# The Effects of Oxytocin on Social Behaviour: The Influence of Context and Individual Differences

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## Summary of the Thesis

The current thesis sought to investigate the role of oxytocin (OT) in social and emotional behaviours and whether these effects are moderated by contextual factors and individual differences; and to address some of the methodological issues that arise in studies that administer intranasal OT (IN-OT).

The findings indicate that the social effects of OT extend to third-party behaviour, and that these effects are moderated by contextual factors, although in contrast to previous research there was no evidence that individual difference factors moderate the effect of OT on participants' social or emotional behaviour. The moderating effect of ingroup/outgroup membership lends itself to the theoretical argument that OT plays a role in a biological mechanism, developed over evolutionary time, to promote group-serving as opposed to self-serving behaviour in order to preserve group functioning and therefore provide indirect fitness benefits to the individual.

Findings reported in the second half of this thesis provide evidence of a reliable effect of IN-OT on salivary OT concentrations, and the presence of sizable individual differences in response to IN-OT. While the thesis provides evidence that these individual differences in peripheral concentrations of OT in response to IN-OT are not accounted for by various biological factors (such as gender) that often act as logistical constraints in OT research, there was also no evidence that psychological factors could explain these differences.

Taken together the thesis reports valuable extensions to previous research, demonstrating that OT's effects extend to third-party social and emotional behaviours and that these effects are moderated by contextual factors; and implications for clinical research, by reporting on a novel clinical group to OT research.

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# List of Publications

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Hubble, K., Daughters, K., Manstead, A. S. R., Rees, A., Thapar, A., & van Goozen, S. H. M. Oxytocin increases attention to the eyes and selectively enhances affective empathy for fear. *Neuropsychologia*. *Under review.*

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# Chapter 1

# Introduction

*"Targeted research is needed to increase our understanding of the specific facets of other-concern that are influenced by oxytocin"*

De Dreu, 2012a, p. 423

Human societies are made up of distinct groups; these can vary from family units, school friends, city populations, to groups based on race and gender. Being able to function effectively in a group therefore is essential to individual success. However, living in a group brings challenges and requires good 'group psychology' – the ability to negotiate complicated social environments. There is an innate struggle between the 'all for one' mentality required for effective group-living and all the advantages that come with forming a group, and the 'one for all' evolutionary instinct. It makes sense therefore that over evolutionary time humans (and other mammalian species) have developed specific neural and endocrine mechanisms that promote behaviours that are advantageous for group-living. Although there are several hormones that have been shown to influence social behaviour, including cortisol (i.e., Essex, Klein, Cho, and Kalin (2002)), arginine vasopressin (i.e., Brunnlieb et al. (2016)), and testosterone (i.e., Eisenegger, Naef, Snozzi, Heinrichs, and Fehr (2010)), the neuropeptide oxytocin (OT) has received the most attention over the past decade (Bos, Panksepp, Bluthé, & van Honk, 2012; Van IJzendoorn & Bakermans-Kranenburg, 2012) and has arguably been shown to have the most extensive influence.

Initial research found that OT increased cognitive empathy (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007), trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), and reciprocity (Barraza & Zak, 2009), leading the hormone to be dubbed "the love drug" by the media. However, later studies found inconsistencies in this strictly prosocial influence of OT on social behaviour, finding that OT could decrease social behaviour towards outgroup members (De Dreu, Greer, Van Kleef, Shalvi, & Handgraaf, 2011). These new insights led to the suggestion that the effects of OT on social behaviour are moderated by contextual factors and individual differences (Bartz, Zaki, Bolger, & Ochsner, 2011; Shamay-Tsoory & Abu-Akel, 2016).

In 2012 a leading researcher in the field of OT suggested that further research was required to establish the effect of OT on empathy-related behaviour (see opening quote; De Dreu, 2012a, p. 423). This thesis aims to address this question, examining the effect of social context and individual differences on the effect of OT on social behaviour and emotion processing.

*Oxytocin – An Introduction*

The Nobel laureate Vincent du Vigneaud won the prestigious chemistry award in 1955 for isolating, defining, and subsequently synthesising the nine amino acid neuropeptide OT in a laboratory (Vigneaud et al., 1953). In nature, OT is synthesised in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus (Swaab, Nijveldt, & Pool, 1975). Vesicles of OT produced in the magnocellular neurons of the PVN and SON are relayed to the posterior pituitary gland where OT is released into the bloodstream. Once in the bloodstream OT is defined as a hormone, interacting with distal targets over relatively long time scales. OT produced in the parvocellular neurons of the PVN projects to several other areas in the brain, including the amygdala, hippocampus, striatum and brainstem, where it acts as a

neuromodulator exerting effects on social behaviour (Castel & Morris, 1988; Peñagarikano et al., 2015; Swanson & Sawchenko, 1980).

Hormones serve specific functions in the body; the physiological role of OT was originally thought to be limited to initiating uterine concentrations during parturition and, in combination with prolactin, enabling lactation (Russell, Leng, & Douglas, 2003). Research has recently found that OT also plays an important role in the digestive system, regulating appetite and, indirectly, body weight (Blevins & Baskin, 2015). However, it was as a result of OT's well-known role in ante- and postnatal functions that researchers began to investigate OT's role in mother-offspring bonding.

The first investigations into the behavioural effects of OT were carried out in animal models. Insel and Shapiro (1992) found that although the projection of OT neurons was the same across a range of prairie vole species, showing either monogamous or polygamous breeding strategies, the distribution of OT receptor (OTR) sites in the brain was significantly different across species. Monogamous species had the highest density of OTRs in the prelimbic cortex, nucleus accumbens and lateral aspects of the amygdala (among others), while the polygamous species had the highest density of OTRs in the cortical nucleus of the amygdala, ventromedial nucleus of the hypothalamus and lateral septum. They were subsequently able to isolate the genetic sequence in the promoter region of the gene that regulated OTR expression. Transgenic research has since shown that the distribution of OTRs and disruption to the OT system can significantly affect not only sexual behaviour (Young et al., 1997) but also parenting behaviour (Winslow & Insel, 2002). These results have now been found in a range of mammalian species, including humans (for a review see Donaldson and Young (2008)).

Leading on from the finding that OT significantly affects a range of sophisticated behaviours in parents, researchers began to investigate whether OT also exerts effects on

adult social behaviour in other social contexts, studying whether OT would affect social interactions between genetically unrelated individuals in typical social scenarios (see *The Influence of Contextual Factors*, below). To do this, psychologists needed to experimentally manipulate OT concentrations in human participants. Although OT is often given intravenously to augment parturition in women, it can also be given in the form of a nasal spray; in order to avoid invasive research techniques and to infer cause and effect relationships, experimenters began using nasal sprays of OT to investigate how elevated concentrations of OT would affect participants' social behaviour. Over the last three years, however, critics have raised concerns over the efficacy and poor understanding of the delivery mechanism of this administration technique (Leng & Ludwig, 2015; McCullough, Churchland, & Mendez, 2013).

Although a consensus on how IN-OT exerts its effect on social cognition has yet to be reached, there are a number of proposed (and not mutually exclusive) mechanisms by which IN-OT may work, and these are consistent across of a number of recent publications (Evans, Dal Monte, Noble, & Averbeck, 2014; Grinevich, Knobloch-Bollmann, Eliava, Busnelli, & Chini, 2016; Quintana, Alvares, Hickie, & Guastella, 2014; Veening & Olivier, 2013). In addition, a number of papers have attempted to address concerns about the physiological influence of IN-OT in a range of measures, including saliva, plasma, urine, and cerebrospinal fluid (CSF), finding that IN-OT does lead to both a peripheral and central increase in OT (Dal Monte, Noble, Turchi, Cummins, & Averbeck, 2014; Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013; Striepens et al., 2013). Further Leng and Ludwig (2015) suggest that CSF measures of OT may not be the best measure of the central effects of IN-OT because CSF concentrations of OT degrade rapidly, and thus a measure of its metabolic products might provide a more accurate insight. This eased their own concerns that 'only' 0.01% of IN-OT may cross the blood-brain barrier.

I nevertheless agree that it would be extremely useful to identify the specific mechanism(s) through which IN-OT exerts effects on the brain. Although the current thesis does not directly address this issue, three chapters are devoted to the methodological considerations of OT research, investigating the effect of IN-OT on salivary OT concentrations in males and females, and assessing endogenous concentrations of OT in two clinical populations. Finally, I share many of the sentiments expressed at the end of several of the highly cited critiques, namely that the issues raised recently do not detract from the validity of OT research, a view recently summarised by Quintana and Woolley (2016).

*The Influence of Contextual Factors*

In 2005 a paper was published in *Nature* (Kosfeld et al., 2005) claiming that OT increased trust in humans. Since then the paper has been cited over 2500 times, making it one of the most famous OT studies to be carried out, and this popularity (in combination with a book published by one of the authors) led to OT's media nickname 'the love drug'. Subsequent papers also found prosocial effects of OT. Barraza and Zak (2009) found that empathy led to a significant increase in endogenous OT and also subsequent increases in generosity during the Ultimatum Game. The same research group later found that IN-OT administration increased charitable donations made by participants at the end of their study, regardless of how much money they had won during the study (Barraza, McCullough, Ahmadi, & Zak, 2011). These results led to the conclusion that OT increases prosocial behaviour, and a consensus developed that OT increases prosocial behaviour in all people, in all contexts.

Crucially, however, these studies had all used a homogenous group context, opening up the question of whether OT would also trigger prosocial behaviour towards outgroup members as well as ingroup members. The term 'parochial altruism' refers to the natural tendency in humans (and many other species) to limit altruistic acts to members of one's own

group. For example, species that demonstrate cooperative breeding only extend this benefit towards ingroup members, i.e., a lioness will only allow cubs from the same pride to suckle, whereas cubs from outside the pride are typically killed on sight. While this behaviour was originally thought to be driven by kin selection (indirect fitness benefits by perpetuating familial genes), recent theories (Clutton-Brock, 2002) now argue that parochial altruism in many species, including humans, is shown among unrelated individuals provided they share the same group identity, e.g., donating blood after a terrorist attack. A key part of group psychology is being able to identify ingroup and outgroup members (Tajfel, 2010) and being able to limit group-benefitting behaviours to one's own group. This enables individuals to target time, energy, and resources towards individuals who provide a fitness benefit, thereby increasing the likelihood of successful group-serving behaviours being passed on over evolutionary time. Thus it is possible that there is a biological mechanism underpinning these behaviours. The theoretical argument presented in the current thesis proposes that OT, an evolutionarily ancient, highly conserved neuropeptide, present in all mammalian species, is a candidate for this biological mechanism.

De Dreu and colleagues have carried out a number of studies providing support for this theory that OT plays an important role in the mechanism underpinning group- serving behaviour (De Dreu, 2016). In a three study paper (De Dreu et al., 2010) De Dreu and colleagues demonstrated that the prosocial effects of OT were moderated by contextual factors, specifically the group identity of interaction partners. In Study 1, participants who were given IN-OT contributed significantly more financial resources to a within-group pot (all ingroup members gained money), but had no effect on contributions to a between-group pot (all ingroup members gained money, all outgroup members lost money): Participants given OT and placebo (PL) invested the same amount in the between-group pot. In Study 2 the researchers investigated the effect of the participants' social value orientation in the same

iterated Prisoners' Dilemma paradigm, finding that both cooperative and non-cooperative individuals given IN-OT invested more in the within-group pot, but invested the same amount (as those given PL) in the between-group pot. In Study 3 participants completed the Prisoners' Dilemma paradigm with either ingroup or outgroup members, but were also exposed to financial options that reflected both a high and low manipulation of fear and greed. In line with their previous findings, OT increased non-cooperation when playing with outgroup members (an ingroup benefitting behaviour), and this occurred to a greater extent in decisions in which the outgroup member was represented as highly fearful by ingroup members. Moreover, they later found that this OT-induced increase in non- cooperation with outgroup members was moderated by ingroup vulnerability: When the ingroup was vulnerable non-cooperation was higher, compared to when vulnerability was low, but this only occurred when the participants' own personal vulnerability was low (De Dreu, Shalvi, Greer, Van Kleef, & Handgraaf, 2012).

