlations between questionnaire scores for the overall population, females only, and males only.

		SPQ-BR: cog-percep	IRI	EQ	SQ	SQ:EQ[1]
Autism Quotient	Both sexes	0.30 **	-0.22 **	-0.41 **	0.03	0.26**
	Females	0.28 **	-0.21 *	-0.47 **	0.03	0.42**
	Males	0.29 *	-0.11	-0.28*	-0.15	0.06
Schizotypal Personality: cognitive perceptual	Both sexes		0.12	-0.12	0.03	0.03
	Females		0.12	-0.17	0.04	0.12
	Males		0.20	-0.03	-0.06	-0.06
Interpersonal Reactivity Index	Both sexes			0.33**	-0.02	-0.12
	Females			0.43**	0.13	-0.23*
	Males			0.10	0.13	0.04

* $p < 0.05$, ** $p < 0.01$

[1] SQ:EQ was calculated as the normalized (using sex-specific mean and SD) EQ score subtracted from the normalized SQ score; a positive SQ:EQ value thus represents a Systemizing bias relative to Empathizing

3.4.5. Psychological-hormonal associations

As initial tests of the relationships between the hormonal and psychological variables in this study, correlational analyses were performed. Table 3.7 presents the Pearson product-moment correlation coefficients of questionnaire scores with baseline hormone levels and pre- to post-video percent change in hormone levels. Correlations are given for the overall population, females only, and males only. Significance values given in the table have not been adjusted for multiple comparisons; if the level of statistical significance were adjusted to account for multiple comparisons (e.g. subscale analysis), none of the correlations maintain statistical significance.

Pre- to post-video change in salivary oxytocin was not associated with the two main measures of empathy, the IRI empathic concern subscale and EQ, in the overall population. For females only, there was a trend toward a positive relationship between oxytocin change and empathic concern ($r = 0.18$, $p < 0.10$). In addition, for the overall population, oxytocin change was positively correlated with IRI-Perspective Taking ($r = 0.18$, $p < 0.05$) and negatively correlated with SQ ($r = -0.13$, $p < 0.10$). Change in salivary testosterone was not associated with any psychological variables in the overall population, though did show a negative correlation with SQ in females only ($r = -0.24$, $p < 0.05$).

Baseline oxytocin showed no significant associations with psychological traits in the overall population. For females only, there was a negative correlation between baseline oxytocin and empathic concern ($r = -0.22$, $p < 0.05$). For males only, balance of baseline testosterone relative to oxytocin (T:OT) was negatively correlated with EQ ($r = -0.23$, $p < 0.05$). For the overall population, T:OT (normalized to account for sex differences) was positively ($r = 0.16$, $p < 0.05$) correlated with Systemizing relative to Empathizing (SQ:EQ, normalized for sex differences in questionnaire scores). All other relationships between hormones and psychological variables were statistically non-significant.

Table 3.7 Correlations between psychological questionnaire scores and hormone variables for the overall population, females only, and males only.

		Baseline T	Baseline OT	Baseline T:OT [1]	% Change T	% Change OT (A - B)	% Change OT (A - C)
Autism Spectrum Quotient	Both sexes	-0.03[1]	-0.07	0.06	-0.06	-0.05	-0.07
	Females	0.05	-0.14	0.17	-0.12	0.01	-0.15
	Males	-0.12	-0.07	-0.05	-0.03	-0.07	0.09
Schizotypal Personality	Both sexes	0.07[1]	-0.01	0.10	-0.03	-0.10	-0.12
	Females	0.09	-0.02	0.09	-0.06	-0.02	-0.15
	Males	0.08	-0.05	0.11	-0.03	-0.11	-0.03
Interpersonal Reactivity Index	Both sexes	0.14[1]	-0.01	0.11	0.03	0.04	0.08
	Females	0.08	-0.13	0.21*	0.09	0.11	0.13
	Males	0.19	0.18	0.02	0.01	-0.19	-0.05
- empathic concern	Both sexes	-0.13[1]	-0.08	0.11	-0.02	0.04	0.06
	Females	0.00	-0.22*	0.21*	0.09	0.06	0.18†
	Males	0.14	0.11	0.03	-0.09	-0.10	-0.15
- fantasy scale	Both sexes	0.10[1]	-0.02	0.10	0.03	0.04	0.02
	Females	0.12	-0.13	0.24*	0.05	0.09	0.05
	Males	0.06	0.12	-0.05	0.05	-0.09	-0.05
- personal distress	Both sexes	0.04[1]	-0.05	0.03	0.03	-0.04	-0.08
	Females	-0.14	-0.08	-0.05	0.06	0.04	-0.11
	Males	0.15	0.02	0.13	0.03	-0.22	-0.07
- perspective taking	Both sexes	0.12[1]	0.09	0.06	0.05	0.01	0.18*
	Females	0.24*	0.06	0.18†	0.05	0.03	0.19
	Males	0.09	0.17	-0.07	0.09	-0.08	0.14
Empathy Quotient	Both sexes	-0.09[1]	0.04	-0.09	0.10	0.02	0.09
	Females	0.00	-0.01	0.01	0.11	0.02	0.07
	Males	-0.15	0.13	-0.23*	0.12	-0.03	0.12
Systemizing Quotient	Both sexes	0.00[1]	0.00	0.09	-0.05	-0.13†	-0.07
	Females	0.11	-0.12	0.22*	-0.24*	-0.07	-0.12
	Males	-0.06	-0.02	-0.02	0.05	-0.07	0.08
SQ:EQ[2]	Both sexes	0.09[1]	-0.09	0.16*	-0.18	-0.07	-0.12
	Females	0.06	-0.07	0.12	-0.26*	-0.08	-0.16
	Males	0.11	-0.13	0.20	-0.08	-0.06	-0.06

[1] due to significant sex differences in testosterone levels, correlations between testosterone and psychological variables for the overall population were calculated in SPSS as partial correlations controlling for sex

[2] baseline T:OT and SQ:EQ balance were calculated using sex-specific means and SDs, due to significant sex differences in the hormone and questionnaire variables.

† $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, unadjusted for multiple comparisons.

Correlation analyses were also performed to test for relationships between hormonal and questionnaire variables and self-reported emotional response to the video (Tables 3.8 and 3.9). The Empathy Response and Distress Response composite scores showed high internal reliability (Cronbach's alpha of 0.842 and 0.759, respectively). Empathy Response showed no significant associations with hormone variables; however, Empathy Response was positively correlated with IRI and EQ scores, and negatively with AQ and SQ scores.

Distress Response was negatively correlated with pre- to post-video change in testosterone in the overall population, meaning that individuals reporting higher levels of the six distress emotions showed larger decreases in testosterone. Distress Response also trended toward a negative correlation with pre- to post-video change in oxytocin the overall population and in females only. Distress Response was positively correlated with IRI and the SPQ cognitive perceptual subscale, and trended toward a positive correlation with AQ.

Table 3.8 Correlations between emotional response to the video and hormone variables for the overall population, females only, and males only.

		Baseline T	Baseline OT	Baseline T:OT	T % Change	OT % Change (A – B)	OT % Change (A – C)
Empathy Response	Both sexes	-0.06[1]	-0.038	-0.054	-0.063	-0.021	-0.054
	Females	-0.10	-0.097	0.008	-0.037	-0.11	-0.13
	Males	-0.034	0.079	-0.10	-0.029	-0.063	-0.041
Distress Response	Both sexes	-0.05[1]	-0.13	0.054	-0.16*	-0.13†	-0.13†
	Females	-0.023	-0.11	0.077	-0.14	-0.18†	-0.20†
	Males	-0.09	-0.12	0.026	-0.17	-0.10	-0.038

† p < 0.10, * p < 0.05, ** p < 0.01, unadjusted for multiple comparisons.

[1] due to significant sex differences in testosterone levels, correlations between testosterone and psychological variables for the overall population were calculated in SPSS as partial correlations controlling for sex

Table 3.9 Correlations between emotional response to the video and questionnaire scores for the overall population, females only, and males only.

		AQ	SPQ-BR cog percep	IRI	EQ	SQ	SQ:EQ
Empathy Response	Both sexes	-0.17*	-0.26	0.50**	0.24*	-0.20*	-0.18*
	Females	-0.25*	-0.30	0.42**	0.36**	0.077	-0.23*
	Males	0.029	0.019	0.46**	0.019	-0.14	-0.15
Distress Response	Both sexes	0.14†	0.23*	0.26**	0.077	0.036	0.027
	Females	0.13	0.24*	0.20†	0.13	0.24*	0.090
	Males	0.21†	0.23*	0.28*	-0.058	-0.086	-0.070

† p < 0.10, * p < 0.05, ** p < 0.01, unadjusted for multiple comparisons.

3.4.6. Genetic associations

Analysis of variance (ANOVA) was used to test associations of genes with hormonal and psychological variables, and if these relationships were mediated by sex. Three genetic models were tested: a co-dominant model comparing the dependent variable between the three genotype groups (Genotype 1 vs. Genotype 2 vs. Genotype 3), a dominant model (Genotype 1 + Genotype 2 vs. Genotype 3), and a recessive model (e.g., Genotype 1 vs. Genotype 2 + Genotype 3). Frequencies for the five oxytocin receptor (OXTR) and one cluster of differentiation 38 (CD38) single nucleotide polymorphisms (SNPs) examined in this study are presented in Table 3.10.

Table 3.10 Genotype frequencies for five OXTR SNPS and one CD38 SNP in the study population

		GT1	GT2	GT3	GT1+GT2	GT2+GT3
OXTR rs53576	**Both sexes**	52	71	49	123	120
	Females	30	38	25	68	63
	Males	22	33	24	55	57
OXTR rs1042778	**Both sexes**	97	65	11	162	76
	Females	49	40	5	89	45
	Males	48	25	6	73	31
OXTR rs7632287	**Both sexes**	2	24	145	26	169
	Females	2	14	78	16	92
	Males	0	10	67	10	77
OXTR rs2254298	**Both sexes**	7	57	109	64	166
	Females	4	27	63	31	90
	Males	3	30	46	33	76
OXTR rs237887	**Both sexes**	47	78	48	125	126
	Females	29	44	21	73	65
	Males	18	34	27	52	61
CD38 rs3796863	**Both sexes**	70	79	24	149	103
	Females	45	32	17	77	49
	Males	25	47	7	72	54

GT = genotype
OXTR rs53576: GT1 = AA, GT2 = AG, GT3 = GG
OXTR rs1042778: GT1 = GG, GT2 = GT, GT3 = TT
OXTR rs7632287: GT1 = AA, GT2 = AG, GT3 = GG
OXTR rs2254298: GT1 = AA, GT2 = AG, GT3 = GG
OXTR rs237887: GT1 = AA, GT2 = AG, GT3 = GG
CD38 rs3796863: GT1 = GG, GT2 = GT, GT3 = TT

Tables 3.11 – 3.16 present the ANOVA results (F and p values) testing variation in hormonal variables between genotypes for OXTR rs53576, OXTR rs1042778, OXTR rs7632287, OXTR rs2254298, OXTR rs237887, and CD38 rs3796863. After accounting for variation in baseline testosterone due to sex, CD38 rs3796863 genotype was significantly associated with baseline testosterone under the dominant model, and significant sex by genotype interactions were present in the codominant and dominant models. Post-hoc analyses (Figure 3.3) found that males with the TT genotype had significantly higher testosterone levels than males with GG or GT genotypes (mean testosterone = 153.1 pg/ml for GG+GT, 216.6 pg/ml for TT; t = -3,22, p = 0.15); testosterone levels did not vary significantly between females with different genotypes (t = 0.32, p = 0.75).

Baseline oxytocin was not associated with any of six SNPs ($p > 0.16$, all models), nor were any significant sex by genotype interactions present in the data ($p > 0.06$, all models). Pre- to post-video percentage change in salivary testosterone showed a significant sex by genotype interaction under the codominant and dominant models for OXTR rs1042778 ($p < 0.02$, both tests): the mean percentage changes in testosterone for females with GG, GT, and TT genotypes were -1.7%, -8.7%, and -15.5%, respectively; the mean percentage changes in testosterone for males with GG, GT, and TT genotypes were -2.3%, -5.5%, and +14.2%, respectively. Percentage change in oxytocin, regardless of the timepoints used, was not associated with any of the genetic variables tested here.

