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Table of Contents

1. Introduction

This thesis explores the potential effects of the hormone Oxytocin on social eye gazing behaviour in autism spectrum condition (ASC) individuals. By conducting an experimental eye tracking study, the aim was to find differences in the extents of eye contact participants exhibited in different conditions – but why the focus on eye gaze?

The eyes are a key component of human social and communication processes. Eye gaze conveys a large amount of nonverbal information and is important for the development of social cognition and functioning (Adams & Nelson, 2016). In ASC, one typical feature is the deficit in social functioning and interactions which often goes along with aberrant eye contact behaviour (American Psychiatric Association; APA, 2013). Researchers have therefore theorised that eye gaze and social competence deficits could be connected – proposing that a change in the former could lead to improvements in the latter (Stuart et al., 2022). But how could this behavioural modification be achieved? Oxytocin may be of help.

The neuropeptide Oxytocin (OXT) is relevant for a wide variety of social behaviour in humans and animals. Since researchers started to investigate the effects of exogenously administrated OXT and observing changes in social cognition and behaviour, the hormone has been proposed as a possible adjuvant in the therapy of psychiatric conditions with social deficits, one of them being ASC. Studies exploring OXT effects on eye gazing in ASC individuals are still scarce – this thesis study aims to contribute novel findings to this research area by employing an innovative eye tracking task and novel intranasal administration technique.

To establish the theoretical background of this study, the state of knowledge on ASC and OXT are described, followed by the pivotal chapter discussing their connection. The research gap, research question and hypotheses are then stated, followed by the materials and methods used. Results are then presented, leading to the discussion and final conclusion.

2. Autism Spectrum Condition (ASC)

2.1. Terminology – ASD or ASC?

The term autism was first used by psychiatrist Leo Kanner in 1943 to describe the irregular development of children showing “autistic aloneness” and an “obsessive insistence on the preservation of sameness” (Strathearn et al., 2018). Since then, considerable progress in research has been made and interdisciplinary knowledge acquired about the neurodevelopmental condition that is now known as autism spectrum disorder (ASD). Information and awareness about the condition have also been spreading among the public

and along with it the discontent about its official ASD terminology. Self-advocates of autism, relatives, but also psychologists like Simon Baron-Cohen have been using and encouraging the use of the term autism spectrum condition in its stead (ASC; TheResearchAutism, 2014). The word 'disorder' has a negative connotation and signifies that something is lacking order or is in disarray. The DSM, as it is the *Diagnostic and Statistic Manual of Mental Disorders*, commonly labels conditions with this term to confer the need for treatment or simply due to habit. As plentiful research has been done on autism across different disciplines and new findings are constantly added, autism has started to be viewed not as a disordered way of thinking but rather as a different, neurodiverse way of processing information and seeing the world (Leadbitter, 2021). People with autism do not necessarily view themselves as being disordered and may want to emphasize the positive sides and assets of the condition without the label of a disorder. Referring to the diagnosis as autism spectrum condition ensures respect towards all people on the spectrum and enables to subsume both strengths and difficulties that go along with neurodivergence under that name.

Language shapes the way people think and behave – therefore, to promote further change in the mindset of people and normalizing its usage, this thesis will also be employing the term ASC.

2.2. Definition of ASC

Autism spectrum condition (ASC; also known as autism spectrum disorder) is a neurodevelopmental condition that is characterised by deficits in social communication and social interactions as well as by the display of restricted, repetitive behaviours, activities, and interests (APA, 2013). In the two prominent diagnostic manuals, the DSM-5 and ICD-11, ASC is diagnosed based on these two core categories of impaired social skills and distinct behavioural patterns, thereby reducing diagnostic complexity compared to earlier manual editions (e.g. DSM-IV and ICD-10). With the updated ICD-11, the manuals mostly converge in diagnostic criteria and guidelines. Hence, for the sake of clarity and legibility, the DSM-5 manual will serve as the exemplary source for this definition of ASC.

In addition to the two key diagnostic criteria that need to be met, symptoms must exist in the early developmental period – even if not yet fully manifested – and cause significant impairment in social, occupational or other important domains of functioning. Furthermore, clinicians need to determine if the symptoms are not better explained by an intellectual developmental disorder or a global developmental delay (APA, 2013). This criterion does not exclude co-occurrence of ASC with intellectual disability as comorbidity estimates range

from 11-65% in children with ASC (Lord et al., 2020). The wide range of reported co-occurrence numbers is likely due to modified manual criteria and growing diagnostic sensitivity over the years. Inclusion of this differentiating criterion emphasizes the need for clinicians to be thorough in their diagnostic evaluation and distinctive between the two conditions as they have partially overlapping symptoms. Besides intellectual disability, ASC commonly occurs with other developmental, medical or psychiatric diagnoses. Research suggests that up to 70% of ASC individuals have at least one comorbid mental disorder including ADHD, anxiety disorders and depression (APA, 2013).

As stated in its name, ASC is defined as a spectrum condition, meaning the expression and severity of symptoms can be highly heterogenous across diagnosed individuals. The global prevalence of ASC is currently estimated at around 1%. Reported numbers of diagnoses have increased in recent years which, instead of an actual rise in the prevalence of the condition, is likely due to changes in the diagnostic definition of autism, a heightened public and clinical awareness as well as improved identification of ASC in certain populations that were under-diagnosed before (Zeidan et al., 2022). Male individuals are three to four times more likely to be diagnosed with ASC. Besides the theory that female gender has a protective effect (Zeidan et al., 2022), the high difference in ratio could also be due to a female ASC phenotype that does not fit the standard clinical diagnosis or due to women being able to mask their social deficits better by "camouflaging" their symptoms (Hodges et al., 2019; Bölte et al., 2019).

2.3. Behavioural Characteristics in ASC

As already described in the introduction, ASC is diagnostically defined by deficits in social communication and interaction as well repetitive behavioural patterns and restricted interests. In the following sections, the diagnostic criteria of the DSM-5 are elaborated in more detail for better understanding of the condition's idiosyncrasies before delving into the ASC symptom most relevant for this thesis: eye gaze.

2.3.1. Repetitive behaviour and restricted interests/activities

Behavioural patterns of individuals with ASC often express themselves in repetitive body movements (e.g. repeating a certain hand motion or gesture, finger-flicking), repetitive use of and play with objects (e.g. arranging toys in lines, handling objects in a certain manner) and repetitive speech (e.g. echolalia, palialia). They may insist on the same routines, food or daily structure and experience distress when routines are interrupted or new elements are introduced. The trait of restricted interests manifests itself in as intense focus dedicated to a

particular object or activity of interest and a general narrowing to a few selected ones (APA, 2013).

Another behavioural particularity in individuals with ASC is the alteration in their experience of sensory stimuli. Reported in up to 87% of individuals, sensory hypersensitivity, hyposensitivity or sensory seeking are common occurrences in ASC. This criterion has only recently been added to the DSM-5, even though it is a well-known concomitant (APA, 2013; Baum et al., 2015). Sensory hypersensitivity expresses itself in strong or negative reactions to low-level environmental stimuli (e.g. sound, touch) that are generally considered to be innocuous. Sensory hyposensitivity shows itself in reduced or absent responses to stimuli, sometimes also pain. Lastly, sensory seeking is described as the longing for a particular kind of sensation (e.g. touching and wearing specific textures) and may also occur as a result to hyposensitivity to get more sensory input. People with ASC may experience a combination of these sensory alterations which can differ depending on the type of sensory input (e.g. visual or auditive; Baum et al., 2015).

2.3.2. Impairments in social communication and social interaction

Deficits in social communication are characteristic for people with ASC but can be highly variable in the extent of manifestation. They can be experienced in all manners of social-emotional situations, for example in the reciprocity of emotions, affect or interest or the struggle to maintain a conversation. Initiating or responding to social interactions can present genuine difficulties. Associated with these deficits are social impairments related to relationship development and maintenance. Individuals with ASC may have trouble with understanding the intricacies that go along with a relationship, like adjusting their behaviour to suit a social context or to accommodate the other person. Furthermore, people may not appear interesting to individuals with ASC who prefer objects over social stimuli which further complicates the establishment of connections (APA, 2013).

Another important factor of social interactions that is frequently impaired in people on the spectrum is nonverbal communication. This deficit becomes apparent through the absent, reduced or unusual use of gestures, speech intonation, facial expressions or eye contact. For example, integrating nonverbal gestures spontaneously into conversations may be difficult or may not be done at all, which could result in a complete lack of facial expressions or body language (APA, 2013). Depending on the situation and symptom severity, behaviours may also be subtle and not noticeable at all; or they could be masked. Masking, also known as camouflaging, is a social coping strategy by ASC individuals where specific techniques are used to convey social competence and to prevent others from detecting social difficulties

(Hull et al., 2017). Camouflaging is a phenomenon usually associated with but not exclusive to the ASC population. Common compensatory techniques are the monitoring of other's reactions, mimicking others' behaviour and forcing and maintaining eye contact as much as possible (Hull et al., 2017). Atypical eye gazing behaviour is an idiosyncrasy commonly found in individuals with autism. Explanations on why it plays such an important part in socio-emotional cognition and theories on why it diverges from neurotypical individuals will be discussed in the following section.

2.3.3. Eye gaze

The eyes are an essential component of social and communication processes in humans as well as in other species. They are an important source of perceiving and conveying nonverbal signals and information about one's internal state. In consequence, they play a critical role in the perception and understanding of facial cues and emotional expressions which is the basis of the ability to understand the intentions and mental state of others (Adams & Nelson, 2016). This social cognition, also known as Theory of Mind (ToM) is impaired in people with ASC, making it one of the key characteristics of the condition (Baron-Cohen, 2000). Research has been investigating if decreased eye contact, a commonly displayed behaviour in ASC, is connected to this social skill deficit (Stuart et al., 2022).

Attenuated eye gaze is the most consistently replicated early predictor of ASC with studies showing that babies later diagnosed with ASC show significantly less eye contact and gaze-following mannerisms (Klin et al., 2020; Tiede & Walton, 2020). This observation of decreased eye contact is also found in adolescents and adults on the spectrum and has become a well-researched phenomenon of the condition (Stuart et al., 2022; Dalton et al., 2005; Neumann et al., 2006; Klin et al., 2002). With eye tracking technology becoming more accessible in recent decades, studies have been able to explore the connection between ASC, eye gaze and accompanying theories on a new level. Within the large number of eye tracking studies published over the years, a variety of methodological features and stimuli has been employed to investigate the subject matter. In experimental settings, social stimuli have mainly been displayed on computer screens as static (picture) or dynamic (video) images; in recent years, real-life interactions have started to be more common as well. The most relevant studies will be elaborated now, for an extensive review of eye tracking studies with ASC individuals, see Frazier and colleagues (2017) or Guillon and colleagues (2013).

The first eye tracking study on diverging eye gazing patterns in ASC individuals used video clips of complex social situations as stimuli (Klin et al., 2002). Eye movements were recorded with a head-mounted eye tracking set-up placed on the bill of a baseball cap. Results

showed a significant difference between the ASC group and the control group with the latter fixating on the eye region twice as much. Additionally, higher fixation time on the mouth region correlated with lower levels of autistic social impairment and higher object fixation time was oppositely associated with higher levels. In 2013, Jones and Klin (2013) conducted another eye tracking study with a prospective longitudinal design and a sample of infants. There were ten data collection time points from 2 to 24 months. Results showed that babies that are later diagnosed with ASC exhibited a mean decline in eye fixation within the first 2-6 months of life compared to infants that did not develop the condition. To collect the eye tracking data, infants were shown video scenes of a female actor playing a caregiver while being recorded with concealed eye tracking cameras set into the monitor. This method of showing video clips was also used by Speer and colleagues (2007) who explored face processing in ASC children with four types of stimuli conditions: social static, social dynamic, isolated static, and isolated dynamic. Notable results were found only in the social dynamic condition where ASC individuals spent significantly less time looking at the eyes of the persons depicted in the video clips than the control group. This finding suggests that aberrant eye gazing behaviour in ASC could be at least partially dependent on the nature of stimuli displayed, with dynamic social stimuli evoking a stronger reaction in eye gazing patterns.

