KU LEUVEN

FACULTEIT GENEESKUNDE
BIOMEDISCHE WETENSCHAPPEN

De effecten van oxytocine op de herkenning van emoties, sociaal functioneren en hechting

(Effects of intranasal oxytocin on emotion recognition, social functioning and attachment scales)

Masterproef voorgedragen tot het behalen van de graad van Master in de biomedische wetenschappen door

Emmely BERRA

Promotor: Prof. dr. Kaat ALAERTS
Faculteit Bewegings- en Revalidatiewetenschappen
Departement Revalidatiewetenschappen
Onderzoeksgroep Bewegingscontrole en Neuroplasticiteit
Onderzoeksgroep Neuromotorische Revalidatie

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Preface

Na vijf jaar biomedische wetenschappen is het einde van mijn academische studiecarrière in zicht. Tijd dus om even stil te staan en enkele mensen te bedanken. Eerst en vooral wil ik mijn vader bedanken voor de financiële steun en alle andere hulp, bedankt papa! Daarnaast wil ik mijn vrienden bedanken voor de leuke momenten. Eline, mijn beste kotgenootje, mijn beste maatje uit Leuven, bedankt! Elise en Marie, het beste gezelschap tijdens de lessen én daarbuiten, bedankt! Saartje en Sandy, mijn beste vrienden thuis, bedankt voor alle succes-smsjes! Ulrich, mijn steun en toeverlaat, bedankt voor al je geduld en begrip!

Bovenal wil ik mijn begeleidster en promotor professor Kaat Alaerts bedanken om mij de kans te geven mijn thesis te mogen uitvoeren in de onderzoeksgroep bewegingscontrole en neuroplasticiteit. Kaat, bedankt om mij negen maanden lang te begeleiden. Het was een genoegen om een eigen projectje te mogen leiden. Bedankt om uw kennis en inzichten met mij te delen en voor de vele interessante discussies tussendoor. Bedankt om mijn thesis na te lezen en feedback te geven. De thesis is er, het resultaat is er, bedankt Kaat voor alle hulp om dit te bereiken!

Daarnaast wil ik Claudia bedanken voor haar hulp tijdens mijn project.

Voorts bedank ik professor Bart Boets en professor Marleen Vanvuchelen voor het nalezen en jureren van mijn thesis.

Aan iedereen een welgemeende dankuwel!

Summary

Over the past years, interest in the neuropsychological effects of oxytocin has increased remarkable. Oxytocin is a natural hormone which plays a key role in socio-emotional behavior and interpersonal bonding. Also, first trials have emerged, indicating that oxytocin can ameliorate social and communication deficits in individuals with psychiatric disorders. Especially for autism spectrum disorders, which are characterized by particular impairments in social cognition, oxytocin is considered a highly promising target for the development of new therapeutic treatments.

We conducted several randomized placebo-controlled pilot studies with young neurotypical males to further elucidate the immediate single-dose and two-week multiple-dose effects of intranasal oxytocin administration on a battery of emotion recognition tasks and several social functioning and attachment scales.

A single dose of oxytocin significantly improved accuracy on bodily emotion recognition. Also, multiple-dose treatment improved performance on the bodily emotion recognition task, as indicated by faster response times. Tasks priming facial emotion recognition and social interaction detection were not significantly affected by nasal spray administration.

Further, results on the social responsiveness scale showed a trend towards decreasing social impairment in both the placebo and oxytocin group. However, for the subscale on social motivation, improvements were significantly more pronounced in the oxytocin group. In terms of the attachment scales, we found a significant reduction on avoidance state attachment in the oxytocin group, yet no effects were found on parent and peer trait attachment.

In conclusion, the effects of oxytocin on socio-emotional behavior of healthy adult males were mild, but present. Future research is necessary to explore whether the current findings will replicate in larger samples. Exploration of the underlying neurobiological mechanism of oxytocin will also facilitate the interpretation of the oxytocin effects. Furthermore, current study encourages future oxytocin research on autism spectrum disorders to implement bodily emotion recognition tasks and attachment scales.

List of abbreviations

An	anxiety
ASD	autism spectrum disorders
Av	avoidance
ERT	emotion recognition task
FERT	facial emotion recognition task
fMRI	functional magnetic resonance imaging
HSD	honestly significant difference
IPPA	inventory of parent and peer attachment
MNS	mirror neuron system
OT	oxytocin
PL	placebo
PLD	point light display
POMS	profile of mood states
RBS-R	restrictive behaviors scale – revised
RCT	randomized controlled trial
RMET	reading the mind in the eyes test
RT	reaction time
SAAM	state adult attachment measure
Se	security
SRS	social responsiveness scale
WHOQOL	world health organization quality of life

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1 Introduction

Oxytocin is a natural hormone which is involved in mother-child bonding, however, oxytocin is also thought to be implicated in the establishment of attachment and close relationships later in life. As such, oxytocin is one of the key players for or social intelligence or social cognition.

Therefore, interest in the neurophysiological effects of oxytocin in individuals that lack appropriate socio-emotional behavior has increased remarkable. In this light, individuals with autism spectrum disorders are characterized by social and communication problems as a core symptom. Two theories indicate that individuals with autism spectrum disorders have a deficit in the social brain and the mirror neuron system. The latter means that people with autism spectrum disorders have difficulties with reading someone's emotions from facial expressions or body language.

Researchers hypothesize that oxytocin may influence the regulation of the social brain and the mirror neuron system brain circuits. Moreover, it is hypothesized that oxytocin may recover the inappropriate brain connectivities and this way, alleviate individuals with autism spectrum disorders from their social impairments. Several studies examined the effects of oxytocin on emotion recognition and social functioning. Still, there remains a gap in neurological studies with oxytocin treatment.

Today, few long-term, multiple-dose studies with oxytocin are conducted with autism spectrum disorder patients. Therefore, we want to perform a two-week pilot study in neurotypical adult males, where several emotion recognition tasks and social functioning and attachment scales will be assessed. This pilot study frames in a phase three clinical trial of a month with individuals diagnosed with autism spectrum disorders.

2 Overview of the literature

2.1 Oxytocin

2.1.1 Neuroanatomical localization

Oxytocin is a nonapeptide produced by the magnocellular cells of the paraventricular and supraoptic nucleus of the hypothalamus and is further secreted by the posterior pituitary gland or neurohypophysis into the peripheral bloodstream. Next to this hormonal role, oxytocin also acts as neurotransmitter as it is released by several other nerve terminals in the brain. Oxytocin receptors were found in the rat brain in the hippocampus, nucleus accumbens, amygdala and bed nucleus of the stria terminalis (1, 2). In humans, expression of oxytocin receptors has also been demonstrated in the hypothalamus and posterior pituitary, the basal nucleus of Meynert, the nucleus of the vertical limb of the diagonal band of Broca, the ventral part of the lateral septal nucleus, the substantia nigra pars compacta, and the substantia gelatinosae of the caudal spinal trigeminal nucleus and of the dorsal horn of the upper spinal cord and in the medio-dorsal region of the nucleus of the solitary tract (3, 4). However, despite these initial demonstrations, the exact location and distribution of oxytocin receptors in the human brain remains largely unknown.

2.1.2 Physiological functioning of oxytocin

Oxytocin is commonly known for its major role at the end of pregnancy, during parturition and lactation for stimulating muscle contraction of the uterus and facilitating milk ejection (5).

Also after birth, oxytocin is known to play an important role in maternal behavior and pair-bonding. The latter can be described as the creation of the unique intimate bound between mother and child, i.e., by enabling the development of attachment between parent and child (6-8). Although direct measurements of care-giving behavior or emotional connection between mother and child are difficult to obtain, considerable evidence indicates that oxytocin is a crucial factor to establish emotional bounds and facilitate appropriate maternal behavior (9-11). For example, one study used functional magnetic resonance imaging (fMRI) to investigate neural responses in mothers when presented with pictures of their smiling or crying firstborn child, and results showed that activity in oxytocinergic brain regions, such as the hypothalamus and pituitary, were significantly higher in secure mothers compared to insecure or dismissing mothers (9). Another fMRI study explored the effects of intranasal oxytocin administration on the auditory processing of crying infant sounds or control sound, and showed that after oxytocin administration activity in the insula and inferior frontal gyrus, two regions related to mother-infant bonding and empathy, was higher during infant crying sounds compared to control sound (10). One other study assessed plasma oxytocin levels in mothers and fathers during the first weeks after birth of their firstborn child and six months later, and demonstrated that increased oxytocin plasma levels were related to parenting behavior (11).

To sum up, these and other studies consistently demonstrated the importance of oxytocin for establishing attachment during early life. However, also in adulthood, oxytocin is thought to play an important role for inducing attachment. Although experimental evidence is rather scarce, several studies suggest that oxytocin might well play a key role in social bonding and affiliation between individuals, including both friendship and romantic partnerbonds (12-14). For example, one study showed that people who are in the first six months of a relation present higher plasma oxytocin levels compared to single persons (12). Further, a placebo-controlled double-blinded randomized controlled trial (RCT) revealed that after intranasal oxytocin administration, adult men with high attachment avoidance scores show an increase in trust towards others and were more cooperative in a social dilemma task (13). Another study reported that couples who received oxytocin showed more approach behavior, better verbal and nonverbal communication, compared to those who received placebo (14).

Aside its role in prosocial behavior, there is also a growing body of evidence that oxytocin plays a role in stress reactions by blocking the hypothalamic–pituitary–adrenal axis, thereby inhibiting the release of corticosterone, a major stress hormone. An experiment in which rats were given an infusion of oxytocin yielded significant reductions in anxiety behavior (15, 16). Also an fMRI study in humans showed reduced right amygdala activity in response to happy, angry and fearful facial expressions after nasal oxytocin administration, suggesting a reduction in uncertainty about the emotional value of social stimuli (17). Another study showed that intranasal oxytocin administration significantly reduced anxiety levels during completion of the Trier Social Stress Test, a test in which participants are required to speak in front of an audience (18). These results indicate that oxytocin can also reduce stress for social interaction and communication.

Figure 1 summarizes the distinct roles of oxytocin in different species. As indicated, in humans, oxytocin stimulates social cognition, eye-contact, generation of trust and anxiety relief. The neurobiology of bonding formation in humans is yet to be further explored, nonetheless, it is believed that interpersonal bonding rests on the same principles of maternal behavior. The latter has been linked to oxytocin, as described earlier (19).

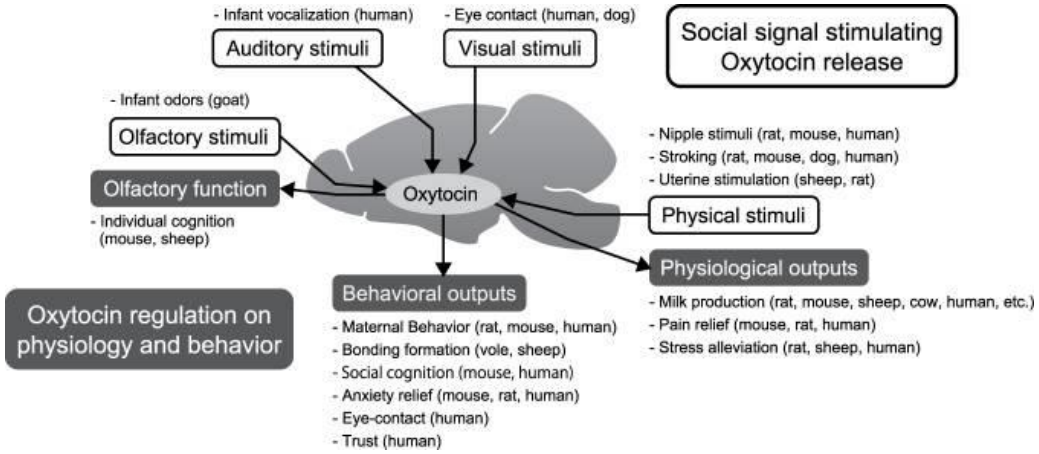


Figure 1. *Oxytocin is involved in several types of communicative behavior, initiated by multiple sensory inputs. Adapted from Nagasawa et al., 2012 (19).*

2.1.3 Oxytocin trials in neurotypical populations assessing effects on emotion recognition

In the following section, we will specifically highlight experiments assessing effects of oxytocin on emotional processing tasks.

One study tested the effects of oxytocin on the processing of briefly presented facial expressions masked by a neutral face after 18, 35 or 53 ms in 56 healthy participants and showed that the oxytocin group performed significantly better, compared to the placebo group (20). Oxytocin administration also resulted in better performance on the Reading the Mind in the Eyes Test (REMT) in a crossover study with 30 males (21). Similarly, accuracy for recognizing happy faces on the Facial Emotion Recognition Task (FERT) improved in the oxytocin group compared to placebo in a study with 50 participants, whereof 29 males (22), without any sex differences. However, another placebo-controlled study in 27 males assessing the effects of oxytocin on a similar task with the six basic emotions (happy, anger, sad, surprised, disgust and anxiety) revealed only a significant effect of accuracy for the emotion of fear (23). These apparent inconsistencies may be related to the differential distribution of males and females in the two studies. A meta-analysis of seven studies in healthy study populations using accuracy on the recognition of facial emotions as primary outcome measure found a Hedge's *g* effect size of 0.291, indicating a small effect size of oxytocin on the performance on the emotion tasks (24). A study with 96 participants, half of them males, examined judgment of facial trustworthiness and attractiveness of images that had to be rated on a seven-point scale with a higher score indicating higher trustworthiness or attractiveness. The oxytocin group scored the images higher compared to the placebo group with no difference in sex (25). These results therefore indicate that oxytocin may have a positive influence on approach behavior towards others.

Of note, in all of the aforementioned studies, oxytocin was administered intranasally. This route is preferred over intravenous administration because of the difficulty to cross the blood-brain-barrier and this way oxytocin levels seem to reach a higher level up to seven hours post administration (26). A dose of 24 IU is most often used.

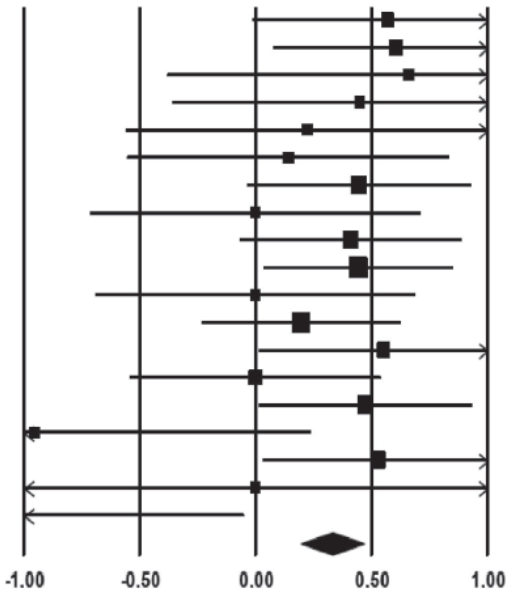
In parallel to the first reports of the facilitating role of oxytocin in emotion recognition in the neurotypical population, oxytocin has gained an increasing interest to study its potentials for treatment of the social-emotional deficits in several psychiatric populations.

2.1.4 The role of oxytocin in psychiatric disorders

Over the past years, interest in exploring the effects of oxytocin in neuropsychiatric disorders has increased enormously, with a particular interest in exploring whether pharmacological management with oxytocin can alleviate patients with a psychiatric disorder from symptoms such as social anxiety, communication difficulties, emotion understanding and related behaviors. A meta-analysis of Bakermans-Kranenburg and van Ijzendoorn (2013) analyzed 19 randomized oxytocin trials in several

neurological disorders including autism spectrum disorders (ASD), schizophrenia, depression, anxiety, obsessive compulsive disorder, bipolar disorder and revealed the greatest effect size (Cohen's *d*) in the studies with ASD populations (**Figure 2**). Most of these studies examined the effect of oxytocin on emotion processing tasks, such as the REMT, FERT, emotion discrimination tasks, but also other symptom and behavioral assessments were included (26).

Author	*Disorder*	*N*	*d*	*se*	*lower*	*upper*	*p*
Andari 2010	Autism	13	0.57	0.30	-0.01	1.16	0.056
Guastella 2010	Autism	16	0.61	0.27	0.07	1.14	0.026
Hollander 2007	Autism	15	0.66	0.53	-0.38	1.70	0.214
Tauber 2011	Prader-Willi	24	0.45	0.41	-0.36	1.26	0.275
Guastella 2009	Anxiety	25	0.22	0.40	-0.56	1.01	0.576
Hall 2012	Anxiety	8	0.14	0.36	-0.55	0.84	0.690
Labuschagne 2010	Anxiety	18	0.45	0.25	-0.04	0.93	0.071
Pitman 1992	PTSD	30	0.00	0.37	-0.72	0.72	1.000
Yatzkar 2010	PTSD	18	0.41	0.25	-0.07	0.89	0.095
Mah 2012	Depression	25	0.44	0.21	0.03	0.86	0.034
Pincus 2010	Depression	8	0.00	0.35	-0.69	0.69	1.000
Averbeck 2011	Schizophrenia	21	0.20	0.22	-0.24	0.63	0.373
Feifel 2010	Schizophrenia	15	0.55	0.28	0.01	1.10	0.046
Goldman 2011	Schizophrenia	13	0.00	0.28	-0.54	0.54	1.000
Pedersen 2011	Schizophrenia	20	0.47	0.24	0.01	0.94	0.044
Den Boer 1992	OCD	12	-0.95	0.61	-2.15	0.24	0.118
Epperson 1996a	OCD	7	0.53	0.26	0.03	1.03	0.038
Epperson 1996b	OCD	2	0.00	0.71	-1.39	1.39	1.000
Bartz 2011	BPD	14	-1.19	0.59	-2.34	-0.05	0.041
Total		*304*	*0.32*	*0.07*	*0.18*	*0.47*	*0.000*



Note. PTSD = Post Traumatic Stress Disorder; OCD = Obsessive Compulsive Disorder; BPD = Borderline Personality Disorder

Figure 2. *Effect sizes of oxytocin administration in randomized controlled trials in groups of different psychiatric disorders. Adapted from Bakermans-Kranenburg and van Ijzendoorn, 2013 (26).*

2.2 Autism spectrum disorders

Work of the present thesis is part of a larger research project exploring the effects of oxytocin on social cognition and behavior in patients diagnosed with ASD. In this section, we will discuss the general disease characteristics of ASD, followed by some of the major theories of the disease mechanism of ASD, and finally, we will discuss the current pharmacological interventions for ASD and the potential of oxytocin as an ASD disease-modifying drug.

ASD, formerly termed pervasive developmental disorders, is an umbrella term for three mental disorders: autistic disorder, pervasive developmental disorder–not otherwise specified (including atypical autism) and Asperger's syndrome (27). The term 'autism' was introduced in 1911 by Paul Eugen Bleuler, a Swiss psychiatrist, who recognized autistic behavior as a fundamental symptom of schizophrenia. Here, autism was originally described as detachment from reality and a rich inner fantasy (28). However, in his report of eleven child cases, Leo Kanner was the first to describe autism as a

syndrome characterized by aberrant behavior, an increased habit for being alone, attachment towards certain objects and disrupted communication (29).
Nowadays, ASD is generally accepted in the Diagnostic and Statistical Manual of Mental Disorders 5 as a lifelong neurodevelopmental disorder in which social-communicative impairment and non-social symptoms are the core features or domains. Social-communicative impairment is manifested in deficits in social-emotional reciprocity, difficulties in maintaining relationships, lack of nonverbal communication and conversation. Non-social symptoms are restricted and repetitive behaviors and interests (27), which are divided in four subdomains: repetitive behaviors and unusual responses to sensory input, rituals and insistence on sameness (30), circumscribed interests (31) and self-injurous behavior (32, 33). In addition, the expressive language level can vary within both domains. Also, intellectual disability often co-occurs with ASD. All symptoms are variable and are defined as a spectrum from a mild to severe phenotype (27).

Prevalence estimates indicate that ASD affects 1% of the population worldwide (34) and epidemiologic follow-up studies indicate that this number continues to increase (35). Males are affected up to five times more than females, with an even higher ratio for high functioning individuals (36). Centers for Disease Control and Prevention have funded several surveillance programs in the United States such as the Autism and Developmental Disabilities Monitoring Network and the Centers for Autism and Developmental Disabilities Research and Epidemiology. Their strategy of monitoring the same population over time with the same methodology in all sites will lead to a better understanding of the epidemics of ASD (37). Children are typically diagnosed around the age of 4 years (38). The corner stone of ASD diagnosis is clinical examination since there is no ASD-specific biomarker (39).

2.2.1 Theories of ASD

To date, the neural basis of autism remains uncertain, although several theories have been proposed. Two of the major theories will be discussed below.

a) Amygdala theory of autism

Brothers (1990) suggested the existence of a social brain of which the orbitofrontal cortex, temporal cortex and the amygdala form the core areas (**Figure 3**) (40). This so-called social brain is the owner of our social cognition or social intelligence, *i.e.*, our ability of interpreting other's mental states and knowledge about how to interact appropriately, verbal and nonverbal, with others (40). The amygdala is a structure related to the appraisal of facial expressions. An increased amygdala activity was observed when faces were presented with emotions of fear (41) and untrustworthiness (42) to normal participants. Accordingly, subjects with bilateral amygdala damage gave unnatural social judgments in terms of approachability and trustworthiness to the projected faces, more specifically in respect to negative facial expressions (43).