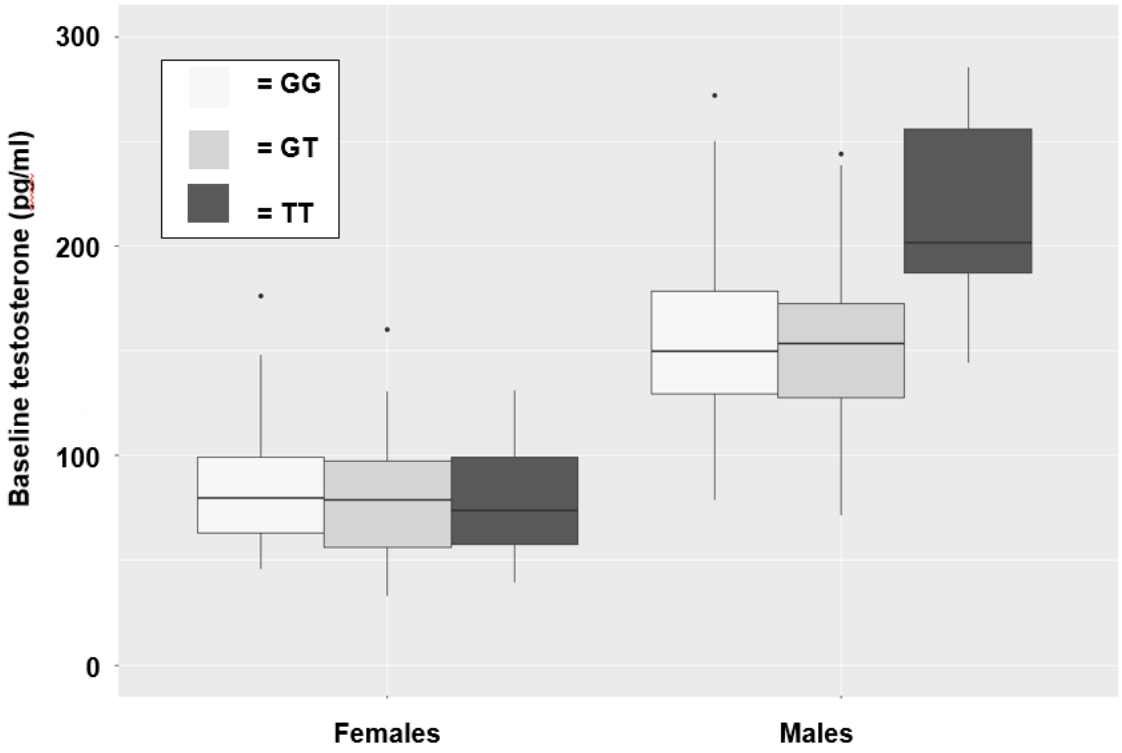

Figure 3.3 **Variation in baseline testosterone between CD38 SNP rs3796863 genotype groups for females and males.**

Table 3.11 ANOVA results testing variation in hormone variables between OXTR rs53576 genotypes.

		CODOM		DOM		REC	
		F	p	F	p	F	p
Baseline Testosterone	Sex	185.60	(0.00**)	186.39	(0.00**)	188.38	(0.00**)
	Genotype	0.31	(0.58)	0.21	(0.65)	1.92	(0.17)
	Sex*Genotype	0.00	(0.99)	0.80	(0.37)	0.89	(0.35)
Baseline Oxytocin	Sex	3.40	(0.07)	3.44	(0.07)	3.37	(0.07)
	Genotype	0.01	(0.93)	0.38	(0.54)	0.21	(0.65)
	Sex*Genotype	2.41	(0.12)	3.69	(0.06)	0.46	(0.50)
Baseline T:OT	Sex	0.01	(0.94)	0.01	(0.94)	0.01	(0.94)
	Genotype	0.49	(0.49)	0.20	(0.66)	2.54	(0.11)
	Sex*Genotype	2.13	(0.15)	5.51	(0.02*)	0.01	(0.91)
Testosterone % Change	Sex	1.52	(0.22)	1.51	(0.22)	1.53	(0.22)
	Genotype	0.30	(0.59)	0.08	(0.77)	0.40	(0.53)
	Sex*Genotype	0.37	(0.54)	0.05	(0.82)	1.59	(0.21)
OT % Change (A-B)	Sex	3.81	(0.05)	3.82	(0.05)	3.86	(0.05)
	Genotype	0.14	(0.71)	1.12	(0.29)	0.18	(0.68)
	Sex*Genotype	0.67	(0.41)	0.16	(0.69)	2.98	(0.09)
OT % Change (A-C)	Sex	0.97	(0.33)	0.97	(0.33)	0.97	(0.33)
	Genotype	1.92	(0.17)	2.59	(0.11)	0.52	(0.47)
	Sex*Genotype	0.90	(0.34)	0.13	(0.72)	1.43	(0.23)
OT % Change (A-BC Avg)	Sex	3.24	(0.07)	3.25	(0.07)	3.26	(0.07)
	Genotype	1.10	(0.30)	2.58	(0.11)	0.03	(0.87)
	Sex*Genotype	1.17	(0.28)	0.00	(0.97)	3.23	(0.07)

* $p < 0.05$, ** $p < 0.01$

Table 3.12 ANOVA results testing variation in hormone variables between OXTR rs1042778 genotypes.

		CODOM		DOM		REC	
		F	P	F	p	F	p
Baseline Testosterone	Sex	179.54	(0.00**)	178.54	(0.00**)	180.44	(0.00**)
	Genotype	0.05	(0.82)	0.01	(0.94)	0.06	(0.80)
	Sex*Genotype	0.94	(0.33)	0.04	(0.83)	1.78	(0.18)
Baseline Oxytocin	Sex	2.98	(0.09)	3.01	(0.08)	2.98	(0.09)
	Genotype	0.07	(0.79)	0.03	(0.86)	0.17	(0.68)
	Sex*Genotype	0.10	(0.76)	1.65	(0.20)	0.09	(0.77)
Baseline T:OT	Sex	0.00	(0.99)	0.00	(0.99)	0.00	(0.99)
	Genotype	0.08	(0.78)	0.00	(0.95)	0.14	(0.70)
	Sex*Genotype	1.21	(0.27)	0.85	(0.36)	0.77	(0.38)
Testosterone % Change	Sex	1.48	(0.23)	1.48	(0.23)	1.46	(0.23)
	Genotype	1.27	(0.26)	0.40	(0.53)	2.79	(0.10)
	Sex*Genotype	5.57	(0.02*)	6.87	(0.01*)	2.38	(0.13)
OT % Change (A-B)	Sex	3.74	(0.06)	3.74	(0.05)	3.73	(0.06)
	Genotype	0.43	(0.51)	0.28	(0.60)	0.31	(0.58)
	Sex*Genotype	0.01	(0.93)	0.37	(0.54)	0.04	(0.83)
OT % Change (A-C)	Sex	1.02	(0.31)	1.00	(0.32)	1.02	(0.31)
	Genotype	0.87	(0.35)	0.02	(0.90)	1.20	(0.27)
	Sex*Genotype	2.67	(0.10)	1.04	(0.31)	2.08	(0.15)
OT % Change (A-BC Avg)	Sex	3.22	(0.07)	3.21	(0.08)	3.22	(0.07)
	Genotype	0.02	(0.90)	0.07	(0.80)	0.08	(0.77)
	Sex*Genotype	0.79	(0.38)	0.05	(0.83)	0.94	(0.33)

* $p < 0.05$, ** $p < 0.01$

Table 3.13 ANOVA results testing variation in hormone variables between OXTR rs7632287 genotypes.

		CODOM		DOM		REC	
		F	P	F	p	F	p
Baseline Testosterone	Sex	188.99	(0.00**)	188.87	(0.00**)	189.52	(0.00**)
	Genotype	0.07	(0.80)	0.01	(0.90)	0.30	(0.58)
	Sex*Genotype	0.77	(0.38)	0.71	(0.40)	n.a.	n.a.
Baseline Oxytocin	Sex	2.40	(0.12)	2.39	(0.12)	2.41	(0.12)
	Genotype	2.03	(0.16)	1.47	(0.23)	1.55	(0.21)
	Sex*Genotype	0.13	(0.71)	0.12	(0.73)	n.a.	n.a.
Baseline T:OT	Sex	0.13	(0.72)	0.13	(0.72)	0.13	(0.72)
	Genotype	0.64	(0.42)	0.47	(0.49)	0.48	(0.49)
	Sex*Genotype	0.05	(0.82)	0.07	(0.80)	n.a.	n.a.
Testosterone % Change	Sex	0.95	(0.33)	0.95	(0.33)	0.95	(0.33)
	Genotype	0.09	(0.77)	0.04	(0.83)	0.16	(0.69)
	Sex*Genotype	0.20	(0.66)	0.18	(0.67)	n.a.	n.a.
OT % Change (A-B)	Sex	3.94	(0.05)	3.94	(0.05)	3.90	(0.05)
	Genotype	0.46	(0.50)	0.36	(0.55)	0.27	(0.60)
	Sex*Genotype	2.15	(0.14)	2.38	(0.12)	n.a.	n.a.
OT % Change (A-C)	Sex	1.01	(0.32)	1.00	(0.32)	1.01	(0.32)
	Genotype	2.01	(0.16)	1.57	(0.21)	1.17	(0.28)
	Sex*Genotype	0.28	(0.59)	0.31	(0.58)	n.a.	n.a.
OT % Change (A-BC Avg)	Sex	3.37	(0.07)	3.36	(0.07)	3.34	(0.07)
	Genotype	1.59	(0.21)	1.24	(0.27)	0.92	(0.34)
	Sex*Genotype	1.53	(0.22)	1.68	(0.20)	n.a.	n.a.

* $p < 0.05$, ** $p < 0.01$

n.a. = analysis not performed due to lack of GT1 males

Table 3.14 ANOVA results testing variation in hormone variables between OXTR rs2254298 genotypes.

		CODOM		DOM		REC	
		F	P	F	p	F	p
Baseline Testosterone	Sex	182.38	(0.00**)	182.55	(0.00**)	179.36	(0.00**)
	Genotype	3.66	(0.06)	3.82	(0.05)	0.53	(0.47)
	Sex*Genotype	0.01	(0.92)	0.00	(0.96)	0.30	(0.59)
Baseline Oxytocin	Sex	3.00	(0.09)	2.99	(0.09)	3.00	(0.09)
	Genotype	0.65	(0.42)	0.39	(0.53)	0.61	(0.43)
	Sex*Genotype	0.70	(0.40)	0.44	(0.51)	0.62	(0.43)
Baseline T:OT	Sex	0.00	(0.99)	0.00	(0.99)	0.00	(0.99)
	Genotype	0.83	(0.36)	1.19	(0.28)	0.00	(0.98)
	Sex*Genotype	0.13	(0.72)	0.10	(0.75)	0.02	(0.88)
Testosterone % Change	Sex	1.44	(0.23)	1.44	(0.23)	1.43	(0.23)
	Genotype	0.00	(0.98)	0.16	(0.69)	0.77	(0.38)
	Sex*Genotype	2.58	(0.11)	2.68	(0.10)	0.28	(0.60)
OT % Change (A-B)	Sex	3.74	(0.05)	3.73	(0.06)	3.75	(0.05)
	Genotype	0.02	(0.90)	0.04	(0.84)	0.74	(0.39)
	Sex*Genotype	0.53	(0.47)	0.33	(0.56)	0.55	(0.46)
OT % Change (A-C)	Sex	1.01	(0.32)	1.01	(0.32)	1.00	(0.32)
	Genotype	1.15	(0.29)	0.91	(0.34)	0.58	(0.45)
	Sex*Genotype	0.80	(0.37)	1.55	(0.21)	0.17	(0.68)
OT % Change (A-BC Avg)	Sex	3.21	(0.07)	3.21	(0.07)	3.23	(0.07)
	Genotype	0.50	(0.48)	0.18	(0.67)	0.97	(0.33)
	Sex*Genotype	0.00	(0.95)	0.13	(0.72)	0.50	(0.48)

* $p < 0.05$, ** $p < 0.01$

Table 3.15 ANOVA results testing variation in hormone variables between OXTR rs237887 genotypes.

		CODOM		DOM		REC	
		F	p	F	p	F	p
Baseline Testosterone	Sex	181.66	(0.00**)	183.74	(0.00**)	180.15	(0.00**)
	Genotype	0.02	(0.90)	1.46	(0.23)	0.99	(0.32)
	Sex*Genotype	2.97	(0.09)	3.49	(0.06)	0.58	(0.45)
Baseline Oxytocin	Sex	2.99	(0.09)	3.01	(0.08)	2.99	(0.09)
	Genotype	0.25	(0.62)	0.00	(1.00)	0.68	(0.41)
	Sex*Genotype	0.63	(0.43)	2.11	(0.15)	0.05	(0.83)
Baseline T:OT	Sex	0.00	(0.99)	0.00	(0.99)	0.00	(0.99)
	Genotype	0.07	(0.79)	0.81	(0.37)	0.21	(0.65)
	Sex*Genotype	0.56	(0.45)	0.02	(0.89)	0.97	(0.33)
Testosterone % Change	Sex	1.43	(0.23)	1.43	(0.23)	1.44	(0.23)
	Genotype	1.00	(0.32)	0.22	(0.64)	1.42	(0.23)
	Sex*Genotype	0.76	(0.38)	0.57	(0.45)	0.64	(0.42)
OT % Change (A-B)	Sex	3.73	(0.06)	3.74	(0.05)	3.74	(0.05)
	Genotype	0.31	(0.58)	0.45	(0.50)	0.06	(0.81)
	Sex*Genotype	0.09	(0.76)	0.04	(0.84)	0.44	(0.51)
OT % Change (A-C)	Sex	1.00	(0.32)	1.00	(0.32)	1.01	(0.32)
	Genotype	0.06	(0.80)	1.13	(0.29)	2.18	(0.14)
	Sex*Genotype	0.01	(0.92)	0.01	(0.90)	0.00	(0.97)
OT % Change (A-BC Avg)	Sex	3.21	(0.08)	3.21	(0.08)	3.23	(0.07)
	Genotype	0.24	(0.62)	0.04	(0.84)	1.03	(0.31)
	Sex*Genotype	0.02	(0.90)	0.00	(0.95)	0.16	(0.69)

* $p < 0.05$, ** $p < 0.01$

Table 3.16 ANOVA results testing variation in hormone variables between CD38 rs3796863 genotypes.