Despite Speer and colleagues (2007) only finding significant gaze alterations in the social video condition, there have been several studies using static images that have reported differences in ASC eye gazing behaviour (Sterling et al., 2008; Neumann et al., 2006; Dalton et al., 2005). For example, Hernandez and colleagues (2009) used pictures of neutral, emotional, and virtual faces as social stimuli. The eye tracker was embedded in the casing of the computer screen displaying the images. In a sample of 23 neurotypical males and 11 ASC males they found that the subjects with autism spent almost half as much time looking at the eye region of the presented stimuli compared to the control group. Another eye tracking study using static images wanted to investigate the influence of competing non-social objects on eye gaze toward social stimuli (Cai et al., 2022). While being recorded with an eye tracker set into the screen casing, ASC and neurotypical male children viewed stimulus pairs of social (face) versus non-social (object) stimuli being presented in black-and-white or colour versions to vary salience. They found a significantly reduced viewing time at the eyes, nose, and mouth in ASC children compared to their neurotypical peers. This difference was observed independent of the salience of the paired non-social objects, indicating a general greater interest towards the non-social compared to the social stimuli.

Although eye tracking studies using static or dynamic social images have produced corroborating evidence for decreased eye gaze in ASC individuals, the study design properties do not resemble real-life social situations. Therefore, it seems likely that passive viewing of pictures or videos does not evoke the same eye gazing behaviour as having an actual interaction with another person. Individuals scan and view faces differently depending on if they are viewed as well or interacting with someone (Cañigueral & Hamilton, 2019; von dem Hagen & Bright, 2017). Thus, in the last decade researchers have started to conduct eye tracking studies with real-life interaction settings to observe more naturalistic social eye gazing patterns of people on the spectrum. As one of the first research teams to do so, Noris and colleagues (2012) created a naturalistic situation to investigate gaze behaviour in ASC children. Subjects wore a head-mounted eye tracking device that simultaneously recorded their eyes and an image of their viewing field while having a 5-10-minute interaction with the experimenter. Results showed that children with ASC viewed the experimenter's face significantly less than their neurotypical peers, exhibiting more downcast and lateral gazing of the visual field. Another study, using an adult sample, explored social interaction difficulties in individuals on the spectrum during a semi-structured interview (Hanley et al., 2015). While wearing a head-mounted eye tracker mounted on a bicycle helmet, subjects had a brief conversation of approximately 3.5 minutes with the experimenter. The authors found significantly reduced attention to others' eyes for the ASC group, alongside increased fixation time towards the mouth, supporting older findings (e.g. Klin et al., 2002) and extending them to social interaction settings beyond screen viewing. The employment of naturalistic experimental settings is an important advance in eye tracking, because it allows researchers to generalise results to genuine social situations and to provide causal theories with more ecologically valid findings.

While decreased eye contact has been established in various eye tracking studies and across different social stimuli modes, the reason as to why individuals on the spectrum display atypical eye gazing behaviour remains uncertain. One popular theory is that social stimuli, particularly the faces and eyes of others, bear less saliency for people with autism (Stuart et al., 2022; Baron-Cohen, 2000). Following this thinking, reduced social interest typical for ASC could then stem from social features being less informative or rewarding, resulting in individuals being less motivated to focus on them (Neumann et al., 2006; Grelotti et al., 2002). Another possible explanation has been supported by consistent reports as well as neuroscientific measures: The "eye avoidance" hypothesis suggests that eye contact causes hyperarousal and emotional distress, resulting in the avoidance of eye contact (Trevisan et al.,

2017; Tanaka & Sung, 2013). Neural substrates show an increased activation of the fusiform gyrus and amygdala in ASC individuals when concentrating their attention on the eyes compared to neurotypical subjects. The amygdala plays a central role in the detection, processing and interpretation of social and emotional stimuli, causing responses to emotionally salient or threatening cues. The hyperarousal of these brain regions could be experienced as unpleasant, consequently leading to eye aversion (Tanaka & Sung, 2013; Dalton et al, 2005).

While theories are still being investigated, research has concurrently started to explore possible interventions for the eye gazing behaviour in ASC. Besides various therapy forms (e.g. CBT or music therapy; Sharma et al. 2018) and other pharmacological treatment, one promising approach is the administration of oxytocin (OXT) which will be the focus of the next chapter.

2.4. Genetic Factors in ASC

ASC is a neurobiological disorder and, as with every other mental phenomenon, it is a complex challenge to infer causal elements or definite markers of the condition. Since the implementation of imaging techniques, there has been a lot of research done on a structural, functional and genetic level with the aim of identifying specific differences that could explain neurodivergence. For this thesis, only the genetic background of ASC bears relevance and will therefore be closer elaborated with a subsequent focus on the oxytocin receptor gene (OXTR).

Determining the aetiology of ASC has been a central research point ever since its first description in 1943 (Strathearn et al., 2018). Today, there is consensus that the condition develops through complex interactions between genetics and environment. Heritability studies have shown significant concordance rates between monozygotic twins and considerably lower but still elevated rates between siblings, resulting in an estimated heritability rate of 40-80% (Rylaarsdam & Guemez-Gamboa, 2019). Hundreds of genes have been linked to autism, yet no definite genetic pattern or pathogenic variation has been identified so far, indicating that ASC presumably is a highly polygenic condition and there is no one gene causing it. Multiple genetic factors interplay with multiple non-genetic factors, causing unique genetic modulations – possibly along with other health conditions – which lead to the highly heterogenous genotype and phenotype that is found among the population of ASC individuals (Genovese & Butler, 2020).

Genetic modulations, for instance, can be caused by copy number variations (CNV). CNVs are small structural alterations in chromosomes that entail duplications, deletion, translocations and inversions of genes that can be concentrated on one DNA strand or stretch over several strands. They can be inherited or occur *de novo*, meaning that they arise due to epigenesis which is the alteration of genetic material due to the interaction between internal and environmental influences (Rylaarsdam & Guemez-Gamboa, 2019). There are a number of CNVs that have been found to be significantly correlated with ASC. However, detecting CNV mutations does not automatically imply the presence of ASC as there are individuals with gene alterations that do not have autism as well as there are ASC individuals that do not have gene mutations (Marshall et al., 2008). Furthermore, individuals with autism that have identical CNVs can still vastly differ in phenotypic presentation and severity of symptoms. This highlights the extent of the impact that interactions with other susceptible genes and the environment have on the expression of ASC (Rylaarsdam & Guemez-Gamboa, 2019).

One specific gene that has been linked to autism regularly is the oxytocin receptor gene (OXTR). The gene is expressed in various parts of the human body. It has been located in reproductive structures, the mammary glands, as well as in several areas of the central nervous system (CNS) like the hypothalamus, amygdala, hippocampus, frontal cortex, and olfactory nucleus (Moerkerke et al., 2021; Loparo & Waldman, 2014). The OXTR gene has been particularly interesting in relation to ASC because of the functions of the hormone it regulates: oxytocin (OXT). OXT is involved in a wide array of human processes such as birth labour, breastfeeding, stress regulation, attachment processes, as well as social interaction and facial expression recognition (de Oliveira Pereira Ribeiro et al., 2017; Feldman et al., 2011; Lee et al., 2009). The latter three depict socio-cognitive aspects that are impaired to varying degrees in individuals with ASC, which has subsequently led to the hypothesis that OXT could be involved in the aetiology and expression of autism.

Focusing on just the receptor gene in this chapter, research has found several gene variations within the OXTR gene that have been significantly associated with ASC. Variations within a gene are called single nucleotide polymorphisms (SNP) and represent changes within a gene that commonly happen in humans. SNPs usually have descriptive marker names made up of letters and numbers that signify different locations within the genome where the variation happens (Vallejos-Vidal et al., 2020). Polymorphisms that have been successfully linked to ASC are rs2268493, rs7632287, rs237887, rs2268491, rs53576 and rs2254298 (Loparo & Waldman, 2014; Di Napoli, 2014; Liu et al., 2010; Harony & Wagner, 2010).

Another genetic modification that could have significant impact on the development of ASC is DNA methylation. A recent review by Moerkerke et al. (2021) that looked closer at the specific workings of OXTR gene variation mechanisms, summarised study findings stating that gene methylation is associated with autism and connected traits. DNA methylation is an epigenetic mechanism that is used by cells to control gene transcription and occurs by adding a methyl group to the DNA. As a result, it can change the function of the affected genes and the amount of genetic factors being expressed. The meta-analysis reported a significant correlation between OXTR hypermethylation and ASC traits in adults. Increased methylation causes a decrease in OXTR gene expression, meaning that less OXT receptors are generated, possibly affecting social behaviour in individuals with ASC (Gregory et al., 2009). Contradictory to the hypermethylation findings, hypomethylation was found in children with ASC, implying that there could be a change at some developmental stage where overproduction of the OXTR gene in childhood switches to underproduction in adulthood. The authors suggested that initial hypomethylation could explain aversive sensations ASC children experience during social encounters that are then countered gradually by developing hypermethylation of the OXTR system (Moerkerke et al., 2021). These theories, however, are yet to be tested in further studies and any causative or consequent effect of OXTR gene alterations are speculative at this point. Nonetheless, these findings seem encouraging for research on behavioural and interventive levels.

2.5. Environmental Factors in ASC

While genetics do play a substantial role in the aetiology of ASC as a neurodevelopmental condition, the impact of environmental factors should not be underestimated. Circumstances during pre-, peri-, and postnatal periods represent another area that has been thoroughly examined for potential elements facilitating or impeding the presentation of ASC. In this chapter, discussion of risk factors and protective resources will be limited to the most consistently evidenced and established ones in currently existing literature.

2.5.1. Risk Factors

One big risk factor that has been explored in depth is parental age. Studies have consistently found an elevated risk with progressing age, meaning that older parents are at an increased risk for ASC development in their offspring (Bölte et al., 2019; Gialloretti et al., 2019; Modabbernia et al., 2017). A meta-analysis of 27 studies found an elevated risk by 41% for higher maternal age and by 55% for higher paternal age (Wu et al., 2017). However, higher age was not closer defined in this review, leaving the exact age from which chances

increase open for interpretation. The review by Gialloretti et al. (2019) summarised an elevated risk in advanced parental age at ≥ 35 years.

Regarding the prenatal period, hypertension seems to be a relevant risk factor. In a review that analysed 20 independent studies, elevated blood pressure in pregnant women was found to yield a small but significant increase in the chances of ASC development compared to controls without hypertensive health issues (Maher et al., 2018). Another prenatal risk factor that has been explored in detail is maternal infection or immune activation during pregnancy. The meta-analysis by Bölte and colleagues (2019) summarises a twofold risk for ASC for maternal influenza and an increased risk by 30% for any other infection during pregnancy. However, it is unclear if the viruses or bacteria are responsible for this elevated risk or if the maternal immune response could play the greater role. Two human studies (Brown et al., 2015; Brown et al., 2014) and several animal studies (Bauman et al., 2014; Malkova et al., 2012) support the theory that maternal immune reactions and elevated antibody levels are the underlying risk factor for offspring with ASC.

There are several perinatal factors that have been discussed as potential risk factors (e.g. caesarean delivery, induced labour, gestational age ≤ 36 weeks) but the most consistent findings have been reported on complications ensuing hypoxia which is the deprivation of sufficient oxygen supply. It is important to note here that substantial oxygen at this critical stage does not just elevate the risk for ASC but also for many other neurodevelopmental, neurological and psychiatric disorders later in life (Bölte et al., 2019).