Considering the social impairments associated with ASDs, an amygdala anomaly is suggested as underlying neuropathofysiology of ASD. First evidence supporting this account came from an animal model of Klüver and Bucy (1937) who generated a rhesus monkey with ablated temporal lobes. Rhesus monkeys with lacking amygdala responded abnormal to anger and fear and had an autistic-like behavior (44). Also patients with amygdala damage have been shown to display symptoms that resembled those of autism patients (45). An fMRI study comprising the REMT with normal and ASD participants confirmed lower activation of the amygdala in autism patients compared to normal individuals (46).
Overall, the amygdala theory of autism hypothesizes an abnormality in amygdala functioning, amongst other abnormal brain regions part of the social brain (47).

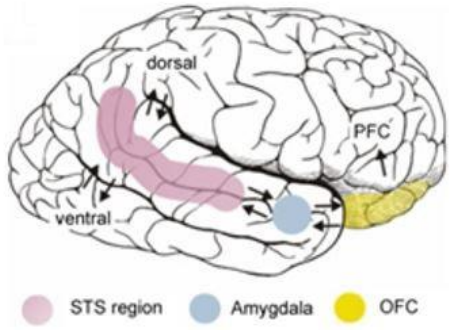


Figure 3. Presentation of the different brain regions which form the social brain. *STS = superior temporal sulcus, PFC = prefrontal cortex and OFC = orbitofrontal cortex. Adapted from Allison et al., 2000 (48).*

b) 'Broken mirror' theory of autism

Neurons in the inferior frontal gyrus of the motor cortex (49) and the inferior parietal lobule (50) have been shown to fire when an individual performs an action as well as when the individual merely observes actions performed by others (51) (**Figure 4**). Based on these remarkable properties, these so-called 'mirror neurons' have been linked to a variety of socio-communicative functions, such as action understanding, imitation, but also for emotion recognition and empathic skills. This is in accordance with the direct-matching hypothesis, suggesting a direct comprehension of observed behaviors of others without the need of cognitive processing (52, 53). In other words, the mirror neuron system (MNS) is crucial in understanding what others do and what the intended goal of their actions is. Along the same line, also empathic skills or reading the emotions of others have been linked to the MNS. Considering that individuals with autism display particular difficulties within these domains, the 'broken mirror theory' has been put forward, suggesting a problem with the direct matching of others emotions and actions within the MNS (27, 54). Mimicking or imitating someone's actions and behaviors, especially someone's emotions, is a manner of learning, critical for mental and social development (53, 55). Next to the fronto-parietal regions encompassing the MNS, the superior temporal sulcus (STS) is linked to the MNS for cognitive processing of emotions and social cues (56).
Furthermore, language is linked to MNS and can be explained by the involvement of Broca's area in the MNS, a frontal lobe region responsible for speech production, crucial for social interaction (57).

To date, several studies addressed the 'broken mirror' theory experimentally, and although several studies found evidence supporting the account, a recent review by Hamilton (2013), listing 25 studies (including electroencephalography, magnetoencephalography, transcranial magnetic stimulation and fMRI studies),provided indications that in general, results are mild or inconclusive (58). Particularly for fMRI studies, it seems that only those studies that included the processing of an emotional component, found group differences between the ASD and control group in brain activity, whereas studies without emotional component were more variable, with some studies finding even more activity in the ASD compared to the control group.

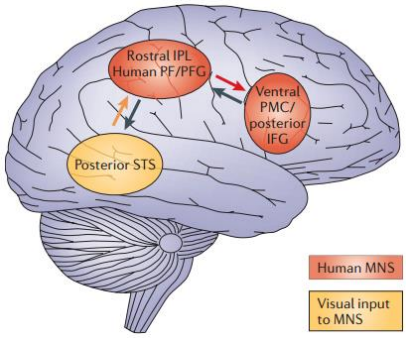


Figure 4. The fronto-parietal regions of the MNS and the link with the STS. *IPL = inferior parietal lobule, PFG = posterior fusiform gyrus, PMC = premotor cortex, IFG = inferior frontal gyrus, STS = superior temporal sulcus and MNS = mirror neuron system. Adapted from Iacoboni et al., 2006 (59).*

2.2.2 Pharmacological treatment of ASD

Previous parts emphasized on potential underlying neural mechanisms of the social deficits in patients with ASD. Since psychiatric disorders are multifactorial diseases it is not straightforward to find a specific biomarker which can be used for diagnosis and later on be targeted with therapeutics. Still, substantial research has already been performed in the search for ASD treatment. First, the only two drugs approved for ASD will be discussed whereafter promising trials with oxytocin in health and disease will be discussed.

a) Approved drugs for ASD

The first drug approved by the Food and Drug Administration for autism is the second generation antipsychotic *risperidone*. Risperidone's mechanism of action is suggested to be antagonism against dopamine type-2 and serotonin type-2 receptor. Since 2006 risperidone can be prescribed, however, exclusively for irritability associated with ASD. Irritability meaning unusually sensitive, resulting in uncontrolled behavior such as aggression, self-injury, hyperactivity, rapid mood changes and temper tantrums (60, 61).

Efficacy of risperidone for irritability in children and adolescents with ASD was examined in multiple placebo-controlled double-blinded randomized controlled trials (RCT's) (62-71). Despite the proven efficacy of risperidone, the drug shows several side-effects. Weight gain and sedation are both

frequently reported adverse events. Sedation is usually only present in the beginning of pharmacotherapy and ceases after initiation. On the contrary, weight gain remains an issue that is characteristic of all atypical antipsychotics. Risperidone results in moderate weight gain and limited risk for metabolic complications, such as diabetes and dyslipidemia (72). This points out why weight gain is an important concern when risperidone is taken continuously. Additionally, increased prolactin levels have been described, albeit hyperprolactinemia-related adverse events such as oligomenorrhea, galactorrhea and gynecomastia, were rare (67, 73-76). Kent et al. (2013) investigated if a dose lower than the minimal recommended dose still exhibited efficacy with the intension to reduce adverse events, but unfortunately, no significant results were found (67).

Another atypical antipsychotic approved in 2009 by the Food and Drug Administration for treating ASD accompanied by irritability is *aripiprazole*. Aripiprazole is assumed to be a partial dopamine type-2 and serotonin type-1A receptor agonist and works antagonistically at serotonin type-2A receptors (77). Two placebo-controlled RCT's demonstrated aripiprazole is efficacious as it significantly decreased the Aberrant Behavior Checklist irritability subscale score (78, 79). Nevertheless, aripiprazole also induced weight gain and sedation, but possesses a more favorable safety profile regarding weight gain when comparing placebo controlled RCT's of aripiprazole and risperidone separately (80). Moreover, aripiprazole does not lead to higher prolactin levels, rather lower prolactin levels were measured (78, 79). Because of uncertainty about long-term effects of weight gain and high prolactin levels, atypical antipsychotics are recommended only for individuals with serious behavioral problems.

Altogether, there is need for longer RCT's with risperidone that will monitor weight gain and prolactin levels, as so far only three RCT's were published with a duration of six months (66, 68, 70). Also, larger RCT's that will compare risperidone against other promising drugs such as aripiprazole are interesting in the search for the drug with the most favorable benefit risk profile. One RCT in which a comparison of aripiprazole and risperidone was performed, showed similar results of efficacy and a comparable safety profile (81). Besides placebo-controlled studies, there is a lack of RCTs comparing pharmaceutical intervention with behavioral therapy to evaluate the added value of one over another.

b) Oxytocin as a potential therapeutic drug treatment for ASD

Since the two approved drugs for ASD, risperidone and aripiprazole, do not target the core symptoms of ASD, it is not a curative treatment. Social and communicative problems still rely on cognitive behavioral therapy and parent training, however, the true effect of these interventions remains precarious (82-85).

Nowadays, oxytocin receives attention as a potential pharmacological intervention for the treatment of the core symptoms of ASD, repetitive behaviors and social-communicative disability. Examination of plasma oxytocin levels in autistic children revealed a decrease in oxytocin and an increase in altered oxytocin peptide forms compared to neurotypical controls (86, 87). The latter indicates that oxytocin, amongst others, could be part of the underlying mechanism of ASD.

Multiple double-blind placebo-controlled RCTs in autism populations have been conducted with promising results. First, we will review single-dose studies, followed by a limited number of long-term, multiple-dose trials.

A crossover study with 15 adults diagnosed with ASD assessed difference in repetitive behavior after a placebo or an oxytocin infusion for four hours, starting from 10 ml/h until 700 ml/h (10 u/ml). Results indicated decreased severity and decreased number of the measured repetitive behaviors (88). Researchers of the aforementioned study investigated with the same study design and patients the effect of oxytocin infusion on the ability to assign affective meaning to speech. Again, positive results were reported in which oxytocin administration leaded to increased comprehension of affective speech (89). The disadvantage of a crossover study design is that often carryover effects are detected, meaning that subjects who first received the actual intervention, here oxytocin infusion, do not return to baseline on the data recorded. The latter was true for the second study of Hollander et al (2007), which was the first oxytocin study investigating social cognition in ASD (89). Also, the low number of patients included in both studies demand a cautious interpretation of the results, as acknowledged by the authors. A crossover study with 18 young adolescent males diagnosed with ASD receiving an intranasal dose of 18 or 24 IU (lower dose for younger patients) showed greater performance on the REMT when given a single-dose of oxytocin compared to placebo (90).

Based on the previous described single administration studies, already small but significant effects of oxytocin on social and emotional tasks were reported in ASD patients. Assessing effects of longer multiple-dose trials in the ASD population are however essential to demonstrate the true therapeutic potential of oxytocin, but to date only a few long-term trials have been conducted (91-94). A clinical trial of six weeks with 19 male adults diagnosed with ASD administering twice a day oxytocin or placebo demonstrated improvement on the REMT and better quality of life in the oxytocin group (91). A seven month study with eight male adolescents examined the effect of three different oxytocin dosages (8 IU, 16 IU and 24 IU). Each dose was administered for two months with a one- to two-week placebo period in between the two dose changes. Significant effects were found in six participants on the communication and social interaction subscales of the Autism Diagnostic Observation Schedule-Generic (92). Nevertheless, a four-day trial did not have any benefit in 38 young males regarding emotion recognition or social behavior (93). In addition, a recent study by Guastella and collegues (2015) did not result in improvement of social functioning assessed via the Social Responsiveness Scale questionnaire and the Clinical Global Impressions-Improvement scale after two months of oxytocin administration to 50 young males compared to placebo (94).

2.3 Aims of the study

The hypothesis that oxytocin stimulates prosocial behavior is an intriguing, not completely unraveled question. Within the present study, we conducted several placebo-controlled RCTs with young neurotypical males to further elucidate the immediate (single-dose) and long-term (multiple-dose) effects of oxytocin administration. To assess the long-term effects on social emotional behavior, oxytocin or placebo will be administered once daily for two weeks. Behavioral effects will be examined using multiple computer tasks and social functioning and attachment questionnaires, which will be discussed in detail in the method section.

We hypothesize that oxytocin will:

1. Increase the accuracy and decrease the reaction time on a bodily emotion recognition task based on point light displays, the Facial Emotion Recognition task and the social task.
2. Decrease the score on the Social Responsiveness Scale indicating a decrease in social impairment.
3. Increase the score on the World Health Organization Quality of Life short form questionnaire, indicating higher quality of life.
4. Increase the score on a trait attachment measure: the Inventory of Parent and Peer Attachment.
5. Increase the score on *security* attachment and decrease the score on *anxiety* and *avoidance* attachment, as measured by a state attachment measure: the State Adult Attachment Measure.

This master thesis project fits in a phase three placebo-controlled RCT in which adult males, diagnosed with ASD, will be administered oxytocin intranasally once daily for four weeks. Within this project, the effects of oxytocin will not only be assessed behaviorally, but also at the neural level using non-invasive fMRI.

Inside the fMRI scanner, a behavioral computer task, the bodily emotion recognition task, will be used to assess the influence of oxytocin on behavior and changes in activations related to the task will be assessed. Besides task-based fMRI, resting-state fMRI will be analyzed in order to pick up any abnormalities in several resting-state functional brain networks, including the default-mode network, which significantly overlaps with brain areas relevant for social cognition (95). Next, structural MRI and diffusion tensor imaging MRI will be performed to visualize structural anatomy and structural functioning, respectively. This way, functional and structural changes in connectivity in the social brain, MNS and default mode network areas can be analyzed.

The results of the current experiments with neurotypical participants will provide important insights in the sensitivity of the included tasks and questionnaires in response to the oxytocin treatment, and will be relevant for selecting the most sensitive outcome measures for inclusion in the patient study.

3 Experimental work

3.1 Materials and methods

Ethics Statement

Written informed consent was obtained from all participants prior to the experiment. Consent forms and study design were approved by the local Ethics Committee for Biomedical Research KU Leuven in accordance to The Code of Ethics of the World Medical Association (Declaration of Helsinki) (96).

Participants

Typically developed adult males were recruited to participate in one of four experiments (**Table 1**). Some of the subjects participated in multiple experiments.

Experiment	Number (N)		Mean age (years) ± Standard deviation	
	PL	*OT*	*PL*	*OT*
1	16	16	21.50 ± 2.06	21.50 ± 2.03
2	8	8	21.37 ± 2.83	20.75 ± 3.86
3	8	8	22.13 ± 2.09	21.00 ± 1.22
4	16	16	21.75 ± 2.87	20.88 ± 2.51

Table 1. *Summary of the four experiments.*

All participants were right-handed (self-reported). A list of exclusion criteria is attached (see **Appendix**, **Addendum 1**).

3.1.1 Drug protocol

Preparation – Nasal sprays were provided by the pharmacy of UZ Leuven. Oxytocin (OT) (40 international units (IU) per ml, Syntocinon®, Sigma-tau), and placebo (PL) nasal sprays (saline natriumchloride solution) had similar packaging and were randomized such that in each of the experiments, half of the participants received oxytocin spray, and the other half placebo spray. Participants and researchers were blinded to the content of the spray. All participants received the same daily dose of 24 IU of oxytocin or three puffs per nostril (six puffs in total).

Administration – All participants received clear instructions, adopted from Dadds et al. (2014) (93), about the use of the nasal spray. Before the first use of the spray, each nasal spray was pumped until a fine mist was observed in order to remove air present in the tube. Participants were asked to keep one nostril closed and to take a deep breath through the nose while tilting their head slightly back during nasal administration to minimize gravitational loss of the spray. Next, participants administered the nasal spray three times in each nostril while the experimenter observed and reported any remarks on the procedure to the participants. The first dose of all subjects was administered in front of the study

experimenter to assure proper usage of the spray and validate tolerability. Thereafter, participants were asked to describe smell and taste of the spray. All participants were asked to keep a daily record of the time point of nasal spray administration and to note if they were alone or in company the first two hours after administration. Subjects were recommended to take the nasal spray in the morning, preferably before breakfast.

Experiment 1 - Participants administered a single dose of 24 IU.
Experiment 2 - Participants administered a daily dose of 24 IU for fifteen days.
Experiment 3 - Participants administered a daily dose of 24 IU for fourteen days.
Experiment 4 - Participants administered a daily dose of 24 IU for fourteen days.

The reason for an extra day of nasal spray administration in *Experiment 2*, is because of the measurement of an immediate oxytocin effect also on the last day, in contrary to *Experiment 3* and *Experiment 4*, where only a two-week effect was examined.

3.1.2 *Experiment 1*: Immediate effect measure of oxytocin on emotion recognition from point light displays with novel stimuli

The Point Light Display (PLD) task was assessed during a single session. Subjects had no previous experience with PLDs. All tests (*Experiment 1* to *Experiment 4*) were assessed in a quiet room, on the same computer monitor. Instructions were provided verbally and on the computer monitor at the start of each test. No feedback was given during the actual tasks.

Point light displays – In all tests, stimuli were based on motion capture data of moving point light displays as previously described (97). Briefly, twelve reflective markers attached to joints of the ankles, the knees, the hips, the wrists, the elbows and the shoulders of a male and female actor were tracked using an eight-camera VICON system (capturing system measuring at 100 Hz, Oxford Metrics, Oxford, UK). Marker points were visualized as twelve moving white spheres on a black background. These moving dots subtended 11 x 12 degrees visual angle at an approximate viewing distance of 50 cm and each dot subtended 0.25 degrees (note that subjects were free to make small trunk and head movements). Movie files were visualized from three different viewpoints (front view (0°), side view (90°) and intermediate view (45°)) (**Figure 5**).

The actors in the PLDs performed two actions: walking and kicking a ball using the right leg. The emotional states were happy and angry. All movie files (duration of 3 or 4 seconds) were presented to the observing subjects either in an upright or inverted position. As such, a grand total of 48 PLDs were presented to all subjects (2 genders x 3 perspectives x 2 actions x 2 emotions x 2 positions).
Moreover, for each of these 48 PLDs, a scrambled version was created. The latter consisted of the same twelve dots undergoing identical local trajectories as in the original PLD, with the difference that the starting position of each dot was randomly assigned between the twelve individual trajectories.

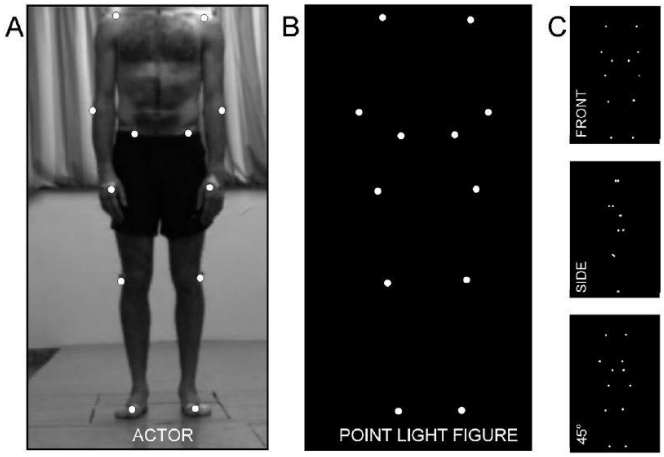


Figure 5. Image of an actor with twelve reflective markers attached to the joints. These markers were tracked using a Vicon motion-capture-system in order to design a PLD. *(A) An exemplary photograph of the male actor with twelve markers attached to the body. (B) The corresponding point light figure of A. (C) Examples of point light figures, viewed from different perspectives, i.e., the front, the side, and the intermediate (45°) view. Adapted from Alaerts et al., 2011 (97).*

Procedure and experimental tests

All subjects participated in a single session, consisting of two tasks: the 'Emotion Task' and the 'Color Task'. The PLD task was performed before and thirty minutes after nasal spray administration to test immediate effects of oxytocin. Importantly, a different set of PLD stimuli were presented to the participants before and after the nasal spray administration, to minimize learning effects. Due to technical problems, no results were obtained from two participants in the second test (1 PL, 1 OT).

Emotion Task. Subjects were presented with a series of PLDs (24 in each test) that depicted a point light figure in a happy or angry emotional state. Participants had to indicate as fast and accurate as possible the emotional state of the PLDs by pressing the respective response buttons. Results of two participants belonging to the oxytocin group were discarded because they were not able to recognize the emotion of the PLDs above chance level.

Color Task. In this control task, subjects were presented with the scrambled version of the PLDs (24 in each test) used in the Emotion Task but one of the dots changed its color to either red or green at an unpredictable time point. Participants were instructed to indicate as fast and accurate as possible which color was shown by pressing different buttons on a keyboard.

All participants started the test with a short practice version which consisted of ten trials of the Emotion Task and twelve trials of the Color Task. The actual test was composed of four blocks of each twelve trials: two Emotion Task blocks and two Color Task blocks, presented in an alternating fashion, with a pause of 16 seconds between blocks.

Data analysis

Reaction times (RTs) to indicate emotion or color, as well as accuracy rates (percentage correct answers) were assessed for all participants. Accuracy rates and RTs were calculated across all trials and separately for recognizing the 'happy', 'angry', 'green' and 'red' PLD. E-Prime software (Psychological Software Tools) was used for stimulus presentation and RT/response logging.
RTs were considered outliers when they exceeded third quartile ± 2 x (third quartile – first quartile), with quartiles calculated across all trials. Following this procedure, 2.28% of trials across all subjects were excluded from the analysis in the first test (baseline measure) and 0.97% of trials were excluded from the immediate measure. Few trials were discarded due to no recorded response (baseline test: 1.82% of trials across all subjects; immediate test: 0.69%). In addition, in the Color Test, trials were excluded if responses were made before change of color (baseline test: 0.52% of trials across all subjects, immediate test: 0.42%). For all tests, only RTs of correct trials were considered.

Repeated measures analysis of variance (ANOVA) were performed on the accuracy rates and mean RTs of each test, with the between-subject factor '*group*' (OT, PL), and the within-subject factor '*time point*' (Baseline and Immediate Effect). We also included the factor '*task*' (Emotion, Color), to assess whether identified effects are specific for the Emotion Task.
Additionally, percentage change from baseline was calculated for the accuracy and RT on the PLD task, where the baseline measurements were attributed value zero. One-way ANOVA was performed on the percentage change from baseline values with the between-subject factor '*group*'.

3.1.3 *Experiment 2*: Effects of two weeks of daily oxytocin administration on emotion recognition from point light displays, using identical stimuli

The PLD task was assessed during two sessions, separated by two weeks: The first day of nasal spray administration and the day of the last administration. Participants performed the PLD task before and thirty minutes after nasal spray administration in both sessions, meaning a total of four tests were assessed.