		CODOM		DOM		REC	
		F	p	F	p	F	p
Baseline Testosterone	Sex	186.29	(0.00**)	200.58	(0.00**)	179.32	(0.00**)
	Genotype	1.38	(0.24)	5.47	(0.02*)	0.00	(0.95)
	Sex*Genotype	5.97	(0.02*)	15.33	(0.00**)	0.78	(0.38)
Baseline Oxytocin	Sex	2.99	(0.09)	3.01	(0.08)	2.98	(0.09)
	Genotype	0.76	(0.38)	1.98	(0.16)	0.06	(0.80)
	Sex*Genotype	0.00	(0.97)	0.03	(0.86)	0.01	(0.92)
Baseline T:OT	Sex	0.00	(0.99)	0.00	(0.98)	0.00	(0.99)
	Genotype	0.03	(0.86)	0.01	(0.94)	0.10	(0.76)
	Sex*Genotype	3.74	(0.05)	10.06	(0.00**)	0.45	(0.51)
Testosterone % Change	Sex	1.45	(0.23)	1.43	(0.23)	1.44	(0.23)
	Genotype	1.73	(0.19)	0.58	(0.45)	1.75	(0.19)
	Sex*Genotype	1.40	(0.24)	0.61	(0.44)	0.82	(0.37)
OT % Change (A-B)	Sex	3.73	(0.06)	3.73	(0.06)	3.73	(0.06)
	Genotype	0.30	(0.58)	0.25	(0.61)	0.18	(0.67)
	Sex*Genotype	0.00	(0.97)	0.06	(0.80)	0.02	(0.88)
OT % Change (A-C)	Sex	1.01	(0.32)	1.01	(0.32)	1.01	(0.32)
	Genotype	2.12	(0.15)	1.12	(0.29)	1.72	(0.19)
	Sex*Genotype	0.22	(0.64)	0.45	(0.50)	0.13	(0.71)
OT % Change (A-BC Avg)	Sex	3.23	(0.07)	3.22	(0.07)	3.23	(0.07)
	Genotype	1.43	(0.23)	0.87	(0.35)	1.07	(0.30)
	Sex*Genotype	0.06	(0.80)	0.06	(0.81)	0.10	(0.76)

* $p < 0.05$, ** $p < 0.01$

Tables 3.17 – 3.22 present the ANOVA results (F and p values) testing variation in psychological variables between genotypes for OXTR rs53576, OXTR rs1042778, OXTR rs7632287, OXTR rs2254298, OXTR rs237887, and CD38 rs3796863. As significant sex differences were present in questionnaire scores, sex has again been included as an independent variable.

OXTR rs53576 showed significant associations with the SPQ-BR cognitive perceptual subscale, IRI, and IRI empathic concern subscale. Post-hoc analyses showed that males, but not females, with AA+AG genotypes had higher SPQ-BR cognitive perceptual scores (females: 36.6 AA+AG, 38.6 GG, $p > 0.20$; males: 39.9 AA+AG, 34.7 GG, $p < 0.05$). IRI scores also showed a sex by genotype effect under the codominant and dominant models, with mean IRI scores of 69.1, 72.7, and 76.5 for females with AA, AG, and GG genotypes, respectively, and mean IRI scores of 65.9, 67.9, and 59.6 for males with AA, AG, and GG genotypes, respectively. IRI empathic concern scores also differed significantly ($p = 0.01$) under the recessive model, with a mean score of 18.1 for AA+AG and 20.0 for GG.

After accounting for sex differences, SPQ-BR and SPQ-BR cognitive perceptual scores differed significantly between OXTR rs1042778 genotype groups under the dominant model ($p < 0.05$, both tests). Mean SPQ-BR and SPQ-BR cognitive perceptual scores were 88.7 and 37.4 for the GG+GT group, respectively, and 99.9 and 43.6 for the TT group. Autism spectrum quotient scores showed a significant sex by genotype interaction under the codominant and dominant models for OXTR rs2254298 ($p < 0.05$, both tests): the mean AQ scores for females with AA, AG, and GG genotypes were 19.3, 18.3, and 16.7, respectively; the mean AQ scores for males with AA, AG, and GG genotypes were 17.3, 18.6, and 20.4, respectively.

CD38 rs3796863 was significantly associated with Systemizing Quotient and Systemizing relative to Empathizing (SQ:EQ) in males, but not females, under codominant and dominant models. Mean SQ scores were 24.9, 23.2, and 13.3 for males with GG, GT, and TT genotypes, respectively, and 13.9, 14.9, and 16.0 for females with GG, GT, and TT genotypes, respectively. This pattern persisted in SQ:EQ scores, with TT males, but not females, showing lower Systemizing relative to Empathizing.

Table 3.17 ANOVA results testing variation in psychological variables between OXTR rs53576 genotypes.

		CODOM		DOM		REC	
		F	p	F	p	F	p
Autism Spectrum Quotient	Sex	7.49	(0.01*)	7.45	(0.01*)	7.54	(0.01*)
	Genotype	0.84	(0.36)	0.05	(0.83)	1.71	(0.19)
	Sex*Genotype	0.14	(0.71)	0.00	(0.99)	0.36	(0.55)
Schizotypal Personality	Sex	8.69	(0.00**)	8.88	(0.00**)	8.60	(0.00**)
	Genotype	1.45	(0.23)	2.28	(0.13)	0.28	(0.60)
	Sex*Genotype	0.71	(0.40)	3.29	(0.07)	0.15	(0.70)
SPQ-BR cog percep	Sex	0.83	(0.36)	0.86	(0.35)	0.82	(0.37)
	Genotype	0.42	(0.52)	0.77	(0.38)	0.05	(0.82)
	Sex*Genotype	1.91	(0.17)	7.22	(0.01*)	0.10	(0.76)
Interpersonal Reactivity Index	Sex	15.22	(0.00**)	15.36	(0.00**)	14.86	(0.00**)
	Genotype	0.23	(0.63)	0.10	(0.76)	1.20	(0.27)
	Sex*Genotype	7.07	(0.01*)	8.75	(0.00**)	2.09	(0.15)
IRI: Empathic Concern	Sex	19.07	(0.00**)	18.64	(0.00**)	19.29	(0.00**)
	Genotype	3.16	(0.08)	0.21	(0.65)	6.29	(0.01*)
	Sex*Genotype	3.02	(0.08)	2.11	(0.15)	1.93	(0.17)
Empathy Quotient	Sex	3.73	(0.06)	3.70	(0.06)	3.73	(0.06)
	Genotype	1.65	(0.20)	0.71	(0.40)	1.69	(0.20)
	Sex*Genotype	0.62	(0.43)	0.24	(0.63)	0.69	(0.41)
Systemizing Quotient	Sex	39.75	(0.00**)	39.68	(0.00**)	39.58	(0.00**)
	Genotype	0.01	(0.90)	0.05	(0.82)	0.00	(0.98)
	Sex*Genotype	1.83	(0.18)	1.50	(0.22)	1.11	(0.29)
SQ:EQ	Sex	0.02	(0.88)	0.02	(0.88)	0.02	(0.88)
	Genotype	1.13	(0.29)	0.41	(0.52)	1.26	(0.26)
	Sex*Genotype	0.03	(0.86)	0.14	(0.71)	0.01	(0.93)

* $p < 0.05$, ** $p < 0.01$

Table 3.18 ANOVA results testing variation in psychological variables between OXTR rs1042778 genotypes.

		CODOM		DOM		REC	
		F	p	F	p	F	p
Autism Spectrum Quotient	Sex	7.21	(0.01*)	7.25	(0.01*)	7.21	(0.01*)
	Genotype	0.04	(0.84)	1.39	(0.24)	0.68	(0.41)
	Sex*Genotype	0.91	(0.34)	0.56	(0.46)	0.40	(0.53)
Schizotypal Personality	Sex	7.87	(0.01*)	8.20	(0.00**)	7.84	(0.01*)
	Genotype	0.24	(0.62)	4.79	(0.03*)	0.22	(0.64)
	Sex*Genotype	0.55	(0.46)	2.72	(0.10)	0.00	(0.99)
SPQ-BR cog percep	Sex	0.79	(0.37)	0.82	(0.37)	0.79	(0.38)
	Genotype	0.53	(0.47)	5.79	(0.02*)	0.08	(0.78)
	Sex*Genotype	0.26	(0.61)	0.11	(0.74)	0.11	(0.74)
Interpersonal Reactivity Index	Sex	15.45	(0.00**)	15.54	(0.00**)	15.47	(0.00**)
	Genotype	0.00	(0.95)	0.68	(0.41)	0.11	(0.74)
	Sex*Genotype	0.01	(0.92)	0.29	(0.59)	0.10	(0.75)
IRI: Empathic Concern	Sex	19.09	(0.00**)	19.01	(0.00**)	19.15	(0.00**)
	Genotype	0.45	(0.50)	0.00	(0.96)	0.74	(0.39)
	Sex*Genotype	0.29	(0.59)	0.01	(0.94)	0.51	(0.48)
Empathy Quotient	Sex	3.72	(0.06)	3.69	(0.06)	3.73	(0.06)
	Genotype	0.07	(0.80)	0.01	(0.91)	0.07	(0.80)
	Sex*Genotype	1.07	(0.30)	0.02	(0.88)	1.46	(0.23)
Systemizing Quotient	Sex	41.31	(0.00**)	42.35	(0.00**)	41.06	(0.00**)
	Genotype	3.37	(0.07)	3.70	(0.06)	1.81	(0.18)
	Sex*Genotype	0.02	(0.89)	3.91	(0.05)	0.56	(0.46)
SQ:EQ	Sex	0.05	(0.83)	0.05	(0.83)	0.05	(0.83)
	Genotype	1.83	(0.18)	1.90	(0.17)	1.01	(0.32)
	Sex*Genotype	0.75	(0.39)	1.88	(0.17)	0.17	(0.68)

* $p < 0.05$, ** $p < 0.01$

Table 3.19 ANOVA results testing variation in psychological variables between OXTR rs7632287 genotypes.

		CODOM		DOM		REC	
		F	p	F	p	F	p
Autism Spectrum Quotient	Sex	7.18	(0.01*)	7.16	(0.01*)	7.20	(0.01*)
	Genotype	0.04	(0.84)	0.02	(0.89)	1.46	(0.23)
	Sex*Genotype	2.05	(0.15)	1.59	(0.21)	n.a.	n.a.
Schizotypal Personality	Sex	7.58	(0.01*)	7.55	(0.01*)	7.71	(0.01*)
	Genotype	0.56	(0.45)	0.11	(0.75)	2.84	(0.09)
	Sex*Genotype	0.65	(0.42)	0.34	(0.56)	n.a.	n.a.
SPQ-BR cog percep	Sex	0.83	(0.36)	0.82	(0.37)	0.86	(0.36)
	Genotype	0.96	(0.33)	0.11	(0.75)	6.54	(0.01*)
	Sex*Genotype	1.72	(0.19)	0.92	(0.34)	n.a.	n.a.
Interpersonal Reactivity Index	Sex	15.00	(0.00**)	14.91	(0.00**)	15.03	(0.00**)
	Genotype	2.38	(0.12)	1.55	(0.22)	2.48	(0.12)
	Sex*Genotype	0.71	(0.40)	0.61	(0.44)	n.a.	n.a.
IRI: Empathic Concern	Sex	17.97	(0.00**)	17.88	(0.00**)	17.82	(0.00**)
	Genotype	3.83	(0.05)	2.88	(0.09)	2.51	(0.11)
	Sex*Genotype	1.12	(0.29)	1.16	(0.28)	n.a.	n.a.
Empathy Quotient	Sex	3.92	(0.05)	3.91	(0.05)	3.95	(0.05)
	Genotype	0.31	(0.58)	0.07	(0.79)	1.39	(0.24)
	Sex*Genotype	0.97	(0.33)	0.74	(0.39)	n.a.	n.a.
Systemizing Quotient	Sex	43.78	(0.00**)	43.85	(0.00**)	43.29	(0.00**)
	Genotype	1.65	(0.20)	2.40	(0.12)	0.14	(0.71)
	Sex*Genotype	1.31	(0.25)	0.81	(0.37)	n.a.	n.a.
SQ:EQ	Sex	0.21	(0.65)	0.21	(0.65)	0.20	(0.65)
	Genotype	0.75	(0.39)	1.81	(0.18)	1.52	(0.22)
	Sex*Genotype	3.07	(0.08)	2.00	(0.16)	n.a.	n.a.

* $p < 0.05$, ** $p < 0.01$

n.a. = analysis not performed due to lack of GT1 males

Table 3.20 ANOVA results testing variation in psychological variables between OXTR rs2254298 genotypes.