Other risk factors such as medication, pesticides or psychosocial circumstances have been investigated, yet reports on their effects in regard to ASC are relatively inconsistent and need further research.

2.5.2. Protective Factors

There has been far less research done on potentially protective resources in regard to ASC, although in recent years more findings have emerged. Still, unlike the mentioned risk factors, these following protective factors are not as well established and need further examination.

Complementary to the risk factor of advanced parental age above, low parental age has been discussed to be a potential protective component. The meta-analysis by Wu et al. (2017) has found a 10% decreased risk of autism linked with lower maternal age and a 20% reduced risk with lower paternal age. The number of children a woman gives birth to could also have a protective effect. A Finnish study investigated a large sample with data from the Finnish Prenatal Study of Autism (FIPS-A) and found a decreasing risk with increasing parity of four or more children compared to firstborns (Cheslack-Postava et al., 2014).

Prenatal vitamin nutrition levels could be an important protective agent in reducing the chances of ASC. In the literature vitamin D, fatty acids like omega-3 and folate in particular have been investigated more closely. Folate is a vitamin B nutrient that is important for cell production and maintenance, including DNA synthesis and methylation. Supplementation of folic acid before and during pregnancy is known to support neurobehavioral development and ensuing social, cognitive and verbal functioning (Bölte et al., 2019; Gialloretti et al., 2019; Karimi et al., 2017). Another group of nutrients that could be a protective influence during development are fatty acids. Sufficient fatty acid levels during pregnancy are important for foetal brain development, signal transduction, and cell functioning. Especially critical during the first two months of pregnancy, supplementation of fatty acids like omega-3, omega-6, and linoleic acid correlated with a 34% reduction in autism risk (Lyall et al., 2013; Karimi et al., 2017). Even though research on protective factors has increased in the past few years, there is still a great level of unexplored elements that could have a positive developmental influence regarding ASC, conveying the need for more resource centred studies.

3. Oxytocin (OXT)

Oxytocin is an evolutionary important neuropeptide that has been produced over centuries in humans and animals alike (MacDonald & MacDonald, 2010). This has enabled past research to conduct a wide variety of studies on animals to investigate its neurological workings. Although animal studies have contributed tremendously to the scientific knowledge of today, to stay in scope, this thesis will solely focus on human research. Thanks to the advances in human-based neurobiological research, there is a sufficient number of clinical studies by now to found the following segments about OXT and its effects, particularly in regard to ASC.

3.1. Properties and physiological distribution

OXT is a versatile player of the CNS, working both as a hormone and neuropeptide transmitter in the human organism. Containing nine amino acids, it is a polypeptide that is mainly produced in the hypothalamus and deposited in and secreted by the pituitary gland. From there it is distributed to many regions of the body and mainly functions as a neuromodulator, meaning that it influences the transmission between neurons by enhancing or inhibiting their neural signals (Florea et al., 2022). As a result, the hormone plays a complex and important role in many neural and biological processes which will be discussed further in the following section. Besides the hypothalamus, OXT is also generated in numerous other areas such as the amygdala and paraventricular nucleus but also peripheral sites like the

pancreas and the gastrointestinal tracts. In accordance with the widespread production of it, OXT shows its effects in various body localities such as the male and particularly female reproductive organs, the digestive system, cardiovascular system but also in hydro-electrolytic regulation, fatty and muscular tissues (Florea et al., 2022).

3.2. Natural functions in humans

Before discussing the investigated effects of OXT via exogenous administration in *Chapter 4*, this segment will look closer at studies that explored the naturally prevalent roles the hormone plays in human behaviour. Starting with the biological event that has gifted OXT its name (oxytocin derives from the Greek words for "quick birth"), the peptide mediates childbirth and related processes like labour and breastfeeding (Macdonald & Macdonald, 2010). Along with other hormones like oestrogen, progesterone and prostaglandins, OXT initiates the onset of labour by being secreted in rhythmic pulses that are triggered by CNS and mechanoreceptor signals when the foetus is exerting pressure on the cervix and vaginal walls. This causes increasingly intense uterine contractions necessary for the progression and completion of birth. By the end of labour, OXT concentrations in the blood have increased 3-4 times compared to concentrations at labour onset. After birth, OXT remains to be important, initiating the mechanism of breastfeeding by causing milk release from the mammary glands when mechanosensitive receptors are activated by suckling of the infant (Walter et al., 2021).

Presumably the best-known behavioural aspect connected to OXT is social bonding. The high OXT levels during birth are associated with elevated bonding between mother and infant. Furthermore, higher plasma and salivary OXT levels in both mothers and fathers were positively correlated with the social engagement of the parent and child. The high levels also coincided with more parent-infant affect synchrony and positive communicative sequences between parent and child (Feldman et al., 2011).

Besides parent-child bonds, OXT also has importance for attachment in romantic relationships. Tops et al. (2007) found that in women OXT plasma levels were positively correlated with self-reported feelings of attachment on the *Temperament and Character Inventory* (TCI). Another study that looked at both sexes examined plasma OXT in 163 individuals and found that the subjects in love had significantly higher levels than singles. Incidentally, OXT levels stayed consistently high at a follow-up six months later. It was significantly associated with the couples' interactive reciprocation, defined by measures like affectionate touch, positive affect and synchronized affective states which mirrored the findings from parent-infant bonding sequences (Schneiderman et al., 2012). Furthermore,

warm touch, affiliation and emotional support – all important for interpersonal relationships – corresponded with elevated OXT levels, further indicating the importance of the hormone in affiliative behaviour (Gonzaga et al., 2006; Lunstad et al., 2008).

Apart from exploring the connection of OXT and affiliative behaviour, research has also been investigating its link to stress regulation. While the function of OXT has been well established in animal stress models, there are few human studies that have examined the subject of endogenously released OXT in relation to stress. Segueing from the bonding segment, Feldman and colleagues (2011) found that OXT levels were positively correlated with relationship distress in women along with stress in the maternal role and maternal attachment anxiety. The authors hypothesized that this OXT increase during stressful moments could activate a behavioural response that would lead to increased affiliative social actions meant to reduce distress. Another finding that only applied to women reported an increase in plasma OXT of female participants after they were exposed to uncontrollable noise (Sanders et al., 1990). Investigating both male and female participants, two recent studies found significantly elevated levels of OXT in response to psychosocial stress in both sexes (Bernhard et al., 2018; Engert et al., 2016). Bernard et al. (2018) found that higher baseline OXT levels seemed to have an anxiolytic effect and led to less increased hormone responses while the opposite applied to lower baseline OXT levels. Additionally, Engert et al. (2016) reported that a higher stress-induced OXT secretion resulted in faster vagal rebound, indicating that secretion of the hormone could expedite recovery from stress.

Lastly, one more human behaviour that endogenous OXT could play a role in is aggression. Although difficult to ethically observe in human subjects, studies that have used competitive computer games or questionnaires have repeatedly reported a negative association between OXT levels and aggressive tendencies. One study found that basal plasma OXT levels in boys with ADHD were negatively correlated with aggression, rated via the *Buss-Perry Aggression Questionnaire* and positively with empathy (Demirci et al., 2016). Measuring the OXT level in the cerebrospinal fluid, Lee et al. (2009) observed a significant negative correlation between aggression and OXT base levels, assessed with *Life History of Aggression* interviews, in both healthy subjects and individuals with various psychiatric diagnoses.

All of these findings on endogenous OXT functions in human behaviour have been further supported by a variety of clinical studies working with administrated OXT to gain further insight into the possible effects of the hormone. Before going into detail on that, the

prevailing application method in behavioural peptide research that is also used in this thesis study will be introduced.

3.3. Intranasal OXT application

In research, there are various ways to administrate substances of interest, the most common ones being oral, intravenous, and intranasal application. Regarding OXT, clinical testing and practice has mainly employed the latter two application methods. Initially used in the 1950s and habituated since then, intravenous OXT is frequently found in clinical contexts to control the process of labour (Kendrick et al., 2018). Apart from its effects on the physical level however, human-based research on the behavioural effects of OXT was for a long time limited. This was due to the hormone's structural properties and the main application method being intravenous. When administered intravenously, the blood-brain barrier keeps the majority of the exogenous OXT from reaching the brain and CNS which impeded research focusing on the neuropeptide's effect on neural functioning. Following novel testing on animals, where invasive intracerebroventricular infusion (ICV) had been the common method to examine peptide functions on the cerebral level thus far, intranasal application was finally introduced to human-based research as an innocuous alternative to ICV. By using the intranasal route, the blood-brain barrier problem is bypassed as substances can enter the brain via the olfactory and trigeminal nerve, as well as some other peripheral routes whose mechanisms are not fully established at this point (Yao & Kendrick, 2022).

Since the first milestone paper that reported successful and direct access of neuropeptides to the cerebrospinal fluid in humans via intranasal application (Born et al., 2002), multitudes of studies have been published that have been employing the intranasal method. The predominantly used administration technique has been via nasal spray devices but in recent years nebulizer devices have entered the field as an effective and potentially superior instrument. Proportionally few studies have conducted their substance administrations with a nebulizer device but the existing findings suggest that this technique could be more efficient in transporting the neuropeptide directly into the brain (Yao & Kendrick, 2022). It is generally suggested that the ideal method for intranasal peptide application is to propel the droplets into the most posterior part of the nasal cavity for uptake via the olfactory and trigeminal nerve. As a result, nebulizer devices may be more efficient as they create smaller droplets of neuropeptide particles and have a larger force in moving the particles farther up the nose (Djupesland, 2013). This advantage in efficiency could consequently lead to a lower required

dose of substance for similar functional effects when using a nebulizer compared to the standard nasal spray device.

4. OXT and ASC

The intersection of OXT and ASC in the socio-behavioural context constitutes the core of this master thesis and is presented by first discussing findings on endogenous OXT levels in ASC individuals and then focusing on the exogenous administration effects of the hormone. Research on OXT effects is first reviewed for neurotypical samples before finally concentrating on the ASC population.

4.1. Endogenous levels of OXT in ASC individuals

Since starting the exploration of the link between OXT and ASC, research has also looked at potential serological differences – meaning variations in plasma, urine or saliva levels – compared to neurotypicals (= individuals with typical neurological development and functioning). The first report of diverging plasma levels was made by Modahl et al. (1998) who detected lower blood OXT concentrations in autistic boys relative to the control group. Since this seminal report, findings have been mixed, with some studies confirming lower OXT levels in ASC samples while others have failed to reproduce it. A meta-analysis by John and Jaeggi (2021) summarised the results of 31 studies and found relatively consistent evidence that OXT levels tend to be lower in autistic children. In addition to diverging OXT levels, 19 articles reported a negative correlation between OXT levels and ASC symptom severity, indicating a mediation of the socio-cognitive function by the peptide. However, these significances were only found in children samples.

There are considerably fewer studies that examined levels in adults and of the six articles included in the review by John and Jaeggi (2021), no significant differences were found between neurotypical and neurodiverse OXT levels. The authors hypothesized that the varying findings between ASC children and adults could suggest an increase in OXT levels as they grow older. Along with that, they cited a review of longitudinal studies in autistic samples showing improvement in severity of symptoms and adaptive functioning and communication over time until adulthood (Magiati et al., 2014). However, no OXT measures were collected in the studies that were included in that review and, considering all environmental and social influences that accompany human development, drawing conclusions on possible connections to the hormone based on these findings would not be on par with scientific standards. Another possible explanation for the differences in childhood OXT levels that John and Jaeggi (2021) stated may be that neurotypical children have

increased levels of the hormone, promoting social interaction and functioning, that decline over time with maturing.