The PLD task of *Experiment 2* differed from the PLD task used in *Experiment 1* concerning the following features: (i) in *Experiment 1*, the PLD task consisted of 48 different trials (including a variation of 2 genders, 3 perspectives, 2 actions, 2 emotions and 2 positions), whereas in *Experiment 2*, the PLD task consisted of a total of 72 different trials, due to the inclusion of an additional action, namely jumping (2 genders, 3 perspectives, 3 actions, 2 emotions and 2 positions); (ii) in *Experiment 1*, the Emotion and Color Task consisted of two blocks of 12 trials in each test, whereas in *Experiment 2*, each session consisted of six blocks of 12 trials (for each task); and finally, (iii) in *Experiment 1*, PLD stimuli at baseline and at the immediate measure were novel, whereas in *Experiment 2*, an identical set of PLD stimuli was presented in all tests (baseline, immediate, post-baseline, post-immediate). In each test, blocks of the Emotion Task were alternated with blocks of the Color Task, with stimuli presented in a randomized

order. Similar as in *Experiment* 1, accuracy scores and corresponding RTs were deleted when RT was considered as an outlier, i.e., exceeding third quartile ± 2 x (third quartile – first quartile), with quartiles calculated across all trials (baseline test: 1.82% of trials across all subjects; immediate test: 1.87%, post-baseline test: 2.13%, post-immediate test: 1.87%). Few trials were discarded due to no recorded response (baseline test: 0.91% of trials across all subjects; immediate test: 0.65%, post-baseline test: 0.26%, post-immediate test: 0.13%). In addition, in the Color Task, trials were excluded if responses were made before change of color (baseline test: 0.26% of trials across all subjects; immediate test: 0.17%, post-baseline test: 0.95%, post-immediate test: 0.17%). For all tests, only RTs of correct trials were considered.

Four different repeated measures ANOVA were performed in *Experiment 2*: (i) Immediate (comparing Baseline with the Immediate Effect measure), (ii) Post (comparing Baseline with the Post Effect measure), (iii) Post Immediate (comparing Post Effect with the Post Immediate Effect measure) and (iv) all four time points together.

3.1.4 *Experiment 3*: Effects of two weeks of daily oxytocin administration on a facial emotion recognition task and a social interaction processing task

For a part of the participants, we additionally tested effects of two weeks of daily oxytocin administration on the following two tasks. Note that no immediate effects were tested for these tasks.

Facial Emotion Recognition Task – Short movies of facial expressions with different emotions were displayed on a screen in the Facial Emotion Recognition Task (FERT, DiagnoseIS, Metrisquare Europe GmbH) (98). Movies consisted of photographs of frontal faces of individuals who appeared neutral and subsequently expressed one of the six basic emotions: happy, anger, sad, surprise, disgust and fear. Faces of the actors were delineated to create pictures restricted to the contour of the faces. A computer-generated program was used to create intermediate expressions from the available neutral (0%) and full-blown expression (100%) photographs of the same actor. The number of frames whereof a movie was composed varied depending on the intensity of emotion. Emotional intensities of 0–40%, 0–60%, 0–80%, and 0–100% corresponded to 8, 12, 16 and 20 frames respectively (**Figure 6**). Photographs were presented on a black background. Movie duration ranged from 1 to 3 seconds, approximately, depending on number of frames. A total of 96 movies were presented with equal distribution of the six facial expressions, going from low to high emotional intensity. No time restrictions on RT were implemented. No control task was included in the FERT.

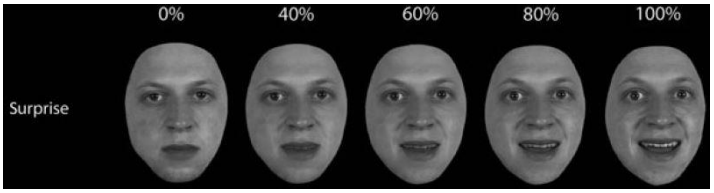


Figure 6. Example of gradual facial expressions in the Facial Emotion Recognition Task. *The actor in the example showed gradual expression of the emotion 'surprise' from neutral (0%) to full emotional expression (100%). Adapted from Kessels et al., 2015 (99).*

Procedure and experimental tests

Participants performed the FERT, once in each session and before nasal spray administration in the first session. Subjects were presented with movies of facial emotional expressions and had to indicate as accurate as possible one of the six possible emotions by indicating the correct answer on the screen with a computer mouse. The FERT was initiated with three practice trials including the emotions anger, happy and disgust.

Data analysis

RTs to indicate facial emotional expression and accuracy rates were assessed for all participants. Accuracy rates and RT were calculated across all trials and separately for recognizing the six different emotions. DiagnoseIS software was used for stimulus presentation. RT and response data were automatically assembled in Microsoft Office Excel.

Due to the fact that one of the possible six answers needs to be chosen by clicking on the right icon on the screen with a computer mouse, reaction time measurements are less relevant compared to the accuracy measure. Accordingly, only the accuracy measure will be discussed in the result section.

Repeated measures ANOVA were performed on the accuracy rates of each session, with the between-subject factor '*group*' (OT, PL), and the within-subject factor '*time point*' (Baseline and Post Effect). ANOVA's were calculated across all trials and for all six emotions separately.

Also here, percentage change from baseline was calculated for the accuracy rates on the FERT task, where the baseline measurements were attributed value zero. One-way ANOVA was performed on the percentage change from baseline values with the between-subject factor '*group*'.

Social task – The third task was a so-called rapid categorization task in which participants were presented with color pictures (apparent size: 28 x 19°) for an ultra-short duration (83 ms) (100). Pictures depicted real-life scenes outdoor and indoor in which two persons were either neutral, friendly or unfriendly going around with each other (**Figure 7**). All pictures were preceded by a 500 ms fixation cross with an inter-trial-interval ranging between 1000-1500 ms. Participants received a maximum of 1000 ms to give a response. The task was divided in two identical parts, each consisting of the same 100 stimuli (50% targets) in a randomized order. The first part was the Social Task followed by the Control Task. Each part was interrupted with a break in the middle of a duration of participant's choice.

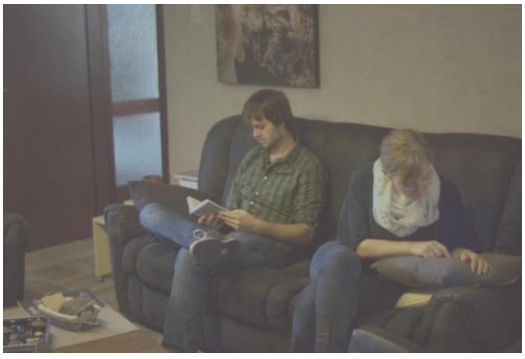


Figure 7. Example of a picture that flashes on the screen during the socialt. *Pictures in the Social Task and the Control Task were identical, always representing two actors in a scene, either indoor or outdoor and actors behaving neutral, friendly or unfriendly with each other.*

Procedure and experimental tests

Participants performed the social task once in both sessions and before nasal spray administration in the first session. Subjects had to answer a question depending on the task for each picture that appeared on the screen.

Social Task. Participants were asked to answer the following question: 'Is there a positive, friendly interaction present in the scene?' Results of two participants belonging to the placebo group were discarded because they were not able to recognize social interaction above chance level.

Control Task. Participants had to respond on the question 'Is the scene happening indoor?' Questions appeared in Dutch on the screen. In both tasks, spacebar was pressed if the answer was yes, otherwise no response execution was needed.

Before the start of the actual test, participants practiced this task. The practice version comprised eight images of the Social Task and eight images of the Control Task. After each trial, participants were given feedback with a green or red circle, indicating correct and not correct respectively.

Data analysis

RTs and accuracy rates were assessed for all participants. Accuracy rates and RTs were calculated across all trials and separately for indoor/outdoor and neutral/friendly/unfriendly scenes in the Social Task and the Control Task, respectively. PsychoPy2 software was used for stimulus presentation. RT and response data were automatically assembled in Microsoft Office Excel.
Only RTs of correct trials were considered. Repeated measures ANOVA were performed on the accuracy rates and mean RTs of each session, with the between-subject factor '*group*' (OT, PL), and the within-subject factors '*time point*' (Baseline and Post Effect), and task (Social, Control), to assess whether effects were specific for the Social Task.

ANOVA's were calculated across all trials, as well as separately for each category, i.e., 'neutral', 'friendly' and 'unfriendly' scenes of the Social Task.
In addition, percentage change from baseline was calculated for the accuracy and the RT on the social task, where the baseline measurements were attributed value zero. One-way ANOVA was performed on the percentage change from baseline values with the between-subject factor '*group*'.

3.1.5 *Experiment 4*: Effects of two weeks of daily oxytocin administration on several questionnaires

Effects of two weeks of daily oxytocin administration were additionally assessed on the following questionnaires. Participants who were also included in *Experiment 2* filled in the questionnaires before the two immediate measures of the PLD task. Questionnaires that deal with assessments over a longer

period than two weeks, were in the second session evaluated over a two-week period, in order to examine differences due to treatment. All questionnaires were self-reported. Only for the Social Responsiveness Scale, an informant version was additionally assessed.

Social Responsiveness Scale (SRS) – A questionnaire of 65 items to measure social impairment related to ASD was assessed. Higher scores indicate a more severe social deficit with a cut-off point of 70 for males. Next to the self-report version, an informant version of the SRS was filled in by partner, close friend or parent of the participant. SRS consists of four subscales: *social communication* (22 items), *social consciousness* (20 items), *social motivation* (11 items) and *rigidity/repetitiveness* (12 items). A four-point Likert-scale was used in which responses range from 0 (not true) to 3 (almost always true).

Restrictive Behaviors Scale-Revised (RBS-R) – Participants completed a questionnaire of 43 items, which examines a heterogynous set of repetitive behaviors, with six subscales: *stereotypic behavior* (6 items), *self-injurious behavior* (8 items), *compulsive behavior* (8 items), *ritualistic behavior* (6 items), *sameness behavior* (11 items) and *restricted interests behavior* (4 items). Ratings range from 0 (behavior does not occur) to 3 (behavior occurs and is a severe problem).

World Health Organization Quality of Life-BREF (WHOQOL-BREF) – The short version of the WHOQOL-100 was used as a measure of change of quality of life during the course of the intervention. This 26-item brief version is divided in four domains: *physical health* (7 items), *psychological health* (6 items), *social relationships* (3 items) and *environment* (8 items). The two remaining questions querying general quality of life and health. Ratings vary from 1 (very bad, very unsatisfied, totally not or never) to 5 (very good, very satisfied, totally or always).

State Adult Attachment Measure (SAAM) – SAAM is likely to capture temporary fluctuations in state attachment. This questionnaire contains 21 statements about how you feel at the moment and consists of three subscales: *security*, *anxiety* and *avoidance*. Participants had to choose an answer from 1 (totally not agreed) to 7 (totally agree).

Inventory of Parent and Peer Attachment (IPPA) – A second measure of attachment was assessed, which deals with attachment to parents, both *mother* and *father*, and *peers*. A fourth part was added in which the same questions of parent's section were asked but about attachment to an *important person* of choice. This last part was not mandatory to fill in. One participant who belonged to the oxytocin group did not fill in the subscale of *father*. Participants had to indicate a number from 1 (almost never) to 4 (almost always).

Profile of Mood States (POMS) – A 32-item POMS version (full-length 65 items questionnaire) was assessed as measurement of transient affective states in order to assess whether mood levels of participants changed over the course of the study. This instrument comprises emotional adjectives subdivided in five domains: *tense*, *strong*, *tired*, *depressive* and *angry* that have to be rated on a five-point scale ranging from 0 (absolutely not) to 4 (very good). Participants were asked to answer how they feel at that moment.

For half of the participants (N = 16 of total 32), the POMS was assessed five times: (i) at the start of the experiment (Baseline); (ii) after the first oxytocin administration (Immediate Effect); (iii) after one week of oxytocin administration (Middle) (not filled in by one placebo participant); (iv) after two weeks of oxytocin administration (Post Effect); and finally, (v) immediately after the last oxytocin administration (immediate effect of oxytocin after two weeks of trial).
However, for the other half of the participants (N = 16 of total 32), the POMS was only assessed at baseline and after two weeks of trial (post).

Data analysis and statistics

For each questionnaire a score was calculated. Participants were not included in the final analyses when test scores were considered as outliers, i.e., when test scores exceeded third quartile ± 1.5 x (third quartile – first quartile) of scores across all participants. If no total score was available, outlier detection was performed separately for each subscale (SRS: 1 PL; SAAM *security*: 1 PL; IPPA *mother*: 1 OT; POMS *tired*: 1 PL, POMS *depressive*: 2 PL, 2 OT, POMS *angry*: 1 PL, 2 OT)
Next, a repeated measures ANOVA was conducted on the total scores of the SRS, RBS-R and WHOQOL-BREF questionnaire with the between-subject factor '*group*' and the within-subject factor '*time point*' (Baseline and Post Effect). Additionally, all questionnaires were analyzed with repeated measures ANOVA for the score on each subscale.
Furthermore, percentage change from baseline was calculated for the score on the two-week measurement of each questionnaire, where the baseline measurements were attributed value zero. One-way ANOVA was performed on the percentage change from baseline values with the between-subject factor '*group*'.

Apart from these questionnaires, a list of general questions was completed by all participants with questions about weight, length, education of the participant and their parents, diet (meat, vegetarian or vegan), smoking, alcohol consumption and drug use.
Also, a side-effects questionnaire was assessed after one week of the two-week nasal spray administration and at the end of the study (see **Appendix**, **Addendum 2**).

All statistics were calculated with Statistica 10 (StatSoft. Inc. Tulsa, USA) and Microsoft Office Excel 2013. A significance criterion of $p < 0.05$ was used in all analyses.

3.2 Results

Only main effects of emotion (meaning happy and angry together) will be discussed for *Experiment 1* and *Experiment 2*. Similarly for the social task in *Experiment 3*, only main effects of social interaction (friendly and unfriendly together) will be discussed.

3.2.1 *Experiment 1*: Immediate effect measure of oxytocin on emotion recognition from point light displays with novel stimuli

To examine the immediate, or single-dose, effect of oxytocin on bodily emotion recognition, participants of both groups, oxytocin (OT) and placebo (PL), performed the point light display (PLD) task before (Baseline) and 30 minutes after nasal spray administration (Immediate Effect). For each test, accuracy rates and reaction times were evaluated for the Emotion Task and a control Color task. Sixteen subjects of each group, oxytocin and placebo, participated in this experiment. Results of thirteen oxytocin and fifteen placebo participants were included in the analysis.

A two-way ANOVA analysis was conducted with the within-subject factor *time point* (Baseline/ Immediate Effect), and the between-subject factor *group* (oxytocin or placebo) to specifically explore the effect of oxytocin on performance on the emotion recognition task.
Also, a three-way ANOVA analysis was performed with the additional within-subject factor *task* (Emotion Task and Color Task) to evaluate whether effects of time point and group were differently modulated depending on the tasks.
For clarity we will only show the results of the three-way ANOVA analysis, those of the two-way ANOVA analysis will be shortly described.

a) Accuray

The three-way ANOVA analysis revealed a two-way interaction of *time point* x *group*, indicating that irrespective of task, performance tended to increase after nasal spray administration in the oxytocin group, not in the placebo group [$F(1,26) = 9.289$, $p < 0.01$]. As seen in **Figure 8, above**, this effect was more pronounced on the Emotion Task, compared to the Color Task, although the three-way interaction *task* x *time point* x *group* failed to reach significance [$F(1,26) = 1.886$, $p = 0.181$].

Group differences in percentage change from baseline immediately after nasal spray administration also showed that only on the Emotion Task, performance specifically increased in the oxytocin group (± 12%), not in the placebo group (± 1%) [$F(1,26) = 6.141$, $p < 0.05$)]. For the Color Task, percentage change from baseline were not significantly different for the placebo and oxytocin group [$F(1,26) = 2.0824$, $p = 0.161$] (**Figure 8, below**).

Direct exploration of effects of nasal spray administration separately for each task showed a significant *time point* x *group* interaction effect only on the Emotion Task [$F(1,26) = 6.4$, $p < 0.05$], not on the Color Task [$F(1,26) = 2.0387$, $p = 0.165$], again, indicating that only in the oxytocin group, performance improved immediately after nasal spray administration [post-hoc Tukey's HSD (honestly significant difference): $p < 0.01$)]. No such performance increase was observed in the group receiving placebo nasal spray ($p = 1.00$).

No main effect of *group* was revealed [$F(1,26) = 0.626$, $p = 0.436$]. For the emotion task, an additional main effect of *time point* [$F(1,26) = 7.158$, $p < 0.05$], but not of *group* [$F(1,26) = 0.626$, $p = 0.436$] was revealed. However, as seen from the interaction term, the *time point* effect was mainly driven by the sharp performance increase in the oxytocin group on the Emotion Task.

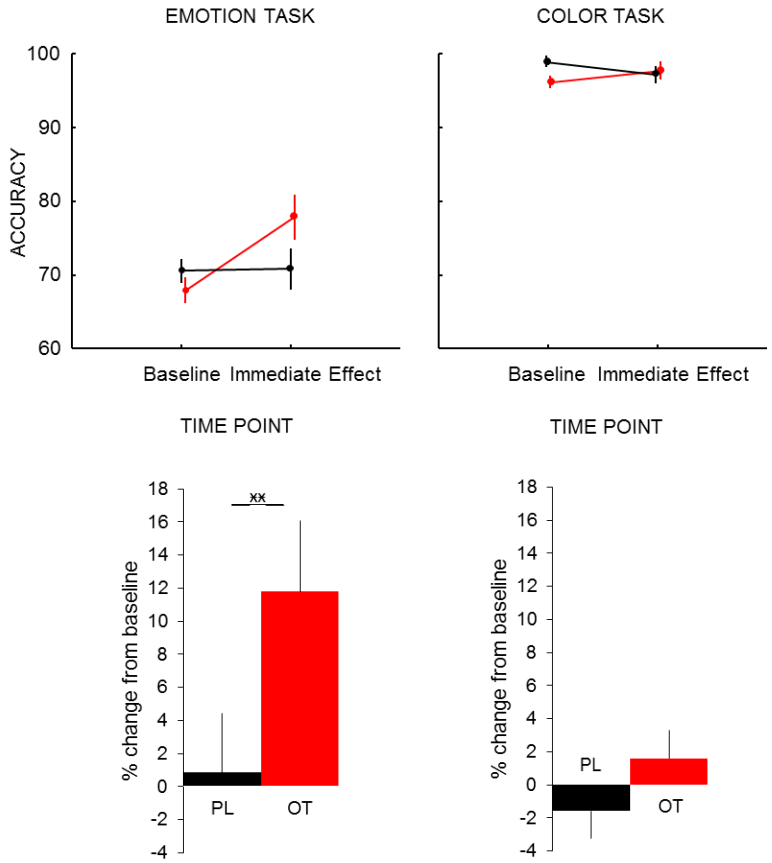


Figure 8. *The effect of oxytocin on the accuracy on the PLD task was nor time point, neither task dependent [$F(1,26) = 1.886$, $p = 0.181$] (above). Group differences in percentage change from baseline were significant only for the Emotion Task [$F(1,26) = 6.141$, $p < 0.05$)] (below). Red = OT, black = PL. Data are expressed as means ± SEM. **: $p < 0.01$*

b) Reaction time

Analysis of the interaction between *task* x *time point* x *group* for the reaction time on the PLD task did not result in a significant effect [F(1,26) = 2.93, p = 0.0988] (**Figure 9, above**). As shown in **Figure 9 below**, the percentage differences from baseline were minimal, and not significantly different between groups (Emotion Task [F(1,26) = 2.35, p = 0.137]; Color Task [F(1,26) = 0.533, p = 0.472]).

Also, direct analysis of *time point x group* interaction effects separately for each task did not reveal significant effects, neither on the Emotion Task [F(1,26) = 2.264, p = 0.144], nor on the Color Task [F(1,26) = 0.446, p = 0.51].

These results indicate that compared to placebo, a single-dose of oxytocin administration did not differentially affect response times on neither task.

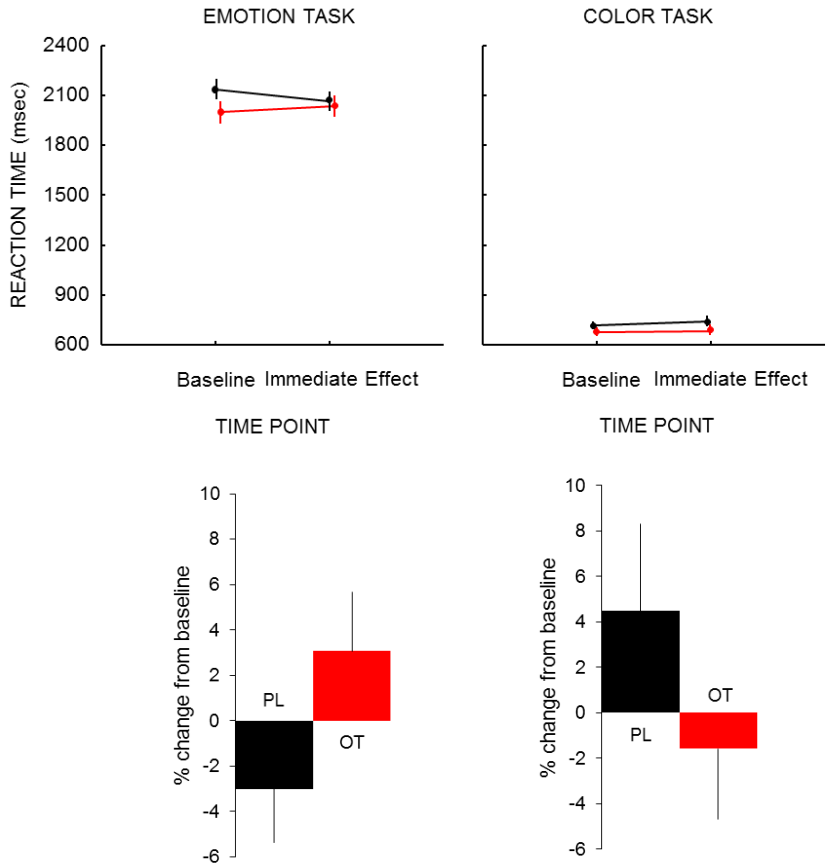


Figure 9. *Three-way interaction plot for the reaction time on the PLD task was not significant [F(1,26) = 2.93, p = 0.0988] (above) and percentage change from baseline revealed neither an immediate significant effect (below). Red = OT, black = PL. Data are expressed as means ± SEM.*

Table 1 and **Table 2** of the **Appendix**, **Addendum 3** summarize the percentage of responders and non-responders of the immediate effect analysis for the accuracy and reaction time on the PLD task, respectively.