		CODOM		DOM		REC	
		F	p	F	p	F	p
Autism Spectrum Quotient	Sex	7.36	(0.01*)	7.35	(0.01*)	7.21	(0.01*)
	Genotype	0.00	(0.97)	0.01	(0.94)	0.01	(0.94)
	Sex*Genotype	4.33	(0.04*)	4.14	(0.04*)	1.03	(0.31)
Schizotypal Personality	Sex	7.91	(0.01*)	7.86	(0.01**)	7.95	(0.01*)
	Genotype	0.06	(0.80)	0.01	(0.93)	0.24	(0.62)
	Sex*Genotype	1.47	(0.23)	0.70	(0.41)	2.17	(0.14)
SPQ-BR cog percep	Sex	0.80	(0.37)	0.80	(0.37)	0.79	(0.38)
	Genotype	0.78	(0.38)	0.73	(0.39)	0.21	(0.65)
	Sex*Genotype	0.55	(0.46)	0.53	(0.47)	0.17	(0.68)
Interpersonal Reactivity Index	Sex	15.60	(0.00**)	15.56	(0.00**)	15.63	(0.00**)
	Genotype	0.01	(0.91)	0.23	(0.63)	0.81	(0.37)
	Sex*Genotype	1.58	(0.21)	0.92	(0.34)	1.09	(0.30)
IRI: Empathic Concern	Sex	19.33	(0.00**)	19.28	(0.00**)	19.31	(0.00**)
	Genotype	0.28	(0.60)	0.90	(0.35)	0.61	(0.44)
	Sex*Genotype	2.57	(0.11)	1.53	(0.22)	2.10	(0.15)
Empathy Quotient	Sex	3.73	(0.06)	3.71	(0.06)	3.75	(0.05)
	Genotype	1.41	(0.24)	0.57	(0.45)	2.63	(0.11)
	Sex*Genotype	0.04	(0.84)	0.09	(0.76)	0.00	(0.98)
Systemizing Quotient	Sex	40.84	(0.00**)	40.80	(0.00**)	40.93	(0.00**)
	Genotype	0.74	(0.39)	0.21	(0.65)	1.83	(0.18)
	Sex*Genotype	0.74	(0.39)	1.11	(0.29)	0.02	(0.89)
SQ:EQ	Sex	0.05	(0.83)	0.05	(0.83)	0.04	(0.83)
	Genotype	0.00	(0.94)	0.00	(0.96)	0.01	(0.94)
	Sex*Genotype	0.96	(0.33)	1.41	(0.24)	0.02	(0.90)

* $p < 0.05$, ** $p < 0.01$

Table 3.21 ANOVA results testing variation in psychological variables between OXTR rs237887 genotypes

		CODOM		DOM		REC	
		F	p	F	p	F	p
Autism Spectrum Quotient	Sex	7.19	(0.01*)	7.20	(0.01*)	7.22	(0.01*)
	Genotype	0.40	(0.53)	0.10	(0.75)	0.53	(0.47)
	Sex*Genotype	0.01	(0.92)	0.69	(0.41)	0.57	(0.45)
Schizotypal Personality	Sex	7.89	(0.01*)	7.95	(0.01*)	7.83	(0.01*)
	Genotype	0.00	(0.95)	0.02	(0.89)	0.00	(0.97)
	Sex*Genotype	1.25	(0.27)	2.33	(0.13)	0.10	(0.75)
SPQ-BR cog percep	Sex	0.79	(0.37)	0.79	(0.37)	0.79	(0.38)
	Genotype	0.01	(0.93)	0.07	(0.79)	0.01	(0.91)
	Sex*Genotype	0.71	(0.40)	0.52	(0.47)	0.41	(0.52)
Interpersonal Reactivity Index	Sex	15.47	(0.00**)	15.49	(0.00**)	15.47	(0.00**)
	Genotype	0.00	(0.97)	0.06	(0.80)	0.10	(0.75)
	Sex*Genotype	0.26	(0.61)	0.33	(0.56)	0.11	(0.74)
IRI: Empathic Concern	Sex	19.09	(0.00**)	19.15	(0.00**)	19.06	(0.00**)
	Genotype	0.45	(0.50)	1.29	(0.26)	0.00	(0.99)
	Sex*Genotype	0.29	(0.59)	0.01	(0.92)	0.46	(0.50)
Empathy Quotient	Sex	3.71	(0.06)	3.71	(0.06)	3.71	(0.06)
	Genotype	0.43	(0.51)	0.14	(0.71)	0.50	(0.48)
	Sex*Genotype	0.21	(0.64)	0.38	(0.54)	0.04	(0.84)
Systemizing Quotient	Sex	40.60	(0.00**)	40.74	(0.00**)	40.80	(0.00**)
	Genotype	0.51	(0.47)	0.42	(0.52)	0.29	(0.59)
	Sex*Genotype	0.01	(0.91)	0.65	(0.42)	1.04	(0.31)
SQ:EQ	Sex	0.04	(0.83)	0.05	(0.83)	0.04	(0.83)
	Genotype	0.14	(0.71)	0.01	(0.92)	0.26	(0.61)
	Sex*Genotype	0.26	(0.61)	2.60	(0.11)	0.50	(0.48)

* $p < 0.05$, ** $p < 0.01$

Table 3.22 ANOVA results testing variation in psychological variables between CD38 rs3796863 genotypes.

		CODOM		DOM		REC	
		F	p	F	p	F	p
Autism Spectrum Quotient	Sex	7.23	(0.01*)	7.18	(0.01*)	7.26	(0.01*)
	Genotype	0.02	(0.90)	0.25	(0.62)	0.30	(0.59)
	Sex*Genotype	1.50	(0.22)	0.10	(0.75)	1.86	(0.17)
Schizotypal Personality	Sex	7.93	(0.01*)	7.94	(0.01*)	7.88	(0.01*)
	Genotype	0.51	(0.48)	0.63	(0.43)	0.20	(0.65)
	Sex*Genotype	1.48	(0.23)	1.51	(0.22)	0.78	(0.38)
SPQ-BR cog percep	Sex	0.79	(0.38)	0.79	(0.37)	0.79	(0.38)
	Genotype	0.32	(0.57)	0.08	(0.78)	0.36	(0.55)
	Sex*Genotype	0.05	(0.82)	1.10	(0.30)	0.08	(0.78)
Interpersonal Reactivity Index	Sex	15.80	(0.00**)	15.55	(0.00**)	15.88	(0.00**)
	Genotype	1.62	(0.21)	0.66	(0.42)	1.52	(0.22)
	Sex*Genotype	2.11	(0.15)	0.41	(0.52)	2.97	(0.09)
IRI: Empathic Concern	Sex	19.39	(0.00**)	19.27	(0.00**)	19.35	(0.00**)
	Genotype	2.99	(0.09)	2.37	(0.13)	1.86	(0.17)
	Sex*Genotype	0.43	(0.51)	0.00	(0.95)	1.21	(0.27)
Empathy Quotient	Sex	3.70	(0.06)	3.71	(0.06)	3.70	(0.06)
	Genotype	0.42	(0.52)	0.50	(0.48)	0.17	(0.68)
	Sex*Genotype	0.01	(0.94)	0.01	(0.93)	0.01	(0.90)
Systemizing Quotient	Sex	42.20	(0.00**)	42.83	(0.00**)	41.10	(0.00**)
	Genotype	0.50	(0.48)	0.96	(0.33)	0.10	(0.75)
	Sex*Genotype	6.52	(0.01*)	8.60	(0.00**)	2.44	(0.12)
SQ:EQ	Sex	0.05	(0.83)	0.05	(0.83)	0.05	(0.83)
	Genotype	0.73	(0.39)	1.17	(0.28)	0.19	(0.66)
	Sex*Genotype	5.95	(0.02*)	5.66	(0.02*)	2.93	(0.09)

* $p < 0.05$, ** $p < 0.01$

Lastly, to test combined genotype effects on hormonal and questionnaire variables, genetic indices were created by summing the number of OXTR and CD38 alleles or genotypes previously associated with variation in oxytocin levels and/or psychological traits. A broadly “Prosocial” phenotype index was created by summing alleles linked to greater empathy, generosity, and pair-bonding, and protective of autism risk [22,53,69–71]: “G” for rs53576; “G” for rs1042778; “G” for rs7632287; “G” for rs2254298; “G” for rs237887; “T” for rs3796863). A “High Oxytocin” index was created by summing the alleles associated with higher plasma oxytocin in a study by Feldman et al. [22]: “G” for rs1042778, “A” for rs2254298, and “T” for rs3796863. Consistent with previous work [72] employing such genetic indices, both an additive model (summing alleles) and a dominant model (summing genotypes) were tested. Under the dominant models, the “Prosocial” genotypes were AA/AG for rs53576, GG/GT for rs1042778, GG for rs7632287, GG for rs2254298, and GT/TT for rs3796863; the “High Oxytocin” genotypes were GG/GT for rs1042778, AA/AG for rs2254298, and GT/TT for rs3796863.

The results of correlation analyses between the genetic indices and questionnaire scores are presented in Table 3.23, with adjustments for sex differences in questionnaire scores. There was a trend ($p < 0.10$) toward a positive correlation between the Interpersonal Reactivity Index-Personal Distress subscale (IRI:PD) and the additive models of the Prosocial and High Oxytocin genetic indices. Systemizing and Systemizing relative to Empathizing were negatively correlated with both models of the Prosocial index ($p < 0.05$), meaning that individuals with a greater number of prosocial OXTR and CD38 alleles or genotypes self-reported greater empathizing relative to systemizing. Table 3.23 presents the correlations between the genetic indices and the hormonal variables. While there was a weak positive relationship between baseline salivary oxytocin and the Prosocial genetic index ($r = 0.11$), this relationship—as well as all of the other relationships tested—did not approach the level of statistical significance.

Table 3.23 Correlations between "Prosocial"and "High Oxytocin" OXTR and CD38 genetic indices and questionnaire scores.

		AQ	SPQ-BR cog percep	SPQ-BR	IRI: EC	IRI: PD	IRI	EQ	SQ	SQ:EQ
Prosocial Index - Additive	r	0.01	-0.03	-0.02	0.05	0.15	0.03	0.09	-0.19	-0.23
	p	(0.87)	(0.73)	(0.84)	(0.51)	(0.07)	(0.68)	(0.30)	(0.02*)	(0.01*)
Prosocial Index - Dominant	r	-0.02	-0.04	-0.03	0.08	0.10	0.05	0.05	-0.21	-0.22
	p	(0.80)	(0.64)	(0.75)	(0.32)	(0.25)	(0.54)	(0.55)	(0.01*)	(0.01*)
Oxytocin Index - Additive	r	-0.01	0.05	0.02	0.02	0.15	0.03	0.06	-0.08	-0.12
	p	(0.95)	(0.53)	(0.77)	(0.79)	(0.07)	(0.71)	(0.44)	(0.31)	(0.15)
Oxytocin Index - Dominant	r	-0.03	0.04	-0.01	0.02	0.11	0.01	0.03	-0.06	-0.08
	p	(0.71)	(0.61)	(0.92)	(0.86)	(0.17)	(0.93)	(0.72)	(0.44)	(0.36)

* $p < 0.05$, ** $p < 0.01$

due to significant sex differences in questionnaire scores, correlations were calculated in SPSS as partial correlations controlling for sex

Table 3.24 Correlations between "Prosocial" and "High Oxytocin" OXTR and CD38 genetic indices and hormone variables.

		Baseline T[1]	Baseline OT	T:OT	T % Change	OT & Change (A-B)	OT & Change (A-C)	OT & Change (A-BC Avg)
Prosocial Index - Additive	R	0.12	0.11	0.01	-0.04	-0.06	0.03	-0.02
	P	(0.12)	(0.14)	(0.93)	(0.57)	(0.45)	(0.67)	(0.83)
Prosocial Index - Dominant	R	0.14	0.08	0.07	-0.08	-0.09	0.02	-0.04
	p	(0.07)	(0.31)	(0.38)	(0.28)	(0.27)	(0.76)	(0.62)
High Oxytocin Index - Additive	r	-0.03	0.03	-0.05	0.00	-0.07	-0.07	-0.08
	p	(0.67)	(0.70)	(0.50)	(0.95)	(0.40)	(0.35)	(0.28)
High Oxytocin Index - Dominant	r	-0.11	-0.01	-0.07	-0.05	-0.04	-0.12	-0.10
	p	(0.14)	(0.95)	(0.39)	(0.56)	(0.58)	(0.13)	(0.22)

* $p < 0.05$, ** $p < 0.01$

[1] due to significant sex difference in testosterone levels, correlations between testosterone and psychological variables for the overall population were calculated in SPSS azs partial correlations controlling for sex

3.5. Discussion

The current study investigated if and how endogenous levels of oxytocin and testosterone jointly changed in response to an empathy-inducing video. Additionally, we tested if hormone variables (baseline hormone levels, empathy-induced changes in hormone levels) were associated with psychological variables or genetic variables, as predicted by previous studies.

The results showed that, on average, viewing the empathetic video triggered a 15% increase in salivary oxytocin and a 4% decrease in salivary testosterone. The result regarding oxytocin replicated the work of Barraza & Zak [39], who reported a 47% increase in plasma oxytocin in individuals who watched the same empathetic video. The observed changes are consistent with our predictions from the hormone administration literature, which reported that oxytocin and testosterone increase and decrease empathy, respectively [reviewed in 73]. As the subject of the video was a child—one who may be perceived as particularly vulnerable due to illness—the results of this study can also be interpreted as consistent with the literature on hormones and parental care, which supports positive associations between parenting and oxytocin and negative associations between parenting and testosterone. The similarities in the roles of oxytocin and testosterone across administration studies and our naturalistic empathy-induction experiment highlight the interactive relationship between hormones and sociality, where changing hormone levels may cause changes in social behaviour or occur as a response to social stimuli.