These results have since been replicated in De Dreu's laboratory using various paradigms. OT enhanced ingroup favouritism during an Implicit Association Task, demonstrated by faster reaction times when positive words were paired with ingroup names, compared to outgroup names (relative to the PL condition); OT enhanced ingroup favouritism during an infrahumanisation task, demonstrated by greater anticipation of ingroup members experiencing secondary emotions (e.g., admiration and humiliation) compared to outgroup members (relative to the PL condition); and OT enhanced sacrificing of outgroup members rather than ingroup members during moral dilemmas, compared to the PL condition (De Dreu, Greer, Van Kleef, et al., 2011). OT has also been shown to manipulate the selection of group members, such that OT increases selection of high threat members, relative to low threat members, in a bid to bolster group competitiveness against possible outgroups (De Dreu, Greer, Handgraaf, Shalvi, & Van Kleef, 2011). This was later found to be moderated

by exposure to testosterone during foetal development (as indicated by the digit ratio between one's index finger and ring finger (Manning, 2002)), such that OT increased selection of high threat members but only in individuals who had been exposed to high concentrations of testosterone during foetal development (those with low testosterone exposure, given IN-OT, selected significantly more low threat targets) (Kret & De Dreu, 2013). These results have also been replicated in other laboratories (Baumgartner, Götte, Gügler, & Fehr, 2012; Baumgartner, Schiller, Rieskamp, Gianotti, & Knoch, 2013; Sheng, Liu, Zhou, Zhou, & Han, 2013).

De Dreu concluded the OT increases 'ingroup love' but does not affect 'outgroup derogation', a theory that has been termed the "tend-and-defend hypothesis" (De Dreu, 2012a). The hypothesis builds on social psychological research demonstrating a tendency towards parochial altruism and the importance of self and other identification (Jetten, Spears, & Manstead, 1996, 1997), suggesting that OT enhances the tendency towards parochial altruism, resulting in behaviours that benefit the ingroup. OT does not, however, increase antisocial behaviour towards outgroups – levels of non-cooperation are consistent whether participants are given OT or PL unless the outgroup is deemed to pose a threat to the ingroup, in which case OT enhances defence-motivated behaviours that directly or indirectly benefit the ingroup.

These studies provide evidence that the social effects of OT are moderated by contextual factors; however, given that the majority of research has been conducted by one group of researchers, the present thesis aims to replicate these findings. In addition, all of the studies detailed above investigated the effect of OT on direct (one-to-one) social interactions. Thus a secondary concern in the current thesis was to investigate whether these findings extend to third-party behaviour (witnessing one-to-one interactions). There is some research investigating this hypothesis (Hu et al., 2016; Riem, Bakermans-Kranenburg, Huffmeijer, &

van IJzendoorn, 2013), but further research is required to support these initial findings.

*The Influence of Individual Differences*

The social effects of OT have also been found to be moderated by individual differences. Shamay-Tsoory and Abu-Akel (2016) recently summarised a number of individual difference factors that have been found to moderate the effects of OT, including gender, attachment style, and psychopathology. Indeed 5 years earlier Bartz et al. (2011) published a similar review highlighting that 33 of 52 studies investigating the influence of IN-OT on social behaviour and cognition showed that it was moderated by either a contextual or individual difference factor.

Given OT's role in the physiological and psychological aspects of parenting, it is unsurprising that this close association has a persistent effect on the relationship between OT and social behaviour across the lifespan. In adults attachment style has been found to moderate the behavioural effects of OT, typically finding that only participants with a certain type of insecure attachment demonstrate significant effects of OT. However, these results vary across studies. A recent study found that only participants low in attachment avoidance demonstrated more prosocial behaviour after IN-OT (Fang, Hoge, Heinrichs, & Hofmann, 2014), whereas another found that only participants high in attachment avoidance demonstrated more prosocial behaviour after IN-OT (De Dreu, 2012b), and a third study found that IN-OT increased positive recollections in less anxiously attached participants, but actually decreased positive recollections in more anxiously attached participants (Bartz, Zaki, Ochsner, et al., 2010).

The moderating effect of attachment style on behavioural outcomes associated with OT may be mediated by OT's effect on specific neural pathways. Strathearn and colleagues found that mothers with a secure attachment style (compared to mothers with an insecure

attachment style) showed greater activation in the mesocorticolimbic regions of the brain, associated with reward, when viewing pictures of their own infant smiling (Strathearn, Fonagy, Amico, & Montague, 2009). Moreover, securely attached mothers had a positive OT response when interacting with their own infant, which also correlated positively with activation in the reward regions of the brain. In contrast, insecurely attached mothers demonstrated a decrease in OT concentrations during interaction with their own infant. Similar results in another laboratory found that insecurely attached mothers demonstrated hypersensitivity to their infants' crying (Riem, Bakermans-Kranenburg, van IJzendoorn, Out, & Rombouts, 2012). This was assessed via greater activation of the amygdala, a higher irritation score, and more force used on a handgrip (used to measure participants' stress response), compared to securely attached mothers.

Riem and colleagues also investigated whether parenting style would moderate the social effects of OT. In a double-blind, placebo controlled, randomised trial, women who received OT demonstrated more prosocial behaviour towards an excluded individual during a game, compared to those in the PL condition (Riem et al., 2013); however, this was only true for women who also reported low levels of maternal love withdrawal (a discipline technique applied by participants' mothers during childhood). This replicated a pattern found in an earlier study (Van Ijzendoorn, Huffmeijer, Alink, Bakermans-Kranenburg, & Tops, 2011). Maternal love withdrawal also moderated the effect of IN-OT on functional brain connectivity: OT triggered connectivity between the posterior cingulate cortex, cerebellum and postcentral gyrus, but only for women who reported low levels of maternal love withdrawal (Riem, Van Ijzendoorn, et al., 2012). Thus parenting style, as well as attachment style, seems to moderate behavioural and neural responses to OT administration, but further research is required to clarify the current consistency of these effects.

Trait empathic ability has also been found to moderate the behavioural effects of OT.

This is a factor that was not discussed by Shamay-Tsoory and Abu-Akel (2016). For example, participants who report low trait empathy demonstrated increased prosocial effects (as assessed by an increase in accuracy during a cognitive empathy task) after IN-OT, whereas participants who report high trait empathy showed no increase (Feeser et al., 2015). Furthermore, participants with high versus low levels of self-reported empathic concern recruited candidates with opposing qualities to their teams after OT administration (Kret & De Dreu, 2013). These findings have been supported by clinical studies (e.g., Andari et al., 2010) in which individuals with impaired social cognition skills showed an increase in empathic ability after OT administration, but to a lesser extent compared to healthy participants. Indeed, other social psychological variables such as social values (Pfundmair, Aydin, Frey, & Echterhoff, 2014) and personality types (Human, Thorson, & Mendes, 2016) have also been found to moderate the behavioural effects of OT.

Finally, and crucially, research has demonstrated that the relationship between OT and psychological individual differences is bidirectional: Early life events can trigger adaptive physiological changes that influence the oxytocinergic system and these changes have been found to have a sustained effect on both physiological and psychological outcomes. For example, women exposed to childhood abuse have been found to have lower CSF concentrations of OT (Heim et al., 2009), and similar results have also been found in monkeys (Winslow, Noble, Lyons, Sterk, & Insel, 2003). Furthermore, men who experienced early separation (before 13 years of age) from one or both of their parents demonstrated weaker anxiolytic effects of IN-OT (Meinlschmidt & Heim, 2007).

These findings were supported in a recent meta-analysis which found a significant association between childhood abuse and methylation of the OTR gene, which significantly predicted the intensity of psychiatric symptoms (Smearman et al., 2016). Thus exposure to specific psychological environments triggers epigenetic changes that regulate the expression

of OTRs in the brain, thereby altering the oxytocinergic system which consequently alters an individual's sensitivity to OT, ultimately dictating their physiological and psychological responses later in life. It is therefore important to consider individual difference factors both in psychological variables, such as attachment style or empathic ability, and in physiological variables, such as OTR polymorphisms or OT concentrations, if research is to pinpoint for whom and under what circumstances OT effects may be beneficial. This is a vital research question to address if OT is to be used as a therapeutic tool in the treatment of various psychopathologies (including ASD, depression, postnatal depression, schizophrenia, and ADHD). The current thesis aims to examine the replicability of findings that the social and emotional effects of OT are moderated by individual difference factors.

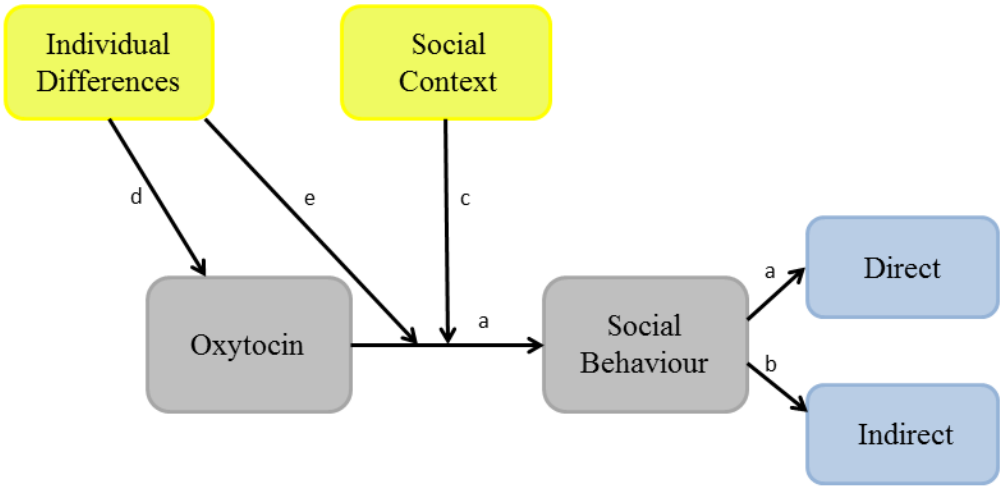
*Thesis Overview*

The aim of this thesis is to investigate the influence of contextual factors and individual differences on the effects of OT on social behaviour. In Chapters 2-4 I first seek to validate methodological techniques and address methodological norms in the OT literature. In Chapters 5-7 I investigate the role of group identity on the anticipated prosocial effects of OT in healthy volunteers (Chapters 5 and 7) and in a clinical population (Chapter 6). Finally, in Chapter 8 I investigate the role of OT in emotion processing, in both a healthy and a clinical population.

Figure 1.1 illustrates the theoretical framework that guided the research reported in this thesis. In the first half of the thesis I address key methodological issues in the OT literature, in particular with respect to IN-OT administration. I first wanted to confirm that IN-OT administration had a significant effect on salivary OT concentrations in 40 male undergraduates from Cardiff University (Chapter 2; path d in Figure 1.1). I then aimed to replicate this in a larger, mixed-gender sample of 216 undergraduates from the University of

Amsterdam, in addition to providing an empirical response to recent critiques of IN-OT administration studies (Chapter 3, path d in Figure 1.1). Finally, I empirically tested the prediction that a clinical sample of patients with Cranial Diabetes Insipidus would demonstrate an OT deficiency (Chapter 4, path d in Figure 1.1) and, if so, whether this deficiency had an effect on their emotion processing skills (Chapter 8; paths e & a in Figure 1.1) or on the influence of group identity as a moderating factor of social behaviour (Chapter 6; paths d, a, c, & d in Figure 1.1).

*Figure 1.1 – Theoretical model examined in the thesis*




In the second half of the thesis I aimed to replicate previous findings that OT affects social and emotional behaviour. I also wanted to address whether the effect of oxytocin on social behaviour is limited to direct interactions, or whether they extend to indirect or third-party interactions. I therefore investigated whether IN-OT administration would affect participants' behaviour and empathy towards an unknown excluded individual during an online game (Chapter 5; paths a, & b in Figure 1.1). The two key questions addressed in the thesis concern the influence of contextual factors and individual differences; specifically,

whether the effects of IN-OT on participants' behaviour and empathy is moderated by group identity (i.e., the social context) (Chapter 5-7; paths a, c, & b in Figure 1.1), and whether this is dependent upon individual difference factors (Chapter 5-7; paths d, a, & b in Figure 1.1). I also examined the role of OT in empathy by recruiting a clinical population with anticipated OT deficits. Specifically, I investigated whether this anticipated OT deficit would affect participants' emotion processing skills, more specifically their ability to infer emotional states from just the eye region (using the Reading the Mind in the Eyes Task) or the whole face (using the Facial Emotion Recognition task) (Chapter 8; paths d & a in Figure 1.1).