3.2.2 *Experiment 2*: Effects of two weeks of daily oxytocin administration on emotion recognition from point light displays, using identical stimuli

In *Experiment 2*, we explored whether similar effects of oxytocin could be measured for performance on the PLD task, when identical stimuli are shown pre- and immediately post oxytocin administration.

In addition to testing immediate, single-dose effects of oxytocin, we explored potential long-term or multiple-dose effects, i.e., by measuring performance on the PLD task pre- and post two weeks of daily oxytocin administration. At the post two-week session, participants also performed the PLD task pre- and immediately post nasal spray administration, to assess possible changes in immediate effects related to the long-term administration. As such, in total, performance accuracy and reaction time on the PLD task were assessed on four different time points, i.e., Baseline, Immediate Effect (single-dose), Post Effect (multi-dose) and Post Immediate Effect, respectively.
Note that sample sizes were small, with only eight subjects in each group (oxytocin and placebo), such that results should be considered preliminary.

For clarity, we first assessed potential immediate, single-dose effects of nasal spray administration, comparing time point 1 (Baseline) with time point 2 (Immediate Effect) (similar to those in *Experiment 1*); next, we assessed potential multiple-dose effects comparing time point 1 (Baseline) with time point 3 (Post Effect); and finally, we assessed immediate effects of nasal spray administration during the post-session, comparing time point 3 (Post Effect) with time point 4 (Post Immediate Effect).

For completeness, **Figure 10** and **Figure 11** visualizes changes in performance for the oxytocin and placebo group for all four time points, for the Emotion and Color Task separately.

a) Accuracy

Figure 10, above shows that the interaction of *task* x *time point* x *group* for all four time points did not reach significance for the accuracy on the PLD task [$F(3,42) = 1.886$, $p = 0.147$], although one can appreciate the increasing trend in accuracy of both groups from Baseline to the Immediate Effect measure on the Emotion Task. However, only the oxytocin group had an increasing trend of accuracy from the Post Effect to the Post Immediate Effect. In addition a significant *task* x *time point* interaction was revealed [$F(3,42) = 7.7$, $p < 0.001$)], indicating that, regardless of group, accuracy improved more from baseline on the Emotion Task, compared to the Color Task.
Participants of both groups had overall the same accuracy scores on the control Color Task.

Group differences in percentage change from baseline graphs show again clearly that, for the Emotion Task, the oxytocin group performed best on the last time point, the Post Immediate Effect measure, while the placebo group had comparable values on the three time points following Baseline (**Figure 10, below**).

A main significant effect of *time point* is revealed [(F(3,42) = 4.853, p < 0.01)]. The interaction analysis indicates this is due to the performance on the Emotion Task and mainly because of the oxytocin group.

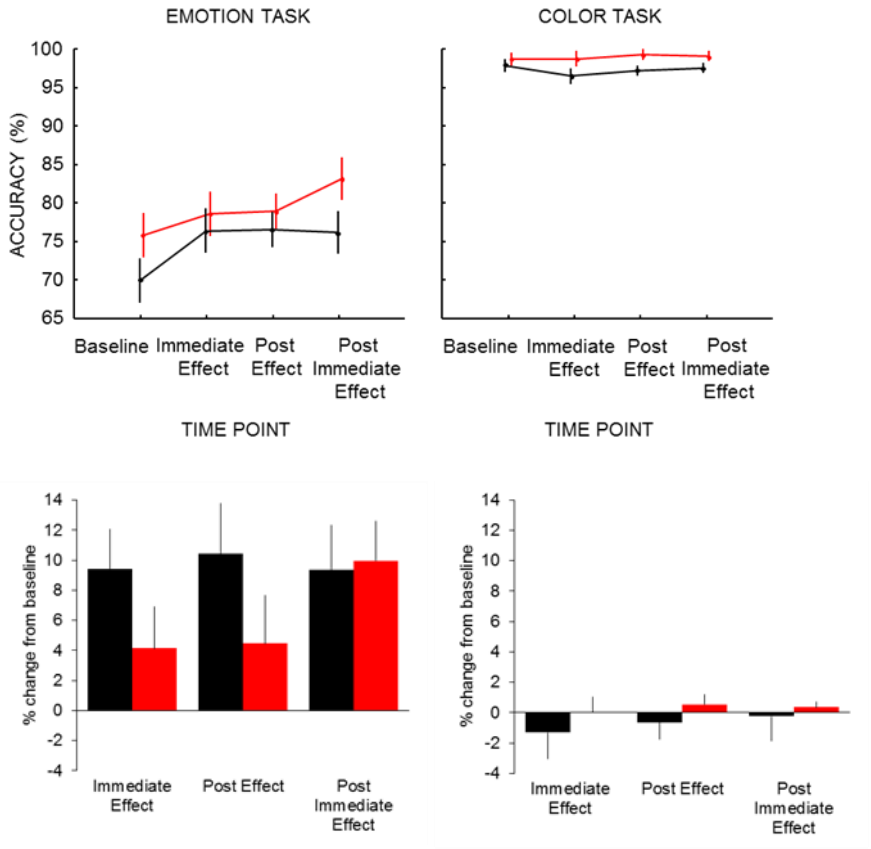


Figure 10. *Three-way interaction analysis of the accuracy on the PLD task between all four time points was not significant [F(3,42) = 1.886, p = 0.147] (above). Group differences in percentage change from baseline were not significant (below). Red = OT, black = PL. Data are expressed as means ± SEM.*

b) Reaction time

Figure 11, above shows the results of the reaction time during the two-week study. Evaluation of the latter revealed that all participants have a decreasing trend in reaction time, meaning both groups were responding faster across the four time points on the PLD task, but without significance [F(3,42) = 1.816, p = 0.159]. Still, the oxytocin group had the greatest improvement or the largest decrease in reaction time, as also depicted in the percentage change from baseline graphs (**Figure 11, below**). In addition a significant *task* x *time point* interaction was revealed [F(3,42) = 32,651, p < 0.000001], indicating that, irrespective of group, reaction time decreased more from baseline on the Emotion Task, compared to the Color Task.

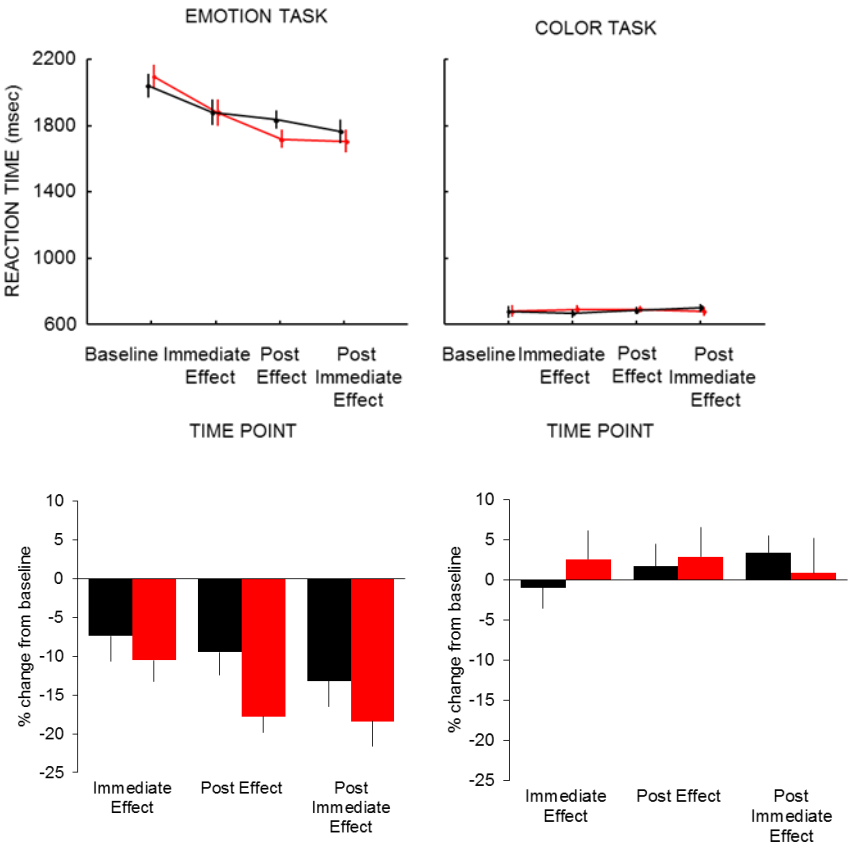


Figure 11. *Three-way interaction analysis of the reaction time on the PLD task between all four time points was not significant [F(3,42) = 1.816, p = 0.159] (above). Group differences in percentage change from baseline were not significant (below). Red = OT, black = PL. Data are expressed as means ± SEM.*

Session 1: Immediate, single-dose effect

a) Accuracy

Examination of the three-way interaction of *task* x *time point* x *group* (including time point 1 and 2 of **Figure 10, above**) revealed no significant Immediate Effect on the accuracy measure on the PLD task [F(1,14) = 2.945, p = 0.108] (**Figure 12, above**). Yet, a significant *task* x *time point* interaction was revealed [F(1,14) = 13.03, p < 0.01)], indicating that, irrespective of group, performance improvements from baseline were more pronounced on the Emotion Task, compared to the Color Task. Also, group differences in percentage change from baseline were for both tasks not significant (Emotion Task [F(1,14) = 1.875, p = 0.192]; Color Task [F(1,14) = 0.441, p = 0.517]) (**Figure 12, below**).

Exploration of the effects of nasal spray administration for each task separately, revealed no significant *time point* x *group* interaction, neither for the Emotion Task [F(1,14) = 1.771, p = 0.205] nor for the Color Task [F(1,14) = 0.519, p = 0.483].

These results are in contrary with *Experiment 1*, where a single-dose of oxytocin differentially affected accuracy performance only on the Emotion Task, immediately after nasal spray administration.

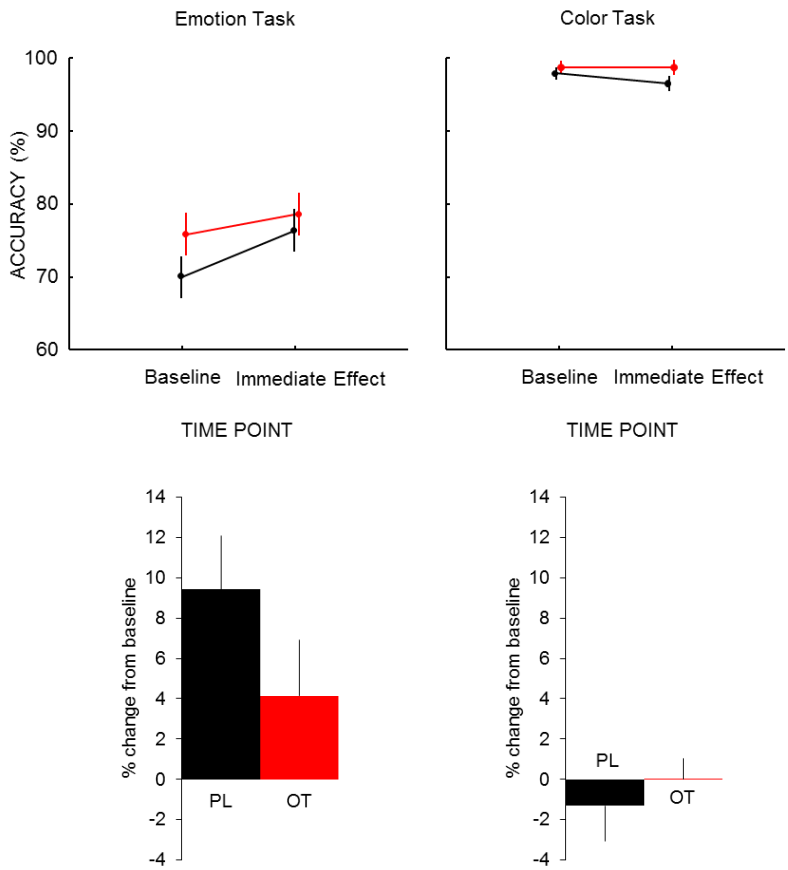


Figure 12. *The effect of oxytocin on the accuracy on the PLD task was nor time point, neither task dependent [F(1,14) = 2.945, p = 0.108] (above). Group differences in percentage change from baseline were not significant (below). Red = OT, black = PL. Data are expressed as means ± SEM.*

b) Reaction time

Analysis of the *task* x *time point* x *group* interaction (including time point 1 and 2 of **Figure 11, above**) for the reaction time revealed no significant Immediate Effect compared to baseline for both groups [F(1,14) = 0.811, p = 0.383] (**Figure 13, above**). Further, as for the accuracy measure, also the reaction time measure showed a significant two-way ANOVA interaction between *task* x *time point* [F(1,14) = 20.447, p < 0.001], indicating that performance improvements from baseline were more pronounced on the Emotion Task, compared to the Color Task. Group differences in percentage change from baseline were not significant for the Emotion Task [F(1,14) = 0.537, p = 0.476] or the Color Task [F(1,14) = 0.624, p = 0.442] (**Figure 13, below**).

Also for the reaction time measure, no significant *time point* x *group* interaction effects were revealed when performance effects were explored separately for each task (Emotion Task [F(1,14) = 0.393, p = 0.541]; Color Task [F(1,14) = 0.519, p = 0.483]). This indicates that compared to placebo, a single-dose of oxytocin administration did not differentially affect response times on neither task.

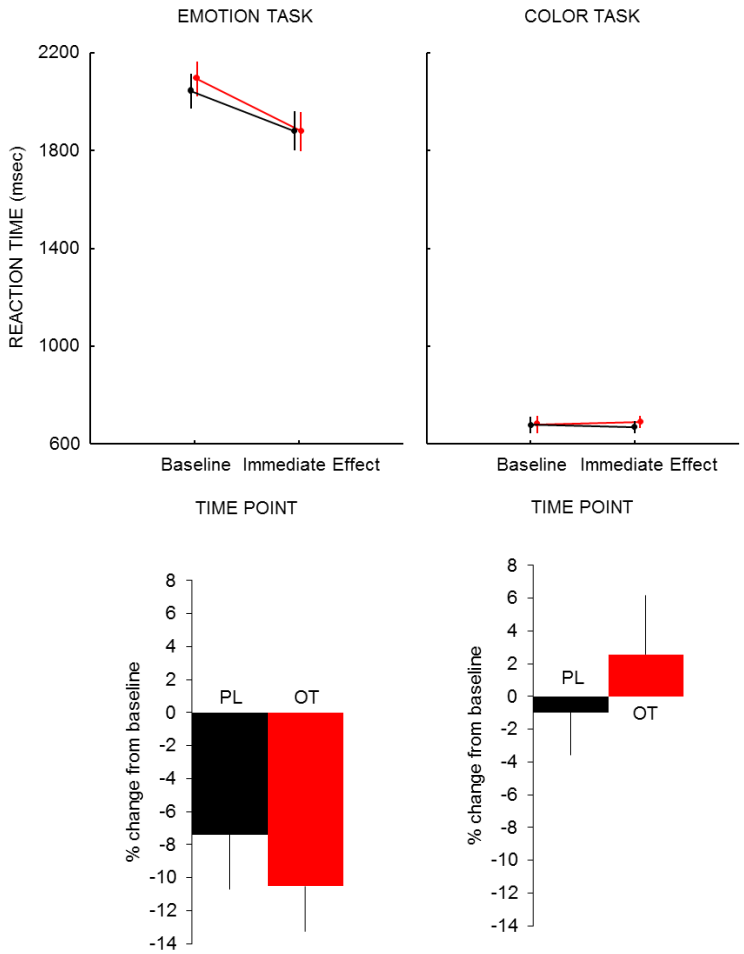


Figure 13. *Reaction time on the PLD task showed no significant three-way interaction effect [F(1,14) = 0.811, p = 0.383] (above). Group differences in percentage change from baseline were not significant (below). Red = OT, black = PL. Data are expressed as means ± SEM.*

Table 1 and **Table 2** of the **Appendix**, **Addendum 4** show the percentage responders and non-responders of the comparison between the Baseline and Immediate Effect measure on the PLD task for the accuracy and reaction time, respectively.

Effect of two weeks oxytocin administration

a) Accuracy

Three-way ANOVA analysis did not result in a *task* x *time point* x *group* interaction (considering time point 1 and 3 of **Figure 10, above**) [F(1,14) = 2.227, p = 0.158] (**Figure 14, above**). Yet, a significant *task* x *time point* interaction was revealed [F(1,14) = 9.927, p < 0.01)], indicating that, irrespective of group, performance improvements from baseline after two weeks of nasal spray administration were more pronounced on the Emotion Task, compared to the Color Task. Also, percentage change from baseline did not differ between both groups after two weeks of nasal spray administration on the Emotion Task [F(1,14) = 1.65, p = 0.22] and the Color Task [F(1,14) = 0.835, p = 0.376] (**Figure 14, below**).

Direct exploration of effects of nasal spray administration separately for each task revealed no significant *time point* x *group* effects (Emotion Task [F(1,14) = 1.282, p = 0.277]; Color Task [F(1,14) = 0.935, p = 0.35]).

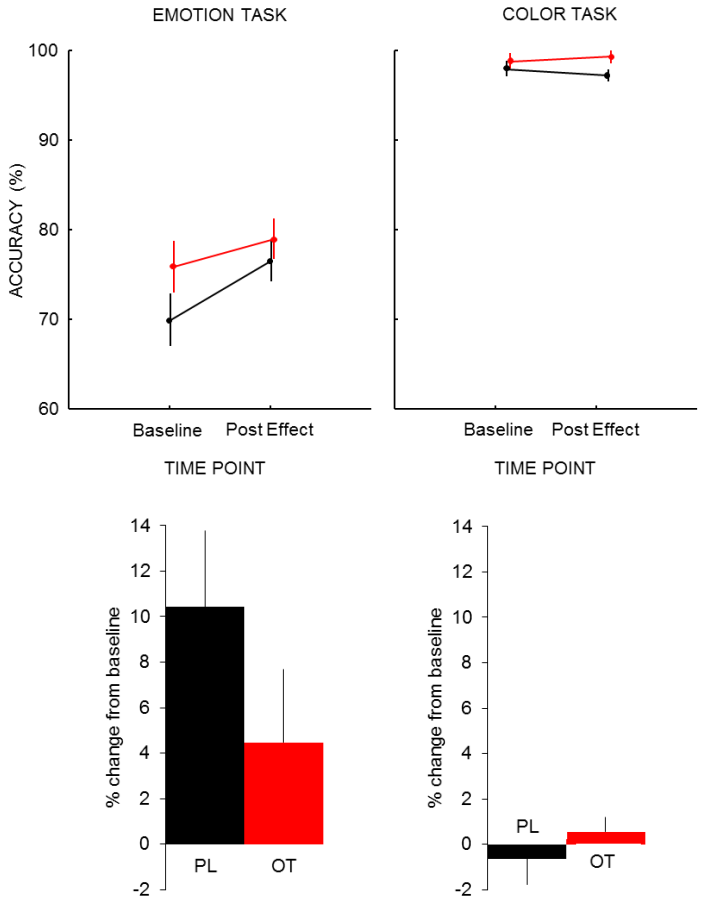


Figure 14. *The effect of two weeks oxytocin nasal spray administration on the accuracy on the PLD task was nor time point, neither task dependent [F(1,14) = 2.227, p = 0.158] (above). Group differences in percentage change from baseline were not significant (below). Red = OT, black = PL. Data are expressed as means ± SEM.*

b) Reaction time

Although no significant *task* x *time point* x *group* interaction (considering time point 1 and 3 of **Figure 11, above**) was revealed for the accuracy on the PLD task, we did found such a three-way interaction effect for the reaction time measure [F(1,14) = 4.722, p < 0.05] (**Figure 15, above**), indicating that, although both groups improved performance, reductions in reaction times were more pronounced for the oxytocin group (p < 0.001), compared to the placebo group (p < 0.01).

Also group differences in percentage change from baseline were only significant for the Emotion Task (± 20%) [F(1,14) = 5,335, p < 0.05] and not for the Color Task (± 12%) [F(1,14) = 0.0697, p = 0.796] (**Figure 15, below**).

In addition to the significant three-way interaction, we also revealed a two-way interaction of *task* x *time point*, indicating that, irrespective of group, performance improved specifically on the Emotion Task, not on the Color Task [F(1,14) = 54.525, p <000001]. Also a trend towards significance of the two-way *time point* x *group* interaction [F(1,14) = 3.17, p = 0.0967], and a significant a main effect of *time point* was revealed [F(1,14) = 36.117, p < 0.0001].

Direct exploration of effects of nasal spray administration separately for each task revealed a borderline significant *time point* x *group* effect for the Emotion Task [F(1,14) = 4.385, p = 0.0549], but no significance for the Color Task [F(1,14) = 0.0246, p = 0.878].