To summarise, the connection between endogenous OXT levels and autism spectrum condition is not completely clear with lower plasma levels being relatively consistently reported in children with ASC, yet do not seem to persist until adulthood with no differences being found in comparison to neurotypical individuals. Research seeking to explore this association should focus on longitudinal studies as well as sampling across all age groups to gather new insights and improve understanding of the existing studies so far.

4.2. Effects of exogenous OXT

4.2.1. Effects in Neurotypical individuals

In the past decades and especially since the new century there has been a plethora of publications in research that employed intravenous and intranasal OXT to investigate its functional and potentially therapeutic effects for translational medicine. The studies span a wide range of social behaviour, reaching from investigations about bonding to exploring the role of the hormone in connection to envy and other negative social feelings.

Beginning with bonding behaviour, there have been some studies investigating it after intranasally administrating the hormone. A study by Scheele et al. (2013) explored the behavioural ratings and neural responses of men for face stimuli of long-term romantic partners. Participants were shown photographs of their partner, of an unfamiliar attractive woman or of a nonface control stimulus after having received either intranasal OXT or placebo. The results showed that OXT significantly enhanced the attractiveness of the loved one's face compared to other women, endorsing evidence that the hormone contributes to romantic pair bonding in humans. Another study focused on couple communication and the effects of exogenous OXT during conflict (Ditzen et al., 2009). 45 minutes after randomly administrating the neuropeptide or placebo, couples were instructed to discuss a conflict topic for ten minutes that they had selected prior to the testing session. The results showed that OXT significantly increased the amount of positive relative to negative behaviour during the conflict discussion, adding to positive relationship behaviour and bonding style.

Another area of sociability that has been focused on for studying OXT effects is trust. The well-known first paper on increased trust in others due to administration of the hormone was published by Kosfeld and colleagues (2005). Participants had to play the *Trust game*, a decision-making game with real monetary stakes where one plays either the part of the trustee or investor. Both roles need to make choices about money – the option with the biggest

benefit requires the biggest risk taking and trusting in the other player. Study results showed that the subjects' willingness to accept social risks and to trust the other participant was significantly enhanced after the administration of intranasal OXT compared to the placebo group. A few years later, Baumgartner and colleagues (2008) conducted a similar trust game and additionally found that exogenous OXT affected behavioural adaptations in participants when being informed that their trust had been broken. While the placebo group decreased their trusting behaviour towards the other party after receiving feedback of the betrayal, OXT subjects did not. They also needed significantly less time to make a trusting decision, indicating that the hormone leads to a reduced fear of social betrayal.

A reduction of fear seems to be a general effect of exogenous OXT as several neuroscientific studies have shown over the years. The forerunner study by Kirsch et al. (2005) found significantly decreased activation of the amygdala after OXT administration when viewing fearful visual stimuli. As mentioned before, the amygdala is an important brain region for the detection, processing and interpretation of social and emotional stimuli and is also activated in situations of possible danger. Its reduced activation therefore suggests a diminished fear response. Another more recent neuroscientific paper (Kreuder et al., 2020) backed these early findings, reporting that OXT induced lower responses of the amygdala to fearful faces compared to neutral faces. Two other studies that explored the effects of OXT on psychosocial stress showed that the hormone significantly decreased saliva cortisol levels after being exposed to a social stress task, further speaking for an anxiolytic effect of administrated OXT (Linnen et al., 2012; Heinrichs et al., 2003).

Focusing on prosocial effects again, OXT seems to promote empathic behaviour in humans. Having been explored over the last few decades (e.g. Hurlemann et al., 2010), two recent intranasal OXT studies have once again found evidence for the hormone's involvement in empathy, specifically emotional empathy. Le and colleagues (2020) as well as Geng and colleagues (2018) found that after administration of OXT, emotional empathy was significantly increased, assessed by conducting the *Multifaceted Empathy Task* (MET). Results were significant across both sexes and across cultures (Caucasian and Asian; Geng et al., 2018). Another study that investigated cognitive empathy reported that, additionally to improving empathic accuracy, OXT effects were greater in individuals that were less socially skilled (Bartz et al., 2010). This speaks for a selective benefit of OXT and highlights the complexity of working with and understanding the workings of the neuropeptide.

OXT effects can be modified by various factors like a person's sex, context or genotype as well as their personality traits or current emotional state, affiliation style or variables based on

the situation. Even though much progress has been made in this domain, reliable prediction as well as replication of exogenous OXT effects can be challenging as they are subject to this variety of influencing factors (Kendrick et al., 2018). This varying susceptibility to OXT effects has been tried to be explained with an impactful theory, the *social salience hypothesis* of OXT. It suggests that the hormone acts by amplifying the salience of social cues which, based on what the individual considers to be salient, can differ depending on person and context. As a result of this increased salience for certain stimuli, it can promote a wide range of emotions and behaviour (Shamay-Tsoory & Abu-Akel, 2016). This theory is also in line with findings on administrated OXT that led to non-prosocial behaviour and feelings. In a study by Shamay-Tsoory et al. (2009), feelings of envy and gloating were increased after intranasal OXT and De Dreu et al. (2016) showed that it promoted out-group discrimination and defensive aggression in favour of in-group love and cooperation, meaning that negative feelings toward the out-group were motivated based on in-group preference rather than hate towards outsiders.

Social salience is also involved in social cognition and recognition, two more mental processes that have been investigated with great interest in regard to OXT. The recognition and memory of social stimuli is an evolutionary important aspect of interpersonal relationships, helping to build and maintain them. To find out if the neuropeptide was connected to the recollection of social stimuli, Rimmele et al. (2009) conducted an intranasal OXT study where they presented pictures of human faces and non-social stimuli after the administration of the hormone or placebo. The following day, they were asked if they remembered or knew any of the randomly displayed stimuli. Results showed a significant increase in the recognition for faces but not non-social stimuli in the OXT condition compared to the control group. Similarly, several studies have reported improved recollection of emotional facial expressions in humans after intranasal OXT, although findings on whether negative or positive emotions were enhanced differed across studies (Lischke et al., 2012; Marsh et al., 2010; Fischer-Shofty et al., 2010; Di Simplicio et al., 2009).

Intranasal OXT also seems to affect the socio-cognitive process of “mind reading”. The ability to infer other peoples’ state of mind and their intentions by reading social cues like body language or facial expressions is an essential part of human interaction. As already mentioned in *Chapter 2.5.3*, eye gaze appears to play an important part in obtaining this social competence. A seminal study by Domes and colleagues (2006) was the first to show that administrated OXT significantly improved subjects’ ability in affective mind-reading by interpreting subtle cues from the eye region. It was measured with the widely used *Reading*

the Mind in the Eyes test (REMT) where subjects have to match pictures showing only the eye region with various mental or emotional descriptors. Similar findings followed with Guastella and colleagues (2008) and Domes and associates (2013) who found that OXT increased the number of fixations and total gazing time toward the eye region in neurotypical individuals compared to the placebo condition. One recent study (Le et al., 2020) further reported a significant shift of eye gaze towards faces due to intranasal OXT facilitating emotional empathy, corroborating the earlier findings that demonstrated enhanced social processing and competence by intranasal administration of the hormone.

Summarising the information from above, OXT overall appears to be a promising tool for emphasizing social cognition and functioning which has led research to explore the possibility of it becoming a therapeutic adjuvant in treating various psychiatric conditions, one of them being ASC.

4.2.2. Effects in ASC individuals

Arriving at the most significant theoretical segment of this thesis, this chapter will focus on the findings that have been made in relation to the effects of OXT on social functioning in Autism Spectrum Condition individuals. People with autism often have considerable deficits in social skills which affect their relationships, education, and employment opportunities as well as overall quality of life. Of course, as the condition is located on a spectrum and degrees of severity vastly differ, there are individuals that subjectively do not feel impairment in any given life situation. Nonetheless, for more severe cases of ASC where individuals or their family do sense a need for intervention, OXT has been speculated as a supportive element for interpersonal therapy options in the future. Even though an increasing amount of clinical studies has been conducted in the last few decades to investigate the effects of administrated OXT on ASC individuals, there is still substantial demand for further research with more refined methods and implementation. Before introducing the present study pursuit and research question, the current state of knowledge will be reviewed.

The first two studies investigating the effects of exogenous OXT in autistic individuals were conducted by Hollander and colleagues (2003; 2007). Contrary to the following studies discussed thereafter, they used an intravenous application method of OXT in both studies, indicating that intranasal application had not yet become the predominant practice it is today. In the first study, a within-subject design was applied with 15 male ASC subjects receiving either OXT or placebo infusions over a four-hour period and the other condition on the second procedure day. Results showed that, after OXT administration, the number and strength of repetitive behaviour symptoms significantly decreased and social cognition significantly

increased compared with the placebo condition (Hollander et al., 2003). The second study by the research team examined the effects of intravenous OXT on the processing and retention of social information. Sample size and study design were the same as in the study from 2003 with additional measurements pre- and post-infusion on each day. Here they found a significant increase in social information processing and retaining after OXT administration. Additionally, they reported a carryover effect, saying that individuals who had received OXT first, seemed to maintain their ability level from the first testing point whereas those that had received placebo first, did not.

The first OXT study with ASC individuals that used intranasal application via nasal spray wanted to investigate affective mind-reading and emotion recognition via the *REMT* (Guastella et al., 2010). In the sample of 15 male adolescents, the hormone significantly increased the ability to read emotions of facial expressions based on pictures of just the eye region compared to the placebo treatment. Domes et al. (2014) also looked at emotion recognition in ASC individuals. Compared to neurotypical individuals, they initially showed a significantly decreased performance in the placebo condition which significantly improved after intranasal OXT administration. They also reported that the OXT effect was larger for emotion identification from the eye region relative to the mouth region, an effect that was not observed in the neurotypical group.

One more study that focused on this specific socio-emotional competence in ASC individuals was conducted by Aoki et al. (2014). They employed an intriguing study design to examine the effects of OXT on the inference of others' social emotions and beliefs: Other than the previous studies (Guastella et al., 2010; Domes et al., 2014) that used direct emotional cues, they created a task based on the well-known *Sally-Anne test* that implicitly tested these measures. This test was initially developed to examine children's theory of mind, the ability to understand how other people behave, think and feel based on their mental states. As mentioned before, this social maturing seems to be impaired in ASC individuals (Baron-Cohen, 2000), making this study particularly relevant and innovative for investigating ASC social cognition by using a *Sally-Anne test* inspired task. Results showed that initial low accuracy in inferring other's social emotions compared to the neurotypical control group was significantly improved after intranasal OXT administration in 20 male ASC individuals, corroborating the preceding findings.

To examine decision making and affect in a social interaction, Andari and colleagues (2010) conducted a simulated ball game where ASC participants had to interact with fictitious play partners that exhibited different amounts of reciprocation in tossing the ball back,

making them good, neutral or bad cooperative players. The authors found that after receiving OXT intranasally, participants engaged significantly more often with the good player compared to the bad one and tossed significantly more balls to them. After completing the task, they also reported a stronger trust and preference towards the good player than the bad player compared with the placebo condition. Additionally, the researchers conducted a computer eye tracking task where participants were presented with pictures of human faces and were asked to name either gender or gaze direction, depending on the instruction. Analysis of the eye tracking data showed that OXT significantly increased the amount of time participants spent scanning the faces, particularly the eye region. This study marked the first instance where increased eye gazing behaviour after administration of OXT in ASC individuals was reported, making it a critical mention for this thesis.