# Chapter 2

## Salivary oxytocin concentrations in males following intranasal administration of oxytocin: A double-blind, cross-over study

Adapted from:
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### Abstract

The use of intranasal oxytocin (IN-OT) in research has become increasingly important over the past decade. Although researchers have acknowledged a need for further investigation of the physiological effects of intranasal administration, few studies have actually done so. In the present double-blind cross-over study we investigated the longevity of a single 24 IU dose of IN-OT measured in saliva in 40 healthy adult males. Salivary OT concentrations were significantly higher in the OT condition, compared to placebo (PL). This significant difference lasted until the end of testing, approximately 108 minutes after administration, and peaked at 30 minutes. Results showed significant individual differences in response to IN-OT administration. To our knowledge this is the largest and first all-male within-subjects design study to demonstrate the impact of IN-OT on salivary OT concentrations. The results are consistent with previous research in suggesting that salivary OT is a valid medium for OT measurement. The results also suggest that the post-administration 'wait-time' prior to starting experimental tasks could be reduced to 30 minutes, from the 45 minutes still occasionally used, thereby enabling testing during peak OT concentrations. Further research is needed to ascertain whether OT concentrations after intranasal administration follow similar patterns in females, and different age groups.

## Introduction

The use of IN-OT in scientific research has become increasingly popular over the past decade. According to a recent review, 230 papers have reported using IN-OT since 1958 (Veening & Olivier, 2013). This scientific interest spans several fields, from clinical psychology, with respect to autism spectrum disorder (Hollander et al., 2007; Hollander et al., 2003) and schizophrenia (Fischer-Shofty et al., 2013), to social psychology, with respect to intergroup relationships (Riem et al., 2013) and emotional processing (Shahrestani et al., 2013). Despite this flourishing interest, concern has been expressed that the assumptions upon which this line of research depends have not been securely established. In particular, there is a lack of evidence concerning both the longevity of the effects of intranasal spray on peripheral OT concentrations and the pattern of concentrations during these effects (Veening & Olivier, 2013). Few studies have addressed these questions, and many IN-OT administration studies do not include any assessment of participants' OT concentrations. The aim of the present study was to provide evidence that IN-OT has a significant impact on salivary OT concentrations in healthy adults (which cannot be explained by 'spiking' alone; see below), and the nature of this impact. We used a double-blind, cross-over design.

We begin by addressing questions about the validity of saliva testing (McCullough et al., 2013). Although others (Carter et al., 2007; Grewen, Davenport, & Light, 2010; White-Traut et al., 2009) have addressed these concerns in detail, we note that there have been recent improvements in the preferred commercial saliva ELISA (enzyme linked immunosorbent assay) that is commonly used in OT research ("Product Technical Bulletin," 2014). These improvements have sought to address the main concern raised by McCullough et al. (2013), namely that earlier ELISAs had a high rate of non-specific binding (when non-OT compounds bind to 'OT-specific' antibodies), leading to artificially

elevated concentrations of OT. The latest ELISA kit ("Product Technical Bulletin," 2014) has reduced non-specific binding, and thereby alleviates this problem. We used this latest kit.

How long IN-OT remains elevated in saliva remains unclear. According to Veening and Olivier (2013), nearly 80 papers that reported using IN-OT administration were published in 2012. To our knowledge only three of these investigated the patterns of OT concentrations in saliva in healthy adults after IN-OT administration. One study (Van IJzendoorn et al., 2012) found that salivary OT was still elevated 7 hours after administration in a double-blind, between-subjects study (n = 46; all female). Participants in both the high dose (24 IU; n = 10) and low dose (16 IU; n = 18) IN-OT conditions still had significantly higher salivary OT concentrations after 7 hours, compared to participants in the PL condition. Concentrations in both OT conditions ranged from tenfold to one hundredfold the average PL concentration. However, there was no statistically significant difference between the high and low dose OT conditions at any point in the study.

There are reasons to question the generalizability of these findings concerning longevity, because there is no other evidence that IN-OT causes elevated OT concentrations for such an extended period of time. Weisman, Zagoory-Sharon, and Feldman (2012) sampled salivary OT concentrations over a 4-hour period after IN-OT administration in 10 participants (5 female; within-subjects design). Samples were taken at baseline and 15, 30, 45, 60, 80, 100, 120, 180, 240 minutes after administration. OT concentrations were significantly higher 240 minutes after administration, compared to the PL condition. There was a significant decrease in salivary OT between 30 minutes and 45 minutes post-administration, followed by a plateau phase lasting from 45 minutes to 120 minutes after administration. Due to the time intervals between samples, it is difficult to state exactly when this plateau ceased. It is possible that the plateau lasted for some time

past 120 minutes before salivary OT decreased significantly at 180 minutes.

Although Weisman et al. (2012) reported a significant increase in salivary OT at 15 minutes post-administration, the precise values should be treated with caution. Given the mode of delivery, is it possible that at 15 minutes what is actually being measured is Syntocinon (synthetic OT) spray that has trickled down from the nasal cavity to the back of the throat, causing an artificial 'spike' in OT concentrations in the saliva. This can occur if some of the OT spray is not absorbed across the nasal membrane during administration. In this instance, the fine hairs in the nasal cavity move the substance from the front of the nasal cavity, to the back, and down the throat. Here the synthetic OT spray could then be brought forward into the mouth when participants are asked to provide a saliva sample.

This process of clearing substances from the nasal cavity to the back of the throat, called mucociliary transport, takes 12-15 minutes in healthy individuals (Marttin, Schipper, Verhoef, & Merkus, 1998). Hence, taking saliva samples at 15 minutes creates the risk of collecting saliva that is 'spiked' with IN-OT (indeed this may be why other saliva studies have chosen 30 minutes as their first measurement, as this allows almost double the length of time of average mucociliary transport (Marttin et al., 1998)). Importantly, when substances are brought to the back of the throat, they do not remain there indefinitely; instead, the swallow reflex is activated and moves substances from the throat into the oesophagus and down to the stomach. Therefore IN- OT that is not absorbed across the nasal membrane is moved to the back of the throat, where it is then swallowed, and can therefore no longer 'spike' saliva. Because this process takes up to 15 minutes on average, spiking cannot account for the sustained and highly significant effects of IN-OT on salivary OT concentrations reported in the literature.

It is nonetheless worth noting that concentrations may peak earlier than 30

minutes, as is the case for many other small peptides (Veening & Olivier, 2013), and can be reliably measured in other matrices. A recent study (Striepens et al., 2013) investigating the relationship between IN-OT and its impact on OT concentrations in plasma and cerebrospinal fluid found that plasma OT was significantly elevated 15 minutes post-administration. A second study (Andari et al., 2010) also found significantly elevated plasma OT concentrations at 10 minutes post-administration.

One of the largest studies to empirically test the longevity of IN-OT in saliva was conducted by Huffmeijer et al. (2011). Fifty-seven females took part in the study, which had a between-subjects design. Salivary OT concentrations were still significantly elevated 2.25 hours after administration, compared to PL concentrations. However, only three samples were taken during the study (baseline, 1.25 and 2.25 hours after administration) so few conclusions can be drawn regarding the pattern of OT concentrations during this elevation, except to corroborate other evidence that the impact of IN-OT lasts for at least 90 minutes in both plasma and saliva (Gossen et al., 2012).

*The Present Study*

We predicted that IN-OT would lead to a significant increase in salivary OT concentrations, compared to PL, in healthy adult males. With just a handful of studies having found significantly elevated concentrations at approximately 120 minutes, we set out to establish both the pattern of the intranasal administration effects on salivary OT, and to confirm that these effects can last up to and beyond 90 minutes.

To achieve this aim we administered either a PL or a single dose of 24 IU of OT to male participants in a double-blind, cross-over design. Saliva samples were collected at baseline, and then at 30, 60, 90, 105, and 108 minutes after administration. Participants completed a series of psychological tasks during the post-baseline period. These tasks were

matched in the OT and PL conditions with respect to timing and content. Performance on these tasks is not the central concern of this chapter, which focuses solely on salivary OT concentrations (see Chapter 5 and Hubble (2015) for behavioural data).

## Method

### *Participants and Ethics*

Forty healthy male students ($M_{age}$ = 20.98; *SD* = 4.55) from Cardiff University took part in the study. The majority were Psychology students (n = 31); those studying other disciplines were Chemistry, Engineering or Journalism students. Psychology students were awarded course credits; non-Psychology students received financial compensation for their time.

The study was approved by both the School of Psychology Ethics Committee at Cardiff University, and the Research and Development Office at Cardiff and Vale University Health Board. All participants completed medical pre-screening forms and signed statements of health before leaving each testing session. They were also cleared to participate in the study by a medical professional. Participants gave written informed consent at the start of both testing sessions, and were fully debriefed after their second session (see Appendix 1 for a flow diagram of the study).

### *Procedure*

Each of the two testing sessions lasted approximately 3 hours. There was a 2-week interval between the two sessions (for practical reasons seven participants had to be tested at later dates; the longest interval between the two sessions was 35 days). The two sessions were timed to take place at the same time of day to control for any potential

diurnal effects. Participants were instructed to abstain from alcohol for 24 hours, and caffeine for 2 hours prior to testing. All participants were non-smokers. Participants were only allowed to drink water during the sessions; if any food had been consumed before the start of a session, participants were asked to rinse their mouths thoroughly before any saliva samples were taken.

On arrival participants completed a series of questionnaires and provided their baseline saliva sample 30 minutes after arrival, to allow for acclimatisation to the testing facility. Participants then self-administered 24 IU (three puffs per nostril, at their own pace) of synthetic OT or an independently manufactured PL nasal spray that chemically matched the OT spray for all compounds, except OT. Both sprays were manufactured by St Mary's Pharmaceutical Unit, Cardiff (http://www.wales.nhs.uk/sites3/home.cfm?orgid=828). Recommendations regarding administration procedures made by Guastella et al. (2013) were followed, although participants' nasal cavities were not medically examined. A doctor was present during administration and for the next 15 minutes. Half an hour after administration participants provided their second saliva sample, after which they began completing the experimental tasks.

To assess whether the effect of the OT spray remained detectable in saliva for up to 90 minutes (based on previous literature), two further samples were taken at 30 min intervals: 60 min and 90 min post-administration. The final two saliva samples were taken at approximately 105 and 108 minutes after administration, immediately before and immediately after a video excerpt that was intended to evoke an empathic response. The 108-minute saliva sample marked the end of the testing session.

*Oxytocin Sampling and Analysis*

Saliva samples were collected in pre-chilled polypropylene 5ml tubes (Sarstedt, Leicester, UK) that were stored on ice throughout the session (the type of plastic used for saliva collection is important as different proteins bind more strongly to certain types of plastic, which can result in inaccurate sampling (Goebel-Stengel, Stengel, Taché, & Reeve, 2011)). For each sample, participants were asked to produce 2ml of passive drool. Samples were frozen as quickly as possible during testing, and were left on ice for no longer than 1 hour. Samples were frozen at -80˚C to ensure that they remained stable during long-term storage (the first samples collected were stored for 6 months; the final samples were frozen for a day). Once all samples had been collected, they were thawed and centrifuged at 4˚C at 1600 x g for 15 min; 1ml of supernatant was transferred to a new tube before being frozen again at -80˚C. To ensure PL and baseline saliva samples would be above the minimum sensitivity of the ELISA (15pg/ml) the samples had to be concentrated. Although the kit manual provides instructions for a chemical concentration process, it is also possible to lyophilize (freeze dry) samples, effectively achieving the same outcome (Carter et al., 2007; White-Traut et al., 2009). Lyophilization has also been found to significantly increase the validity of measuring OT via ELISA (Christensen, Shiyanov, Estepp, & Schlager, 2014; Leng & Ludwig, 2015; McCullough et al., 2013). Therefore, we used the lyophilisation process instead of the chemical process outlined in the manual. Samples were freeze-dried overnight (for approximately 15 hours), until all samples were dehydrated. The length of freeze-drying required depends upon the volume added. Because samples were freeze-dried in batches due to a limited space, some batches contained more total volume than others, therefore requiring a slightly longer time to achieve the same outcome, compared to other batches. After samples were lyophilized they were stored at -20˚C until analysis. It was appropriate to store freeze-dried samples at

-20˚C for two reasons: first, samples become more stable when they have been freeze-dried; second, samples were to be analysed within 2 weeks of freeze- drying, and therefore only required short-term storage.