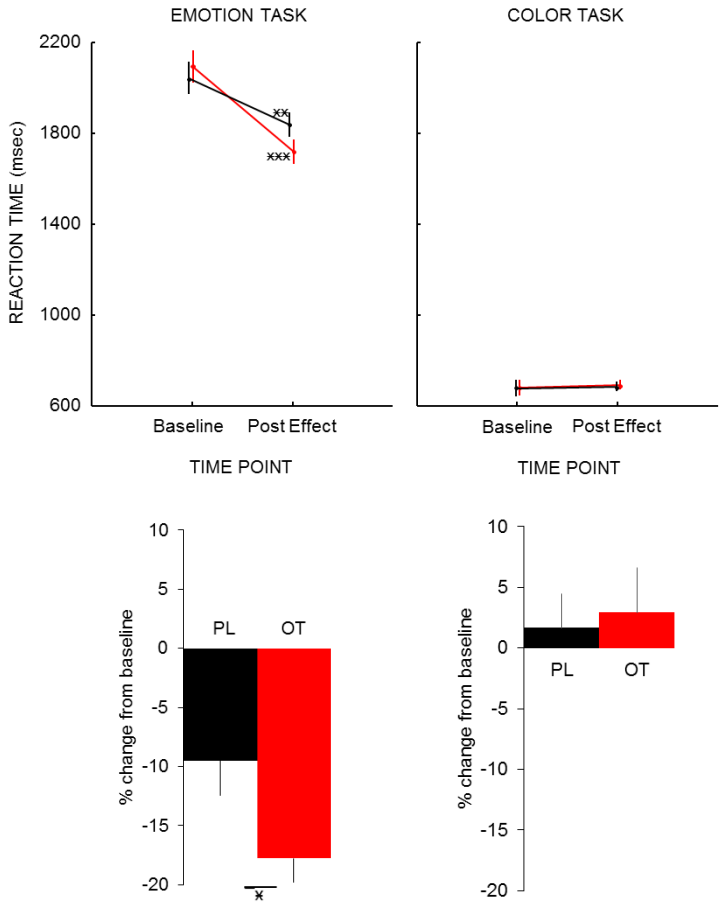


Figure 15. *The effect of oxytocin on the reaction time of the PLD task was time and task dependent [F(1,14) = 4.722, p < 0.05] (above). Group differences in percentage change from baseline were only significant on the Emotion Task [F(1,14) = 5,335, p < 0.05] (below). Red = OT, black = PL. Data are expressed as means ± SEM. *: p < 0.05, **: p < 0.01, ***: p < 0.001*

Table 3 and **Table 4** of the **Appendix**, **Addendum 4** show the percentage responders and non-responders of the comparison between the Baseline and Post Effect measure of the accuracy and reaction time on the PLD task, respectively.

Session 2: Effect of two weeks oxytocin administration on single-dose administration

a) Accuracy

A significant three-way interaction effect of accuracy was revealed between *task* x *time point* x *group* (considering time point 3 and 4 of **Figure 10, above**), indicating that only in the oxytocin group, not in the placebo group, performance still increased after two weeks of nasal spray administration, only on the Emotion Task, not on the Color Task [F(1,14) = 5.151, p < 0.05]. Also a post-hoc Tukey's HSD test was significant only for the oxytocin group (p < 0.05), not for the placebo group (p = 1.00) (**Figure 16, above**). Yet, group differences in percentage change from baseline only showed a trend towards significance on the Emotion Task [F(1,14) = 2.64, p = 0.126] and no significance on the Color Task [F(1,14) = 0.166, p = 0.69] (**Figure 16, below**).

In addition, assessing the *time point* x *group* interaction effect directly for the Emotion Task, revealed only a tentative effect, despite of the steep increase in performance improvement in the oxytocin group [F(1,14) = 2.745, p = 0.12]. For the Color Task, the effect was not significant [F(1,14) = 0.135, p = 0.719].

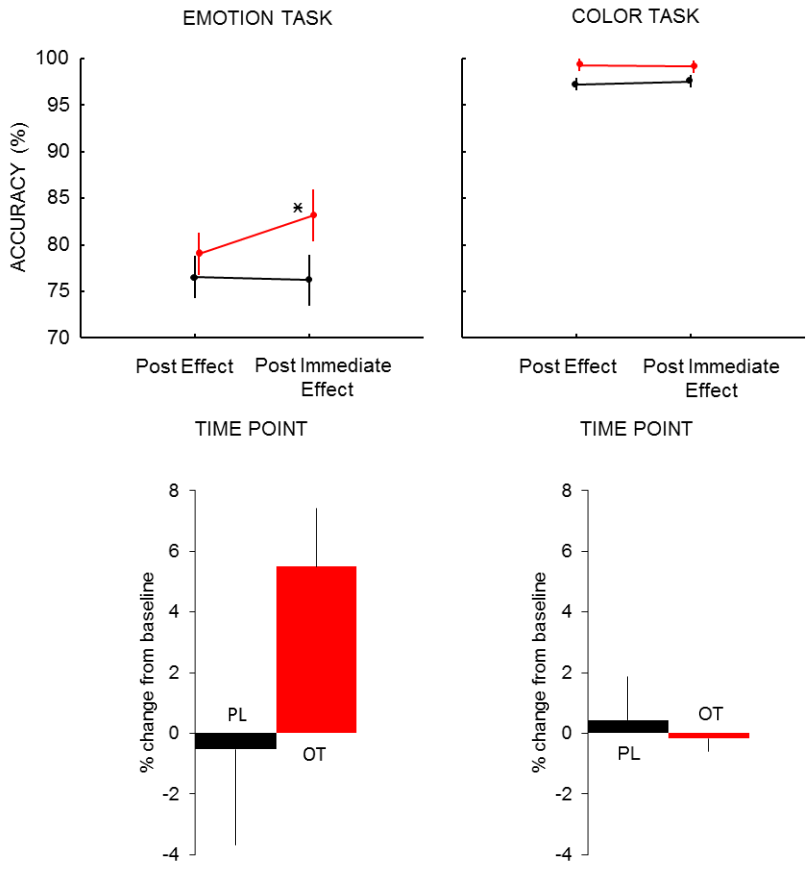


Figure 16. *The effect of oxytocin on the accuracy on the Emotion Task of the PLD task was time point and task dependent on the Post Immediate Effect measure [F(1,14) = 5.151, p < 0.05] (above). Group differences in percentage change from baseline were not significant (below). Red = OT, black = PL. Data are expressed as means ± SEM. *: p < 0.05*

b) Reaction time

Examination of the reaction time on the PLD task of both groups between the Post Effect and Post Immediate Effect measure (considering time point 3 and 4 of **Figure 11, above**) revealed no significant *task* x *time point* x *group* interaction effect [F(1,14) = 2.803, p = 0.116] (**Figure 17, above**). Also, group differences in percentage change from baseline were not significant for both tasks (Emotion Task [F(1,14) = 1.229, p = 0.286]; Color Task [F(1,14) = 2.47, p = 0.138]) (**Figure 17, below**).

Further, direct explorations of *time* x *group* interactions separately for each task revealed no significant results (Emotion Task [F(1,14) = 1.492, p = 0.242]; Color Task [F(1,14) = 2.38, p = 0.145]).

These results indicate that compared to placebo, a single-dose of oxytocin administration after two weeks of daily nasal spray administration did not differentially affect response times on neither task.

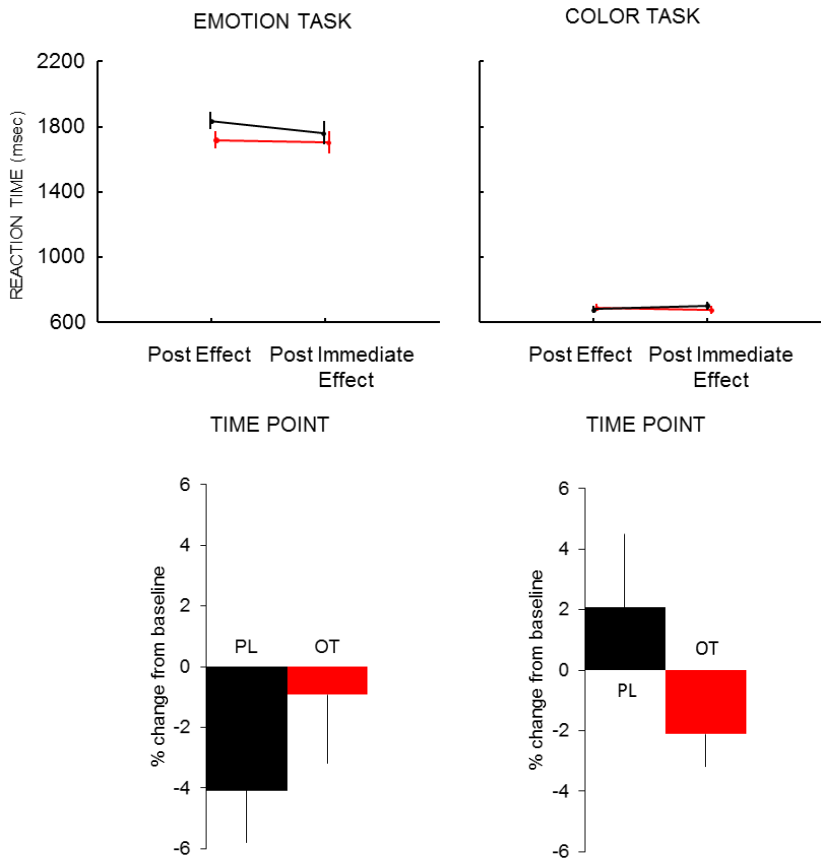


Figure 17. *Reaction time on the PLD task showed no significant three-way interaction effect [F(1,14) = 2.803, p = 0.116] (above). Group differences in percentage change from baseline were not significant (below). Red = OT, black = PL. Data are expressed as means ± SEM.*

Table 5 and **Table 6** of the **Appendix**, **Addendum 4** show the percentage responders and non-responders of the comparison between the Post Effect and Post Immediate Effect measure on the PLD for the accuracy and reaction time, respectively.

3.2.3 *Experiment 3*: Effect of two weeks of daily oxytocin administration on a facial emotion recognition task and a social interaction processing task

To further explore the multiple-dose effects of oxytocin on emotional and social functioning, two additional tasks were included in the study: the Facial Emotion Recognition Task (FERT) and the social task. Both tasks were assessed before and after two weeks of daily nasal spray administration. No immediate single-dose effects were assessed for these tasks.

Facial Emotion Recognition Task

In order to examine the multiple-dose effects of oxytocin on facial emotion recognition, participants (8 PL, 8 OT) performed the FERT. Only accuracy measures, no reaction time measures were assessed from the FERT, due to the computer mouse scrolling to indicate answers.

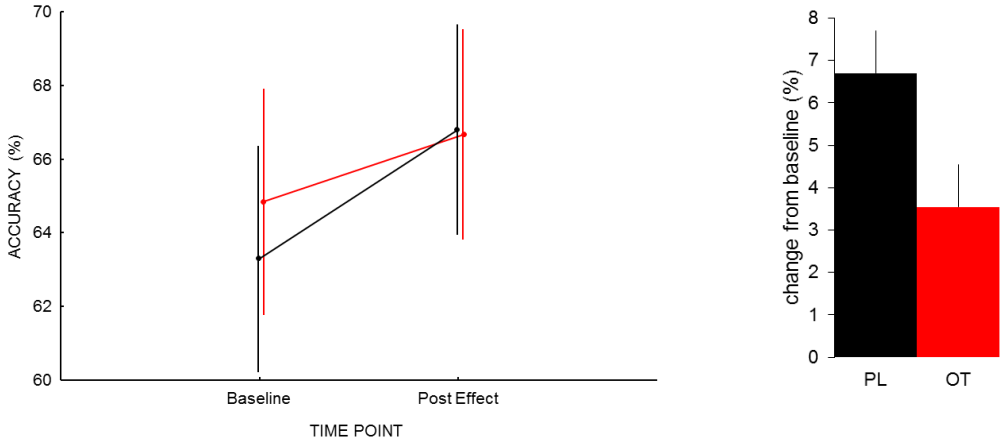


Figure 18. *Two-way interaction analysis between group and time point for the accuracy on the FERT task was not significant [F(1,14) = 0.2, p = 0.662] (left). Red = OT, black = PL. Data are expressed as means ± SEM.*

No significant *time point* x *group* interaction was revealed, indicating that two weeks of daily oxytocin administration did not specifically enhance performance on the FERT, compared to the placebo group [F(1,14) = 0.2, p = 0.662] (**Figure 18, left**). Although both groups showed a trend towards higher performance, on the post, compared to the baseline session, the main effect of *time point* did not reach significance [F(1,14) = 1.989, p = 0.18]. The latter indicates that, irrespective of group, performance on the FERT did not improve or decline after two weeks of nasal spray administration. Further, group difference in percentage change between baseline and two weeks of nasal spray administration was not significant [F(1,14) = 0.24, p = 0.632] (**Figure 18, right**).

Table 1 of the **Appendix**, **Addendum 5** summarizes the percentage of responders and non-responders for the accuracy on the FERT after two weeks of nasal spray administration.

ANOVA analysis for the six emotions separately revealed only a significant effect of *happy* (happy [F(1,14) = 5,744, p < 0.05]; other, all F < 2.333, all p > 0.149), however, the placebo groups shows a trend towards better performance while the oxytocin group performed worse compared to baseline.

Social task

To study the multiple-dose effects of oxytocin on social functioning, participants performed the social task. Reaction times are the most important measure of this task, but differences in performance accuracy were also assessed. Results of six PL and eight OT participants were included in the analysis.

a) Reaction Time

For the reaction time measure, we did not reveal a significant three-way interaction between *task* x *time point* x *group* [F(1,12) = 0.296, p = 0.596] (**Figure 19, left**). In addition, group differences in percentage change from baseline were neither significant (Social Task [F(1,12) = 0.278, p = 0.608]; Control Task [F(1,12) = 0.0186, p = 0.894]) (**Figure 19, below**).

Also no significant two-way interactions between *time point* and *group* were revealed, when effects were explored separately for each task (Social Task [F(1,12) = 0.328, p = 0.577]; Control Task [F(1,12) = 0.00082, p = 0.978]). Further, no general effects of *time point* were found, indicating that irrespective of group, performance on the social task did not improve or decline after two weeks of nasal spray administration (Social Task [F(1,12) = 1.438, p = 0.254]; Control Task [F(1,12) = 0.00082, p = 0.978]).

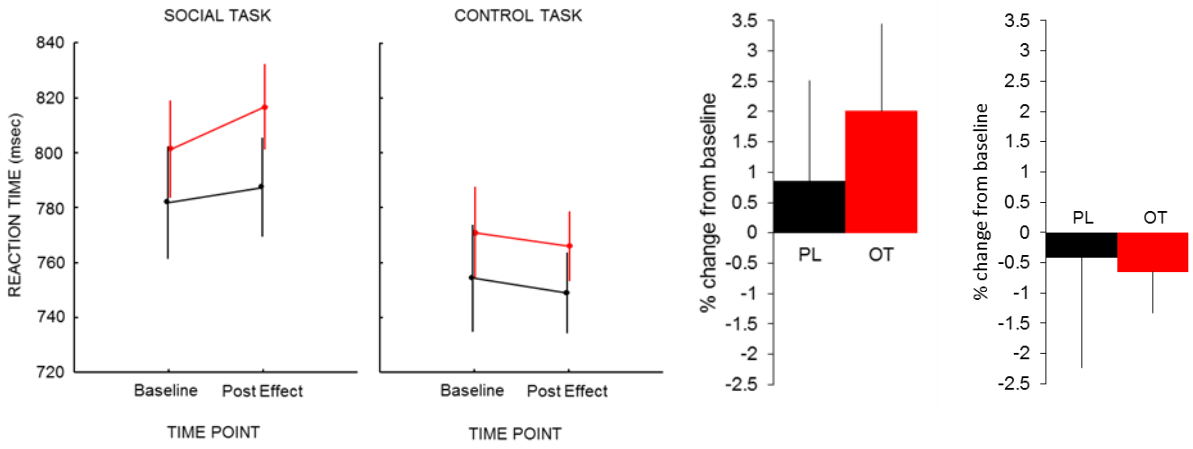


Figure 19. *Three-way interaction analysis of the reaction time on the social task was not significant [F(1,12) = 0.296, p = 0.596] (left). Red = OT, black = PL. Data are expressed as means ± SEM.*

Table 2 of the **Appendix**, **Addendum 5** summarizes the percentage of responders and non-responders for the reaction time performance on the social task after two weeks of nasal spray administration.

b) Accuracy

Similarly as the three-way ANOVA analysis on the reaction time, no significant interaction was revealed on the accuracy measure on the social task [F(1,12) = 0.566, p = 0.466]. In addition, there were no group differences in percentage change from baseline (Social Task [F(1,12) = 0.185, p = 0.675]; Control Task [F(1,12) = 0.448, p = 0.516]).

3.2.4 *Experiment 4*: Effects of two weeks of daily oxytocin administration on several questionnaires

In *Experiment 4*, several questionnaires were administered at baseline, and after two weeks of daily oxytocin administration to examine long-term, multi-dose effects of oxytocin on social functioning and attachment scales.

A two-way ANOVA analysis was conducted for each questionnaire with the within-subject factor *time point* (Baseline and Post Effect) and the between-subject factor *group* (placebo and oxytocin).

Social Responsiveness Scale (SRS)

Changes in social functioning were assessed using the SRS questionnaire. Both a self-report and informant version (50% partner, 31.25% close friend, 18.75% parent) were administered.

a) Self-reported SRS

No significant *time point* x *group* interaction effect was revealed for the total score on the SRS [F(1,29) = 2.662, p = 0.114] (**Figure 20**). Only a significant main effect of *time point* was revealed [F(1,29) = 16.646, p < 0.001)]. These findings indicate that irrespective of type of nasal spray, oxytocin or placebo, all participants tended to have lower SRS scores, indicating less social impairment, after the two-week trial, compared to baseline. Percentage change from baseline were also not significant different between both groups [F(1,29) = 0.982, p = 0.33] (**Figure 21**).

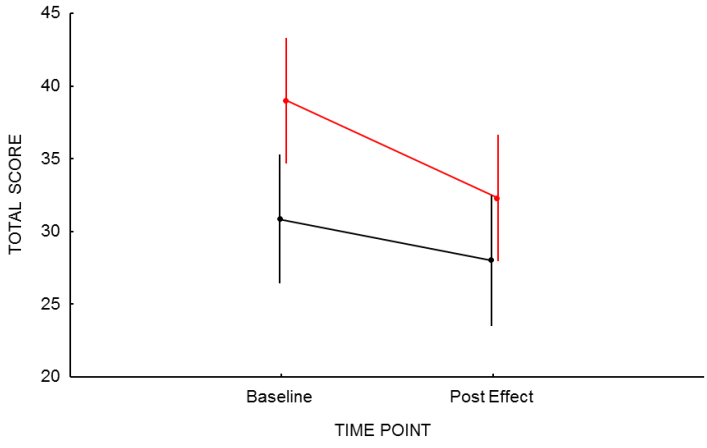


Figure 20. *No interaction effect was found between group and time point on the SRS total score [F(1,29) = 2.662, p = 0.114]. Red = OT, black = PL. Data are expressed as means ± SEM.*

Table 2 shows that the oxytocin group includes mostly responders on the total SRS score. The same is true for the placebo group, still the oxytocin group has more responders compared to the placebo group.

	Responders (%)	Non-Responders (%)	
		No change (%)	*Increase (%)*
PL (N = 15)	9 (60%)	0 (0%)	6 (40%)
OT (N = 16)	14 (87.5%)	0 (0%)	2 (12.5%)

Table 2. *For each group, PL and OT, the percentage of responders (decrease of score) and non-responders (no change or increase of score) is given for the total SRS score.*

Next, effects of oxytocin were explored for each of the four subscales of the SRS. As shown in **Figure 22 A**, the subscale on *social motivation* revealed a significant interaction effect between *group* and *time point*, which indicates a significant improvement in the oxytocin group but not in the placebo group after the two-week trial [$F(1,29) = 8.337$, $p < 0.01$]. A post-hoc Tukey's HSD test of the *social motivation* subscore was highly significant for the oxytocin group ($p < 0.001$) and not significant for the placebo group ($p = 0.948$). However, direct exploration of the percentage change from baseline showed no significant difference between both groups [$F(1,29) = 1.841$, $p = 0.185$] (**Figure 21**).

As visualized in **Figure 21**, no significant group differences in percentage change from baseline were revealed for the other three subscales (*social consciousness* [$F(1,29) = 1.242$, $p = 0.274$]; *social communication* [$F(1,29) = 0.254$, $p = 0.618$]; *rigidity/repetitiveness* [$F(1,29) = 0.09$, $p = 0.766$].
Also two-way ANOVA analyses of the *time point* x *group* interaction were not significant. (*social consciousness* [$F(1,29) = 0.466$, $p = 0.50$]; *social communication* [$F(1,29) = 0.603$, $p = 0.444$]; *rigidity/repetitiveness* [$F(1,29) = 0.0835$, $p = 0.775$].