After this initial finding, only few other studies have explored this OXT-induced gazing behaviour change towards the eye region of humans in individuals on the spectrum. Starting with the most relevant study for this thesis as its design strongly resembles the present one and served as inspiration to conceptually replicate it, Auyeung et al. (2015) conducted an eye tracking task to explore this intersection between intranasal OXT, ASC and eye gazing behaviour. What distinguishes it from other published eye tracking studies is that instead of showing remote video clips or images to participants for data collection, the authors examined gazing behaviour during a real-time interaction with a research assistant. By having a real interpersonal conversation as experimental task, potential limitations of computer tasks can be avoided and external validity is increased. 45 minutes after administration of a placebo or OXT nasal spray, subjects were asked several questions in a semi-structured interview style while wearing the eye tracker and being recorded. The final sample was comprised of 32 male ASC individuals and 34 male control subjects, matched for IQ and age. Analysis of the placebo condition showed that ASC individuals exhibited significantly less gazing time and fewer fixations to the eye region of the face compared to the control group. Administration of OXT resulted in a significant increase of total fixations and gazing time to the eyes for both the ASC and control group. Additionally, within the ASC group the hormone had a greater effect on gazing time in those individuals that displayed lower eye contact in the placebo condition. This finding suggests a treatment effect of OXT that could be especially beneficial for autistic individuals that have greater issues with eye contact.

Another study that explored eye gazing behaviour in a clinical trial conducted an eye tracking experiment with 73 individuals scoring high on the *Autistic-Spectrum-Quotient* (AQ; Marsh et al., 2021). They were presented with different categories of black-and white pictures

of human faces to explore if OXT administration modulated gazing preference. The four categories were pictures of their romantic partner's face, the face of a close friend, of themselves or a stranger's face. Results showed that participants spent significantly more time looking at the eye region of familiar faces, namely their partner's or friend's face, in the OXT condition. Moreover, they found a negative interaction between gazing time towards the eyes and AQ scores, meaning that individuals with higher autistic traits spent less time looking at the eye region of faces.

This connection between AQ score and gazing behaviour was also reported in a recent study by Le and colleagues (2020). They explored the eye gaze of individuals towards social vs. non-social stimuli and various facial expressions and showed that there was a negative association between ASC traits and gaze towards social stimuli in both the placebo and OXT condition. For facial expressions they found that the neuropeptide only enhanced gazing time towards the eyes of fearful faces but not towards other expressions (happy, angry, neutral). This bias for fearful faces, however, was not replicated and even countered in another study that was led by the same researcher (Le et al., 2022). The study team conducted a six-week program of administrating OXT every other day in young autistic children (three-eight years) that incorporated positive social interactions with caregivers after each dose. Results showed that OXT administration increased the time spent looking at the eyes of happy, angry, and neutral faces while actually looking less at the eyes of fearful faces. Besides the effects on eye gazing behaviour, the neuropeptide treatment significantly reduced autistic symptoms, measured with the universally recognized *Autism Diagnostic Observation Schedule-2* (ADOS-2) and the *Social Responsivity Scale-2* (SRS-2). The subsequent period of social interaction with a caregiver after administration may have been one of the key elements for the treatment efficacy of this study.

Having introduced this most recent study of clinical longitudinal trials by Le and colleagues (2022), several other studies have been published before that which have also looked at the effects of long-term OXT administration in autistic subjects. The earliest one by Anagnostou and colleagues (2012) employed a six-week treatment trial of intranasal OXT in adults with ASC. Participants received placebo or OXT twice a day over the span of six weeks via a self-applicated intranasal spray. After the end of the trial, results showed a significant improvement in measures of social cognition (REMT), lower-order repetitive behaviours like stereotypies (*Repetitive Behaviour Scale-Revised*; RBS-R) and emotional well-being as an aspect of quality of life (*World Health Organization Quality of Life Questionnaire;* WHOQOL). No significant side effects were reported, making it the first

longitudinal study showing potential and safe therapeutic admission of intranasal OXT with effects in socio-behavioural domains. Following these findings, an Australian research team conducted a five-week clinical trial in ASC children to explore the hormone's potential effects on social interaction deficits (Yatawara et al., 2016). Being the first ones to examine longitudinal OXT effects in an ASC children sample, they found that a twice-daily administration of OXT over the course of five weeks resulted in significantly improved scores on the *Social Responsiveness Scale* (SRS-P) that were further backed by ratings of the experimenters evaluating clinical global improvement in OXT condition subjects. Contrary to the findings by Anagnostou et al. (2012), there was no observed reduction in repetitive behaviour measured via the *RBS-R* or other secondary measures. In concordance with the significant findings on the *SRS* improvement however, another more recent longitudinal study also reported an increase in the *SRS* score in children with autism after an intranasal OXT treatment trial of four weeks with two doses per day (Parker et al., 2017). Furthermore, they discovered that pre-treatment plasma OXT levels predicted treatment outcome, meaning that the individuals with the lowest initial OXT concentrations showed the greatest improvement in social abilities.

Having described the positive findings, it is important to also mention studies that have failed to replicate OXT-induced effects. Dadds and colleagues (2014) reported that the subsequent intranasal administration of the neuropeptide once a day over the span of five days did not significantly enhance socio-emotional behaviour in male children with ASC. Yet, the choice of pre- and post-measures as well as study design may not have been suitable to find effects after a five-day treatment trial. Another study that investigated the hormone's effects in ASC youth was also not able to find significant improvements after repeated application (Guastella et al., 2015). The authors conducted a study over the course of eight weeks where male adolescents with autism received OXT twice a day and did not show significant change in any of the socio-behavioural measures. Lastly, a large-scale study from 2021 that spanned 24 weeks of daily intranasal OXT administration failed to reveal OXT-induced refinement in measures of social or cognitive functioning (Sikich et al., 2021). However, the reliability of this recent treatment trial is not fully convincing as the primary outcome measure had not been validated and dosages differed vastly within and between subjects from 8 to 80 international units (IU), with some receiving steadily increasing OXT doses and others staying on low substance portions.

There are some limitations to all of the mentioned studies, regardless of their outcomes, which complicate direct comparison between studies and generalisability to the larger ASC

population. Firstly, every introduced study recruited high-functioning individuals only (IQ > 70). From an executive research perspective this excluding factor may be reasonable and is usually accompanied by ruling out various psychiatric comorbidities with possible confounding effects as well. Nonetheless, as a result the sample compositions are not representative of the ASC population as a lot of people on the spectrum have at least one co-occurring psychiatric condition or intellectual disability (Hodges et al., 2019).

Another substantial factor impairing generalisability is the predominance of males in clinical samples. A meta-analysis by Alvares and colleagues (2017) reported that of all publications up until 2017 exploring the intranasal OXT-ASC intersection, only 3-7% of participants were female, depicting a stark difference in gender distribution. Stated reasons for this were unknown potential effects of OXT on the hormonal system of women and oppositely possible confounding influences due to their fluctuating female cycles. Because of that, most of the studies have chosen to recruit an all-male sample, limiting the significance of results in regard to the population.

Lastly, as there is still plenty of information left to be discovered concerning the workings of OXT in humans, there is no clarity as to which dosage or application method is the most ideal to induce socio-cognitive and behavioural effects. Most intranasal studies have used 24 IU per administration, yet this amount is due to scientific precedence rather than systematic reasoning and investigation, meaning that researchers chose to employ this dosage only because others before them had used that amount (Martins et al., 2022). It is also uncertain which type of application offers the most efficient absorption of the neuropeptide. Since discovering and establishing the benefits of the intranasal method by it circumventing the blood-brain barrier and directly reaching the neural system, nasal sprays have been the predominant tool of administration. As the most posterior part of the nasal cavity is suggested to be the optimal spot for absorption of neuropeptides, it has been questioned if nasal sprays are capable of sufficiently propelling the particles to this region. Because of that, nebulizer devices have been suggested to present a potentially better alternative, yet not enough studies have been conducted with it so far to be certain.

Additional to implementary limitations, the complexity of the hormone itself and varying personal and contextual conditions further complicate the findings of consistent results and deriving subsequent guidelines for the employment of exogenous OXT in therapeutic settings. Being aware of these obstacles and wanting to contribute to consequential research, the current project underlying this thesis is employing refined methods and materials to provide an improved base frame for testing and meaningful results.

5. Research question

5.1. Research question

Having outlined the current state of knowledge on Autism spectrum condition (ASC), oxytocin (OXT) and their intersection by exploring the latter's effects on cognition and behaviour in the former, the acquired research question of this thesis states: Is there an effect of Oxytocin on the eye gazing behaviour in ASC individuals?

As the past chapters on theoretical background have conveyed, the connection between ASC and OXT and particularly the exogenous effects of the hormone on socio-cognitive functioning have only started to be investigated closer in the last few decades. Despite there being a large translational and scientific interest, research progress has been made haltingly and has not offered enough clear results to procure directions for therapeutic application. Therefore, there is momentous need for further studies with refined designs and improved implementation that add to the current pool of knowledge and enable researchers and clinicians to confidently take first steps in generating directives for translational medicine.

To stay true to the intention of conducting improved and refined research for generalisable results, this thesis project takes inspiration from the notable study by Auyeung and colleagues (2015) and is designed as a conceptual replication. To reiterate details of the original study, the research team carried out a randomized, double-blind, placebo-controlled, within- and between-subjects design which is identical to the current study. The measure they used to repeatedly evaluate the social behaviour of participants in various conditions was a real-life interaction with a researcher, making it the first OXT study to employ an eye tracking task with naturalistic non-digital stimuli. Despite improved transferability of results to real-life situations, no study exploring the intersection between OXT and ASC has applied this social interaction task since. Thus, this thesis study is especially relevant in its demand to replicate and collect more data with this interactive task measure.

While fitting the description of a replication study, there are several aspects of the current project that diverge from the original study design. Firstly, the sample of this study will also include female participants. Back in 2015, Auyeung and colleagues (2015) recruited only male participants, limiting the universal significance of the study results. By adding female subjects to the sample, a greater chance for general inference based on the data is created. Another alteration concerns the method of intranasal administration. The original study used a nasal spray for intranasal application of OXT. As has been mentioned, this application technique may not be the most efficient in transporting the neuropeptide into the CNS. Hence, this study is employing a nebulizer device which has been suggested to be a more efficient

tool for intranasally administrating OXT. Only few studies have used this device and none in connection with ASC (Yao & Kendrick, 2022), making this study consequential not only for the future usage of nebulizers but also in offering first insights on administration efficacy for this population.

One last aspect that significantly differs from the original study is the dosage of OXT. Auyeung et al. (2015) used the most commonly applied amount of 24 IU via an intranasal spray. As this current study employs a nebulizer device instead, a lower amount of 10 IU was chosen as standard dose for OXT administration. This decreased dosage was chosen because one, existing literature suggests that nebulizer devices could be more efficient in administering the neuropeptide into the CNS; and two, this thesis is part of a bigger research project where a second substance – the opioid antagonist naltrexone (NAL) – is dispensed randomly across sessions. Hence, a higher OXT dose was decided against to avoid possible overstimulation of the participants' organism in sessions where both substances are administered.

To summarise, this thesis aims to investigate the effects of exogenous intranasal OXT on the eye gazing behaviour in individuals with ASC by conducting a real-life interaction eye tracking task, conceptually replicating the study of Auyeung and colleagues (2015) but refining it through adapted sampling, administration technique, and dosage. Besides creating the opportunity to see if the original study's results are generalisable, the modified methodological aspects offer improved conditions to obtain qualitative findings and contribute to the current knowledge pool of this still fairly unexplored intersection of OXT, ASC and eye gaze.

5.2. Hypotheses

There are two main hypotheses that have been derived from the presented literature and research aim:

H1: After administration of oxytocin, ASC individuals show increased gaze to the eye region compared to the placebo condition.

H2: In the placebo condition, ASC individuals show less gaze to the eye region than neurotypical individuals.