We also demonstrated that the increase in oxytocin and decrease in testosterone tended to occur together, and used circular statistical techniques in a novel way to demonstrate the presence of underlying directionality in our dataset on simultaneous changes in two hormones. The finding of a paired change supports Crespi's [35] diametric effects hypothesis of the roles of these hormones in human social cognition, which states that oxytocin promotes social cognition, testosterone promotes anti-social or asocial cognition, and increases in both hormones should thus tend be incompatible. Despite these overall patterns, 61% of participants in the current study showed paired hormone level changes that were not in the predicted directions (see chi square test, Table 3.4). To the extent that these results represent real hormone changes, rather than sampling error and random hormonal variation across multiple saliva samples, the sources of

variation in hormonal response remain unclear. The psychological and genetic factors analyzed in this study did not explain a significant amount of the variation in hormonal response. Factors mediating oxytocin and testosterone responses that were not captured in this study may be psychological, physiological, genetic, or interactions between genes and environment.

No strong relationships between hormonal variables and individual questionnaire scores were observed in our data, including the predicted positive correlations of oxytocin with IRI, EQ, and SPQ-BR, and testosterone with AQ and SQ. The lack of significant findings suggests, in principle, that socio-cognitive traits have a complex biological basis, with hormonal variables alone unable to account for a meaningful amount of the variation. Of the correlations that did achieve nominal significance in the overall population, the positive correlation ($r = 0.18$, $p < 0.05$) between oxytocin increase and the perspective taking subscale of the IRI is consistent with the literature showing that oxytocin enhances theory-of-mind ability [6]. A nominal positive correlation ($r = 0.16$, $p < 0.05$) was observed between SQ:EQ and T:OT, such that individuals with higher Systemizing relative to Empathizing tended to have higher baseline testosterone relative to oxytocin, when questionnaire scores and hormone levels were normalized to adjust for sex differences in these variables. By contrast, baseline hormone levels considered separately were not significantly associated with Empathy Quotient, Systemizing Quotient, or SQ:EQ for the overall population. Lastly, the Distress Response of the Post-Video Emotion Rating questionnaire showed a significant negative correlation with pre- to post-video testosterone change, meaning that individuals who rated the video as more distressing showed greater decreases in testosterone. This result is consistent with the finding from Kuo et al. [74] that males' testosterone levels decreased significantly when watching their own infant in a challenging situation, though testosterone response to stress is known to show high variability among males and females [74,75].

Regarding the results of the genetic analyses, the variants in the OXTR and CD38 genes previously associated with plasma oxytocin levels (i.e., rs2254298 and rs1042778 in OXTR, and rs3796863 in CD38 [22]) were not associated with salivary oxytocin in the current study individually or as part of the "High Oxytocin" genetic index. SNP rs3796863 was, however, significantly associated with baseline testosterone among males. To the best of our knowledge, this SNP has not been previously associated with testosterone in

humans. However, a study of CD38 knock-out mice [76] found elevated serum testosterone in males, but not females, relative to wildtype, which was correlated with significantly decreased expression of the androgen receptor. Interestingly, males with the high testosterone rs3796863 genotype were also found to have significantly lower Systemizing Quotient and Systemizing relative to Empathizing scores. This relationship appears to conflict with the hypothesized positive association of testosterone with Systemizing [77], though could be mediated by effects of this SNP on androgen receptors, as reported in [76]. However, given the small number of males (n = 7) with the high-testosterone CD38 SNP rs3796863 genotype, future studies with larger sample sizes are needed to confirm any associations with hormonal or psychological variables. We suggest that future studies with mixed sex populations also include estradiol, as the CD38 knock-out female mice showed elevated levels of this steroid hormone, but not testosterone [76].

A sex difference was also found in the relationship between OXTR rs1042778 genotype and pre- to post-video change in salivary testosterone, with TT females showing a greater testosterone decrease relative to GG+GT females, but TT males showing an average testosterone increase relative to the average testosterone decrease for GG+GT males. Although interpretation of this result is again limited by the small number of TT genotype individuals (5 females, 6 males), the OXTR rs1042778 TT genotype has previously been associated with greater amygdala reactivity to angry facial expressions in males but not females [78]. As testosterone is positively associated with amygdala reactivity [79,80], future studies may want to directly test interactions between amygdala reactivity, hormone levels, and OXTR genotype.

In addition to the associations between individual SNPs and social traits, a “Prosocial” genetic index (sum of OXTR and CD38 alleles or genotypes associated with broadly prosocial phenotypes) showed a significant negative correlation with Systemizing relative to Empathizing, meaning that individuals with a greater number of prosocial alleles or genotypes were more likely to have an empathizing-biased cognitive style [61]. Although the “High Oxytocin” genetic index (which comprised 3 of the 6 SNPs for the Prosocial index) was not significantly associated with psychological traits in our study population, the same genetic index was negatively correlated with Autism Spectrum Quotient subscales in a study by Crespi & Hurd of a larger population [72]. The results of

the current study are indeed congruent with those of Crespi & Hurd as autism spectrum conditions have been conceptualized in terms of an extreme systemizing bias [62], and SQ:EQ was positively correlated with AQ in our population. Taken together, these studies support the hypothesis that individual SNPs contribute to social phenotype via effects on hormones.

The overall findings of this study highlight the value of assessing Systemizing relative to Empathizing, particularly when limited by sample size, as SQ:EQ showed significant associations with hormonal, genetic, and other psychological variables in some cases where SQ and EQ considered separately did not. First, SQ:EQ was positively correlated with T:OT, which is consistent with the hypothesized role of testosterone in promoting systemizing [77]. Individuals with higher Systemizing relative to Empathizing reported significantly lower Empathy Responses to the video and pre- to post-video hormone changes were negatively correlated with SQ:EQ, although only the correlation between SQ:EQ and testosterone change for females was statistically significant. SQ:EQ was also negatively and significantly correlated with both models of the "Prosocial" genetic index, as described above. Lastly, EQ:SQ was positively correlated with AQ for the overall population, and negatively correlated with IRI for females only. Taken together, these results support that Empathizing relative to Systemizing is an important measure of cognitive style with relationships to basal testosterone/oxytocin balance, genotype, and psychological traits linked to autism. Furthermore, the pattern reported here between higher testosterone relative to oxytocin and higher Systemizing relative to Empathizing suggests compatibility of Crespi's [35] diametric oxytocin-testosterone hypothesis with Baron-Cohen's [62] empathizing-systemizing theory of cognition.

Beyond the small sample sizes, limitations of this study include quantification of oxytocin in saliva, the lack of a control video, and limited data on individual response to the video. The validity of oxytocin measurements in unextracted saliva has previously been questioned [65]; however, with careful collection and preparation protocols and newer, highly specific assays, salivary oxytocin measures are expected to correlate with plasma oxytocin [38]. Salivary oxytocin measures do show a greater range compared to oxytocin measurements in extracted plasma [65], which may relate to our lack of significant findings regarding relationships between the oxytocin variables (baseline or percentage change) and psychological or genetic variables. However, undertaking a study with multiple collection times per participant would have been challenging and cost-

prohibitive if hormones were assayed from blood rather than saliva. For similar reasons, we chose to use an empathy-inducing video already validated against a neutral control video [39], which allowed us to direct our resources toward assessing hormones in individuals for whom oxytocin was predicted to increase; certainly not all participants found the video to be equally emotional, which is reflected in the post-video questionnaire responses. Another limitation is that our data on individual response to the video is limited to the 12 emotion ratings on the Post-Video Questionnaire, which showed only weak associations with hormonal variables. The use of software that records and analyzes micro-expressions, such as The FaceReader [81], would give us a richer dataset that more accurately identifies various reactions to the video relative to self-report and includes emotions not covered by the questionnaire. Lastly, although our sample size may be considered large for a hormone study, it is small for genetic analysis where individual SNPs are expected to have small effects, restricting the interpretation of our tests of relationships between genes and hormone and questionnaire variables.

As further tests of the roles of hormones in mediating cognitive trade-offs, future studies could assess oxytocin and testosterone responses to stimuli expected to promote a pattern of change opposite to that associated here with empathizing. Asocial or antisocial stimuli would be predicted to mediate a decrease in oxytocin and an increase in testosterone, and might include playing a strategy game like chess against a computer or independently solving visuo-spatial problems. More broadly, future studies testing the role of a specific hormone in social behaviour should consider incorporating a variable related to a second hormone. The potential value of doing so is attested to by a recent study by Holtfrerich et al. [82], which found that oxytocin administration decreased reaction time to infant stimuli, but only among individuals with high salivary testosterone levels. Even though both oxytocin and testosterone were not directly measured in this study, the result is supportive of the opposing effects of these hormones, whereby altering the balance of oxytocin relative to testosterone is what promotes alterations to social cognition or behaviour.

Further research is also needed to understand the mechanism that connects oxytocin and testosterone response in the central nervous system. Currently, it is not clear if these hormones are independently regulated or if interactions between these hormones generate the negative regulation, as observed in this study. A recent study by Dai et al.

[83] has shed the first light on this question, reporting that pre-incubation with testosterone reduces oxytocin mRNA levels and that the androgen receptor downregulates oxytocin gene expression in post-mortem hippocampal cells. By moving toward research that considers multiple hormones, we can begin to understand the complex interactions between biological variables that promote the diversity of human social behaviour.

3.6. References

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3.7. Supplementary data

3.7.1. Demographics questionnaire

Please fill in the blank or circle the applicable answer.

1. Age: _____
2. Biological sex (as assigned at birth): male / female
3. Gender identity: male / female / transgender / other
4. Sexual orientation: heterosexual / homosexual / bisexual / other
5. Ethnicity: Caucasian / East Asian (examples: Chinese, Japanese, Korean)
   South Asian (examples: Indian) / Other (please specify) _____
6. Are you in a long-term relationship? Yes No
   If yes, please estimate the length of your current relationship: _____
7. Number of children: 0 1 2 3 or more
8. Has a close friend or family member ever been diagnosed with cancer? Yes No
9. Have you or any immediate family members (parents, siblings, grandparents) been diagnosed with the following mental illnesses? Please circle those which apply.
   Autism or autistic spectrum disorder Schizophrenia Depression
   Borderline personality disorder Bipolar disorder

FEMALES ONLY

10. Are you currently pregnant? Yes No
11. Do you currently use a hormonal method of birth control (examples: Depo-Provera, NuvaRing, oral contraceptive pills)? Yes No
12. When was the first day of your last menstrual period?
    Within last 7 days | 1 to 2 weeks ago | 2 to 3 weeks ago
    3 to 4 weeks ago | more than 4 weeks ago | I do not get a period

3.7.2. Post-video questionnaire

Please circle your responses.

1. The child in the video had which condition: autism cancer Down syndrome

2. The narrator of the video was the child's: doctor brother father

Please refer to the word on the left, then circle the number that best represents how strongly you felt that emotion.

	DID NOT FEEL THIS WAY AT ALL				STRONGLY FELT THIS WAY
1. Sympathetic	1	2	3	4	5
2. Warm	1	2	3	4	5
3. Anxious	1	2	3	4	5
4. Annoyed	1	2	3	4	5
5. Compassionate	1	2	3	4	5
6. Sad	1	2	3	4	5
7. Tender	1	2	3	4	5
8. Distressed	1	2	3	4	5
9. Soft-hearted	1	2	3	4	5
10. Frightened	1	2	3	4	5
11. Moved	1	2	3	4	5
12. Disturbed	1	2	3	4	5

3.7.3. Autism spectrum quotient

Please read the following 50 statements and circle the option that best describes you.				
1. I prefer to do things with others rather than on my own.	definitely agree	slightly agree	slightly disagree	definitely disagree
2. I prefer to do things the same way over and over again.	definitely agree	slightly agree	slightly disagree	definitely disagree
3. If I try to imagine something, I find it easy to create a picture in my mind.	definitely agree	slightly agree	slightly disagree	definitely disagree
4. I frequently get so strongly absorbed in things that I lose sight of other things.	definitely agree	slightly agree	slightly disagree	definitely disagree
5. I often notice small sounds when others do not.	definitely agree	slightly agree	slightly disagree	definitely disagree
6. I usually notice car number plates or similar strings of information.	definitely agree	slightly agree	slightly disagree	definitely disagree
7. Other people frequently tell me that what I've said is impolite, even though I think it is polite.	definitely agree	slightly agree	slightly disagree	definitely disagree
8. When I'm reading a story, I can easily imagine what the characters might look like.	definitely agree	slightly agree	slightly disagree	definitely disagree
9. I am fascinated by dates.	definitely agree	slightly agree	slightly disagree	definitely disagree
10. In a social group, I can easily keep track of several different people's conversations.	definitely agree	slightly agree	slightly disagree	definitely disagree
11. I find social situations easy.	definitely agree	slightly agree	slightly disagree	definitely disagree
12. I tend to notice details that others do not.	definitely agree	slightly agree	slightly disagree	definitely disagree
13. I would rather go to a library than a party.	definitely agree	slightly agree	slightly disagree	definitely disagree
14. I find making up stories easy.	definitely agree	slightly agree	slightly disagree	definitely disagree