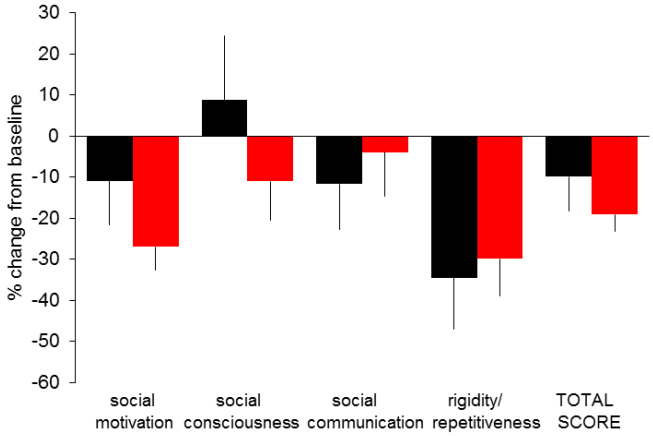


Figure 21. *Group differences in percentage change from baseline for the total SRS and all four subscales were not significant. Red = OT, black = PL. Data are expressed as means ± SEM.*

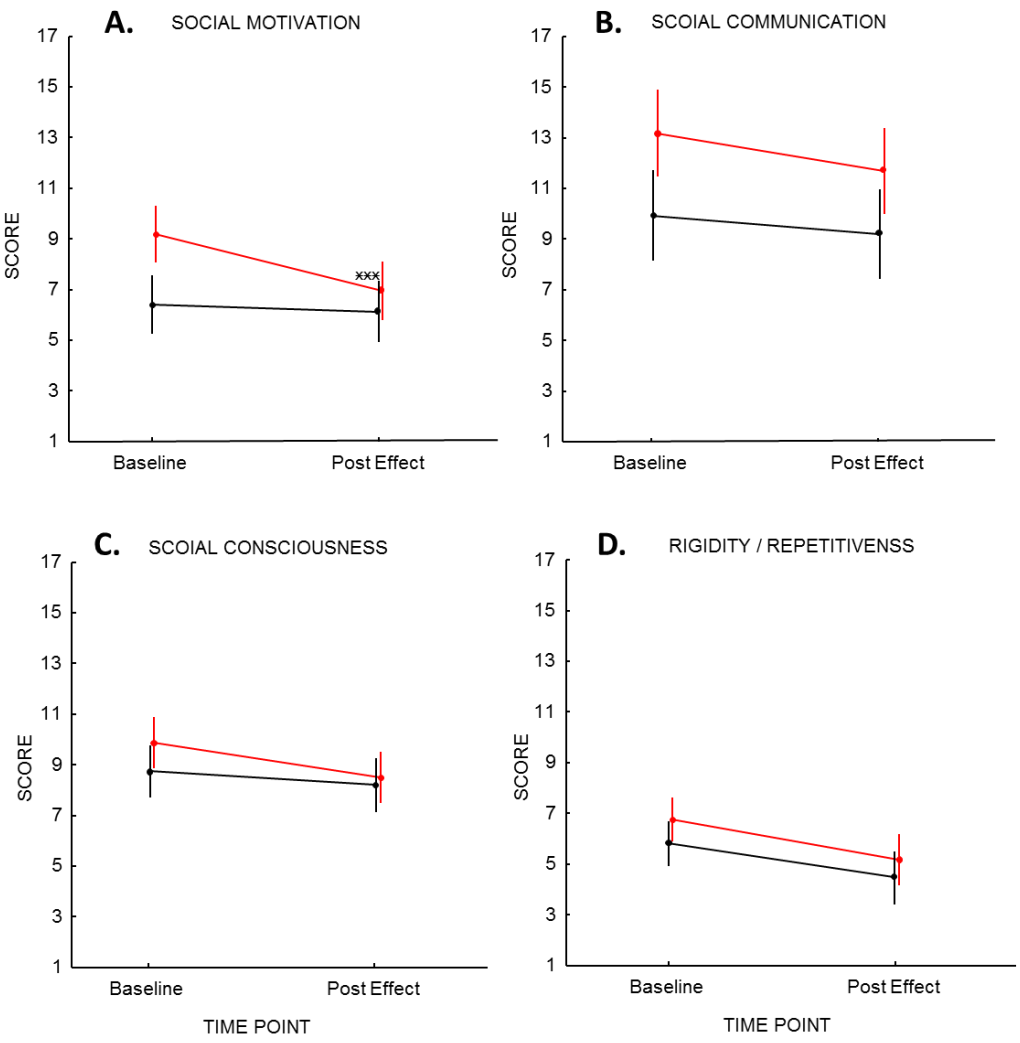


Figure 22. *Two-way ANOVA analysis on all four SRS subscales revealed a significant time point x group interaction only on the social motivation subscale [F(1,29) = 8.337, p < 0.01] (A). Red = OT, black = PL. Data are expressed as means ± SEM. ***: p < 0.001*

b) Informant SRS

Nine informants did not fill in the post measure (4 PL, 5 OT). No significant effects were revealed for the informant SRS scores (total SRS score [F(1,21) = 0.399, p = 0.534]; subscales, all F < 1.859, p > 0.187).

Restrictive Behaviors Scale-Revised (RBS-R)

The RBS-R assesses restricted and repetitive behaviors and interests, which are one of the core features of ASD.

No significant *time point* x *group* interaction was revealed on the total score of the RBS-R [F(1,30) = 0,128, p = 0.723] (**Figure 23, left**). Group differences in percentage change from baseline of the total score and all five subscales were also not significant (all F < 2.901, all p > 0.0992) (**Figure 23, right**).

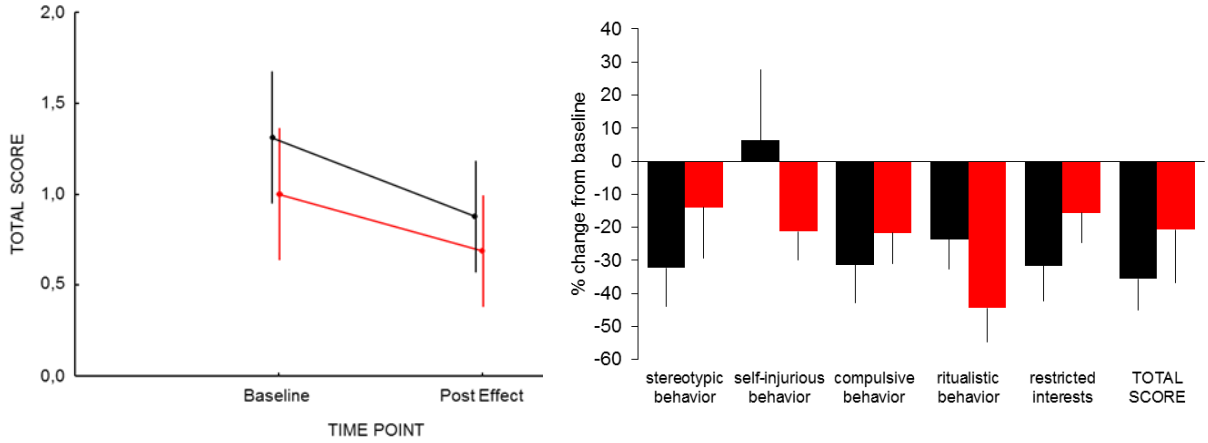


Figure 23. *No significant change was revealed on the total RBS-R score when comparing both groups between baseline and post two weeks of nasal spray administration [F(1,30) = 0.128, p = 0.723] (left). Group differences in percentage change from baseline for all five subscales were neither significant (right). Red = OT, black = PL. Data are expressed as means ± SEM.*

Table 3 shows that for both groups, the majority of the participants were non-responders regarding the total score of the RBS-R, which is in agreement with the results of **Figure 24**.

	Responders (%)	Non-Responders (%)	
		No change (%)	*Increase (%)*
PL (N = 16)	7 (43.75%)	6 (37.50%)	3 (18.75%)
OT (N = 16)	5 (31.25%)	9 (56.25%)	2 (12.5%)

Table 3. *For each group, PL and OT, the percentage of responders (decrease of score) and non-responders (no change or increase of score) is given for the total RBS-R score.*

World Health Organization Quality of Life-BREF (WHOQOL-BREF)

The WHOQOL-BREF questionnaire comprises several general wellbeing questions divided in four subscales, including *physical health*, *psychological health*, *social relationships* and *environment*.

First, statistics of the WHOQOL-BREF total score will be reported, whereafter the subscale *social relationships* will be discussed.

a) WHOQOL-BREF total score

No significant *time point* x *group* interaction effect was revealed on the total WHOQOL-BREF score [F(1,30) = 0.00165, p = 0.968], indicating that two weeks of nasal spray administration did not affect the total score of this questionnaire (**Figure 24**). Similarly, group difference in percentage change from baseline was not significant for the WHOQOL-BREF total score [F(1,30) = 0.00482, p = 0.945] (**Figure 25**). We did reveal a general effect of *group* ([F(1,30) = 4.59, p < 0.05], indicating overall higher quality of life scores in the placebo, compared to the oxytocin group.

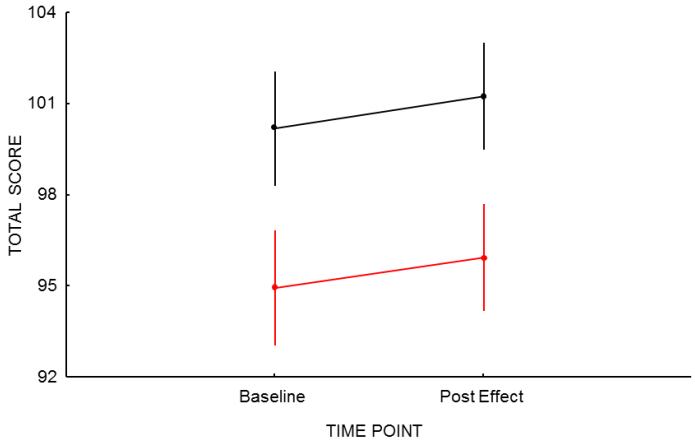


Figure 24. *Two weeks of oxytocin administration did not improve total scores on the WHOQOL-BREF questionnaire compared to placebo since no time point x group interaction was revealed [F(1,30) = 0.00165, p = 0.968]. Red = OT, black = PL. Data are expressed as means ± SEM.*

Table 4 shows that the majority of participants of both groups responded on the total WHOQOL-BREF score two weeks after the Baseline measure.

	Responders (%)	Non-Responders (%)	
		No change (%)	Decrease (%)
PL (N = 16)	11 (68.75%)	1 (6.25%)	4 (25%)
OT (N = 16)	10 (62.50%)	1 (6.25%)	5 (31.25%)

Table 4. *For each group, PL and OT, the percentage of responders (increase of score) and non-responders (no change or decreasing score) is given for the total WHOQOL-BREF score.*

b) WHOQOL-BREF subscales

Two-way ANOVA analysis did not reveal a *time point* x *group* interaction on any of the four subscales (all F < 4.121, all p > 0.0513), although the subscale with the lowest p-value, *psychological health*, was borderline significant. Note though that participants of the placebo group, not of the oxytocin group, showed a trend towards improvement on this subscale. Percentage change from baseline scores were not significantly different between the placebo and oxytocin group for all subscales (all F < 3.0665, all p > 0.0901) (**Figure 25**).

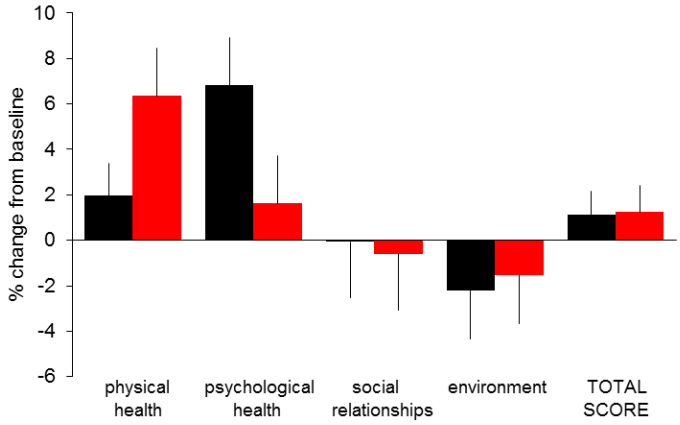


Figure 25. *Group differences in percentage change for the total WHOQOL-BREF score and all four subscales were not significant. Red = OT, black = PL. Data are expressed as means ± SEM.*

State Adult Attachment Measure (SAAM)

In addition to the aforementioned questionnaires, testing changes on core autism-related behaviors, or general life quality, we also assessed whether oxytocin administration affected measures of attachment. The SAAM assesses state attachment, i.e., how safely attached the participant feels at that particular moment. The SAAM questionnaire encompasses three subscales, including *security*, *anxiety* and *avoidance*.

Exploration of the three subscales separately revealed that two weeks of oxytocin administration significantly improved the *avoidance* subscore [F(1,30) = 5.949, p < 0.05] (**Figure 26, left**). A post-hoc Tukey's HSD test revealed a borderline significant effect for the oxytocin group (p = 0.0507), but not for the placebo group (p = 0.882). Similarly, group differences in percentage change from baseline were only significant for the avoidance subscale [F(1,30) = 6.862, p < 0.05] (**Figure 26, right**).

The *security* and *anxiety* subscale showed no *time point* x *group* interaction (all F < 0.0899, all p > 0.766), and no significant group differences between the percentage change from baseline scores (all F < 0.00637, all p > 0.937) (**Figure 26, right**).

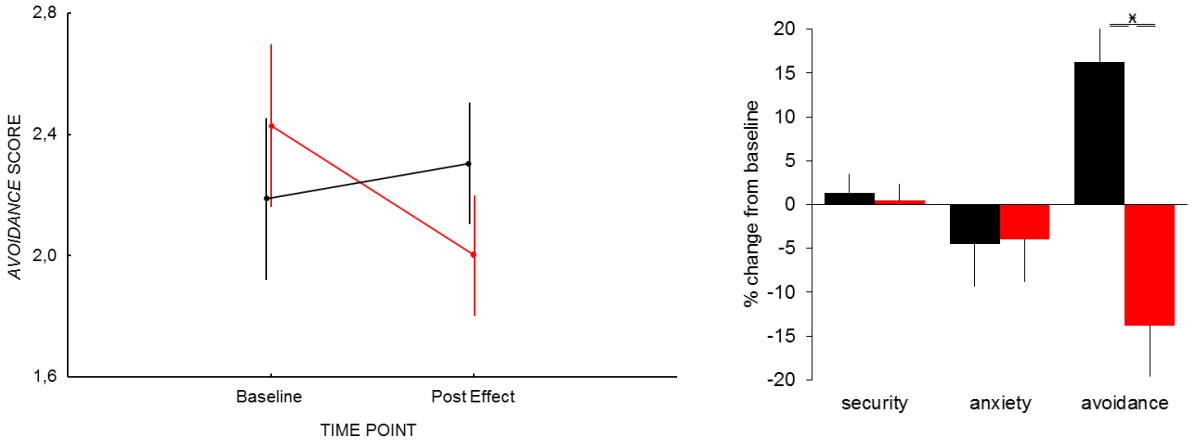


Figure 26. *Two-way ANOVA analysis of time point x group on the three SAAM subscales revealed a significant effect on the avoidance subscale [F(1,30) = 5.949, p < 0.05] (left). Group differences in percentage change from baseline were not significant (right). Red = OT, black = PL. Data are expressed as means ± SEM. *: p < 0.05*

Table 5 shows that for each subscale, the majority of the oxytocin group responded and exceeds the number of responders in the placebo group for the *security* and *anxiety* subscale. Also, the result of **Figure 26** is translated in this table as the greatest number of responders were participants of the oxytocin group for the *avoidance* subscale. The placebo group did not respond on the *security* and *avoidance* subscale.

		Responders (%)	Non-Responders (%)	
			No change (%)	*Decrease (Se) (%) Increase (An, Av) (%)*
Security	PL (N = 15)	7 (46.67%)	1 (6.67%)	7 (46.67%)
	OT (N = 16)	9 (56.25%)	0 (0%)	7 (43.75%)
Anxiety	PL (N = 16)	9 (56.25%)	1 (6.25%)	6 (37.5%)
	OT (N = 16)	9 (56.25%)	2 (12.5%)	5 (31.25%)
Avoidance	PL (N = 16)	5 (31.25%)	2 (12.5%)	9 (56.25%)
	OT (N = 16)	11 (68.75%)	2 (12.5%)	3 (18.75%)

Table 5. *For each group, PL and OT, the percentage of responders and non-responders is given for the security (Se), anxiety (An) and avoidance (Av) subscale.*

Inventory of Parent and Peer Attachment (IPPA)

The IPPA scale assesses trait attachment towards a participant's *peers*, *mother*, *father* (not filled in by 1 OT participant) and an *important person* [50% partner, 21.87% close friend, 28.13% unanswered (4 PL, 5OT)].

Two-way ANOVA analysis of the *time point* x *group* interaction revealed a trend towards significance for the *peers* subscale [F(1,30) = 2.811, p = 0.104] (**Figure 27, left**). Interaction analysis of *mother*, *father* and *important person* subscale were not significant (all F < 1.558, all p > 0.222). A significant effect of *group* is reported on the *important person* subscale, indicating higher attachment of the oxytocin group to this person (p = 0.01). Exploration of the group differences in percentage change from baseline for the IPPA subscales did not reveal significant effects (all F < 2.785, all p > 0.106) (**Figure 27, right**).

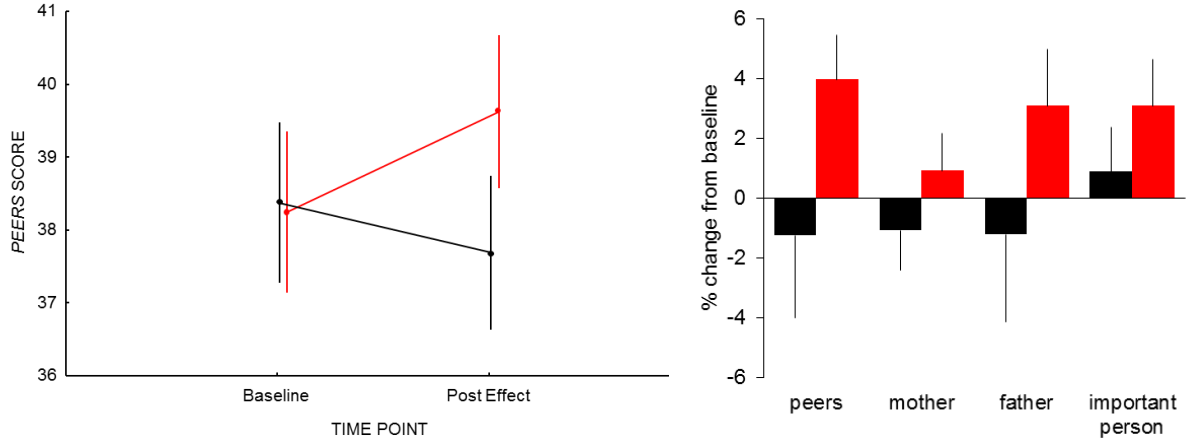


Figure 27. *The subscale of peers showed a trend towards significance [F(1,30) = 2.811, p = 0.104] (left). Group differences in percentage change from baseline are presented for all subscales (right). Red = OT, black = PL. Data are expressed as means ± SEM.*

Table 6 shows that the oxytocin group mostly responded on the *peers* and *father* subscale. For all subscales, half or more of the participants of the placebo group were non-responders.

		Responders (%)	Non-Responders (%)	
			No change (%)	*Decrease (%)*
Peers	PL (N = 16)	5 (31.25%)	5 (31.25%)	6 (37.50%)
	OT (N = 16)	10 (62.25%)	3 (18.75%)	3 (18.75%)
Mother	PL (N = 16)	4 (25%)	4 (25%)	8 (50%)
	OT (N = 15)	5 (33.33%)	4 (26.67%)	6 (40%)
Father	PL (N = 16)	7 (43.75%)	3 (18.75)	6 (37.5%)
	OT (N = 15)	10 (66.67%)	1 (6.67%)	4 (26.67%)
Important person	PL (N = 12)	6 (50%)	2 (16.67%)	4 (8.33%)
	OT (N = 11)	4 (36.36%)	4 (36.36%)	3 (27.27%)

Table 6. *For each group, PL and OT, the percentage of responders (increase of score) and non-responders (no change or decrease of score) is given for the four IPPA subscales.*

Profile of Mood States (POMS)

The POMS was administered to assess the participant's mood at each time point of the experiment. This questionnaire comprises five subscales: *tense*, *strong*, *tired*, *depressive* and *angry*. Half of the participants filled in the POMS twice (Baseline and Post Effect) and the other half five times (Baseline, Immediate Effect, Middle, Post Effect and Post Immediate Effect). Results of the POMS will be discussed in following order:

a) Changes in POMS scores from baseline to post session (including all participants)

Two-way ANOVA analysis revealed a significant effect of *tense* [$F(1,30) = 6.942$, $p < 0.05$], indicating that participants of the oxytocin group were less tense at the Post Effect measure, compared to placebo. None of the other four subscales revealed a significant *time point* x *group* interaction (all $F < 1.834$, all $p > 0.187$). In addition, no main effects of *time point* were revealed, indicating that, irrespective of nasal spray, all participants remained on the same mood level during the study.

b) Changes in POMS scores over all time points (Baseline, Immediate Effect, Middle, Post Effect and Post Immediate Effect (assessed for 16 of total N = 32 participants)

Two-way ANOVA analysis between *group* and *time point* for each subscale of the POMS questionnaire for the participants who filled in this form on five different time points revealed a significant interaction on the subscale *strong* [$F(4,52) = 4.620$, $p < 0.01$] (**Figure 29**), indicating that after one-week of nasal spray administration, participants using oxytocin reported a reduction in 'strong' mood compared to the placebo group (strong mood includes descriptions such as active, lively, energetic). The other four subscales revealed no such interaction effect (all $F < 2.134$, all $p > 0.0896$).

A significant effect of *group* was revealed on the subscale *depressive* and a trend towards significance on the subscale *angry*, indicating that in general, the placebo group had a more 'negative' mood compared to the oxytocin group. However, these differences were already present at baseline.
Also, a main effect of *time point* was revealed on the subscale *tired*, indicating that participants were less tired at later time points during the study [$F(4,48) = 3.933$, $p < 0.01$].