The hypotheses are based on previous studies which found that ASC individuals display decreased gazing to the eye region compared with the control group in the placebo condition and enhanced eye gazing behaviour in individuals with ASC after the administration of

intranasal OXT (Auyeung et al., 2015; Andari et al., 2010). Hypotheses will be investigated in the context of this thesis study and the bigger research study it is embedded in.

6. Materials and Methods

For transparency and better comprehension, it is explicitly stated at this point that this thesis is part of a bigger research study (BRS) that involves administration of the opioid antagonist naltrexone (NAL) and two more tasks. The additional data material collected in conditions where NAL is administrated and the extra tasks will not be analysed and discussed in this paper, as it is subject of the doctoral thesis by Raimund Bühler. Its focus lies on the combined effects of OXT and NAL on social behaviour in ASD individuals compared with a control group of neurotypical persons. Hence, when there is a mention of additional conditions or tasks when describing drug administration or testing procedure in this chapter, it is referring to aspects of the BRS that exceed this thesis' endeavours.

6.1. Participants

The sample for this thesis varies from the final sample of the BRS as their recruitment process continues until after this thesis has been completed. Hence, the sample used for this thesis analysis represents a subsample, differing in size and composition.

Neurotypical participants are recruited via a participant database of the University of Vienna, while neurodiverse subjects are recruited mainly by two means: Firstly, a database consisting of ASC individuals that have already participated in clinical studies before which is accessible to the supervisor of both the BRS and this thesis, Dr. Giorgia Silani. And secondly, by contacting organizations like "Autistenhilfe Vienna" that work closely together with ASC individuals and sharing flyers on Facebook. If interested, participants receive a link for an online screening where inclusion and exclusion criteria are assessed. Individuals needed to be between the ages of 18-65 and be fluent in German. Exclusion criteria included regular drug use or addiction, as well as diagnosis of a psychiatric or neurological disorder apart from ASC. Additionally, eligible ASC participants need to hand in an official attestation of their diagnosis to partake in the research project.

The sample for this thesis consists of 24 participants – 10 individuals with ASC (7 females and 3 males; mean age 30.7 years; *SD* 9.8 years) and 14 neurotypical persons (6 females and 8 males; mean age 26.3, *SD* 5,9 years). Subjects in the ASC group were predominantly female (70%) whereas the controls were skewed slightly male (57%). Out of all 24 participants, one individual (ASC, female) ended participation prematurely without stating reasons or concern.

6.2. Drug Administration

For this thesis, the administration of OXT and its effects on eye gazing in an ASC and a control sample are the subject of interest. In the BRS however, there is one more substance being included to investigate its functions, the opioid antagonist naltrexone (NAL). As a result, there are two time points during each session where substances get administered, once with a pill and once via a nebulizer device. For each session, substances are randomized and participants either receive the pharmacological component of interest or a placebo (PLA). This results in a total of four possible substance combinations for the BRS, namely PLA-PLA, PLA-OXT,PLA-NAL and OXT-NAL. For this master thesis only two of these conditions are of interest, PLA-PLA and PLA-OXT.

Contrary to the prevailing application method via nasal spray, substances will be administered with a nebulizer device as it has been proposed to provide greater efficacy in dispensing neuropeptides into the CNS (Yao & Kendrick, 2022). During each testing session and with the aid of a PARI SINUS nebulizer device (PARI GmbH, n.d.), participants receive either 10 IU (2 ml) of Syntocinon (OXT) or saline solution (PLA) intranasally. The study is double-blinded, so neither participants nor research assistants know in which session the placebo and in which the respective active substances are given. The nebulizer administration lasts ten minutes in total; each nostril receives a nebulised spray of the substance for five minutes. While one nostril is being sprayed, the other one is sealed with a small plug and participants are asked to keep their mouth in a certain position, closing the soft palate, to ensure maximum absorption.

6.3. Interview task

A naturalistic real-time interaction task was used to assess the eye gazing behaviour of subjects in different pharmacological conditions. For this, a half-structured interview was created where participants are asked various questions about their daily life while wearing the eye tracking device and sitting opposite the research assistant. The interview is conducted in German and consists of 13 questions which are phrased progressively more open-ended throughout the interview. For example, question 1 asks ‘Hast du letzte Nacht gut geschlafen?’ (transl.: Have you slept well?), compared to question 8: ‘Könntest du mir bitte erzählen, was du am nächsten Wochenende vorhast?‘ (transl.: Could you please tell me what your plans for the upcoming weekend are?). The interview should last between 8-10 minutes to collect sufficient eye tracking data. Depending on the length of their answers, follow-up questions can be asked to meet the required duration time. At the end, subjects are also able to pose

questions to the research assistant to convey a more realistic situation. The interview objective is to have a natural and relaxed conversation where participants are able to exhibit their normal eye gazing patterns and behaviour.

6.4. Eye tracking measure

A variety of high-quality eye tracking technology is available today to observe the eye gazing behaviour of subjects. The crucial decision for researchers to make, therefore, is how to implement it in a way that is appropriate for answering the research question. As this thesis and the BRS want to recreate a relatively realistic social interaction, a mobile eye tracking device is used during the interview task. Compared to the majority of eye tracking studies on this subject matter which have used remote computer stimuli, this research project uses real-life stimuli by having a research assistant sit opposite the participant and lead a naturalistic, half-structured interview while they are wearing a wearable eye tracker.

The eye tracking device is a *Pupil Core eye tracking headset* from Pupil Labs (Pupil Labs, n.d.) that is equipped with a high speed 60Hz (720p) world camera and two 200Hz (192x192px) binocular eye cameras to simultaneously track eye movements and the corresponding viewing target. Connected via USB to a laptop, the device runs with a software program provided by Pupil Labs, recording the task and fixations with the set of cameras.

The headset is put on prior to the interview for setup and calibration. The eye tracking device needs to be individually adjusted for each subject and each testing session. For that, the three cameras are adjusted so that the full range of pupil movement is captured and in focus while the outward facing camera represents the view the participant has of the research assistant and surroundings. Calibration is done by focusing one's eyes a presented target while moving the head in circular motions for 30-60 seconds. Synchronicity and accuracy of cameras and output are then checked. This calibration process is repeated until feedback is satisfactory which is when the interview can start.

6.5. Procedure

After successful online screening, participants had to select blocks with four respective time slots set approximately two weeks apart. There were four sessions in total in order to complete the experimental tasks under each drug condition. As stated before, for this thesis, only two of the sessions are relevant, PLA-PLA and PLA-OXT. Research assistants stayed the same for each participant across all sessions to avoid possible bias.

Written consent was obtained before and during the first testing appointment. In each session, a urine sample and the first of three saliva samples were taken upon arrival. Participants then answered a short questionnaire, the *Positive and Negative Affect Schedule (PANAS),* to assess their mood. To attain an IQ measure, they additionally had to take a verbal intelligence test (*MWT)* in session 1. Following the assessment(s), placebo or substance was administered via pill and, after a waiting time of 15 minutes, again via nebulizer device. Subsequently, they had another 15-minute waiting period after which a second saliva sample was taken. Subjects then had to answer a questionnaire to document possible experienced side effects. The exact waiting time until the eye tracking task was dependent on the randomised order of the tasks that were conducted as part of the BRS, varying between 15-50 minutes after substance administration. After setup and calibration, the eye tracking task lasted around 8-10 minutes in which participants were asked questions in a semi-structured interview by the research assistant while wearing a mobile eye tracking device. When testing was done, subjects had to assess *Medication guess* and *Instructor likeability rating* on an online rating to control for possible confounding factors. Finally, the third saliva sample was taken and the session was completed with a debriefing sheet and compensation receipt of 40€ minimum. In the last session (Session 4), participants were additionally asked to complete a number of questionnaires (e.g. *Autism Quotient, AQ,* Baron-Cohen et al., 2006*; Interpersonal Reactivity Index; IRI,* Keaton, 2017*)* before receiving the final debriefing and receipt.

6.6. Data Analysis

The number of fixations and total fixation duration were assessed for the eyes, mouth, and other non-eye-or-mouth face areas using the PupilLabs software program (Pupil Labs, n.d.). Minimum fixation duration was 80 ms and maximum fixation duration was 220 ms. Data of participants were normalized for respective lengths of interview with the following calculation:

Normalized number of fixations per second = number of fixations/interview time

Normalized fixation time = total fixation time/interview time

This step was done to guarantee uniform fixation sizes independent of interview length.

To examine the exact locations of fixations on the participant's viewing field, analysis of each interview session recording was analysed frame by frame using openCV in *Python* (cv2 package; Van Rossum & Drake, 2009) and the *facenet-pytorch* package (Esler, 2023). Areas of interest (AOI), or the regions that were looked at by participants and coded into distinct categories, were eyes, mouth, face, and non-face region.

Correcting for positive skewness, a square-root transformation was employed for all of the data. After transformation, data approximated the normal distribution and was used in all subsequent analyses.

For analysis of a possible three-way interaction, a repeated-measures analysis of variance (ANOVA) was conducted between group (two levels – ASC and control; CTRL), substance (two levels - OXT/PLA) and AOI (four levels – eyes, mouth, face and non-face areas) via the statistics program SPSS Version 29.0.1.0 (IBM Corp, 2021). For significant interactions, separate follow-up simple effects analysis was done for each region and tests within variables were done to discern the nature of the interaction.

7. Results

One participant (ASC, female) was excluded from the final analysis because of dropping out after session 2 without stating reasons or concern. The final data set consisted of 23 participants, 9 ASC subjects (6 female, 3 male) and 14 CTRL subjects (6 female, 8 male). Sample age ranged from 18-48 years with a mean of 29.3 years (*sd* = 9.4) in the ASC group and 26.3 years (*sd* = 5.9) in the CTRL group.

7.1. Baseline analysis for looking preferences between groups

To look at baseline group differences, comparisons were made between ASC and CTRL group in the placebo condition by conducting independent samples *t*-tests. There were no significant differences observed between the two groups for looking preferences (number of fixations per second). No significant *p*-values were achieved, yet effect sizes were moderate with AOI face showing the largest effect (eyes: Cohen's d = .557; mouth: d = .537; face: d = .778; non-face: d = -.585). Group differences for total looking time (fixation duration) also failed to be significant, yet showed moderate effect sizes across AOIs again with the face region showing the largest difference and effect, followed by the non-face AOI.

7.2. Effects of OXT

In order to understand the association between group, substance and eye gazing preferences, a three-way repeated-measures ANOVA was performed. Results are presented in Table 1. The error terms stand for the residual variability within/between groups that is unaccounted for after analysis of individual/group differences. The three-way interaction between AOI x substance x group was not significant (F = 0.587, p = .631, partial η^2 = .085). Figure 1 and 2 display the associations between normalized number of fixations for each AOI by substance for each group.