15. I find myself drawn more strongly to people than to things.	definitely agree	slightly agree	slightly disagree	definitely disagree
16. I tend to have very strong interests, which I get upset about if I can't pursue.	definitely agree	slightly agree	slightly disagree	definitely disagree
17. I enjoy social chit-chat.	definitely agree	slightly agree	slightly disagree	definitely disagree
18. When I talk, it isn't always easy to get a word in edgewise.	definitely agree	slightly agree	slightly disagree	definitely disagree
19. I am fascinated by numbers.	definitely agree	slightly agree	slightly disagree	definitely disagree
20. When I'm reading a story, I find it difficult to work out the characters' intentions.	definitely agree	slightly agree	slightly disagree	definitely disagree
21. I don't particularly enjoy reading fiction.	definitely agree	slightly agree	slightly disagree	definitely disagree
22. I find it hard to make new friends.	definitely agree	slightly agree	slightly disagree	definitely disagree
23. I notice patterns in things all the time.	definitely agree	slightly agree	slightly disagree	definitely disagree
24. I would rather go to the theatre than a museum.	definitely agree	slightly agree	slightly disagree	definitely disagree
25. It does not upset me if my daily routine is disturbed.	definitely agree	slightly agree	slightly disagree	definitely disagree
26. I frequently find that I don't know how to keep a conversation going.	definitely agree	slightly agree	slightly disagree	definitely disagree
27. I find it easy to "read between the lines" when someone is talking to me.	definitely agree	slightly agree	slightly disagree	definitely disagree
28. I usually concentrate more on the whole picture, rather than the small details.	definitely agree	slightly agree	slightly disagree	definitely disagree
29. I am not very good at remembering phone numbers.	definitely agree	slightly agree	slightly disagree	definitely disagree
30. I don't usually notice small changes in a situation, or a person's appearance.	definitely agree	slightly agree	slightly disagree	definitely disagree
31. I know how to tell if someone listening to me is getting bored.	definitely agree	slightly agree	slightly disagree	definitely disagree
32. I find it easy to do more than one thing at once.	definitely agree	slightly agree	slightly disagree	definitely disagree

33. When I talk on the phone, I'm not sure when it's my turn to speak.	definitely agree	slightly agree	slightly disagree	definitely disagree
34. I enjoy doing things spontaneously.	definitely agree	slightly agree	slightly disagree	definitely disagree
35. I am often the last to understand the point of a joke.	definitely agree	slightly agree	slightly disagree	definitely disagree
36. I find it easy to work out what someone is thinking or feeling just by looking at their face.	definitely agree	slightly agree	slightly disagree	definitely disagree
37. If there is an interruption, I can switch back to what I was doing very quickly.	definitely agree	slightly agree	slightly disagree	definitely disagree
38. I am good at social chit-chat.	definitely agree	slightly agree	slightly disagree	definitely disagree
39. People often tell me that I keep going on and on about the same thing.	definitely agree	slightly agree	slightly disagree	definitely disagree
40. When I was young, I used to enjoy playing games involving pretending with other children.	definitely agree	slightly agree	slightly disagree	definitely disagree
41. I like to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.).	definitely agree	slightly agree	slightly disagree	definitely disagree
42. I find it difficult to imagine what it would be like to be someone else.	definitely agree	slightly agree	slightly disagree	definitely disagree
43. I like to plan any activities I participate in carefully.	definitely agree	slightly agree	slightly disagree	definitely disagree
44. I enjoy social occasions.	definitely agree	slightly agree	slightly disagree	definitely disagree
45. I find it difficult to work out people's intentions.	definitely agree	slightly agree	slightly disagree	definitely disagree
46. New situations make me anxious.	definitely agree	slightly agree	slightly disagree	definitely disagree
47. I enjoy meeting new people.	definitely agree	slightly agree	slightly disagree	definitely disagree
48. I am a good diplomat.	definitely agree	slightly agree	slightly disagree	definitely disagree
49. I am not very good at remembering people's date of birth.	definitely agree	slightly agree	slightly disagree	definitely disagree
50. I find it very easy to play games with children that involve pretending.	definitely agree	slightly agree	slightly disagree	definitely disagree

3.7.4. Schizotypal personality questionnaire

1	I sometimes jump quickly from one topic to another.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
2	Do everyday things seem unusually large or small?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
3	Do you feel that you cannot get “close” to people?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
4	I find it hard to be emotionally close to people.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
5	Do you sometimes feel that people are talking about you?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
6	I often feel that others have it in for me.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
7	I get anxious when meeting people for the first time.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
8	I have some eccentric (odd) habits.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
9	I sometimes avoid going places where there will be many people because I will get anxious.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
10	Other people see me as slightly eccentric (odd).	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
11	Have you ever felt that you are communicating with another person telepathically (by mind-reading)?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
12	Do you tend to wander off the topic when having a conversation?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
13	Do you believe in telepathy (mind-reading)?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
14	Do you sometimes get concerned that friends or co-workers are not really loyal or trustworthy?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
15	I rarely laugh or smile.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
16	I often ramble on too much when speaking.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree

17	When shopping do you get the feeling that other people are taking notice of you?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
18	Do you believe in clairvoyance (psychic forces, fortune telling)?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
19	Are your thoughts sometimes so strong that you can almost hear them?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
20	Do you sometimes feel that other people are watching you?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
21	Have you had experiences with astrology, seeing the future, UFOs, ESP, or a sixth sense?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
22	I feel very uncomfortable in social situations involving unfamiliar people.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
23	I tend to keep feelings to myself.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
24	Do you feel that there is no one you are really close to outside of your immediate family, or people you can confide in or talk to about personal problems?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
25	I am not good at expressing my true feelings by the way I talk or look.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
26	I often hear a voice speaking my thoughts aloud.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
27	I sometimes forget what I am trying to say.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
28	When you look at a person or yourself in a mirror, have you ever seen the face change right before your eyes?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
29	Do you often feel nervous when you are in a group of unfamiliar people?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
30	Do you often have to keep an eye out to stop people from taking advantage of you?	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
31	People sometimes comment on my unusual mannerisms and habits.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
32	I am an odd, unusual person.	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree

3.7.5. Interpersonal reactivity index

Please read the following statements and circle the option that best describes you.

	DOES NOT DESCRIBE ME AT ALL				DESCRIBES ME VERY WELL
1. I daydream and fantasize, with some regularity, about things that might happen to me.	A	B	C	D	E
2. I often have tender, concerned feelings for people less fortunate than me.	A	B	C	D	E
3. I sometimes find it difficult to see things from the "other guy's" point of view.	A	B	C	D	E
4. Sometimes I don't feel very sorry for other people when they are having problems.	A	B	C	D	E
5. I really get involved with the feelings of the characters in a novel.	A	B	C	D	E
6. In emergency situations, I feel apprehensive and ill-at-ease.	A	B	C	D	E
7. I am usually objective when I watch a movie or play, and I don't often get completely caught up in it.	A	B	C	D	E
8. I try to look at everybody's side of a disagreement before I make a decision.	A	B	C	D	E
9. When I see someone being taken advantage of, I feel kind of protective towards them.	A	B	C	D	E

10. I sometimes feel helpless when I am in the middle of a very emotional situation.	A	B	C	D	E
11. I sometimes try to understand my friends better by imagining how things look from their perspective.	A	B	C	D	E
12. Becoming extremely involved in a good book or movie is somewhat rare for me.	A	B	C	D	E
13. When I see someone get hurt, I tend to remain calm.	A	B	C	D	E
14. Other people's misfortunes do not usually disturb me a great deal.	A	B	C	D	E
15. If I'm sure I'm right about something, I don't waste much time listening to other people's arguments.	A	B	C	D	E
16. After seeing a play or movie, I have felt as though I were one of the characters.	A	B	C	D	E
17. Being in a tense emotional situation scares me.	A	B	C	D	E
18. When I see someone being treated unfairly, I sometimes don't feel very much pity for them.	A	B	C	D	E
19. I am usually pretty effective in dealing with emergencies.	A	B	C	D	E
20. I am often quite touched by things that I see happen.	A	B	C	D	E

21. I believe that there are two sides to every question and try to look at them both.	A	B	C	D	E
22. I would describe myself as a pretty soft-hearted person.	A	B	C	D	E
23. When I watch a good movie, I can very easily put myself in the place of a leading character.	A	B	C	D	E
24. I tend to lose control during emergencies.	A	B	C	D	E
25. When I'm upset at someone, I usually try to "put myself in his shoes" for a while.	A	B	C	D	E
26. When I am reading an interesting story or novel, I imagine how I would feel if the events in the story were happening to me.	A	B	C	D	E
27. When I see someone who badly needs help in an emergency, I go to pieces.	A	B	C	D	E
28. Before criticizing somebody, I try to imagine how I would feel if I were in their place.	A	B	C	D	E

3.7.6. Empathy quotient and systemizing quotient

Please read the following statements and circle the option that best describes you.

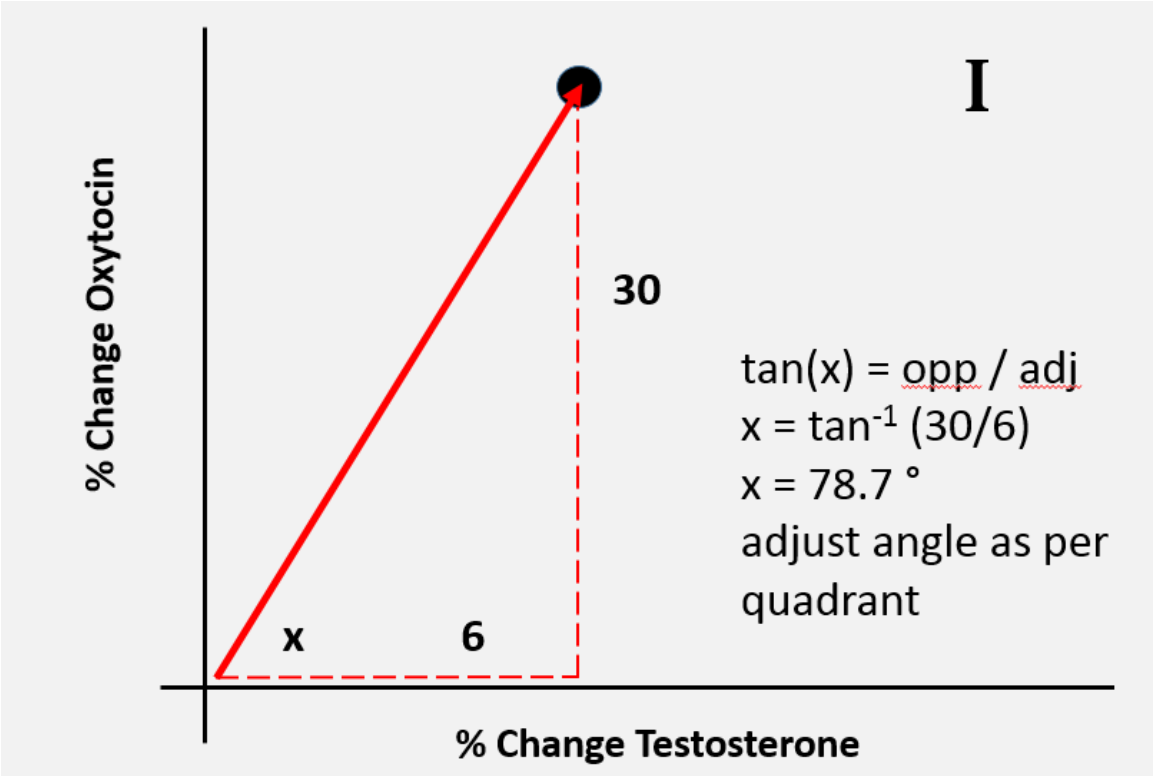
1. I can easily tell if someone else wants to enter a conversation	definitely agree	slightly agree	slightly disagree	definitely disagree
2. I really enjoy caring for other people.	definitely agree	slightly agree	slightly disagree	definitely disagree
3. I find it hard to know what to do in a social situation.	definitely agree	slightly agree	slightly disagree	definitely disagree
4. I often find it difficult to judge if something is rude or polite	definitely agree	slightly agree	slightly disagree	definitely disagree
5. In a conversation, I tend to focus on my own thoughts rather than on what my listener might be thinking.	definitely agree	slightly agree	slightly disagree	definitely disagree
6. I can pick up quickly if someone says one thing but means another	definitely agree	slightly agree	slightly disagree	definitely disagree
7. It is hard for me to see why some things upset people so much.	definitely agree	slightly agree	slightly disagree	definitely disagree
8. I find it easy to put myself in somebody else's shoes.	definitely agree	slightly agree	slightly disagree	definitely disagree
9. I am good at predicting how someone will feel.	definitely agree	slightly agree	slightly disagree	definitely disagree
10. I am quick to spot when someone in a group is feeling awkward or uncomfortable.	definitely agree	slightly agree	slightly disagree	definitely disagree
11. I can't always see why someone should have felt offended by a remark.	definitely agree	slightly agree	slightly disagree	definitely disagree