Samples were analysed using a 96-well OT ELISA kit (Enzo Life Sciences, Exeter, UK). This kit has been used in several OT studies (Van IJzendoorn et al., 2012; Weisman et al., 2012) although as noted above the kit has recently (September 2013) undergone modification and "rigorous validation" ("Product Technical Bulletin," 2014, p. 1) . As a result, the ELISA used in the present study has a greater specificity compared to the earlier version. Freeze dried samples were reconstituted in 250µl of assay buffer, thereby concentrating all samples four-fold. Where possible all samples were run in duplicate. Some participants struggled to produce enough saliva for every sample, in which case only 0.5 ml was frozen after centrifugation. In such cases, samples were then reconstituted in 125µl of assay buffer, such that the concentration was the same as the other samples; however in these cases there was only sufficient volume to run a single analysis. All samples were processed in accordance with the manual's ELISA protocol ("Product Manual: Oxytocin ELISA kit," 2013), with an overnight incubation of 19 hours. Samples were read at 405nm and concentrations were calculated from the standard curve. Finally, the international correction for OT concentrations, devised by the National Institute for Biological Standards and Control and the World Health Organisation was applied.

The ELISA manual ("Product Manual: Oxytocin ELISA kit," 2013) reports that intra-assay and inter-assay coefficients of variability are 12.6 – 13.3% and 11.9 – 20.9%, respectively. The present study obtained intra-assay and inter-assay coefficients of <8% and 10.6 – 14.5%, respectively. Accepted values for coefficients of variability are <10% for intra-assay and <15% for inter-assay variability ("Inter- and intra-assay coefficients of

variability," 2014). To confirm that the process of freeze drying did not significantly degrade samples, a serial dilution series was prepared and freeze dried with the samples. There was a high correlation between the control series and the standards, $r(6) = .96$, $p = .002$.

Because a number of samples were more than three standard deviations above the mean, the data were winsorized prior to data analysis.

*Data Analysis*

A related-samples Friedman's two-way analysis of variance was conducted to assess the effect of IN-OT administration on participants' OT concentrations. Significant results were followed up with Wilcoxon signed rank tests.

## Results

Initial analysis revealed that 9 out of 12 samples violated the assumption of normality (see Supplementary Information 1, p 31). We therefore used non-parametric tests to assess whether there was a significant effect of drug on salivary OT concentrations.

Mean salivary OT concentrations are shown in Table 6.1, broken down by Drug (OT vs PL) and Time (Samples 1- 6). Related-samples Friedman's two-way analysis of variance revealed a significant difference between samples, $\chi^2(11) = 286.75$, $p < .001$.

Follow-up Wilcoxon signed rank tests were then carried out. The results of these tests are also shown in Table 2.1. There was no significant difference between the baseline samples. There were significant differences between all the remaining samples.

*Table 2.1 - Mean salivary OT concentrations (pg/ml) at each time point for OT and PL condition, with reported outcomes of Wilcoxon signed rank test*

| Time | OT Condition Mean (SD) | PL Condition Mean (SD) | *Z* | Sig |
|---|---|---|---|---|
| Baseline | 77.93 (74.46) | 45.96 (33.27) | -1.91 | .056 |
| 30 minutes | 999.52 (813.95) | 37.45 (21.24) | -5.43 | <0.001 |
| 60 minutes | 951.98 (772.96) | 46.43 (33.54) | -5.50 | <0.001 |
| 90 minutes | 531.29 (538.76) | 39.58 (29.77) | -5.38 | <0.001 |
| 105 minutes | 417.41 (366.88) | 44.88 (33.47) | -5.31 | <0.001 |
| 108 minutes | 355.17 (235.69) | 34.08 (25.97) | -5.50 | <0.001 |

Large standard deviations showed that there were considerable individual differences in OT concentrations after In-OT administration. To demonstrate this, the means for each drug condition are presented in Figures 2.1 and 2.2, with additional lines representing +/- 1 standard deviation. All participants' OT concentrations for both conditions are presented in Supplementary Information 1, p 32.

*Figure 2.1 - The mean and ± 1 standard deviation of OT concentrations in the PL condition*

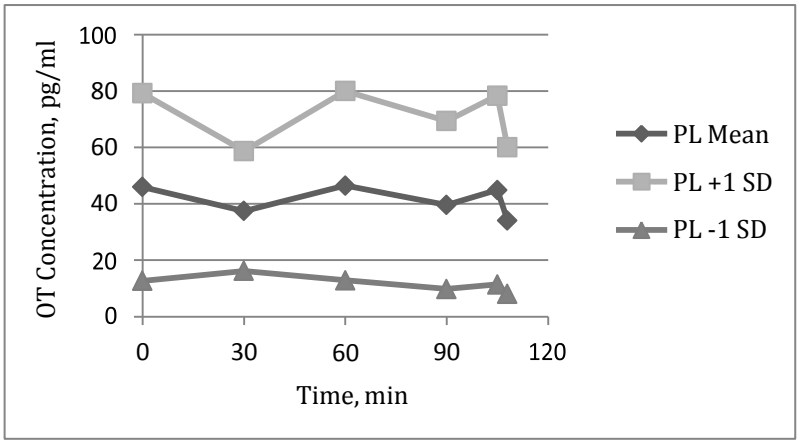
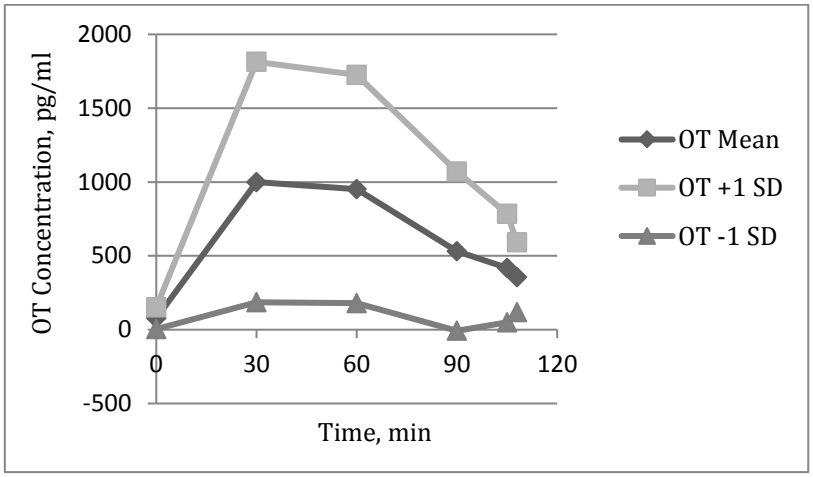


*Figure 2.2 - The mean and ± 1 standard deviation of OT concentrations in the OT condition*




## Discussion

To our knowledge, this is the largest within-subjects design study to date demonstrating that intranasal administration of OT has significant effects on salivary OT concentrations in males. As expected, participants had significantly higher salivary OT concentrations after OT administration, compared to their PL session. In line with previous research (Gossen et al., 2012; Kirkpatrick, Francis, Lee, de Wit, & Jacob, 2014; Neumann et al., 2013; Paloyelis et al., 2014), maximum OT concentrations were detected 30 minutes after administration. When using saliva sampling, 30 minutes may be the first reliable time point at which to measure OT concentrations, given that samples taken prior to this may reflect 'spiking'. Samples taken from 30 minutes onwards, however, cannot reflect spiking, as substances that are not absorbed across the nasal membrane are transported to the back of the throat, and then swallowed within 15 minutes (Marttin et al., 1998). Any remaining IN-OT at 30 minutes could not account for the significant increase seen here,

and also in other studies.

The results are also consistent with previous findings (Huffmeijer et al., 2011; Weisman et al., 2012) in showing that the effect of IN-OT on peripheral (salivary in the present study) OT concentrations lasts beyond 90 minutes. Together, these results support the idea of a positive feedback system between peripheral and central concentrations of OT: "… central regulating mechanisms [control] the duration of the elevated levels of peripheral OT" (Veening & Olivier, 2013, p. 1450). This would explain why a compound with a half-life of several minutes in peripheral bodily fluids remains elevated for such an extended period of time. Although the mechanism by which IN-OT affects both central and peripheral concentrations both directly and indirectly is not fully understood (an issue raised in several papers (Churchland & Winkielman, 2012; Leng & Ludwig, 2015)), the continuing interest in OT research has driven biologists as well as psychologists and clinicians to investigate this further (e.g., Quintana et al. (2014)). A pioneering study (Neumann et al., 2013) showed that IN-OT does reach specific brain regions in rats and mice, demonstrating that IN-OT does cross the blood-brain barrier, thereby addressing one of the main concerns (Churchland & Winkielman, 2012). More recently, Paloyelis et al. (2014) demonstrated that IN-OT also crosses the blood-brain barrier in human males, providing further evidence that IN- OT has direct effects on the brain. In addition, several studies have found that IN-OT leads to a significant increase in OT concentrations in cerebrospinal fluid, which may also be indicative of IN-OT reaching the brain (Dal Monte et al., 2014; Modi, Connor- Stroud, Landgraf, Young, & Parr, 2014). However, we acknowledge that more research is required to corroborate these findings, and evaluate the relationship between central and peripheral concentrations of OT (see Chapter 9 for a discussion).

An important attribute of the present study is its use of a within-subjects design.

The extent of individual differences in response to IN-OT is considerable and thus far unaddressed in the literature. Indeed, this is an important area of focus for research, especially psychological research, partly because these individual differences may be shaped by psychological factors, and partly because individual differences in reactivity to IN-OT are likely to influence subsequent psychological and behavioural variables. In addition to Guastella et al.'s (2013) suggestion that anatomical differences could account for individual differences in response to IN-OT, and the possibility that genetic factors may also influence responses, individual differences in psychological factors might account for a significant proportion of this variance. Several studies have shown that individual differences in social anxiety and early parental relationships can moderate the way in which participants respond behaviourally to IN-OT (Bakermans- Kranenburg, van IJzendoorn, Riem, Tops, & Alink, 2011; De Dreu, 2012b; Riem et al., 2013). Investigating or controlling for these variables in future studies may advance our understanding of their effect on OT-mediated processes.

Furthermore, a recent paper expressed concern regarding the statistical power of many IN-OT studies (Walum et al., 2015), and we have already expressed our own concern that few studies reporting behavioural effects of IN-OT include a measure of peripheral OT. By using the largest, within-subjects, sample size published to date, we aimed to address both types of concerns.

Some limitations of the present research should be acknowledged. First, although the baseline concentrations are statistically similar, the difference does not fall very far short of statistical significance, with OT baseline concentrations being higher than PL baseline concentrations. We have carefully reviewed possible reasons for this (non-significant) difference. The study was double-blind; participants were randomly assigned to their drug order; there were no significant order effects; and all data points were

winsorized (therefore there were no statistical outliers). Within the winsorized dataset, there were four participants with noticeably higher OT baseline concentrations; if their values are removed the OT baseline mean decreases to 51.13 (*SD* = 30.86) pg/ml, much closer to the PL baseline mean of 45.96 pg/ml. However, in absence of any good reason to remove them, we retained their winsorized values in the dataset.

These atypical values may results from individual differences that are either psychological or anatomical/genetic in origin. It is worth emphasizing that this small and non-significant difference in baseline values cannot account for the much larger and highly significant difference in salivary OT concentrations following intranasal administration, and therefore does not detract from the main result of the present study.

One other possible limitation is that the concentrations of OT observed in the PL condition are higher than those previously reported. Typical values observed in previous research suggest that OT concentrations in peripheral bodily fluid are <10 pg/ml under PL conditions (e.g., Grewen, Girdler, Amico, and Light (2005)). In the present study we found concentrations of 30-40 pg/ml across the testing session. It is important to note that although many studies use the same commercial ELISA kit, there is no standard operating procedure for saliva collection (an issue raised by Guastella et al. (2013)). Given that salivary OT can degrade very quickly at room temperature and the lack of a standardised procedure for its collection, it is difficult to compare concentrations between studies. There are three reasons why these apparently elevated PL concentrations may not be of real concern: 1) PL concentrations were highly consistent within participants; 2) there is no methodological reason to question the validity of the concentrations; 3) the primary effects found in the present study are the same as those observed in other studies (see Chapter 3 for a replication of these findings).