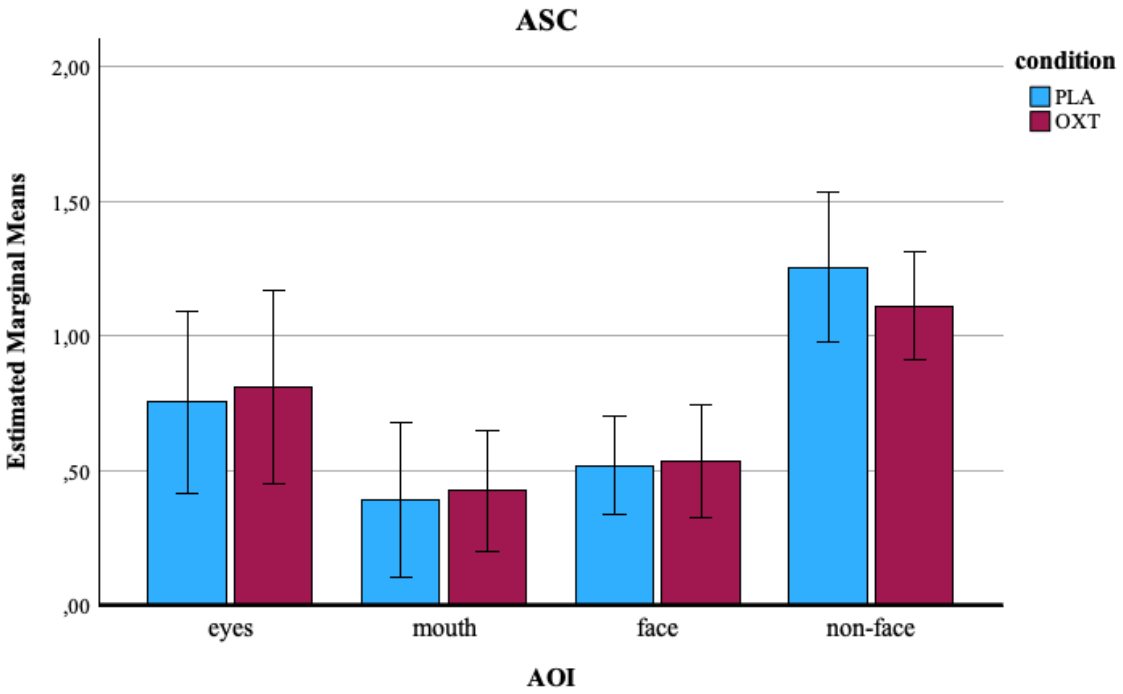

Figure 1. Shows number of fixations per second for each AOI by drug for the ASC group. Error bars show ± 1 sd for this within-subjects comparison.

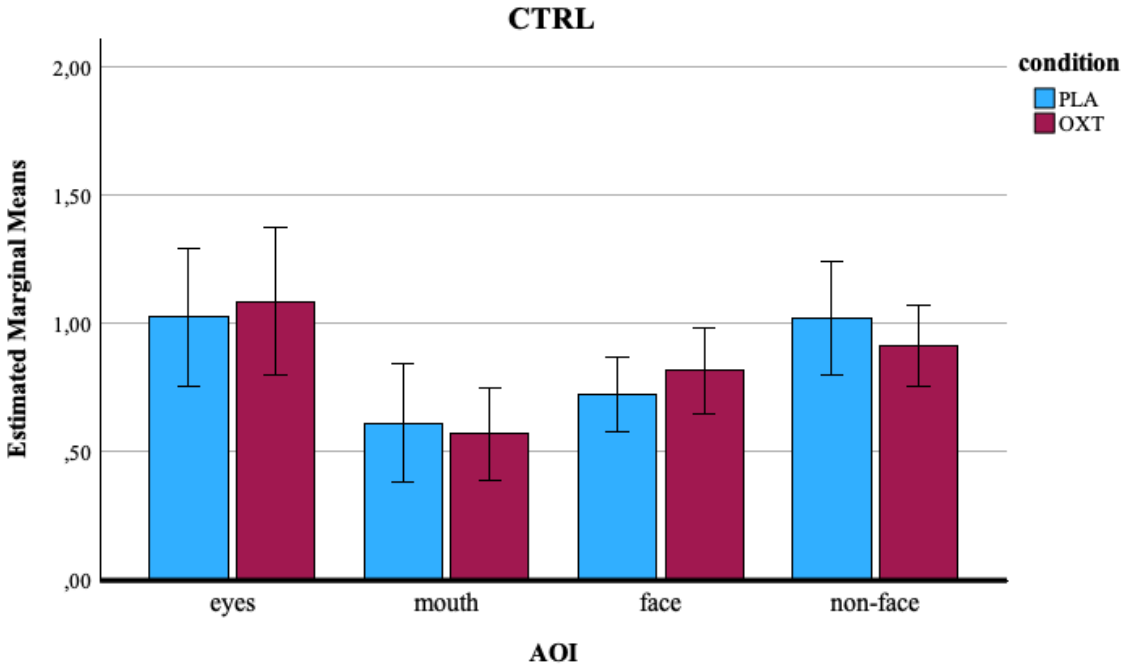

Figure 2. Shows number of fixations per second for each AOI by drug for the CTRL group. Error bars show ± 1 sd for this within-subjects comparison.

Table 1

Three-way interaction ANOVA

Tests of within-subject effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
AOI	Sph. Ass.[1]	8,871	3	2,957	13,601	<,001
AOI * group	Sph. Ass.	1,691	3	,564	2,593	,060
Error(AOI)	Sph. Ass.	13,70	63	,217		
substance	Sph. Ass.	,001	1	,001	,010	,920
substance * group	Sph. Ass.	,001	1	,001	,020	,890
Error(substance)	Sph. Ass.	1,036	21	,049		
AOI * substance	Sph. Ass.	,240	3	,080	2,097	,110
AOI * substance * group	Sph. Ass.	,036	3	,012	,312	,816
Error(AOI* substance)	Sph. Ass.	2,407	63	,038		

[1]Sphericity Assumed

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	108,329	1	108,329	299,048	<,001
group	,628	1	,628	1,733	,202
Error	7,607	21	,362		

The two-way group interaction between AOI x substance was also not significant (F = 2.097, p = .110, partial η^2 = .091). The two-way group interaction between AOI x group narrowly failed to show significance (F = 2.593, p = .06, partial η^2 = .110). Significance was found for the within-subject factor AOI (F = 13.601, p = <.01, partial η^2 = .393). Follow-up pairwise comparisons between the various AOIs revealed significant differences for all regions, presented in Table 2. The region of eyes (mean = .919, std. error = .103) and non-face (mean = 1.076, std. error = .064) showed the highest number of fixations (mouth: mean = .5, std. error = .074; face: mean = .649, std. error = .056).

As the two-way interactions of AOI x substance and AOI x group came close to reaching significant *p*-values, follow-up simple effects analysis for each relationship was conducted. For AOI x substance no significant interactions were found in subsequent pairwise comparisons (PLA condition: mean diff. = .275, $F = 1.552$, $p = .227$; OXT condition: mean diff. = .271, $F = 1.7$, $p = .206$). For AOI x group, no significant differences were found for the placebo AOI conditions. However, in the OXT condition, simple effects analysis revealed a significant mean difference for looking preferences between the groups for the face AOI (mean diff. = .278*, $p = .042$, $d = .924$) and also for total looking time (mean diff. = .116*, $p = .046$, $d = .905$). Looking at Figure 1 and 2, a group difference in fixations per second can be seen for both placebo and OXT condition, although non-significant. After the administration of OXT, the difference between groups was increased with controls showing a larger rise in face fixations than their autistic peers, turning the group difference significant. For the other AOI regions no significant group differences were found, see Table 3.

Table 2

AOI Pairwise Comparisons - Number of fixations

		Mean Difference			95% Confidence Interval for Difference[b]	
(I) AOI	(J) AOI	(I-J)	Std. Error	Sig.[b]	Lower Bound	Upper Bound
eyes	mouth	,419*	,099	<,001	,213	,625
	face	,270*	,075	,002	,115	,425
	non-face	-,157	,148	,301	-,465	,151
mouth	eyes	-,419*	,099	<,001	-,625	-,213
	face	-,149*	,053	,011	-,260	-,038
	non-face	-,576*	,104	<,001	-,793	-,359
face	eyes	-,270*	,075	,002	-,425	-,115
	mouth	,149*	,053	,011	,038	,260
	non-face	-,427*	,092	<,001	-,619	-,235
non-face	eyes	,157	,148	,301	-,151	,465
	mouth	,576*	,104	<,001	,359	,793
	face	,427*	,092	<,001	,235	,619

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 3

Simple effects analysis - Number of fixations - OXT condition

AOI	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
eyes	CTRL	ASC	,275	,221	,227	-,184	.734
mouth	CTRL	ASC	,142	,139	,317	-,147	,431
face	CTRL	ASC	-,278*	,129	,042	,011	,546
non-face	CTRL	ASC	-,198	,122	,120	-,452	,056

Based on estimated marginal means

*. The mean difference is significant at the ,050 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

8. Discussion

8.1. Findings

This double-blind, placebo-controlled within- and between-subjects design study aimed to find effects of exogenously administrated OXT on the eye gazing behaviour in individuals with ASC. The design of the study is a conceptual replication of the eye tracking study by Auyeung and colleagues (2015). They used a novel measure to examine naturalistic looking preferences in a neurotypical and ASC sample. By conducting a real-life interaction eye tracking task to assess eye gazing behaviour instead of remote social image or video displays, enhanced external validity and inferability of results can be achieved. The current study adopted this interactive measurement technique from the original study and modified administration logistics and sample composition in order to offer new insights in addition to replicating the findings from 2015.

In this subsample of 23 participants (9 ASC, 14 CTRL), analysis failed to find significant three-way interaction effects between eye gazing behaviour, group, and substance conditions. The single significant effect observed in the three-way interaction ANOVA was the within-subject factor AOI: the number of fixations per second significantly differed between all four focus regions (eyes, mouth, face, non-face; Table 2). Eyes and non-face regions were the most looked at AOIs. This means that, levelled across groups and conditions, eyes and non-face areas like room surroundings were focused on the most during the interview. As the eye region is a socially essential but also normative focus point in social communication and interactions – at least in Western society –, this finding of larger fixation numbers on the eye AOI across groups is in line with existing literature (Cañigueral & Hamilton, 2019). The large

looking proportion to non-face regions during the interview is also plausible: Participants were asked to answer several open-ended questions that involved, for example, thinking about last week's activities or what their plans for the upcoming weekend are. Conjuring memories and producing an answer requires concentration and cognitive resources. However, maintaining eye contact also takes up cognitive storage, meaning that there may not be enough cognitive resources to think properly while keeping eye contact. By averting one's eyes for short periods during communication, cognitive storage is regained and made available for complex mental processes (Kajimura & Nomura, 2016). This offers an explanation for the high proportion of looking at non-face regions across both the neurotypical and neurodiverse group. For the ASC sample, a larger focus on non-face regions is also in agreement with existing literature. Studies have reported decreased interest in human stimuli and a larger focus on objects or nonsocial stimuli in children and adults with autism (Cai et al., 2022; Frazier et al., 2017).

Follow-up analysis of the two-way interaction of AOI x group revealed a significant group difference with ASC individuals looking less at the face region compared to their neurotypical peers after the administration of OXT. This means that neurotypical subjects exhibited stronger looking preferences towards the face after OXT induction compared to the neurodiverse sample. The group difference for the non-face AOI did not cross the $< .05$ *p*-value in either substance condition, yet it did show a trend towards ASC individuals having a higher number of fixations per second towards regions other than the eyes, mouth or face compared to the control group. When comparing figures and values, it is visible that there is a group difference in both conditions for the non-face AOI. Even though no significance was achieved, both groups appear to show a reduction of fixations to the non-social region in the OXT condition, suggesting a substance-induced larger focus on social areas instead. However, as values did not reach significance, no inferences can be made from the sample data. Interesting to note is that the original study by Auyeung and colleagues (2015) did not observe a trend regarding non-face AOI group differences. Reasons for that, however, can only be speculated on as they are numerous, possibly stemming from personal, procedural or technical factors.

Baseline (PLA) comparisons of the looking preferences (number of fixations per second) and total looking time did not reveal any significant differences between groups. Despite not having reached significance, the moderate sizes of effect across AOIs are worth mentioning in this discussion as they indicate that the findings are more substantial than one might assume at first sight. Nowadays, research is starting to question the predominant chase for statistical

significance as it results in publication bias and the neglection of promising yet non-significant study findings. With the introduction of preregistration measures and emphasising the importance of other indicative values like effect sizes, there is a growing effort to challenge these significance-centred norms. The current study results represent an example of that: Even though there are few *p*-values showing significance, the observation of relatively large Cohen's *d* effect sizes throughout the sample data analysis suggests that there is indeed an effect but sample size did not suffice for values to cross the *p*-benchmark. With more participants and a larger data set for analysis, significance may have been reached. As this thesis study is part of an ongoing BRS that will recruit a sample of 60 participants, it offers the unique chance to see if this assumption holds true by them reporting a significant version of this study's trend findings.