12. I don't tend to find social situations confusing.	definitely agree	slightly agree	slightly disagree	definitely disagree
13. Other people tell me I am good at understanding how they are feeling and what they are thinking.	definitely agree	slightly agree	slightly disagree	definitely disagree
14. I can easily tell if someone else is interested or bored with what I am saying	definitely agree	slightly agree	slightly disagree	definitely disagree
15. Friends usually talk to me about their problems as they say that I am very understanding.	definitely agree	slightly agree	slightly disagree	definitely disagree
16. I can sense if I am intruding, even if the other person doesn't tell me.	definitely agree	slightly agree	slightly disagree	definitely disagree
17. Other people often say that I am insensitive, though I don't always see why.	definitely agree	slightly agree	slightly disagree	definitely disagree
18. . I can tune into how someone else feels rapidly and intuitively.	definitely agree	slightly agree	slightly disagree	definitely disagree
19. I can easily work out what another person might want to talk about.	definitely agree	slightly agree	slightly disagree	definitely disagree
20. I can tell if someone is masking their true emotion.	definitely agree	slightly agree	slightly disagree	definitely disagree
21. I am good at predicting what someone will do.	definitely agree	slightly agree	slightly disagree	definitely disagree
22. I tend to get emotionally involved with a friend's problems	definitely agree	slightly agree	slightly disagree	definitely disagree
23. If I were buying a car, I would want to obtain specific information about its engine capacity.	definitely agree	slightly agree	slightly disagree	definitely disagree

24. If there was a problem with the electrical wiring in my home, I'd be able to fix it myself.	definitely agree	slightly agree	slightly disagree	definitely disagree
25. I rarely read articles or web pages about new technology	definitely agree	slightly agree	slightly disagree	definitely disagree
26. I do not enjoy games that involve a high degree of strategy.	definitely agree	slightly agree	slightly disagree	definitely disagree
27. I am fascinated by how machines work.	definitely agree	slightly agree	slightly disagree	definitely disagree
28. In math, I am intrigued by the rules and patterns governing numbers.	definitely agree	slightly agree	slightly disagree	definitely disagree
29. I find it difficult to understand instruction manuals for putting appliances together.	definitely agree	slightly agree	slightly disagree	definitely disagree
30. If I were buying a computer, I would want to know exact details about its hard disc drive capacity and processor speed.	definitely agree	slightly agree	slightly disagree	definitely disagree
31. I find it difficult to read and understand maps	definitely agree	slightly agree	slightly disagree	definitely disagree
32. When I look at a piece of furniture, I do not notice the details of how it was constructed.	definitely agree	slightly agree	slightly disagree	definitely disagree
33. I find it difficult to learn my way around a new city.	definitely agree	slightly agree	slightly disagree	definitely disagree
34. I do not tend to watch science documentaries on television or read articles about science and nature.	definitely agree	slightly agree	slightly disagree	definitely disagree
35. If I were buying a stereo, I would want to know about its precise technical features.	definitely agree	slightly agree	slightly disagree	definitely disagree

36. I find it easy to grasp exactly how odds work in betting.	definitely agree	slightly agree	slightly disagree	definitely disagree
37. I am not very meticulous when I carry out D.I.Y.	definitely agree	slightly agree	slightly disagree	definitely disagree
38. When I look at a building, I am curious about the precise way it was constructed.	definitely agree	slightly agree	slightly disagree	definitely disagree
39. I find it difficult to understand information the bank sends me on different investment and saving systems.	definitely agree	slightly agree	slightly disagree	definitely disagree
40. When travelling by train, I often wonder exactly how the rail networks are coordinated	definitely agree	slightly agree	slightly disagree	definitely disagree
41. If I were buying a camera, I would not look carefully into the quality of the lens.	definitely agree	slightly agree	slightly disagree	definitely disagree
42. When I hear the weather forecast, I am not very interested in the meteorological patterns.	definitely agree	slightly agree	slightly disagree	definitely disagree
43. When I look at a mountain, I think about how precisely it was formed.	definitely agree	slightly agree	slightly disagree	definitely disagree
44. I can easily visualize how the motorways in my region link up	definitely agree	slightly agree	slightly disagree	definitely disagree
45. When I'm in a plane, I do not think about the aerodynamics.	definitely agree	slightly agree	slightly disagree	definitely disagree
46. I am interested in knowing the path a river takes from its source to the sea	definitely agree	slightly agree	slightly disagree	definitely disagree
47. I am not interested in understanding how wireless communication works.	definitely agree	slightly agree	slightly disagree	definitely disagree

3.7.7. Transformation of testosterone and oxytocin percent change to circular data



Chapter 4.

The Williams syndrome prosociality gene GTF2I mediates oxytocin reactivity and social anxiety in a healthy population

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Biology Letters, doi: 10.1098/rsbl.2017.0051.

4.1. Abstract

The neurohormone oxytocin plays a central role in human social behaviour and cognition, and oxytocin dysregulation may contribute to psychiatric disorders. However, genetic factors influencing individual variation in the oxytocinergic system remain poorly understood. We genotyped 169 healthy adults for a functional polymorphism in GTF2I (general transcription factor II-I), a gene associated with high prosociality and reduced social anxiety in Williams syndrome, a condition reported to involve high oxytocin levels and reactivity. Participants' salivary oxytocin levels were measured before and after watching a validated empathy-inducing video. Oxytocin reactivity, defined as pre- to post-video percentage change in salivary oxytocin, varied substantially and significantly between individuals with different GTF2I genotypes, with, additionally, a trend towards an interaction between genotype and sex. Individuals with more oxytocin-reactive genotypes also reported significantly lower social anxiety. These findings suggest a model whereby GTF2I has a continuum of effects on human sociality, from the extreme social phenotypes and oxytocin dysregulation associated with gene deletion in Williams syndrome, to individual differences in oxytocin reactivity and sociality associated with common polymorphisms in healthy populations.

4.2. Introduction

The neurohormone oxytocin plays central roles in human social cognition, emotionality, and behaviour [1–3]. Moreover, dysregulated oxytocin levels have been reported in psychiatric disorders including major depression [4] and autism [5]. Despite intense interest in social effects of oxytocin, the genetic mechanisms regulating individual variation in oxytocin are poorly understood.

Genetic disorders characterized by atypical social behaviour may provide useful insights into genes influencing the human oxytocinergic system. Williams syndrome, a neurodevelopmental disorder caused by hemizygous deletion of ~25 genes at chromosomal region 7q.11.23, is characterized by high prosociality and low social anxiety, which may be linked with the increased oxytocin levels and reactivity reported in this condition [6,7]. High prosociality in Williams syndrome, and in mouse models of this syndrome, has been linked with reduced expression of the gene general transcription factor II-I (GTF2I) [8,9], which is located within the 7q11.23 Williams syndrome deletion region. Single nucleotide polymorphisms (SNPs) of this gene are also associated with autism risk [10] and, among healthy populations, with variation in social anxiety, autistic-like traits, threat-related amygdala activity, and extraversion [11-13]. These latter findings provide evidence of roles for GTF2I in sociality and anxiety among healthy humans, which notably resemble the roles of oxytocin itself [7, 11, 14].

Convergent lines of evidence thus suggest that the associations of GTF2I with social behaviour—in Williams syndrome and in healthy populations—may be mediated by effects of GTF2I genetic variation on oxytocin. We tested this hypothesis using data on salivary oxytocin levels collected before and after experimental empathy induction, data on self-reported social anxiety, and data from genotyping of rs13227433, the GTF2I SNP previously associated with social anxiety, extraversion, and amygdala reactivity in healthy populations.

4.3. Materials and methods

4.3.1. Study population

Healthy participants were recruited from a Canadian university (92 females, 77 males). The study population was of mixed ethnicity (42% East Asian, 25% Caucasian, 22% South Asian, 11% other or mixed ethnicity) with an average age of 20.3±2.2 years. No participants reported having children. No female participants reported being pregnant, and mean oxytocin levels and reactivity did not differ with use of hormonal contraception or stage of menstrual cycle ($p > 0.24$ for all tests, Tables S1, S2, electronic supplementary material).

4.3.2. Experimental design

Saliva samples were collected before and after participants watched a validated empathy-inducing video of a child with terminal cancer [15]. Oxytocin was quantified in both samples, and reactivity was calculated as pre-video to post-video percentage change in salivary oxytocin. As part of the experiment, participants also completed the Schizotypal Personality Questionnaire-Brief Revised (SPQ-BR-BR) [16,17], which includes a four-item excessive social anxiety subscale. Each high-anxiety item endorsed (“agree” or “strongly agree”) was scored 1; social anxiety scores thus ranged from 0-4 with higher scores indicating greater social anxiety.

4.3.3. Salivary oxytocin collection and analysis

Saliva was collected by passive drool into pre-chilled tubes and immediately frozen at -20°C. Consistent with published protocols for measuring salivary oxytocin [18-20], 0.5 ml of saliva was lyophilized overnight to concentrate the sample twofold. Measurement of oxytocin was performed in duplicate using Enzo Life Sciences enzyme-linked immunosorbent assay kit ADI-901-153 [21]. Samples from the same individual were analyzed on the same plate. Plates were read at 405 nm and oxytocin concentrations were calculated from standard curves. Intra- and inter-assay coefficients of variability were <8% and <18%, respectively, for 16 plates, which were consistent with the manufacturer’s normative variability ranges (12.6–13.3% and 11.9–20.9%).

Debate exists concerning measurement accuracy of oxytocin by ELISA in unextracted compared to extracted fluids, as well as the relationship between salivary and plasma oxytocin [22]. The assay used in this study has undergone rigorous testing and is highly specific to oxytocin (i.e., it does not detect vasopressin) [21]. Furthermore, salivary oxytocin has been shown to correlate positively with plasma oxytocin [23], and any possible methodological measurement effects are expected to affect all samples, rather than being genotype specific in any way.

4.3.4. GTF2I genotyping

Participants were genotyped for SNP rs13227433, which tags a ~73kb haplotype that includes the promoter region of the GTF2I gene (Figure 4.2). DNA, fluorophore-labeled primers (TaqMan® SNP Genotyping Assays), and TaqMan® Master Mix were combined and run on a Roche LightCycler® 96 Real-Time PCR machine. Fluorescence data were analyzed under Endpoint Genotyping with LightCycler® 96 software, version 1.1.0.1320. Genotype frequencies did not deviate from Hardy-Weinberg equilibrium in our sampled population ($\chi 2 = 2.28$, $df = 1$, $p = 0.13$).

4.3.5. Statistical analysis

R (version 3.3.1) was used to analyze all data. Mean differences were tested using t-tests for two-group comparisons and analyses of variance (ANOVA) for comparisons involving more than two groups. Given the rarity of the GG genotype (<5%), GG and GT genotypes were combined and compared to TT genotypes. Results were considered significant if $p < 0.05$.

4.4. Results

Salivary oxytocin increased, on average across all participants, after viewing the empathy-inducing video (paired t-test: $t = -2.95$, $df = 168$, $p = 0.004$). Variation in oxytocin was analyzed using a 2 x 2 x 4 factorial ANOVA (genotype x sex x ethnicity). The analysis indicated a significant main effect of GTF2I rs13227433 genotype on oxytocin reactivity ($F = 5.5$, $p = 0.02$, mean difference: 16.6, 95% confidence intervals of difference: 2.8 – 30.2), with the GG+GT group showing higher reactivity (Figure 1), and a trend towards an interaction between genotype and sex ($F = 3.0$, $p = 0.08$). Social

anxiety was analyzed using a 2 x 2 factorial ANOVA (genotype x sex), which also resulted in a significant main effect of genotype ($F = 4.4$, $p = 0.04$, mean difference: -0.5, 95% confidence intervals of difference: -0.03 – -0.99) with the GG+GT group self-reporting lower levels of social anxiety. Mean oxytocin reactivity and social anxiety scores for each genotype group and sex are presented in Table 1. All other effects and interactions were statistically non-significant (Tables S3, S4, electronic supplementary material), including variation in baseline oxytocin levels between GTF2I genotype groups ($p = 0.44$, means: 101.0 pg/ml GG+GT, 109.9 pg/ml TT).

Given that the more oxytocin-reactive genotype group also reported lower social anxiety, a correlation analysis between oxytocin reactivity and social anxiety was performed. Self-reported social anxiety and oxytocin reactivity to the emotional video were uncorrelated (Pearson product-moment correlation = 0.007, $p = 0.93$).

Table 4.1. Mean oxytocin reactivity and social anxiety scores for GTF2I SNP rs1322743 genotype groups.