A final limitation of the present study concerns the number of saliva samples

taken. Due to logistical and financial restrictions, it was not possible to collect samples beyond 108 minutes. As previously stated, the longevity of IN-OT effects on peripheral bodily fluids is not established. Given the interest in IN-OT studies, and its known effects on behaviour, it is important to address this question in future studies.

In light of the present findings, taken together with those from previous research, it is recommended that the 'wait-time' between intranasal administration of OT and experimental testing should be no longer than 30 minutes. Several studies have now shown that OT concentrations in both plasma (Gossen et al., 2012; Neumann et al., 2013) and saliva (Weisman et al., 2012) peak at 30 minutes, if not earlier. Testing should therefore begin at 30 minutes to ensure that tasks are carried out under peak OT concentrations. This also aids the efficiency of testing. If testing begins 30 minutes post administration then, based on current research, one further hour of testing can be conducted whilst endogenous OT concentrations are significantly elevated above baseline.

As previously noted, however, there is a need to develop standard operating procedures for all aspects of OT research: sampling, processing of samples, intranasal administration techniques, and how OT studies are reported. This would enable precise replications and more accurate comparisons between studies to be made.

In terms of saliva analysis, it would be beneficial if future research could establish whether freeze drying samples rather than using chemical extraction results in higher concentrations. In addition, shorter time intervals between samples (for example, as used by (Weisman et al., 2012)) would enable a more detailed picture of the pattern of OT concentrations after IN-OT administration.

Finally, future research could investigate whether different age groups and whether males and females, differ in responsiveness to IN-OT administration. The present study and the cited literature provide evidence of the effects of IN-OT in young adults.

However, we are unable to assess whether this is representative of responsiveness in older adults or in children. Given that the social brain is now understood to continue developing into the second decade of life (Thompson et al., 2000), there is reason to believe that there may be significant differences in responsiveness over the life span. Although one research group has investigated peripheral OT concentrations in response to OT-related behaviour across two generations (Feldman, Gordon, & Zagoory-Sharon, 2010), those researchers analysed 'natural' OT concentrations before and after social interaction. To our knowledge, no studies have examined whether there are differences in responsiveness to intranasal administration of OT across generations. Given the interest in the therapeutic potential of IN-OT (e.g., Veening and Olivier (2013)), this is a high priority research question.

*Conclusions*

In conclusion, the present study found that intranasal administration of OT resulted in a significant increase in salivary OT concentrations in healthy adult males for at least 108 minutes. The findings underscore the need for a within-subjects design when employing a PL controlled, IN-OT study, because of the large individual differences both in baseline concentrations and especially in response to OT sprays. It would be desirable for future researchers to include a manipulation check for endogenous OT. This would not only add to the relatively small literature on the effects of IN-OT on peripheral OT concentrations, but also enable researchers to identify individuals who are more responsive, compared to others, and to assess whether there are any psychological or behavioural differences between more and less responsive individuals.

## Supplementary Information 1

*SI 1 - Results of Shapiro-Wilks tests for normality for each saliva sample*

| **Saliva sample** | ***D*** | **df** | ***p*** |
|---|---|---|---|
| Placebo Baseline | .150 | 39 | .027 |
| Placebo 30 mins | .110 | 39 | >.05 |
| Placebo 60 mins | .160 | 39 | .013 |
| Placebo 90 mins | .139 | 39 | >.05 |
| Placebo 105 mins | .221 | 39 | <.001 |
| Placebo 108 mins | .200 | 39 | <.001 |
| Oxytocin Baseline | .239 | 39 | <.001 |
| Oxytocin 30 mins | .186 | 39 | .002 |
| Oxytocin 60 mins | .179 | 39 | .003 |
| Oxytocin 90 mins | .200 | 39 | <.001 |
| Oxytocin 105 mins | .139 | 39 | >.05 |
| Oxytocin 108 mins | .170 | 39 | .006 |

*SI 2 – Salivary OT concentrations for PL and OT conditions*

| Participant | Session | Condition | Baseline | 30 | 60 | 90 | 105 | 108 |
|---|---|---|---|---|---|---|---|---|
| **1** | 1 | Placebo | 31.25 | 28.22 | 16.44 | 31.25 | 51.14 | 32.60 |
| | 2 | Oxytocin | 34.82 | 538.60 | 701.51 | 148.96 | 232.32 | 288.40 |
| **2** | 1 | Placebo | 29.08 | 8.85 | 29.26 | 45.90 | 40.95 | 21.80 |
| | 2 | Oxytocin | 269.96 | 1959.14 | 1947.40 | 281.55 | 987.89 | 221.43 |
| **3** | 1 | Placebo | 36.32 | 22.60 | 5.61 | 7.44 | 17.97 | 14.02 |
| | 2 | Oxytocin | 15.95 | 1801.14 | 845.07 | 380.17 | 84.70 | 206.03 |
| **4** | 1 | Placebo | 36.21 | 28.56 | 157.55 | 19.95 | 35.81 | 30.52 |
| | 2 | Oxytocin | 33.39 | 624.10 | 241.60 | 213.99 | 157.11 | 103.87 |
| **5** | 1 | Placebo | 14.57 | 39.12 | 42.49 | 38.69 | 32.78 | 43.44 |
| | 2 | Oxytocin | 27.94 | 799.99 | 421.82 | 177.38 | 289.85 | 288.26 |
| **6** | 1 | Oxytocin | 232.45 | 761.24 | 233.74 | 115.35 | 565.10 | 831.50 |
| | 2 | Placebo | 32.78 | 33.15 | 19.09 | 18.47 | 31.89 | 31.20 |
| **7** | 1 | Placebo | 62.23 | 41.44 | 82.02 | 71.99 | 71.99 | 36.09 |
| | 2 | Oxytocin | 68.23 | 3043.75 | 2846.97 | 1719.60 | 1357.65 | 1137.94 |
| **8** | 1 | Placebo | 17.96 | 38.08 | 17.15 | 37.22 | 37.22 | 18.52 |
| | 2 | Oxytocin | 63.68 | 1423.53 | 2846.97 | 558.32 | 558.32 | 357.80 |
| **9** | 1 | Oxytocin | 78.54 | 2670.42 | 1979.87 | 753.05 | 753.05 | 161.11 |
| | 2 | Placebo | 1.88 | 23.13 | 24.03 | 21.26 | 21.26 | 14.94 |
| **10** | 1 | Oxytocin | 19.39 | 635.01 | 340.22 | 102.82 | 65.96 | 44.26 |
| | 2 | Placebo | 41.23 | 27.50 | 15.92 | 19.43 | 16.44 | 18.81 |
| **11** | 1 | Oxytocin | 301.31 | 1383.11 | 1133.03 | 1231.86 | 561.94 | 389.43 |
| | 2 | Placebo | 135.71 | 67.25 | 100.85 | 115.54 | 47.51 | 34.89 |
| **12** | 1 | Oxytocin | 14.83 | | 128.78 | 1719.60 | 875.95 | 223.95 |
| | 2 | Placebo | 20.19 | 22.38 | 27.15 | 29.90 | 40.98 | 20.85 |
| **13** | 1 | Placebo | 11.46 | 10.68 | 7.08 | 17.19 | 17.30 | 8.39 |
| | 2 | Oxytocin | 61.91 | 106.34 | 44.18 | 16.58 | 79.35 | 25.24 |
| **14** | 1 | Placebo | 18.01 | 18.24 | 15.56 | 30.16 | 12.53 | 11.25 |
| | 2 | Oxytocin | 63.91 | 549.27 | 78.85 | 21.94 | 19.07 | 22.36 |
| **15** | 1 | Placebo | 15.56 | 22.51 | 24.92 | 16.06 | 24.60 | 15.56 |
| | 2 | Oxytocin | 147.11 | 186.17 | 431.28 | 94.83 | 124.68 | 81.92 |
| **16** | 1 | Placebo | 59.26 | 56.93 | 71.81 | 41.67 | 36.08 | 36.08 |
| | 2 | Oxytocin | 99.70 | 209.86 | 344.70 | 260.48 | 75.94 | 33.84 |
| **17** | 1 | Oxytocin | 88.42 | 1201.37 | 1260.47 | 1040.19 | 456.14 | 872.25 |
| | 2 | Placebo | 19.02 | 20.11 | 26.62 | 68.99 | 93.51 | 16.73 |
| **18** | 1 | Placebo | 19.02 | 36.95 | 28.38 | 23.99 | 55.58 | 20.60 |
| | 2 | Oxytocin | 56.03 | 1343.82 | 2053.82 | 796.25 | 650.25 | 511.46 |
| **19** | 1 | Oxytocin | 21.09 | 64.98 | 71.10 | 21.57 | 38.72 | 49.23 |
| | 2 | Placebo | 20.62 | 29.78 | 42.69 | 15.51 | 50.73 | 31.15 |
| **20** | 1 | Placebo | 16.10 | 7.78 | 12.76 | 9.96 | 12.85 | 7.11 |
| | 2 | Oxytocin | 105.82 | 527.04 | 675.10 | 119.32 | 151.70 | 147.21 |
| **21** | 1 | Oxytocin | 25.63 | 32.83 | 96.71 | 89.72 | 72.18 | 1137.94 |
| | 2 | Placebo | 35.39 | 62.12 | 98.17 | 138.64 | 190.54 | 62.44 |
| **22** | 1 | Oxytocin | 301.31 | 245.75 | 688.99 | 548.32 | 43.61 | 217.10 |
| | 2 | Placebo | 47.47 | 37.78 | 23.16 | 36.33 | 28.54 | 140.45 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **23** | 1 | Placebo | 53.74 | 91.16 | 49.05 | 41.66 | 44.18 | 52.01 |
| | 2 | Oxytocin | 57.36 | 1239.48 | 716.50 | 373.12 | 573.95 | 264.04 |
| **24** | 1 | Oxytocin | 34.93 | 1673.35 | 1619.64 | 31.68 | 32.94 | 17.72 |
| | 2 | Placebo | 19.55 | 24.08 | 19.29 | 21.14 | 10.25 | 13.61 |
| **25** | 1 | Placebo | 53.84 | 50.76 | 60.18 | 70.42 | 48.80 | 57.48 |
| | 2 | Oxytocin | 30.66 | 1789.17 | 794.32 | 1719.60 | 383.98 | 611.27 |
| **26** | 1 | Placebo | 71.82 | 50.76 | 34.04 | 69.96 | 47.23 | 54.19 |
| | 2 | Oxytocin | 100.96 | 2340.22 | 2177.57 | 1161.31 | 1357.65 | 979.50 |
| **27** | 1 | Placebo | 84.27 | 15.07 | 84.83 | 18.39 | 76.28 | 18.15 |
| | 2 | Oxytocin | 74.27 | 351.87 | 435.27 | 102.19 | 149.27 | 24.64 |
| **28** | 1 | Placebo | 46.95 | 10.18 | 47.58 | 8.39 | 33.45 | 9.85 |
| | 2 | Oxytocin | 24.97 | 273.32 | 726.20 | 113.66 | 459.05 | 49.19 |
| **29** | 1 | Oxytocin | 40.83 | 631.59 | 1644.95 | 335.87 | 1303.50 | 340.37 |
| | 2 | Placebo | 52.57 | 13.82 | 27.95 | 6.35 | 23.05 | 16.43 |
| **30** | 1 | Placebo | 11.27 | 20.12 | 24.56 | 22.59 | 21.18 | 30.77 |
| | 2 | Oxytocin | 87.35 | 810.94 | 2103.43 | 1719.60 | 282.03 | 376.84 |
| **31** | 1 | Oxytocin | 82.96 | 1885.30 | 639.00 | 731.54 | 469.08 | 93.16 |
| | 2 | Placebo | 39.81 | 69.72 | 98.08 | 31.17 | 40.07 | 26.54 |
| **32** | 1 | Oxytocin | 6.39 | 1402.10 | 1264.66 | 1042.48 | 614.78 | 859.33 |
| | 2 | Placebo | 18.98 | 17.92 | 20.78 | 8.65 | 17.13 | 17.13 |
| **33** | 1 | Oxytocin | 48.68 | 234.14 | 193.47 | 58.54 | 188.62 | 80.45 |
| | 2 | Placebo | 38.48 | 60.82 | 37.27 | 32.61 | 47.76 | 32.20 |
| **34** | 1 | Placebo | 135.71 | 57.80 | 73.60 | 61.60 | 48.99 | 40.74 |
| | 2 | Oxytocin | 45.97 | 185.05 | 439.40 | 508.60 | 486.46 | 349.50 |
| **35** | 1 | Oxytocin | 34.10 | 2086.58 | 1518.30 | 1719.60 | 668.53 | 668.53 |
| | 2 | Placebo | 53.55 | 36.80 | 75.50 | 49.62 | 37.51 | 49.31 |
| **36** | 1 | Placebo | 35.28 | 32.37 | 40.80 | 45.66 | 41.08 | 36.95 |
| | 2 | Oxytocin | 33.02 | 241.88 | 1122.51 | 1191.38 | 769.87 | 394.66 |
| **37** | 1 | Placebo | 105.79 | 70.20 | 72.56 | 50.76 | 57.18 | 34.36 |
| | 2 | Oxytocin | 61.90 | 362.14 | 338.95 | 265.36 | 317.25 | 151.22 |
| **38** | 1 | Oxytocin | 67.46 | 2339.48 | 1199.29 | 490.95 | 545.77 | 687.98 |
| | 2 | Placebo | 101.67 | 80.13 | 93.30 | 68.36 | 104.40 | 95.17 |
| **39** | 1 | Placebo | 92.77 | 42.85 | 33.37 | 20.61 | 21.38 | 20.61 |
| | 2 | Oxytocin | 43.64 | 589.85 | 589.85 | 271.89 | 243.63 | 226.44 |
| **40** | 1 | Oxytocin | 46.67 | 437.49 | 320.55 | 190.89 | 106.95 | 548.23 |
| | 2 | Placebo | 75.09 | 55.36 | 56.38 | 90.17 | 103.11 | 76.95 |