Another indicator that this study had a promising approach, but not enough power, is that the effect sizes are similar to the ones found in the original study by Auyeung and colleagues (2015). The baseline comparisons between groups back then revealed a significant difference for fixation time to the eye region with a Cohen's *d* of .666 which is relatively close to this current study's $d = .557$.

Moving on to discussion of a technical aspect, this study used a novel nebulizer device to test its application efficacy of OXT. Different to the original 2015 experiment, this conceptual replication study altered device and dosage to improve absorption of the neuropeptide. Administration via nebulizer device has been suggested to act more efficient in transporting neuropeptide particles into the most posterior parts of the nasal cave where the olfactory and trigeminal nerves – main gateways into the CNS – are located (Yao & Kendrick, 2022). Not enough studies have been conducted to verify the proposed superior efficacy of the nebuliser until now. However, the results of this study, along with the lower but seemingly equally effective dispensed dosage, support the notion. Most intranasal OXT studies have used 24 IU when administrating the hormone. The current study chose to only administrate 10 IU OXT per session. This was decided because of the nebulizer's proposed efficacy and to keep side effects as minimal as possible for participants. As there is a second substance involved in the BRS that is applied along with OXT in some sessions, the lower dose was settled on to avoid overstimulation of the participants' organisms in joint substance sessions. Despite the decreased amount of administrated OXT, it seems to have reached the CNS sufficiently as this study found significant differences between groups in the OXT condition and showing a positive trend for both the ASC and control group (see Figure 1 and 2).

The results of the present study do not support either of the stated hypotheses. The first statement – "After administration of oxytocin, ASD individuals show increased gaze to the eye region compared to the placebo condition." – cannot be affirmed by the current statistical analysis. H_2 – "In the placebo condition, ASD individuals show less gaze to the eye region than typically developing individuals." – can also not be supported. Yet, the moderate size of Cohen's *d* (*d* = .557) indicates that with a larger sample the baseline group difference could have reached significance.

8.2. Limitations and implications for future research

There are several limitations to this study. Firstly, the sample size was smaller than initially intended due to delays in the testing schedule. The final sample of 23 participants did not hold enough statistical power to detect significant effect sizes in the analysis. While this thesis study failed to produce the desired findings, the overarching bigger research study (BRS) may do so as their final sample will consist of 60 participants (30 ASC, 30 CTRL). Secondly, the composition of the sample is not ideal. As it is only a subsample, there was no possibility to match for gender, IQ or age as the original study by Auyeung and colleagues (2015) did. Of all these confounding variables, IQ is the most influential for this study as high-functioning ASC individuals often compensate idiosyncratic behaviour like atypical eye gaze by camouflaging their manners and adapting normative eye gaze etiquette (Hull et al., 2017). Because we only included autistic individuals with average to high intelligence, the full spectrum of gazing behaviour in the ASC population may not be adequately represented. Furthermore, group sizes for ASC and CTRL group were not equal which has an influence on statistical measures and the variance of results.

Moving on from sample flaws, another aspect of the study that encompassed various limitations is the real-life interaction eye tracking task. The measure represents an improved design for capturing social eye gazing behaviour - nonetheless, testing remains to be a unique experience for participants and cannot constitute a fully ecologically valid situation. While it is superior in creating a realistic social situation compared to remote display tasks, it is accompanied by several procedural factors that are difficult to control. Firstly, the eye tracking device is head-mounted which makes it especially susceptible for interference. Even after instruction to move as little as possible, shaking or nodding the head are hard to prevent as they are deeply internalised non-verbal gestures. Larger movements may have impaired the quality of eye tracking data. Additionally, the eye tracker had technical difficulties in some of the sessions (e.g. recording only one eye) which could have further decreased data quality.

Two situational circumstances of the eye tracking task that were difficult to standardise are lighting conditions and interview contents. As testing is conducted throughout the day, the video quality of sessions conducted later in the day may have been decreased due to artificial lighting. Regarding the interview conversation, the nature of the half-structured interview poses some standardisation. However, depending on the talkability of participants, research assistants were instructed to ask follow-up questions that were not specified and could vastly differ in contents, possibly affecting participants' constitutions in unwanted ways.

Another limitation that came to attention first during testing and then again while analysing the data is the interview concept of the social interaction task. During the interview participants are asked several questions, some of them open-ended and requiring increased thinking to answer. This cognitive engagement often causes humans to look away from the eyes in conversations to free up cognitive resources that are engaged when maintaining eye contact (Kajimura & Nomura, 2016). Therefore, the one-sided concept of an interview may not be ideal to emulate a genuine social interaction – as participants have to produce answers throughout the interview, inducing them to think and consequently avert eye contact for time fragments, eye tracking data may be distorted. When having a real-life conversation, talking and listening are usually distributed equally between interaction partners. Whilst the other person speaks, their facial expressions are observed to understand the conveyed contents better. Because the social interaction task is conceptualized as an interview, valuable eye gazing behaviour that is expressed while listening may not be captured adequately. Future studies employing a real-life social interaction eye tracking task should aim for a task design that allows participants to express observant listening behaviour as well, for example by having research assistants answer the posed questions themselves after subjects have replied.

Also worth mentioning is a possibly confounding factor that concerns the administration logistics of OXT. Due to this thesis study being part of the BRS, the waiting time between administration of the hormone and conduction of the eye tracking task could not be standardised across participants. Depending on the randomised order of the BRS tasks – three in total with the eye tracking task – the waiting periods varied across participants and sessions. Longer durations may have caused the effects of OXT to subside by the time the interview began. Future studies should standardise waiting time and ensure that the eye tracking task is conducted in the optimal absorption period.

Lastly, I want to state a research implication for future studies that came to mind while exploring the genetical background of OXT. Related to the findings on the OXTR gene described in *Chapter 2.4,* it would be interesting to see if the genetic hypomethylation of the

receptor gene that is reported in ASC children coincides with decreased expression of OXT on a hormonal serological level. Hypomethylation causes an increased OXTR expression, meaning there are more receptors for OXT to dock on. If release of the neuropeptide is decreased, this abundance would be superfluous which in turn may lead to a decreased expression of the gene and consequently to hypermethylation that has been reported in adults with autistic traits. To my awareness, this possible connection has not been explored by published studies yet and poses an intriguing research matter to investigate further in the future.

9. Conclusion

This thesis study aimed to find effects of exogenously administrated oxytocin on the eye gazing behaviour in individuals with autism spectrum condition. Compared with a control group of neurotypical subjects, results failed to support the research question and hypotheses. There were no significant OXT-induced effects observed for either group. The single group difference reaching significance was found in regard to looking preference to the face region. Both groups showed a non-significant trend increase in fixations toward the face after the administration of OXT, yet compared to their neurotypical peers ASC individuals looked at the face region significantly less.

Despite no further significant differences of interest being found, analysis did reveal moderate effect sizes for several variables. This indicates that with a larger sample size significance could have been achieved. As this study is part of an ongoing bigger research study that will include a larger sample, this poses a unique opportunity to observe the statistical consequences. It will be exciting to see if the trend that has been found in the current data could manifest to meaningful results with a larger data set and statistical power.

In conclusion, this study did not succeed in producing significant findings regarding the effects of OXT on social eye gazing behaviour in individuals on the autism spectrum. Conceptually replicating an eye tracking study by Auyeung and colleagues (2015) and refining some aspects, the current results failed to reproduce the original findings. A probable reason is the comparably small sample size – the overarching BRS comprising a larger sample may be able to produce results that will corroborate the findings from 2015. All three studies are part of a research directive hoping to contribute to the assessment of oxytocin as a future therapeutic adjuvant for enhancing social behaviour in ASC individuals.

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Tables

Figures

List of Abbreviations

ADHD	Attention deficit hyperactivity disorder
ADOS-2	Autism diagnostic observation schedule-2
AOI	Areas of interest
APA	American Psychiatric Association
AQ	Autism spectrum quotient
ASC	Autism spectrum condition
ASD	Autism spectrum disorder
BRS	Bigger research study
CBT	Cognitive behavioural therapy
CNS	Central nervous system
CNV	Copy number variations
CTRL	Control group (neurotypical sample)
FIPS-A	Finnish prenatal study of Autism
ICV	Intracerebroventricular infusion
IQ	Intelligence quotient
IU	International unit
MET	Multifaceted empathy task
NAL	Naltrexone
OXT	Oxytocin
OXTR	Oxytocin receptor
PLA	Placebo
RBS-R	Repetitive behaviour scale – revised
REMT	Reading the mind in the eyes test
SNP	Single nucleotide polymorphisms
SRS; SRS-2	Social responsiveness scale(-2)
TCI	Temperament and character inventory
ToM	Theory of mind
WHOQOL	World Health Organization quality of life questionnaire

Appendix

English Abstract

Autism spectrum condition (ASC) is a lifelong neurodevelopmental condition characterised by social interaction and communication deficits as well as restricted, repetitive behaviours and interests. Oxytocin (OXT) is a hormone found in humans and animals that plays an important role in social cognition and behaviour. In recent decades, OXT has been explored as a potential psychopharmacological adjuvant for enhancing social behaviour in ASC. This study aimed to explore the effects of intranasally administrated OXT on social eye gazing behaviour in individuals with autism. Employing a real-life interaction eye tracking task instead of remote social stimuli displays to increase external validity, 23 participants completed the task in a placebo and experimental (OXT) condition. This served the purpose to explore effects within and between groups. Analysis did not reveal significant differences of interest, yet results did show a trend in expected directions. Limitations and implications for future studies are discussed. This study is part of an ongoing bigger research project, offering the opportunity to reassess current study results with a larger sample size and statistical power.

Keywords: autism spectrum condition, oxytocin, social eye gaze, eye tracking.

German Abstract

Autismus-Spektrum-Störung (ASC) ist eine lebensbegleitende neurologische Entwicklungsstörung, die durch Defizite in sozialer Interaktion und Kommunikation sowie durch eingeschränkte, sich wiederholende Verhaltensweisen und Interessen gekennzeichnet ist. Oxytocin (OXT) ist ein in Menschen und Tieren vorkommendes Hormon, das eine wichtige Rolle für soziale Wahrnehmung und Verhalten spielt. In den letzten Jahrzehnten wurde OXT als potenzieller psychopharmakologischer Hilfsstoff zur Verbesserung des Sozialverhaltens in ASC erforscht. Ziel der aktuellen Studie war es, die Auswirkungen von intranasal verabreichtem OXT auf das soziale Blickverhalten von Menschen mit Autismus zu untersuchen. Um die externe Validität zu erhöhen, wurde ein Eye Tracking Task mit echter zwischenmenschlicher Interaktion durchgeführt, anstelle von digital dargestellten sozialen Stimuli. 23 Teilnehmer absolvierten die Aufgabe in einer Placebo- und einer experimentellen OXT-Bedingung, um Effekte innerhalb und zwischen Gruppen zu untersuchen. Die Analyse ergab keine signifikanten Unterschiede, jedoch zeigten die Ergebnisse einen Trend in die erwartete Richtung. Limitationen und Implikationen für zukünftige Studien werden diskutiert. Diese Masterstudie ist Teil eines fortlaufenden größeren Forschungsprojekts, was die Möglichkeit bietet, die aktuellen Studienergebnisse mit einer größeren Stichprobe und statistischen Power erneut zu bewerten.

Stichworte: Autismus-Spektrum-Störung, Oxytocin, soziales Blickverhalten, Eye tracking.