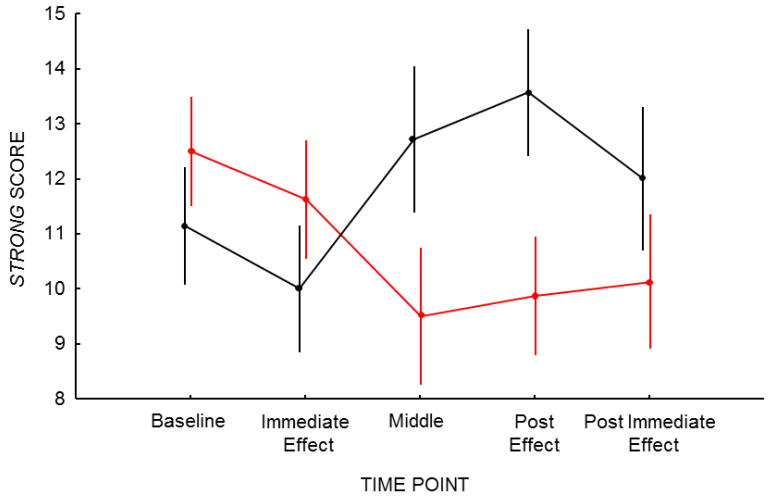


Figure 29. *A significant interaction between group and time point was revealed on the strong subscale of the POMS questionnaire. $F(4,52) = 4.620$, $p < 0.01$. Red = OT, black = PL. Data are expressed as means ± SEM.*

4 Discussion and conclusion

With the current study we aimed to explore the effects of oxytocin on social cognition. To do so, tasks and questionnaires measuring emotion recognition, attachment, communicative abilities, and social interaction were assessed.

4.1 Oxytocin results in immediately improved bodily emotion recognition

Experiment 1 showed a significant immediate effect on the accuracy on the Emotion Task of the point light display (PLD) task with the oxytocin group performing significantly better compared to placebo thirty minutes after nasal spray administration. This indicates that oxytocin can improve bodily emotion recognition, in terms of inferring observed emotional state merely from body kinematics. As such, oxytocin was shown to facilitate the reading of emotional body language of others.
Individuals with an autism spectrum disorder (ASD) have been shown to perform significantly worse compared to neurotypical controls on the same task (101). Therefore, it should be of interest to explore whether patients with ASD also benefit from intranasal oxytocin administration with regard to performance on the bodily emotion recognition task.

Along this line, it would be of interest to test the effects of oxytocin on other bodily emotion processing tasks, such as affective body expression classification tasks, which makes use of full-body grey-scale suits instead of PLDs, with the common feature that no facial expressions are present. Also, this full-body task comprises four emotions (happiness, anger, sadness and neutral) instead of only two (102).

4.2 Multiple-dose oxytocin administration during two weeks facilitates bodily emotion recognition on the longer term

Unlike *Experiment 1*, the PLD task in *Experiment 2* made use of an identical set of PLDs. This increases the chance of learning effects and indeed, a trend towards better performance was revealed in both groups on the single dose examination on day one. However, interestingly, we found a significant immediate effect on the post measurement, indicating that after two weeks of nasal spray administration, only the oxytocin group, not the placebo group still improved performance accuracy immediately after the last nasal spray administration. The fact that the placebo group no longer improves on the accuracy after the second measure indicates that the improvement of the oxytocin group is a consequence of the oxytocin. Similarly for the reaction time on the PLD task, the oxytocin group significantly improved performance after two weeks of nasal spray administration, compared to the placebo group.
To our knowledge, these findings are the first to report long-term effects of oxytocin on emotion recognition in a healthy population, however, trials with larger sample sizes are needed to replicate these results. Also, multiple-dose studies in ASD patients mostly include facial emotion recognition tasks (91, 94) instead of bodily emotion recognition tasks.

4.3 Two weeks of daily oxytocin administration has no effect on facial emotion recognition

Next to the examination of bodily emotion recognition, effects of two weeks of daily oxytocin administration were also assessed for a facial emotion recognition task. Despite the hypothesis of better emotion recognition upon oxytocin administration, we revealed no main significant effect of emotion on the Facial Emotion Recogntion Task (FERT) *(98)*. This is in contrary to the results of two previous oxytocin studies reporting improvements in facial emotion recognition after oxytocin administration, at least for recognizing the emotional states 'happy' and 'fear' (22, 23). Although, one study also did not reveal any effect of oxytocin on facial emotion recognition (103). Here, we only found a significant effect of the emotion 'happy' of the six basic emotions (happy, anger, sad, surprise, disgust and fear), unfortunately, in favor of the placebo group. However, the present data should be considered highly preliminary, considering that the included samples sizes were limited (eight participants in each of the intervention group). Future experiments including larger study samples are therefore necessary to further explore the current findings.

Next to the FERT, some alternative tests exist which are also attractive to examine the effect of oxytocin on facial emotion recognition. First, the Reading the Mind in the Eyes Test (RMET), in which subjects have to read the mind solely on pictures of the eye region (104). A study of Domes et al. (2007) with 30 healthy males showed that oxytocin facilitated the overall score of the RMET (21). The RMET is an advanced emotion recognition task since it includes more complex mental states, such as jealous or arrogant. Second, the Reading the Mind in the Film Task in which participants have to rate the emotions of the actors in a social scene fragment of a movie, representing daily life. Adults diagnosed with ASD score significantly lower on this task compared to healthy controls (105). Last, the Reading the Mind in the Voice Test-Revised includes several spoken fragments which have to be rated on the basis of verbal content and emotion (106). The original Reading the Mind in the Voice Test consists of a neutral sentence that is spoken in different intonations (107). Both versions of the Reading the Mind in the Voice test reported a difference between ASD patients and healthy controls with the latter performing significantly better.

4.4 Two weeks of daily oxytocin administration had no effect on the detection of social interactions

Participants performed the social task in order to verify if oxytocin can facilitate the recognition of social interactions in real life scenes. Oxytocin did not affect performance of the social task, neither on the actual Social Task, where participants have to discriminate between friendly and unfriendly interactions, nor on the Control Task, where participants have to discriminate between indoor and outdoor scenes. Again, due to the limited sample sizes (eight participants in each group), the present data should be considered highly preliminary. A previous study found that ASD participants perform tentatively worse on this task compared to controls (100).

Further, although the results of the social task were not significant, this task could be of relevance in an fMRI paradigm as it is known to increase the recruitment of brain areas involved with social cognition, such as the dorsomedial prefrontal cortex and the temporal poles (108).

4.5 Effect of two weeks of daily oxytocin administration on social functioning as assessed using the SRS questionnaire

Oxytocin and placebo showed a similar non-significant decreasing trend on the total score of the Social Responsiveness Scale (SRS), indicating slight increases in social functioning in both groups. However, when SRS subscale scores were analyzed separately, the oxytocin group was shown to significantly improve on the *social motivation* subscale, indicating that participants who received oxytocin rated themselves to take initiative for social interaction more easily post compared to prior of the two-week oxytocin trial.

A placebo-controlled randomized controlled trial (RCT) of eight weeks with ASD male adolescents who administered twice a day 18 or 24 IU oxytocin (94) and a six-week study (91) with adults receiving twice a day 24 IU did not show any improvements on the total score of the informant SRS questionnaire. Only the study of Guastella et al. (2015) explored additionally the SRS subscales, however, without significant results (94).

These findings are at odds with the current findings showing a significant effect of oxytocin at least on the *social motivation* scale. However differences in dose, duration of the trial and other trial-specific factors might have contributed to these divergent findings. Further, these factors might differ between patients with ASD and neurotypical samples, as well as for different age groups. Additional research might be necessary to define the optimal parameters for each population.

We also included an informant version of the SRS, but here we found no significant effects. One should note though that most of the included participants were students who lived in student dorms for the duration of the trial. Accordingly, informants – who were most often partners – did not have regular contact with the participant, and this might have prevented them from accurately noticing or reporting any changes related to the intervention. This factor might be relevant for future research to take into account.

When participants were asked after termination of the study if they noticed a difference in social behavior, three of sixteen participants who administered oxytocin reported a difference in socio-emotional behavior. One person reported to communicate better with his partner while the other one communicated better with colleagues. The third participant reported that he felt less attached to his partner after termination of the study. None of the participants in the placebo group reported a difference in social behavior.

4.6 Effects of two weeks of daily oxytocin on attachment

Oxytocin administration did significantly decrease the score on the *avoidance* subscale of the State Adult Attachment Measure (SAAM), meaning oxytocin lowers the avoidance of close relationships and intimate bonds (109). The second attachment measure, the Inventory of Parent and Peer Attachment (IPPA), only revealed a trend for increased attachment towards peers. This could be explained because the majority did not or had little contact during the week with both parents or the important person. Further, the IPPA measures *trait* attachment, i.e., the stable characteristic of attachment, while the SAAM is a measure of *state* attachment which likely captures more temporary fluctuations in attachment as a consequence of life events that changed behavior related to attachment or context-dependence (100). Considering that the current trial measured effects over a time period of only two weeks, it can be anticipated that mostly state characteristics, rather than trait characteristics will be affected by oxytocin. However, it would be of great interest to explore whether the administration of oxytocin can also affect trait attachment after longer administration periods, or when retention effects are measured weeks or months after completion of the trial.

To date, specific attachment measures were not included in oxytocin research, so results cannot be compared to previous reports. More than fifteen years ago, some evidence indicates that children with autism have deficits in attachment underlying social interaction and development of attachment is delayed with an altered behavioral pattern (111, 112). However, secure attachment specifically towards the mother of the autistic child seems to be equal as a typically developed child (111, 113). Therefore, it would be of great relevance to investigate attachment behavior in adults with ASD.

4.7 Effects of two-week oxytocin administration on quality of life reports

In addition to the previously discussed questionnaires, the World Health Organization Quality Of Life-BREF (WHOQOL-BREF) questionnaire indicated high physical, mental and social well-being during the whole study of all participants. Although the *social relationships* subscale was the most relevant in this study to show oxytocin related modulations, no significant effects were found. This can be explained by the fact that the *social relationships* subscale constitutes only three items (personal relationships, social support and sexual activity), which is limited to capture changes. Thus, in healthy control subjects the WHOQOL-BREF questionnaire is more applicable to compare baseline scores between both groups and not as an outcome measure.

The WHOQOL-BREF questionnaire is mostly used in a clinical setting to verify the impact of a treatment on all kind of diseases. Hence, quality of life is more probable to be lower in ASD patients because their disability can influence overall well-being. A previous six-week study with adult males diagnosed with ASD who administered 24 IU oxytocin twice a day did found significantly improved quality of life (91).

The POMS questionnaire was included not solely to examine the presence of a stable mood during the study, but also as a measure of consciousness when performing the experiments, as measured by the

tired subscale. The main significant effect of *time point* on this subscale, with decreasing trend, indicated that all subjects actively participated during the experiments.

4.8 Oxytocin is a safe drug

Participants of our study reported few mild side-effects (see **Appendix**, **Addendum 6**). Some of the side-effects in the oxytocin group were rather positive, such as confident and focused, however, frequencies are too low to make valuable conclusions. Moreover, these positive side-effects were present in both the oxytocin and placebo group.
Overall, oxytocin showed no significant side effects in previous performed trials in a meta-analysis of MacDonald et al. (2011). Only mild adverse events were reported in previous studies such as a light headache, nasal irritation or euphoria (114). Up till now, no data are available about the long term side-effects of oxytocin. Also, long-term oxytocin trials should monitor oxytocin abuse, since use of a nasal spray can develop addiction.

4.9 Oxytocin, only a prosocial drug?

Many studies highlight that oxytocin facilitates in-group trust and social relationships (115). Nevertheless, some evidence points a route for opposite effects towards out-group members, such as aggressiveness and defense (116, 117). These data raise the possibility that oxytocin not merely has positive effects when different groups, either culture, race or personal aspects, encounter. Oxytocin is therefore also attributed the 'tend-and-defend' function (118). Next, a review of Bartz and colleagues (2011) discuss the context and person dependency of oxytocin, which both can clarify the inconsistency in reported results oxytocin effects on social cognition (119). In the light of oxytocin as a therapeutic treatment, ideal oxytocin dosages can differ between individuals and demands personalized medicine.

Also, the results of the POMS in our study showed a progressive decrease of the *strong* subscale score in the oxytocin group. This results contradicts the motivation of positive behavior of oxytocin and underpins that oxytocin indeed can have undesired effects.

In addition, one participant of the oxytocin group reported to be more competitive (see **Appendix**, **Addendum 6**). This is in agreement with the hypothesis that oxytocin induces out-group aggressiveness. Studies with larger sample sizes that may replicate this side-effect in a reasonable number of participants belonging to the oxytocin group can give more insights and refine the 'tend-and-defend' theory of oxytocin.

4.10 Limitations

Several factors might contribute to the fact that oxytocin administration in our study did not or only had minor effects on emotion recognition and social-communicative skills. *First*, all participants were neurotypical young male adults. Such a test population is not expected to have major deficits in social cognition. Therefore, the lack of significant findings on several of the included tasks, might be related to the fact that all participants displayed relatively high baseline socio-emotional abilities. Indeed, this is reflected in the total SRS score and the two attachment scales.

Second, the sample size of all four experiments was rather small, which often resulted in relatively large standard error (of the mean) ranges. Future research is necessary to explore whether the current findings will replicate in larger samples. In the larger autism project, more subjects will participate in order to increase power (20 PL, 20 OT).

Third, the ideal oxytocin dose is not known, a dose of 24 IU once a day was used in this study as this is considered the standard dose for adults which is within safety limits. However some studies were conducted with twice-daily oxytocin dosages (91, 120). One might wonder if perchance more oxytocin than 24 IU a day is needed to reach a significant change on social and emotional measures, especially in a typically developed study population. Likewise, it should be of interest to set up studies that examine a criterion for oxytocin dose, such as a dose based on body weight or maybe in function of social functioning scores in autism populations.

Fourth, a two-week study to measure long term effects of oxytocin can be too short. Social interaction and emotional skills are two personal characteristics depending on education, environment and genetics, which are built throughout years. Hence, it can be doubted if these characteristics are able to upgrade in fourteen days. Therefore, the autism project will be conducted during four weeks.
In addition, the age of the study population can also play a role. Especially in patients with ASD it could be useful to start treatment early in life. This should increases the sensitivity of neuromodulation by oxytocin as the brain is thought to have an increased plasticity at younger age (121).

Fifth, it can be questioned if oxytocin administration via the nasal route reaches the receptors in the brain. Still, nasal administration is preferred over intravenous administration because of the difficulty to cross the blood-brain-barrier and this way salivary oxytocin levels seem to reach a higher level up to seven hours post administration (26). In addition nasal administration is an easy way to use.

Sixth, the PLD task was the only task that was assessed for immediate effects. Assessing these effects on the FERT and the social task would also be of interest.

Seventh, a critical remark regarding the control task of the PLD task and the social task is that the performance on these control tasks are already at ceiling at baseline, meaning accuracy rates range between 95% and 100%. This way, only minimal accuracy improvements are expected on the control task, hence lowering the measurement of specificity of effects on the task of interest (Emotion and Social Task).

Last, a critical remark on the FERT. Former task has no limitations on reaction time and no outlier detection was performed on the latter. Although several studies using the FERT did no corrections on the reaction times, answers with high reaction times could maybe differ from what came first in the participant's mind. Therefore, a threshold for or an outlier detection on the reaction time would be reasonable.

4.11 Conclusion

The aim of this project was to examine the prosocial effects and the facilitation of emotion recognition of oxytocin in neurotypical male adults. An immediate effect of oxytocin on bodily emotion recognition was revealed in a set-up that reduced learning effects. Furthermore, a reduction in *avoidance* attachment was reported in the oxytocin group. Today, research is focused on unraveling the modulating effects of oxytocin on socio-emotional cognition and behavior. Despite several promising reports, more research is needed regarding the effects of long-term administration, the possibility for eliciting retention effects, as well as to establish optimal dosages, which might depend on the included age range. To this end, numerous trials on oxytocin in autism and neurotypical populations are ongoing and will be published in the near future and probably fill the gap of inconclusiveness.

With this work and our future project, assessing both neural and behavioral effects of oxytocin in patients with autism, we hope to make a contribution to this quickly evolving and exciting field. The therapeutic value of oxytocin for ASD is predicted to be high, nevertheless, trials that will study the combination of behavioral therapy with oxytocin as pharmacological treatment could reveal synergistic effects that outweigh the separate effects.

5 Nederlandse samenvatting

5.1 Overzicht van de literatuur

5.1.1 Oxytocine en gerelateerde fysiologische functies

Oxytocine is een natuurlijk nonapeptide geproduceerd door de hypothalamus en gescreteerd door de neurohypofyse in de perifere bloedstroom. Naast deze hormonale rol speelt oxytocine ook nog een rol als neurotransmitter. Echter, de exacte locaties en verdeling van de oxytocine receptoren in het humane brein zijn nog niet volledig gekend.

Oxytocine is merendeel gekend door zijn rol aan het eind van de zwangerschap, tijdens de arbeid en borstvoeding doordat oxytocine de spiercontractie van de baarmoeder stimuleert en de melkejectie bevordert. Ook na de geboorte speelt oxytocine nog een rol in de moeder-kind binding en het stimuleren van moedergedrag. Verscheidene functionele magnetische resonantie beeldvorming studies tonen aan dat oxytocinerge hersenregio's meer actief zijn wanneer een moeder visuele of auditieve stimuli te zien krijgt van hun eigen huilende baby.

Naast deze belangrijke fysiologische functies van oxytocine in het vroege leven, wordt vermoed dat oxytocine ook een sleutelrol heeft later in het leven voor de vorming van hechte banden tussen vrienden en partners. Vandaar dat oxytocine vaak de naam 'liefdeshormoon' opgespeld krijgt.

Daarenboven zou oxytocine ook rol toegekend krijgen in stressreacties door het reduceren van het stresshormoon corticosterone. Toediening van nasale oxytocine reduceert ook de amgydala activiteit wanneer angst stimuli gepresenteerd worden.

Samengevat, oxytocine stimuleert sociale cognitie, oogcontact, het genereren van vertrouwen en doet angstreacties dalen. Niettemin, de neurobiologie onderliggend aan het vormen van hechte banden tussen individuen moet nog verder onderzocht worden, maar, men gelooft dat deze banden berusten op dezelfde principes als de moeder-kind binding.

Verscheidene studies in normaal ontwikkelde individuen vonden inderdaad effecten van oxytocine toediening op de prestaties op emotie herkenningstaken.

5.1.2 De rol van oxytocine in psychiatrische stoornissen

De afgelopen jaren steeg de interesse naar oxytocine als potentiële medicamenteuze therapie voor patiënten met neuropsychiatrische aandoeningen die lijden aan symptomen zoals sociale angst, communicatie problemen, problemen met emotiebegrip aan verwante gedragingen. Een meta-analyse toonde aan dat oxytocine als therapie het grootste effect had in personen gediagnosticeerd met autisme spectrum stoornissen.

Autisme spectrum stoornissen

Autisme spectrum stoornissen zijn levenslange neurologische ontwikkelingsstoornissen waarbij sociaal-communicatieve en niet-sociale symptomen de hoofdkenmerken zijn. Het eerste hoofdsymptoom wordt gekenmerkt door socio-emotionele moeilijkheden, moeite met het onderhouden van relaties en gebrek aan non-verbale communicatie. De niet-sociale symptomen zijn de restrictieve repetitieve gedragingen en interesses, zoals het aanhouden van rituelen, vasthouden aan gelijkheid en bepaalde patronen in het dagelijks leven. Daarenboven kan het expressieve taal- en intellectuele niveau verschillen. Alle symptomen zijn variabel en worden gedefinieerd in een spectrum van mild tot ernstig.

Tot op de dag van vandaag is de onderliggende neuropathofysiologie van autisme ongekend, maar bepaalde theorieën werden reeds voorgesteld. Een van deze theorieën is het amygdala of sociale brein deficiet. Het sociale brein bevat verschillende neurale structuren, waaronder de amygdala, frontale en temporale hersencortex en bezit onze sociale cognitie. Meer specifiek is de amygdala van belang voor de waardebepaling van gezichtsexpressies. Autisme patiënten worden verondersteld een abnormale werking te hebben van de amygdala en het sociale brein.

Een tweede theorie is deze van het spiegelneuronensysteem, die frontale en pariëtale hersengebieden omvat. Spiegelneuronen vuren wanneer een individu een actie uitvoert of wanneer een actie uitgevoerd door iemand anders geobserveerd wordt. Gebaseerd op deze merkwaardige eigenschap, werden deze spiegelneuronen gelinkt aan verscheidene socio-communicatieve functies, zoals het begrijpen van acties, imitatie, maar ook voor emotie herkenning en empathie. Dit laatste betekent ook het lezen van de emoties van anderen. Vandaar dat men verwacht dat personen met autisme spectrum stoornissen ‘gebroken’ spiegelneuronen hebben en moeilijkheden met het direct matchen van de emoties en gedragingen van anderen in het spiegelneuronensysteem.

Tot heden zijn er twee medicijnen op de markt voor de behandeling van autisme spectrum stoornissen, risperidone en aripiprazole. Echter, deze twee medicijnen behandelen enkel prikkelbaarheid en dus niet de twee belangrijkste symptomen van autisme spectrum stoornissen. Daarenboven hebben risperidone en aripiprazole het nadeel dat ze gewichtstoename en sedatie als bijwerking hebben.

Onderzoek spitst zich nu voornamelijk toe op oxytocine als potentieel therapeuticum voor de behandeling van de hoofdsymptomen van autisme spectrum stoornissen. Oxytocine zou mogelijks de sociale vaardigheden alsook repetitief gedrag kunnen verbeteren in individuen met autisme spectrum stoornissen.