# Chapter 3

## Factors affecting peripheral responses to intranasal administration of oxytocin: Individual differences, gender, digit ratio and diurnal variation


### Abstract

In recent publications several weaknesses have been noted, including low power and a gender bias, in studies investigating the effects of administering the neuropeptide oxytocin (OT).

Seeking to address these (and other) concerns, in the present study we administered either a chemically matched placebo (PL) or 24 IU of synthetic OT to 216 students (73% female). Saliva samples were collected at baseline, 30 and 60 minutes after administration and analysed for the presence of OT. Results revealed that salivary OT was significantly higher in the OT condition, but also demonstrated large individual differences in participants' responsiveness to intranasal OT (IN-OT). Results further indicated that individual differences in responsiveness were not related to gender (although there was a small gender difference in baseline concentrations), digit ratio (an indicator of prenatal testosterone exposure), or female participants' menstrual cycle. Finally, there was no evidence of diurnal variation in OT concentrations. It follows that several logistical constraints that have been commonly applied to IN-OT studies may not be necessary and could be relaxed or even abandoned.

## Introduction

Over the past decade a wave of research has investigated the psychological effects of the hormone OT. Recently, however, a number of reviews have pinpointed weaknesses in this literature, casting doubt on the reliability of previous findings. Weaknesses cited include a lack of power and gender bias. These are important issues to address, and in the current study we sought to address these concerns in addition to addressing several other factors, specifically focusing on factors that may affect participants' responsiveness to intranasal administration of OT.

Given the continuing interest in the therapeutic potential of OT (Martinetz & Neumann, 2016; Young & Barrett, 2015) it is vital to ensure that this treatment is as effective as possible. However there are individual differences in physiological responses to intranasal administration of OT (Althaus et al., 2016; Daughters et al., 2015) and behavioural responses are moderated by contextual factors (De Dreu et al., 2010; De Dreu et al., 2012), making it difficult to predict the level of benefit a given individual may derive from IN-OT administration. As recently pointed out, however, intranasal administration studies are often conducted on small- or medium-sized and/or unisex samples. The present study aimed to recruit a large, mixed gender sample to replicate previous findings and investigate possible moderating factors of participants' responsiveness to IN-OT. This information will improve our current ability to identify those mostly likely to benefit (or not to benefit) from possible OT treatment.

It is more typical in OT administration studies not to include a physiological 'manipulation check' (checking that exogenous OT has had an impact on endogenous concentrations) than to include one. Recent research has demonstrated that there are individual differences in response to the same intranasal dosage of OT (Althaus et al., 2016; Daughters et al., 2015; Weisman et al., 2012). Two of these studies, however, used

an all-male sample, and although Weisman et al. (2012) used a mixed-gender sample and a within-subjects design, conclusions drawn from a sample of 10 individuals should be treated with caution. Further research is therefore needed to assess whether individual differences are evident in women, as well as men.

Individual differences in both physiological and behavioural responses to IN- OT exist, but it is unclear to what extent these differences are moderated by biological, as opposed to psychological, factors. For example, it was recently noted that previous OT studies "pointed to powerful gender differences" (Evans, Dal Monte, Noble, & Averbeck, 2014, p. 5) but the origins (and extent) of these differences are still unclear. Gender differences may arise as a result of hormone interactions (McCarthy, McDonald, Brooks, & Goldman, 1996) or as a consequence of psychological variables (Kubzansky, Mendes, Appleton, Block, & Adler, 2012).

OT receptor distribution in the brain is regulated by oestrogen and oestrogen receptors (Bale & Dorsa, 1995; Young, Wang, Donaldson, & Rissman, 1998). Thus receptor distribution, as opposed to gender differences in psychological variables, may contribute to gender differences in the OT literature (Francis, Young, Meaney, & Insel, 2002). Although some researchers acknowledge the possibility of gender differences despite their use of unisex (typically all-male) samples (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008; Feeser et al., 2015), it is more often the case that researchers use a unisex sample without commenting on possible gender differences (Kosfeld et al., 2005; Striepens et al., 2013; Van IJzendoorn et al., 2012). As Evans et al. (2014) point out, studying only one gender when there are reasons to expect gender differences does little to clarify the issue. Thus the current study recruited males and females to investigate whether there are physiological gender differences in response to IN-OT administration.

In addition to gender differences, several studies have investigated the influence of hormone variations over the menstrual cycle in female participants. Salonia et al. (2005) found lower OT concentrations during the luteal phase, compared to both the follicular and ovulatory phases, in naturally cycling women (i.e., those not taking oral contraceptives [n = 20]) but no difference among women taking oral contraceptives (n = 10). Cardoso, Ellenbogen, Serravalle, and Linnen (2013) found that menstrual cycle and oral contraceptives did not modulate the behavioural effects of IN-OT (n = 50) and similar results were reported by Morhenn, Park, Piper, and Zak (2008). If there are variations in endogenous OT concentrations as a result of hormone interactions over the menstrual cycle in 'normally' cycling women, it is unclear whether such variations influence behavioural or indeed peripheral responses to IN-OT.

Hormone interactions may also be determined prenatally. During foetal development the ratio of testosterone to oestrogen exposure has an important impact on subsequent development. Studies have investigated whether digit ratio, a reliable indicator of prenatal sex hormone exposure (Manning, Scutt, Wilson, & Lewis-Jones, 1998), is a moderator of behavioural responses to IN-OT. Kret and De Dreu (2013) found that the effect of IN-OT on selection of team members was moderated by digit ratio. Others have found digit ratio to affect cognitive empathy (Van Honk et al., 2011) and prosocial behaviour during economic games (Buser, 2012), variables that have also been found to be affected by OT administration (Barraza & Zak, 2009; Domes, Heinrichs, Michel, et al., 2007). However, to our knowledge, no study has investigated whether behavioural differences associated with OT and digit ratio are related to the possibly moderating influence of digit ratio on physiological responses to IN-OT.

A final point concerns statistical power in behavioural IN-OT studies. The average power in healthy participant studies is 16%, and while this is broadly comparable to

neuroscience studies, which have an average power of 21%, it is sufficiently underpowered to justify reliability concerns raised recently (Walum et al., 2015). Walum and colleagues also noted the presence of a publication bias towards positive findings, which, in combination with the problem of underpowered studies resulting from small sample sizes, increases the risk of false-positives being reported in the literature.

*The Present Study*

The present study aimed to address the issues noted above. Specifically, we focused on factors that could influence participants' responsiveness to IN-OT, as measured by their salivary OT concentrations. Keeping recent papers (Button et al., 2013; Walum et al., 2015) in mind, a large sample size was recruited in order to have sufficient statistical power. We aimed to replicate the findings reported by Daughters et al. (2015) demonstrating individual differences in response to a standard dosage of IN-OT. Thus we hypothesized that although salivary OT concentrations would be larger in the OT group than in the PL group, there would also be large individual differences in OT concentrations within the OT group. By recruiting both males and females we were able to explore the possibility of physiologically based gender differences, but in view of the conflicting evidence in the literature, no specific hypotheses were made. It was hypothesized that there would be no difference in female participants' responsiveness between the follicular and luteal phase of the menstrual cycle. Finally, we explored the relationship between digit ratio, as a marker of prenatal androgen exposure, and participants' salivary OT concentrations.

## Method

### *Participants and Ethics*

We recruited 216 participants ($M_{age}$ = 21.8, *SD* = 3.42) for the study, which was carried out at the University of Amsterdam. Both male and female students (74% female) signed up for the study, which was described as a study investigating medication and decision-making. The study was approved by the University of Amsterdam's Psychology Ethics Committee (file 2015-WOP-4100), and adhered to the Declaration of Helsinki. All participants completed a medical screening form before they could take part in the study. Exclusion criteria included any serious physical or psychological illness, and whether those who signed up for the study were currently taking prescribed medication. Participants were asked not to consume drugs or alcohol the evening prior to testing, and were also asked not to smoke during the 2 hours preceding the study. No deception was involved and participants were financially compensated for their time.

### *Procedure*

Participants arrived at the laboratory in groups of four and were allowed to interact before being seated in individual cubicles. This interaction helped participants to understand that a subsequent group-based decision task would be carried out with real people. The behavioural data collected during these decision-making tasks are not the focus of this chapter, and will not be discussed here. All participants read an information sheet about the study, signed the medical screening form, and gave written informed consent. Once seated in their individual cubicles, participants read brief instructions on a computer screen before providing a baseline saliva sample. Immediately after this, participants self-administered 24 IU (three puffs of 4IU, per nostril) of either Syntocinon spray (Novartis) or a chemically matched PL spray under the supervision of the experimenter. Sprays were

identical with respect to all preservatives; the only difference was the presence or absence of synthetic OT.

After administration, participants spent 25 minutes completing personality measures before providing a second saliva sample 30 minutes after administering the nasal spray. Participants then completed a decision-making task, and provided a final saliva sample. Before participants were debriefed, a scan was taken of their hands so that, at a later date, the digit ratio (2D:4D) of their right hand could be calculated. Digit length was calculated from the crease closest to the finger to the fingertip using photo- editing software, replicating the methods used by Kret and De Dreu (2013); also see Manning (2002); Manning, Baron Cohen, Wheelwright, and Sanders (2001); Manning et al. (1998). Having a longer ring finger relative to index finger (i.e., a low ratio) indicates high testosterone and low oestrogen exposure during foetal development (Brown, Finn, & Breedlove, 2002; Manning, 2002).

Test sessions were run at four different times of day (early morning, morning, early afternoon, afternoon) meaning that OT concentrations were assessed across the day. Currently many studies attempt to control for diurnal variation in OT by running sessions at the same time of day, despite the absence of evidence for a circadian rhythm (specifically changes in OT during daytime, when the vast majority of studies are run) in OT (Gossen et al., 2012; Kirkpatrick et al., 2014; Weisman, Schneiderman, Zagoory-Sharon, & Feldman, 2013b). Thus the current study also investigated whether OT concentrations varied as a function of time of day, providing evidence for or against diurnal variations in OT, and thereby helping to determine whether these logistical constraints are necessary.