		All	GG	GT	GG + GT	TT
% Oxytocin Reactivity mean (SD)	**Females + Males**	14.2 (42.9) *n = 169*	32.2 (56.6) *n = 9*	24.0 (48.6) *n = 46*	25.4 (49.5)† *n = 55*	8.8 (38.4)† *n = 114*
	Females	17.3 (47.1) *n = 92*	29.5 (59.8) *n = 8*	36.9 (50.2) *n = 24*	35.1 (51.8) *n = 32*	7.8 (41.8) *n = 60*
	Males	10.5 (37.2) *n = 77*	53.8 (n.a.) *n = 1*	10.0 (43.8) *n = 22*	11.9 (43.7) *n = 23*	10.0 (34.5) *n = 54*
Social Anxiety mean (SD)	**Females + Males**	1.6 (1.5) *n = 166*	1.1 (1.5) *n = 9*	1.3 (1.4) *n = 46*	1.3 (1.4)† *n = 55*	1.8 (1.5)† *n = 111*
	Females	1.5 (1.4) *n = 90*	1.3 (1.6) *n = 8*	1.0 (1.3) *n = 24*	1.1 (1.3) *n = 32*	1.8 (1.5) *n = 58*
	Males	1.7 (1.6) *n = 76*	0 (n.a.) *n = 1*	1.6 (1.6) *n = 22*	1.6 (1.6) *n = 23*	1.8 (1.5) *n = 53*

3 individuals were excluded from social anxiety analyses due to incomplete questionnaire items.
† Mean oxytocin reactivity and social anxiety differ significantly ($p < 0.05$) between GG+GT and TT genotype groups for Females+Males (see text for ANOVA results).
n.a. = not applicable, due to sample size of 1

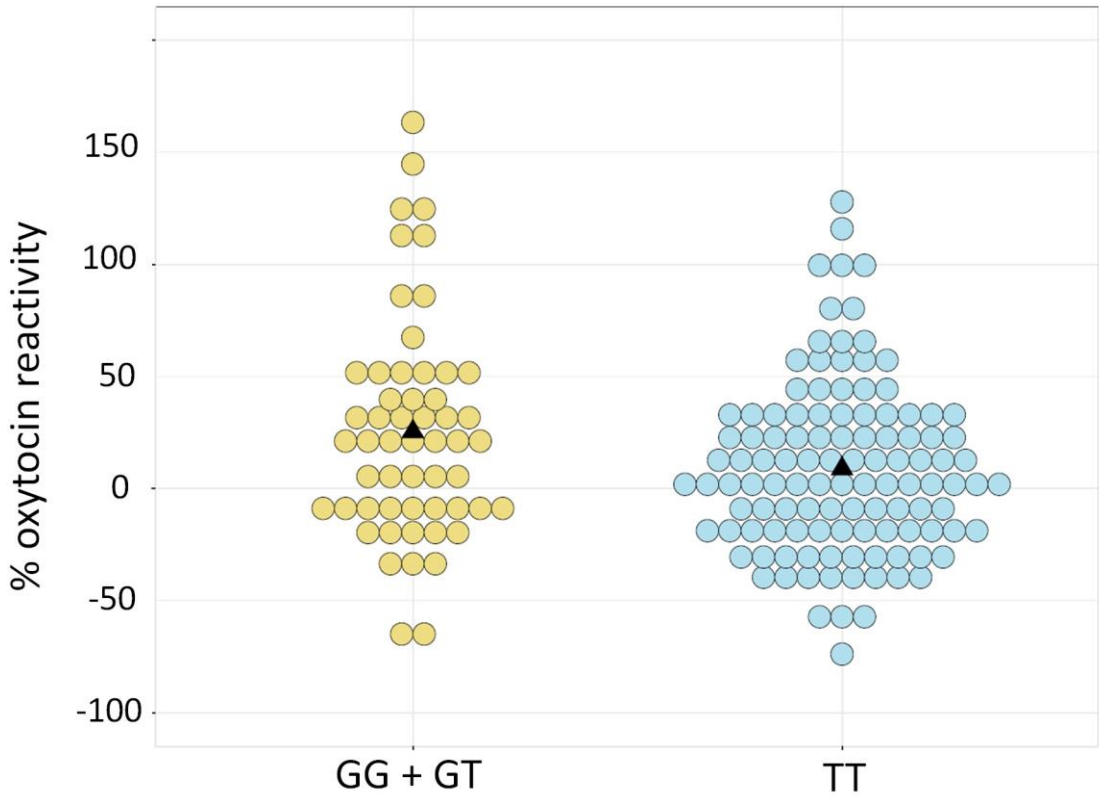

Figure 4.1 Dot plot indicating percentage change in salivary oxytocin for individuals with GG+GT vs. TT genotypes for GTF2I SNP rs13227433.

Each dot represents one individual. Triangles indicate the mean for each genotype group (25.4 for GG+GT, 8.8 for TT).

4.5. Discussion

Our results demonstrate a relationship between oxytocin reactivity and genotypes of the SNP rs13227433, a common polymorphism in the Williams-syndrome associated gene GTF2I. We further show that individuals with more oxytocin-reactive genotypes report lower levels of social anxiety. The lack of correlation between oxytocin reactivity and social anxiety suggests that multiple factors, including some not accounted for in this study, influence these variables, which is not unexpected.

Taken together, the results reported here support a model whereby common genetic variation in GTF2I mediates human sociality and anxiety via effects on oxytocin reactivity. Such a model is consistent with previous studies showing that variation in GTF2I SNPs is associated with social phenotypes in healthy populations, as described above [11-13], and it supports a hormonal basis for the effects. The mechanisms connecting GTF2I with oxytocin remain unknown, but may involve differential methylation of the oxytocin receptor OXTR, which has been reported among individuals with 7q11.23 deletions and duplications [24], and alternative splicing of GTF2I mRNA among individuals with different SNP genotypes, including those analyzed here [25].

The relationship of GTF2I with oxytocin reactivity is relevant to Williams syndrome as it provides the first evidence that a gene subject to hemizygous deletion in this syndrome, and implicated in its characteristic high empathy and prosociality [8,9], modulates oxytocin reactivity. As such, the reported dysregulation of oxytocin levels and reactivity [7], and the high prosociality, high empathy, and low social anxiety [6] found in Williams syndrome, may arise at least in part from reduced GTF2I expression or activity. Further research will increase our understanding of how polymorphisms in GTF2I, and other oxytocin-associated genes, have contributed to the evolution of human sociality and disorders.

4.6. Acknowledgements

We thank Paul Zak for use of the empathy-inducing video and Justin Jagore for assistance with hormone assays.

4.7. References

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4.8. Supplementary data

Figure 4.2 A ~73kb haplotype block in the GTF2I gene, defined by Haploview (Barrett 2009) from the CEU (European) population under the default settings, contains three SNPs (including rs13227433, highlighted) in very high linkage disequilibrium.

These three SNPs have been studied in relation to autism, anxiety, personality, oxytocin reactivity, and Williams syndrome cognitive-behavioural phenotypes. Shown are haplotypes with frequencies of 0.05 and above. This haplotype block includes the promoter region of GTF2I (the regions just upstream to the start of the gene itself).

Figure is adapted, with permission, from Crespi & Procyshyn (2017), *Williams syndrome deletions and duplications: genetic windows to understanding anxiety, sociality, autism, and schizophrenia,* Neuroscience and Biobehavioural Reviews.

Table 4.2 T-test results for differences in oxytocin levels and reactivity with regard to use of hormonal birth control in female research participants.

	T statistic	p		"No" mean (SD)	"Yes" mean (SD)
Baseline Oxytocin	0.169	0.866		99.8 pg/ml (62.9)	97.3 pg/ml (63.9)
Post-video Oxytocin	-0.811	0.422		105.1 pg/ml (65.1)	119.7 pg/ml (82.3)
Oxytocin Reactivity	-1.19	0.240		13.5 (45.8)	26.9 (49.9)

"No", not currently taking hormonal birth control: N = 66
"Yes", currently taking hormonal birth control (oral contraceptives, Depo-Provera, NuvaRing, etc): N = 26

Table 4.3 ANOVA table for differences in oxytocin levels or reactivity across estimated stage of menstrual cycle.

	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Baseline Oxytocin	4	8243	2060.8	0.510	0.728
Post-video Oxytocin	4	12355	3088.9	0.616	0.652
Oxytocin Reactivity	4	5513	1378.2	0.611	0.656

Table 4.4 ANOVA table for differences in oxytocin reactivity by GTF2I genotype, biological sex, and ethnicity.

	Degrees of Freedom	Sum of Squares	Mean Square	F	P
GTF2I	1	10135	10135.3	5.479	0.0205 *
Sex	1	1481	1480.7	0.800	0.372
Ethnicity	3	1474	491.3	0.266	0.850
GTF2I * Sex	1	5602	5602.3	3.028	0.084
GTF2I * Ethnicity	3	3694	1231.2	0.665	0.574
Sex * Ethnicity	3	261	87.1	0.047	0.986
GTF2I * Sex * Ethnicity	3	3421	1140.5	0.616	0.605
Residuals	153	283037	1849.9		

Ethnicity was organized into four categories: Caucasian (25), East Asian (42%), South Asian (22%), and Other/Multiple Ethnicities (11%). $p < 0.05$

Table 4.5 ANOVA table for differences in social anxiety by GTF2I genotype, biological sex, and interaction between genotype and sex.

	Degrees of Freedom	Sum of Squares	Mean Square	F	P
GTF2I	1	9.61	9.6054	4.3916	0.0377 *
Sex	1	1.26	1.2574	0.5749	0.4494
GTF2I * Sex	1	2.13	2.1321	0.9748	0.3249
Residuals	162	354.33	2.1872		

* $p < 0.05$

Chapter 5. Conclusions

The degree to which hormones mediate and motivate human social behaviour has recently become a subject of intense research interest in psychology, endocrinology, neuroscience, and psychiatry. Informed by a social-evolutionary framework, the studies comprising this thesis aimed to explore two central questions regarding the role of hormone levels and hormone-associated genetic polymorphisms in human social behaviour: (i) do hormone-associated genes linked to psychiatric conditions contribute to variation in social traits among non-clinical populations, and (ii) do changes in endogenous hormone levels coordinate adaptive social behaviour with stimuli in the environment?

In support of the first hypothesis, in Chapter 2 I reported a novel association between an AVPR1a microsatellite previously linked to autism risk [1–3] and autistic-like traits (e.g., high attention to detail, reduced social skill, communication, imagination, and attention switching), as measured by the Autism Spectrum Quotient self-report questionnaire [4], in a healthy, mixed-sex study population. Chapter 4 revealed that a common single nucleotide polymorphism in GTF2I, a gene associated with high prosociality in Williams syndrome [5], mediated oxytocin response to an emotional stimulus and social anxiety in healthy individuals, with a trend toward an interaction between genotype and sex. Taken together, these studies support a model whereby genes contribute to variation in social traits—via their effects on hormone systems, in the cases of AVPR1a and GTF2I—along a continuum, mediating typical variation in social phenotypes in non-clinical populations, as well as the extreme social phenotypes characteristic of clinical conditions such as autism and Williams syndrome.

Such a model has important implications for our understanding of the persistence of psychiatric risk genes, and is suggestive that genes contributing to risk of psychiatric conditions are present in healthy individuals where they contribute to normal variation in social traits. Furthermore, it is possible that such psychiatric risk genes in healthy individuals may confer a cognitive advantage. For example, the presence of multiple autism risk genes has been associated with higher intelligence and cognitive functioning in non-clinical populations [6]. It is possible that genetic polymorphisms with links to

hormones and psychiatric conditions, such as the GTF2I genotype studied in Chapter 4, mediate social traits with direct fitness implications, for example, it could be tested if the highly oxytocin reactive, low anxiety GTF2I genotype identified in Chapter 4 is associated with higher quality of parental care.

While Chapters 2, 3, and 4 report significant associations between genes and social traits, it should be noted that the mechanisms through which these polymorphisms mediate social behaviour are poorly understood. In the case of the AVPR1a microsatellite polymorphisms in Chapter 2, there is evidence that shorter repeat lengths result in lower promoter activity, and possibly lower transcription [1]. However, the functional effects of the "target allele" that drove associations between AVPR1a RS3 and social behaviour in our study and others [7,8] has not been specifically examined. Furthermore, although GTF2I SNP variants, including that studied in Chapter 4, can result in abnormal GTF2I mRNA splicing in the brain [9], the mechanism linking GTF2I to oxytocin remains unknown. Further molecular work is needed to elucidate how common polymorphisms mediate variation in social traits via effects on hormone systems.

The second hypothesis, if changes in endogenous hormone levels coordinate adaptive social behaviour with stimuli in the environment, was explored in Chapter 3 by testing hormonal response to an empathy-inducing stimulus. Although there was substantial variation in hormonal responses to the stimulus, on average, the stimulus triggered an increase in oxytocin paired with a decrease in testosterone. This result was consistent with the results of separate hormone administration studies demonstrating that oxytocin and testosterone increase and reduce social cognition, respectively [reviewed in 8]. The paired change in oxytocin and testosterone may thus be interpreted as coordinating adaptive social behaviour with the emotional stimulus, specifically priming the brain for empathetic cognition and increasing the probability of prosocial behaviour.

The methodology employed in Chapter 3 is a departure from the current trend of assessing the role of oxytocin and testosterone in human social behaviour via hormone administration, where hormone levels are inflated independently of social context. We hope our finding that empathy is associated with a joint oxytocin increase and testosterone decrease motivates future studies of endogenous hormone responses to ecologically-valid stimuli, such as a social bonding or social exclusion. With consideration of the specific stimulus, future studies could expand their hormonal

variables to include cortisol, which is known to interact with testosterone [e.g., 11,] and thus may have had explanatory power in our empathy-induction experiment, or estrogen, which is known to interact in a synergistic manner with oxytocin [12]. Lastly, given the evidence of hormonal dysregulation in psychiatric conditions such as autism, it may be a fruitful line of research to assess if clinical and non-clinical populations differ in their endogenous hormonal responses to ecologically-valid social stimuli. The results of such future studies may have implications for the psychotheraputic use of hormones such as oxytocin in treating psychiatric conditions.

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