Verscheidene placebo-gecontroleerde gerandomiseerde studies tonen veelbelovende resultaten, echter, meer studies zijn nodig op de langere termijn om de ware effecten van oxytocine aan te tonen.

5.2 Doel van de huidige studie

De hypothese dat oxytocine prosociaal gedrag stimuleert is een intrigerende, deels nog te ontrafelen vraag. In de huidige studie hebben we verscheidene placebo-gecontroleerde gerandomiseerde piloot studies uitgevoerd in jonge, normaal ontwikkelde volwassen mannen om de directe effecten (eenmalige-dosis) en effecten op de langere termijn (meerdere dosissen) van oxytocine nasale toediening verder te verduidelijken. De effecten op lange termijn werden onderzocht door dagelijks neusspray gebruik voor twee weken. Gedragsmatige effecten zullen onderzocht worden met meerdere computertaken en sociaal functioneren- en hechtingsvragenlijsten.

We gaan uit van volgende vier hypotheses, dat oxytocine zal zorgen voor:

1 Stijging van de accuraatheid en daling van de reactietijd op de lichamelijke emotie herkenningstaak met point-light displays, de gelaatsemotie herkenningstaak en de sociale taak.

2 Daling van de score op de Social Responsiveness Scale (SRS) wat wijst op een daling van sociale moeilijkheden.

3 Stijging van de score op de World Health Organization Quality of Life-bref vragenlijst, wat een hogere kwaliteit van het leven aangeeft.

4 Stijging van de score op de Inventory of Parent and Peer Attachment hechtingsschaal.

5 Stijging van de score op *veiligheid* hechting en daling van de score op *angst* en *mijden* hechting, gemeten door de State Adult Attachment Measure (SAAM).

Deze masterthesis behoort tot een fase drie placebo-gecontroleerde gerandomiseerde studie waarbij volwassen mannen gediagnosticeerd met autisme spectrum stoornissen oxytocine neusspray dagelijks vier weken lang zullen gebruiken. Binnen dit project zullen de effecten van oxytocine niet enkel gedragsmatig nagegaan worden, maar ook neuraal door gebruik van niet-invasieve functionele magnetische resonantie beeldvorming.

De resultaten van de huidige experimenten met normaal ontwikkelde deelnemers zal belangrijke inzichten opleveren in verband met de gevoeligheid van de geïncludeerde taken en vragenlijsten in respons op de oxytocine behandeling, wat relevant kan zijn voor de selectie van de testbatterij in de studie met deelnemers met autisme spectrum stoornissen.

5.3 Resultaten en discussie

5.3.1 Oxytocine resulteert in een onmiddellijke verbetering van de prestatie op de lichamelijk emotie herkenningstaak

Eenmalige toediening van oxytocine verbetert significant de accuraatheid op de emotietaak (blij of boos) van de point-light display taak (weergave van een bewegende acteur op basis van witte bolletjes die 12 markeringen op de gewrichten voorstellen) in vergelijking met de placebo groep, dertig minuten na toediening van de neusspray. Tijdens de tweede test werden nieuwe stimuli gepresenteerd. Dit wijst erop dat oxytocine het herkennen van lichamelijke emoties verbetert, met andere woorden, oxytocine bevordert het lezen van emotionele lichaamstaal.
Een eerdere studie toonde aan dat individuen met autisme spectrum stoornissen slechter presteren op dezelfde taak in vergelijk met normaal ontwikkelde personen, daarom zou het interessant zijn om na te gaan of individuen met autisme stoornissen ook voordeel halen uit oxytocine gebruik op deze taak.

5.3.2 Effecten van meervoudige oxytocine toediening gedurende twee weken op de lichamelijke emotie herkenningstaak

Alle deelnemers voerden de taak uit de eerste dag, voor en na neusspray toediening en twee weken later, voor en na neusspray toediening. Een significant effect werd gevonden op de accuraatheid op het onmiddellijke effect na twee weken, maar niet op het eerste onmiddellijke effect. Dit kan te wijten zijn aan het feit dat hier, in tegenstelling tot vorig experiment, dezelfde stimuli gebruikt werden tijdens alle vier de testen wat de kans op leereffecten vergroot. Niettemin, de reactietijd verbeterde significant na twee weken enkel in de oxytocine groep. Deze resultaten wijzen erop dat de verbeteringen te wijten zijn aan de oxytocine.
Naar onze kennis is dit de eerste studie dat effecten van oxytocine op lange termijn rapporteert op emotie herkenning in een gezonde populatie. Studies bij autisme deelnemers berusten meestal op gelaatsemotie herkenningstaken. Wij stellen voor dat studies in de toekomst bij autisme deelnemers ook lichamelijk emotie herkenningstaken includeren.

5.3.3 Twee weken van dagelijkse oxytocine toediening had geen effect op gelaatsemotie herkenning

Naast het bestuderen van lichamelijke emotie herkenning, werden ook effecten van twee weken dagelijkse oxytocine toediening nagegaan met gebruik van de gelaatsemotie herkenningstaak (waarbij ‘morphed’ gezichten gebruikt worden van de zes basis emoties: blij, boos, droevig, verrast, afkeer en angst). Helaas, geen positieve effecten werden gevonden op geen van de zes emoties. Deze data moeten als voorafgaand onderzoek aanzien worden, vandaar de minieme steekproefomvang (acht deelnemers per interventie groep). Experimenten in de toekomst met een groter aantal deelnemers zijn nodig om de effecten van oxytocine op gezichtsemotie herkenning verder te onderzoeken.

5.3.4 Twee weken van dagelijkse oxytocine toediening had geen effect op de detectie van sociale interacties

De sociale taak werd uitgevoerd om na te gaan of oxytocine het discrimineren tussen vriendschappelijke, positieve interacties en onvriendschappelijke, negatieve interacties in foto's van levensechte scenes vergemakkelijk. Geen effecten werden gevonden op deze taak. De sociale taak kan echter wel interessant zijn om toe te passen in een beeldvorming studie om na te gaan of, na oxytocine toediening, rekrutering van hersenregio's betrokken bij sociale cognitie gestimuleerd wordt.

5.3.5 Effecten van twee weken dagelijkse oxytocine toediening op sociaal functioneren en hechtingsschalen

Beide groepen, oxytocine en placebo, toonden een gelijkaardige, niet-significante dalende trend op de totale score van de SRS, wijzend op een lichte stijging in sociaal functioneren in beide groepen. Maar, de subschaal *sociale motivatie* was enkel en alleen significant in de oxytocine groep, dit wil zeggen dat oxytocine het initiatief nemen tot sociale interactie verbetert, in vergelijking met placebo.
Eerdere oxytocine studies in individuen met autisme spectrum stoornissen faalden in het aantonen van effecten op de SRS. Dosis, duur van de studie en andere factors kunnen bijdragen aan de inconsistente resultaten.

Daarnaast zorgde oxytocine ook voor een significante daling op de 'avoidance' (mijding) subschaal van de SAAM, betekenend dat oxytocine het mijden van intieme banden en relaties doet dalen. Specifieke hechtingsschalen werden tot nog toe niet geïncludeerd in oxytocine onderzoek en worden verwacht interessante positieve resultaten op te leveren in de toekomst.

5.4 Conclusie

Het doel van dit project was om de prosociale effecten en facilitatie van emotie herkenning door oxytocine na te gaan in normaal ontwikkelde volwassen mannen. Enkele effecten werden onthuld zowel op emotie herkenning, sociaal functioneren en hechting. Voorgaand onderzoek rapporteert tegensprekende resultaten, maar deze inconsistentie zal dalen wanneer de ideale oxytocine dosis en tijdsspanne nodig om merkbare effecten te bereiken, gekend zijn.
Het autisme project dat deze pilootstudie opvolgt wordt verwacht belovende resultaten op te leveren. Doordat ook neurologische effecten zullen nagegaan worden, kan men eventueel vroege neuromodulerende veranderingen detecteren die gedragsveranderingen vooraf gaan. De therapeutische waarde van oxytocine in patiënten met autisme spectrum stoornissen wordt hoog ingeschat, niettemin, de combinatie van gedragstherapie met oxytocine dient ook onderzocht te worden.

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7 Appendix

Addendum 1: List of exclusion criteria

Contra-indicatie screening oxytocine onderzoek

*Onderstaand vindt u een lijst van exclusie-criteria voor deelname aan het oxytocine onderzoek. Gelieve de lijst grondig door te nemen. Indien **geen** van deze exclusie-criteria op u van toepassing is, kan u deelnemen aan het oxytocine onderzoek.*

Persoonlijke kenmerken

Vrouw
Linkshandig
Jonger dan 18 jaar en ouder dan 30 jaar

Psychiatrische of neurologische aandoening:

ADHD
ADD (aandachtstoornis)
ASD (autisme spectrum stoornissen: autisme, Asperger syndroom)
Prematuriteit (vroeggeboorte)
Bipolaire stoornis
Psychose
Posttraumatische stressstoornis
Schizofrenie
Depressie
Angststoornis

Nemen van psychotrope medicatie:

Rilatine
Anti-depressiva
Anti-epileptica
Kalmeermiddelen
Slaappillen

Geschiedenis van neurologische ziekte:

Onstabiele epilepsie
Gekende genetische syndromen

Geschiedenis of evidentie van:

Maligniteit (o.a. kanker)
Hematologische ziekte (boedziekte, dysfunctioneren van lymfeklieren)
Endocriene ziekte (o.a. diabetes)
Cardiovasculaire ziekte (inclusief hartritmestoornissen)
Respiratoire aandoening (ademhaling)
Renale aandoening (nieren)
Leveraandoening
Gastrointestinale ziekte (maag, darmen)

Naam deelnemer: Naam onderzoeker:
Datum:
Handtekening: Handtekening:

Addendum 2: Side-effects questionnaire after oxytocin administration

Naam:
Voornaam:
Nummer:
Datum:

Gelieve de vakjes in te vullen (kruisjes) indien de beschreven bijwerking op u van toepassing is.

Symptoom	J/N	Start van de reactie		Duur van de reactie (uren)	Reactie NU nog steeds aanwezig?	Ernst van de reactie		
		Direct na toediening	Binnen de 2 uur			Mild	Matig	Ernstig
Hoofdpijn								
Slaperigheid								
Duizeligheid								
Flauwvallen								
Veranderingen in hartslag of hartkloppingen								
Kortademigheid								
Koorts								
Keelpijn								
Droge keel / droge mond								
Heesheid								
Hoesten								
Ophoesten van slijm								
Verstopte neus								
Niezen								
Irritatie neus								
Lopende neus								
Branderig gevoel in neus en/of oren								
Gevoelig aan geuren								
Tranende ogen								
Misselijkheid en/of overgeven								
Buik- of maagpijn								
Gedaalde eetlust								
Hongerig								
Constipatie								
Diarree								
Spierpijn/krampen								
Huiduitslag								

Gestegen vochtinname								
Waterretentie / opgeblazen gevoel								
Slapeloosheid / moeilijk slapen								
Nachtmerries								
Staren / dagdromen								
Anafylaxis								
Veranderingen in perceptie van de tong								
Rugpijn								
Bedplassen								
Gewichtstoename								
Zweten								
Wazig zicht								
Minder praten met anderen								
Ongeïnteresseerd in anderen								
Aanhoudende gedachten en/of gevoelens								
Ontwikkeling van repititief gedrag								
Stijging in repititief gedrag								
Nagelbijten								
Geïrriteerd, verveeld								
Triestig / ongelukkig								
Gevoelig voor huilen, emotioneler								
Angstig, bezorgd, discomfort								
Gelukkig, tevreden								
Euforisch, ongewoon gelukkig, meer energie								
Kalm, gerelaxeerd, comfortabel								
Meer gefocust								
Meer zelfvertrouwen								

Heeft u andere bijwerkingen ondervonden die niet in bovenstaande tabel vermeld zijn?

Addendum 3: Tables of the responders and non-responders of *Experiment 1*

Table 1 and **Table 2** summarize the percentages of responders and non-responders of the immediate effect analysis for the accuracy and reaction time of the point light display (PLD) task in *Experiment 1*, respectively.

The oxytocin group mostly responded while participants of the placebo group were mostly non-responders for the accuracy on the Emotion Task. This is in agreement with the results of **Figure 8**. Next, accuracy on the Color Task mostly did not change for both groups (**Table 1**).

Reaction time on the Emotion task had less responders than non-responders in the oxytocin group while the placebo group showed the opposite distribution. The Color Task has an almost equal distribution of responders and non-responders (**Table 2**).

ACCURACY			Responders (%)	Non-Responders (%)	
				No change (%)	*Decline (%)*
Immediate Effect	Emotion Task	PL (N = 15)	7 (46.67%)	2 (13.33%)	6 (40%)
		OT (N = 13)	10 (76.92%)	0 (0%)	3 (23.8%)
	Color Task	PL (N = 15)	3 (20%)	7 (46.67%)	5 (33.33%)
		OT (N = 13)	5 (38.46%)	7 (53.85%)	1 (7.69%)

Table 1. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (increasing accuracy) and non-responders (no change or decline in accuracy) is given for the Emotion and Color Task of the PLD task.*

REACTION TIME			Responders (%)	Non-Responders (%)	
				No change (%)	*Increase (%)*
Immediate Effect	Emotion Task	PL (N = 15)	10 (66.67%)	0 (0%)	5 (33.33%)
		OT (N = 13)	6 (46.15%)	0 (0%)	7 (53.85%)
	Color Task	PL (N = 15)	7 (46.67%)	0 (0%)	8 (53.33%)
		OT (N = 13)	7 (53.85%)	0 (0%)	6 (46.15%)

Table 2. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (decline of reaction time) and non-responders (no change or increasing reaction time) is given for the Emotion and Color Task of the PLD task.*

Addendum 4: Tables of the responders and non-responders of *Experiment 2*

Table 1 and **Table 2** show the percentage responders and non-responders of the comparison between the Baseline and Immediate Effect measure for the accuracy and reaction time on the point light display (PLD) task in *Experiment 2*, respectively.

Participants of the oxytocin group mostly responded on the Emotion Task, but likewise did the participants of the placebo group (**Table 1**). Similarly, both groups mostly responded faster on the Emotion Task immediately after nasal spray administration (**Table 2**).
The majority of both groups were non-responders regarding accuracy on the control task (**Table 1**), but reaction time on the control task had just as much responders as non-responders for the oxytocin group and mostly responders for the placebo group (**Table 2**).

ACCURACY			Responders (%)	Non-Responders (%)	
				No change (%)	*Decline (%)*
Immediate Effect	Emotion Task	PL (N = 8)	7 (87.5%)	0 (0%)	1 (12.5%)
		OT (N = 8)	5 (62.5%)	0 (0%)	3 (37.5%)
	Color Task	PL (N = 8)	1 (12.5%)	3 (37.5%)	4 (50%)
		OT (N = 8)	3 (37.5%)	3 (37.5%)	2 (25%)

Table 1. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (increasing accuracy) and non-responders (no change or decline of accuracy) is given for the Emotion and Color Task of the PLD task during session 1.*

REACTION TIME			Responders (%)	Non-Responders (%)	
				No change (%)	*Increase (%)*
Immediate Effect	Emotion Task	PL (N = 8)	6 (75%)	0 (0%)	2 (25%)
		OT (N = 8)	7 (87.5%)	0 (0%)	1 (12.5%)
	Color Task	PL (N = 8)	6 (75%)	0 (0%)	2 (25%)
		OT (N = 8)	4 (50%)	0 (0%)	4 (50%)

Table 2. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (decline of reaction time) and non-responders (no change or increasing reaction time) is given for the Emotion and Color Task of the PLD task during session 1.*

Table 3 and **Table 4** show the percentage responders and non-responders of the comparison between the Baseline and Post Effect measure of the accuracy and reaction time on PLD task, respectively.

The majority of both groups responded on the Emotion task regarding accuracy (**Table 3**). Similarly, both groups mostly responded faster on the Emotion Task immediately after nasal spray administration (**Table 4**). However, all participants of the oxytocin group had faster reaction times after two weeks of nasal spray administration, which is in agreement with the three-way ANOVA analysis (**Figure 15**).
The majority of both groups were non-responders regarding accuracy on the control task (**Table 3**), but reaction time on the control task had just as much responders as non-responders for the oxytocin group and mostly non-responders for the placebo group (**Table 4**).

ACCURACY			Responders (%)	Non-Responders (%)	
				No change (%)	*Decline (%)*
Post Effect	Emotion Task	PL (N = 8)	7 (87.5%)	0 (0%)	1 (12.5%)
		OT (N = 8)	6 (75%)	0 (0%)	2 (25%)
	Color Task	PL (N = 8)	3 (37.5%)	1 (12.5%)	4 (50%)
		OT (N = 8)	3 (37.5%)	3 (37.5%)	2 (25%)

Table 3. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (increasing accuracy) and non-responders (no change or decline of accuracy) is given for the Emotion and Color Task of the PLD task during for the post measure compared to baseline.*

REACTION TIME			Responders (%)	Non-Responders (%)	
				No change (%)	*Increase (%)*
Post Effect	Emotion Task	PL (N = 8)	7 (87.5%)	0 (0%)	1 (12.5%)
		OT (N = 8)	8 (100%)	0 (0%)	0 (0%)
	Color Task	PL (N = 8)	3 (37.5%)	0 (0%)	5 (62.5%)
		OT (N = 8)	4 (50%)	0 (0%)	4 (50%)

Table 4. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (decline of reaction time) and non-responders (no change or increasing reaction time) is given for the Emotion and Color Task of the PLD task during for the post measure compared to baseline.*

Table 5 and **Table 6** show the percentage responders and non-responders of the comparison between the Post Effect and Post Immediate Effect measure of the PLD task for the accuracy and reaction time, respectively.

All participants of the oxytocin group responded on the Emotion Task regarding accuracy (**Table 5**), and almost all participants responded faster on the Post Immediate Effect measure (**Table 6**). Nonetheless, more than half of the participants of the placebo group also responded on the Emotion Task (**Table 5**) and almost all of these subjects had a decrease in reaction time on the second immediate effect measure (**Table 6**).

The Color Task had a majority of no change responsiveness regarding accuracy in the oxytocin group and the placebo group also had mostly non-responders on this control task when both non-responder groups were taken together (**Table 5**). Reaction time on the Color Task had mostly responders in the oxytocin group and an equal distribution of responders and non-responders in the placebo group (**Table 6**).

ACCURACY			Responders (%)	Non-Responders (%)	
				No change (%)	*Decline (%)*
Post Effect	Emotion Task	PL (N = 8)	5 (62.5%)	0 (0%)	3 (37.5%)
		OT (N = 8)	8 (100%)	0 (0%)	0 (0%)
	Color Task	PL (N = 8)	3 (37.5%)	1 (12.5%)	4 (50%)
		OT (N = 8)	1 (12.5%)	6 (75%)	1 (12.5%)

Table 5. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (increasing accuracy) and non-responders (no change or decline in accuracy) is given for the Emotion Task and Color Task of the PLD task during session 2.*

REACTION TIME			Responders (%)	Non-Responders (%)	
				No change (%)	*Increase (%)*
Post Effect	Emotion Task	PL (N = 8)	7 (87.5%)	0	1 (12.5%)
		OT (N = 8)	6 (75%)	0	2 (25%)
	Color Task	PL (N = 8)	4 (50%)	0	4 (50%)
		OT (N = 8)	6 (75%)	0	2 (25%)

Table 6. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (decline of reaction time) and non-responders (no change or increasing reaction time) is given for the Emotion Task and Color Task of the PLD task during session 2.*

Addendum 5: Tables of the responders and non-responders of *Experiment 3*

Table 1 summarizes the percentages of responders and non-responders for the accuracy on the Facial Emotion Recognition Task (FERT) after two weeks of nasal spray administration. The oxytocin group had mostly responders, while the placebo group had the same number of participants of responders and non-responders.

ACCURACY		Responders (%)	Non-Responders (%)	
			No change (%)	*Decline (%)*
Post Effect	PL (N = 8)	4 (50%)	1 (12.5%)	3 (37.5%)
	OT (N = 8)	5 (62.5%)	1 (12.5%)	2 (25%)

Table 1. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (increasing accuracy) and non-responders (no change or decline in accuracy) is given for the FERT.*

Table 2 summarizes the percentages of responders and non-responders for the reaction time performance on the social task after two weeks of nasal spray administration. The table shows that for the actual Social Task, the oxytocin group had just as much responders as non-responders and that the placebo group mostly had non-responders. The Control Task had an equal distribution of responders and non-responders for both groups.

REACTION TIME			Responders (%)	Non-Responders (%)	
				No change (%)	*Increase (%)*
Post Effect	Social Task	PL (N = 6)	3 (50%)	0 (0%)	3 (50%)
		OT (N = 8)	2 (25%)	0 (0%)	6 (75%)
	Control Task	PL (N = 6)	3 (50%)	0 (0%)	3 (50%)
		OT (N = 8)	4 (50%)	0 (0%)	4 (50%)

Table 2. *For each group, placebo (PL) and oxytocin (OT), the percentage of responders (decreasing reaction time) and non-responders (no change or increasing reaction time) is given for the social task.*

Addendum 6: Table of side-effects frequencies

Table 1 shows the frequency of each reported side-effect during the two-week oxytocin study.

Side-effect	PL (N = 32)	OT (N = 32)	Mild		Moderate		Severe	
			PL	*OT*	*PL*	*OT*	*PL*	*OT*
Focused	2	2			2	2		
Confident	2	1			1	1	1	
Competitive		1		1				
Relaxed		1	1					
Sweaty	1	1		1	1			
Itchy nose		1	1					

Table 1. *Number of times each side-effect was reported indicated for each group, placebo and oxytocin.*