*Oxytocin Sampling and Analysis*

Saliva samples were collected in pre-chilled 50ml tubes that were stored on ice throughout the session. For each sample, participants were asked to produce approximately 4-5ml of passive drool. Samples were frozen as quickly as possible during testing, and were left on ice for no longer than 45 minutes. Samples were frozen at -25˚C (which is suitable for short-term storage: the first saliva samples were stored for 3 months; the final samples were frozen for 2 months). The samples were shipped on dry ice from the University of Amsterdam to the University Hospital Wales, Cardiff, where they were analysed. Transit took 24 hours, and thaw checks revealed that samples had not undergone any freeze-thaw cycles during transit.

In preparation for analysis, the samples were thawed and centrifuged at 4˚C at 1600 x g for 15 minutes; 1ml of supernatant was transferred to a new 5ml tube before being frozen again at -25˚C. To ensure PL and baseline saliva samples would be above the minimum sensitivity of the ELISA kit (15 pg/ml), the samples had to be concentrated. They were therefore lyophilized and stored at -25˚C until analysis (for additional information regarding the validity of lyophlization and storage temperature, see Chapter 2).

Samples were analyzed using a 96-well OT ELISA kit (Enzo Life Sciences, Exeter, UK). This kit has been used repeatedly in the OT literature (Daughters et al., 2015; Van IJzendoorn et al., 2012; Weisman et al., 2012). Lyophilized samples were reconstituted in 250µl of assay buffer, thereby concentrating all samples four-fold. Where possible samples were run in duplicate and were processed in accordance with the manual ("Product Manual: Oxytocin ELISA kit," 2013), with an overnight incubation of 19 hours. Thirteen samples could not be run twice because participants had not provided enough saliva to generate 1ml of supernatant. In order to achieve the same four-fold concentration,

0.5ml of these samples were lyophilized and reconstituted in 125µl of assay buffer and only one reading was obtained. Samples were read at 405nm and concentrations were calculated from the standard curve. Finally, the international correction for OT concentrations, devised by the National Institute for Biological Standards and Control and the World Health Organization was applied (in accordance with the guidelines in the manual).

The ELISA manual ("Product Manual: Oxytocin ELISA kit," 2013) reports that intra-assay and inter-assay coefficients of variability are 12.6 – 13.3% and 11.9 – 20.9%, respectively. The present study obtained intra-assay and inter-assay coefficients of 12% and 18-24%, respectively. To confirm that the process of lyophilization did not interfere with the samples, a serial dilution series was prepared and freeze-dried with the samples. There was a high correlation between the control series and the standards, $r(7) = .99$, $p < .001$.

Several samples were more than three standard deviations above the (condition-relevant) mean. The data were therefore winsorized prior to data analysis. Finally, an 'Area Under the Curve' corrected for baseline value was calculated for each participant (AUCi). The formula used (formula 6 from Pruessner et al. (2003)) enables one to control for individual differences in baseline concentrations. Ultimately each participant had one AUCi value indicating their OT concentrations over the testing session, reflecting (in the case of those in the OT condition) their responsiveness to the OT administration.

*Data Analysis*

A 2 (Condition: OT vs PL; between-subjects) x 3 (Time of measurement: baseline vs 30 min vs 60 min; within-subjects) x 2 (Gender: male vs female; between-subjects) mixed ANOVA was conducted to assess the effects of condition, time of measurement, and

gender on participants salivary OT concentrations, with follow-up *t*-tests/simple effects analysis to investigate any significant interactions. A 2 (Phase: luteal vs follicular; between-subjects) x 2 (Condition: OT vs PL; between-subjects) x 3 (Time of measurement: baseline vs 30 min vs 60 min; within-subjects) mixed ANOVA was conducted to determine whether there were any differences in salivary OT concentrations between menstrual cycle phases; an ANCOVA (Digit Ratio: high vs low; between-subjects) was carried out to assess the effect of digit ratio on OT concentrations, while controlling for condition; and finally an ANCOVA (Time of Day: morning vs afternoon; between-subjects) was carried out to determine whether there was any diurnal variation in OT concentrations, while controlling for condition.

## Results

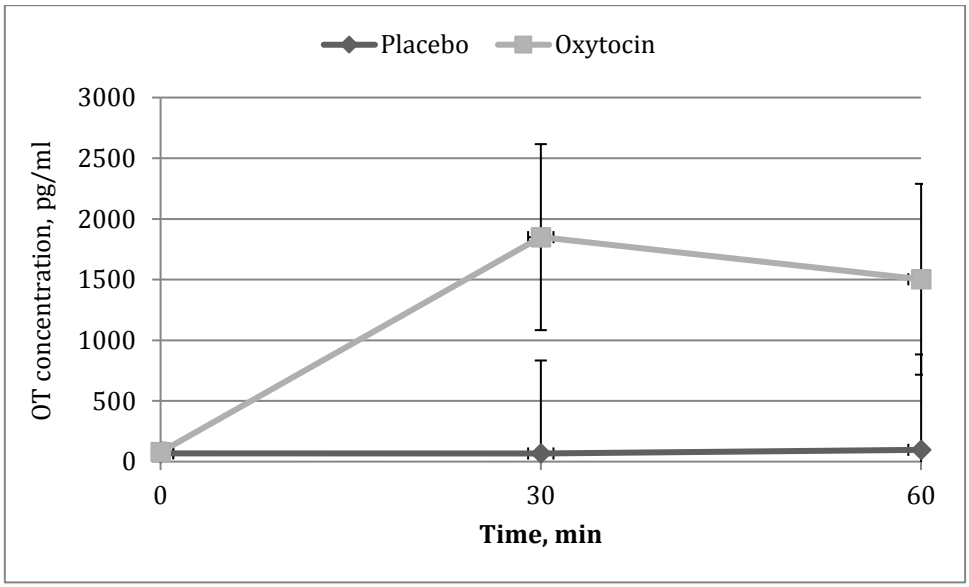
Initial analysis revealed that all samples violated the assumption of normality. A log transformation was carried out, after which the transformed values met the assumption of normality. All statistical analyses reported below were carried out on the transformed data. For ease of interpretation we report untransformed means and standard errors. Where the assumptions of sphericity are not met, Greenhouse-Geisser values are reported.

### *Condition Effects*

There was a significant effect of condition, $F(1, 141) = 334.187$, $p < .001$, $\eta^2_p = .703$, confirming that participants in the OT condition had significantly higher OT concentrations ($M = 458.14$, SE = 1.09) than participants in the PL condition ($M = 53.82$, SE = 1.08). There was also the expected main effect of time of measurement, $F(2, 282) = 240.904$, $p < .001$, $\eta^2_p = .631$. Bonferroni corrected pairwise comparisons showed that

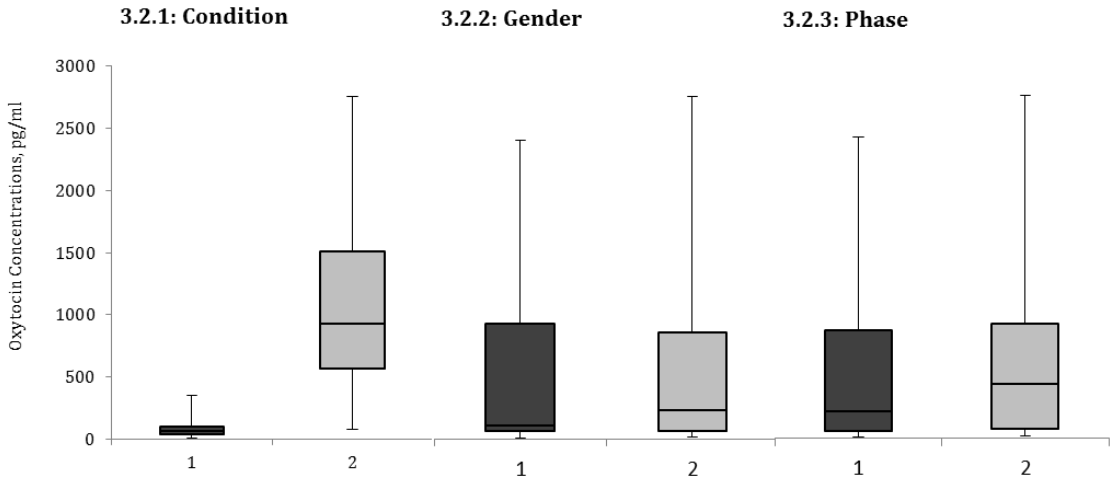
baseline samples were significantly lower ($M$ = 52.36, SE = 1.08) than both the 30-minute ($M$ = 270.40, SE = 1.08) and 60-minute ($M$ = 273.53, SE = 1.08) samples, and that there was no significant difference between the 30 and 60 minute samples. There was a significant interaction between condition and time of measurement, $F(2, 282) = 215.953$, $p < .001$, $\eta^2_p = .605$, and the relevant means are depicted in Figure 3.1. Follow-up paired-sample $t$-tests revealed that there was no significant difference between the baseline and 30 minute sample in the PL condition, $t(84) = .923$, $p = .359$, although there was a significant increase in OT concentration from the 30 to the 60 minute sample, $t(80) = 3.048$, $p = .003$; the difference between baseline and 60 minutes was also significant, $t(83) = 3.512$, $p = .001$). In the OT condition there was a significant increase from baseline to 30 minutes, $t(82) = 32.842$, $p < .001$; a significant decrease from 30 to 60 minutes, $t(71) = 4.217$, $p < .001$; the difference between baseline and 60 minutes was also significant, $t(73) = 23.804$, $p < .001$. Of note, however, is the large range in OT concentrations in the OT condition (see Figure 3.2.1).

*Figure 3.1 - Mean salivary OT concentrations as a function of condition and time (± SD)*



Although there was no main effect of gender, $F(1, 141) = 1.195$, $p = .276$, $\eta^2_p = .008$ (see Figure 7.2.2 for a display of the variance in OT concentrations by gender), there was a significant interaction between time of measurement and gender, $F(1.885, 265.845) = 3.597$, $p = .031$, $\eta^2_p = .025$. Follow-up analysis revealed that this was driven by a significant difference at baseline, $F(1, 141) = 5.578$, $p = .020$, $\eta^2_p = .038$, where males had higher OT concentrations compared to females. There was no difference between males and females at 30 minutes, $F(1, 141) = .383$, $p = .537$, $\eta^2_p = .003$, or 60 minutes, $F(1, 141) = .435$, $p = .511$, $\eta^2_p = .003$. The interactions between gender and condition ($F(1, 141) = .472$, $p = .493$, $\eta^2_p = .003$), and gender, time of measurement, and condition ($F(1.885, 265.845) = 1.069$, $p = .342$, $\eta^2_p = .008$), were not significant.

*Figure 3.2 - Box-and-whisker plot showing salivary OT concentrations as a function of condition, gender, and menstrual cycle phase*




*Menstrual Cycle, Digit Ratio, and Diurnal Effects*

There was no main effect of female participants' menstrual cycle, $F(1, 85) = .002$, $p = .966$, $\eta^2_p = .001$ (see Figure 7.2.3 for a display of the variance in OT concentrations by

phase). There was also no significant interaction between phase and condition, $F(1, 85) = .444$, $p = .507$, $\eta^2_p = .005$; or between phase and time of measurement, $F(1.748,148.542) = 2.249$, $p = .116$, $\eta^2_p = .026$. Finally, the three-way interaction between phase, time of measurement, and condition was also non-significant, $F(1.748, 148.542) = 1.097$, $p = .330$, $\eta^2_p = .013$.

There was no main effect of digit ratio on participants' OT response, $F(1, 114) = 1.529$, $p = .219$, $\eta^2_p = .013$, when controlling for condition. Exploratory correlations also revealed no relationship between participants' digit ratio and their OT concentrations over the testing session (baseline: $r(115) = .15$, $p = .119$; 30 minutes: $r(115) = -.03$, $p > .250$; 60 minutes: $r(115) = -.08$, $p > .250$).

There was no significant main effect of time of day, $F(1, 68) = .004$, $p > .250$, $\eta^2_p = .001$, when controlling for condition. There was also no interaction between time of day and time of measurement, $F(1.763, 119.886) = .435$, $p > .250$, $\eta^2_p = .006$. The main effects of condition and time of measurement and their interaction remained significant in this analysis.

## Discussion

The objective of the present study was to address some of the limitations of IN-OT studies that have been identified in recent publications. Replicating previous findings (Daughters et al., 2015; Van IJzendoorn et al., 2012; Weisman et al., 2012), the results demonstrate that intranasal administration of OT leads to a significant increase in salivary OT concentrations; however, as predicted there were considerable individual differences in response to the same dosage of IN-OT. If there is continued interest in the therapeutic

potential of OT, being able to identify high and low responders (and the factors responsible for these differences) is an important target for future research.

Although there was a small gender difference in baseline OT concentrations, there were no gender differences at later measurement points, in either the PL or the OT condition. There were also no differences in female participants' OT concentrations across their menstrual cycle; and no difference in participants' responsiveness to IN-OT (as measured by AUCi) as a function of digit ratio. Finally, there was no difference in OT concentrations (after controlling for condition) between morning or afternoon sessions. This suggests that there is no diurnal variation in OT concentrations during the day, when the vast majority of IN-OT administration studies are conducted.

These findings run counter to the methodological and logistical assumptions that are often made in planning and conducting OT studies. Despite a small gender difference in concentrations at baseline, males and females had statistically similar OT concentrations in both the OT and the PL condition. Therefore gender differences in OT administration studies must arise from either differences in psychological responses to IN-OT itself (i.e., gender differences in behavioural responses to IN-OT are moderated by psychological, rather physiological, factors), or sex-specific psychological variables (i.e., males and females may report different emotional responses, due perhaps to stereotype conformity, independent of OT administration). In order to investigate the effect of gender further, future studies should avoid recruiting general unisex samples.

Furthermore, there was no difference in OT concentrations between the luteal and follicular phases of the menstrual cycle. Previous research (Salonia et al., 2005) has shown that women taking the contraceptive pill show no difference in plasma OT over the menstrual cycle. Because information regarding female participants' contraceptive use was not available, we were unable to test this hypothesis; it is therefore possible that the

absence of an effect of menstrual phase was due in part to some female participants taking oral contraceptives. Our results nevertheless show that responsiveness to IN-OT does not vary as a function of the menstrual cycle. A replication of this finding would be useful in confirming that in OT administration studies with female participants, controlling for menstrual phase may not be required.

Finally, the present study aimed to address the limitation of small sample sizes, typically using in IN-OT studies, resulting in a lack of statistical power. By conducting one of the largest (n = 216) IN-OT administration studies, and the largest in which hormone analysis (n = 145) was conducted, we achieved a power greater than 0.99 for the main effects of condition, time of measurement and their interaction.

*Conclusions*

In conclusion, the present study investigated the validity of assumptions commonly made in the OT literature, and to evaluate which (if any) factors affected participants' responses to intranasal administration of OT. We found that none of the factors identified as candidate moderators on the basis of previous research had a significant effect on salivary OT concentrations. There was no effect of gender, menstrual cycle, digit ratio or diurnal variation. Instead, the results were consistent with those of our previous study (Daughters et al., 2015) in demonstrating considerable individual differences in response to IN-OT. The factors responsible for these differences are as yet unknown. This represents an important goal for future research if IN-OT is to become an effective and targeted therapeutic tool.

# Chapter 4

## Adults with posterior and anterior hypopituitarism have lower salivary oxytocin concentrations compared to healthy controls


### Abstract

The neuropeptides arginine vasopressin (AVP) and oxytocin (OT) are synthesised and released in the body in the same way. Recent research has demonstrated that several psychopathologies that are characterised by deficits in social behaviour are associated with altered OT systems.

The present study investigated whether a patient group with Cranial Diabetes Insipidus (CDI), who have a known deficiency in AVP, would also demonstrate a deficiency in OT. Fifty-five participants were recruited for the clinical study, including 15 clinical control patients (HP; patients with anterior hypopituitarism) and 20 healthy control (HC) participants who were age- and gender-matched to CDI patients. CDI and HP patients had lower OT concentrations compared to HC participants. Because OT deficits have been associated with increased symptomology in several psychopathologies it is important to establish whether these findings are generalisable, and if they are, to investigate the consequences of this deficiency for patients' social and emotional behaviour.

## Introduction

Over the past decade research has demonstrated the important role that OT plays in social cognition and behaviour. As this line of research has developed, so has the interest in the therapeutic potential of OT, specifically its potential to treat a range of disorders that are characterised by deficits in the social behaviours associated with OT. In recent years researchers have begun to explore this possibility, investigating the impact of OT in autism spectrum disorder (ASD; Husarova et al., (2016)), depression (Jobst et al., 2015), attention deficit hyperactivity disorder (ADHD; Demirci, Ozmen, Kilic, & Oztop (2016)), and schizophrenia (Jobst, Dehning, et al., 2014). It is also important to consider other clinical groups that may also be at risk of an OT deficiency, and to investigate how such a deficiency might affect their social behaviour. The present chapter investigated the possibility of an OT deficiency in a patient group with CDI.

CDI is characterised by a deficiency in AVP, which occurs due to a significant loss (>80%) of function in hypothalamic neurons responsible for AVP synthesis (Ball, 2005). AVP is responsible for maintaining water balance in the body (Ball, 2005; Robertson, 1995); symptoms of AVP deficiency therefore are excessive thirst (polydipsia) and excessive urination (polyuria). More importantly, in the present context, AVP is the sister peptide of OT: They are both highly conserved over evolutionary time; they differ in structure by just two amino acids (Brownstein, 1983); and they are both produced in the paraventricular and supraoptic nuclei of the hypothalamus, where they are relayed to the posterior pituitary gland for release into peripheral circulation (Swaab et al., 1975). Because both peptides are synthesised in the same regions of the brain and released from the same region of the pituitary gland, we hypothesised that a deficiency in AVP, resulting in a diagnosis of CDI, may also be associated with a deficiency in OT.

The aetiology of CDI can be diverse, ranging from genetic heritability to head

trauma, but brain tumours account for approximately 33% of CDI cases (Kovács & Lichardus, 2012). The typical treatment plan for these tumours (often benign non-functioning pituitary adenomas) is transsphenoidal (through the nasal cavity) surgery to remove the tissue. However, CDI can also be acquired through this treatment plan: removing pituitary tumours causes a loss in neuron function and therefore AVP production, resulting in CDI. Because of this risk patients' AVP production is tested after surgery, in symptomatic patients, using the water deprivation test (Makaryus & McFarlane, 2006). When an AVP deficiency is confirmed and a positive diagnosis of CDI is made, patients are then placed on an artificial AVP analogue, desmopressin, which is typically taken for the rest of their lives (Makaryus & McFarlane, 2006).

Patients with CDI are only treated for clinical symptoms associated with an AVP deficiency. Currently there is no protocol for checking OT production in patients with CDI, because a deficiency in OT is not currently known to be associated with adverse symptomatic or clinical sequelae. The only study (to our knowledge) to investigate OT concentrations in medical patients (Daubenbüchel et al., 2016) found that childhood-onset craniopharyngioma patients with lesions to the hypothalamus (the site of AVP and OT synthesis) had significantly lower OT concentrations after fasting compared to healthy controls. However, as previously discussed, psychological research has investigated the association between OT deficiency and behavioural symptoms associated with several psychopathologies.

Husarova et al. (2016) found that children ($M_{age}$ = 4.72 years old) with a diagnosis of ASD had significantly lower levels of OT in their blood (mean OT = 124 pg/ml) than age-matched typically, developing children (mean OT = 268 pg/ml). Moreover participants' OT concentrations were associated with the severity of ASD symptoms relating to reciprocal interactions and social communication, consistent with previous

findings (Green et al., 2001; Modahl et al., 1998). However because the majority of ASD research is carried out on children and young adolescents, to our knowledge no study has yet investigated whether these results are generalisable to adults with ASD.

A recent meta-analysis (LoParo & Waldman, 2015) of genetic studies found several single nucleotide polymorphisms (SNPs) in the OTR gene (and the gene as a whole) to be significantly related to ASD. It is worth noting that two of the 12 studies incorporated in the meta-analysis included data from autistic adults (participants in both studies had a mean age in the early 20s (Chakrabarti et al., 2009; Liu et al., 2010)). Nonetheless, further research is required to investigate whether OT concentrations are also significantly lower in adults with ASD.[1]

Although ASD has received the most attention in relation to the therapeutic potential of OT (Bakermans-Kranenburg & van IJzendoorn, 2013), OT has also been associated with other psychopathologies. Male adolescents with ADHD have also been found to have an OT deficiency, compared to a healthy control group (Demirci et al., 2016). OT concentrations were negatively correlated with a measure of aggression and positively correlated with the number of correct responses during the RMET, a measure of cognitive empathy (see Chapter 8). Similarly, male patients with schizophrenia have also been found to have lower OT concentrations (mean OT = 255.6 pg/ml) compared to healthy individuals (mean OT = 376.0 pg/ml) (Jobst, Dehning, et al., 2014). This deficiency significantly predicted greater negative symptomology, reflecting lower scores on emotional and social withdrawal, consistent with previous findings from a study with a mixed-gender sample (Kéri, Kiss, & Kelemen, 2009). Finally, Goldman, Marlow-O'Connor, Torres, and Carter (2008) found significantly lower OT concentrations in

[1] We also note that a recent study found no difference in OT concentrations between children with ASD and two control samples, although these researchers did find that lower OT concentrations were associated with poorer social cognition (Parker et al., 2014

schizophrenic patients who also had a co-morbid diagnosis of polydipsic hyponatremia (excessive production of AVP). Schizophrenic patients who had no co-morbid diagnosis and those with polydipsia but no hyponatremia (low sodium concentrations) had lower, albeit not significantly lower, OT concentrations compared to the healthy control group.

Given the neuroanatomic similarities between AVP and OT, research has also investigated whether AVP might also play an important role in social behaviour. We note, that although AVP does play a role in certain social behaviours (for a review see Heinrichs and Domes (2008)), studies that have measured or administered both hormones simultaneously more often find significant differences rather than similarities in the social behaviours affected by OT and AVP (Jin et al., 2007; Jobst, Dehning, et al., 2014; Popik & Van Ree, 1991; Rilling et al., 2014).

*The Present Study*

The study was conducted to investigate the role of anatomical factors and individual differences in the OT system. We hypothesised that patients with CDI (and anterior hypopituitarism) would have significantly lower salivary OT concentrations compared to an age- and gender-matched clinical control (with anterior hypopituitarism alone) group and a healthy control group. In this way the study was able to investigate the role of individual differences in the OT system. We also took the opportunity to examine the role of individual differences in empathy, as measured by the IRI (Davis, 1983), attachment, as measured by the ECR-RS (Fraley et al., 2011), and autistic tendencies, as measured by the AQ-S (Kloosterman et al., 2011).

## Method

*Participants and Ethics*

Fifty-five white British adults ($M_{age}$ = 46.54; *SD* = 16.30) took part in the clinical study. Participants were recruited to one of three groups: the CDI group; the clinical control (HP) group; and the healthy control (HC) group. Inclusion criteria for the CDI group were that patients had acquired CDI after transsphenoidal surgery or as a result of a craniopharyngioma. CDI patients were matched by age and gender to HP patients, who had a diagnosis of either full or partial anterior hypopituitarism and were on full hormone replacement medication (see Table 4.2). HC participants were also matched on age and gender to the CDI group. The final number of males and females recruited for each group are reported in Table 4.1; a one-way ANOVA revealed that there was no significant difference in age between the three groups, $F(2, 52) = .983$, $p = .382$, $\eta^2_p = .039$.

The study was approved by the Research and Development Office at Cardiff and Vale University Health Board and by the Cambridge Central Research Ethics Committee. All participants read a detailed information sheet and gave written informed consent at the start of the experiment, and were fully debriefed at the end. Participants were financially compensated £20 for their participation (see Appendix 2 for a flow diagram of the study).

*Table 4.1 - Gender distribution across groups*

| Group | Males | Females |
|---|---|---|
| **CDI** | 8 | 12 |
| **HP** | 6 | 9 |
| **HC** | 7 | 13 |

*Materials*

*Interpersonal Reactivity Index*

The Interpersonal Reactivity Index (IRI; Davis, 1983) is an established questionnaire, with items pertaining to four subscales: empathic concern, fantasy, personal distress and perspective taking. There are 28 items in total, seven for each subscale; nine items are reverse scored. For each item, participants are asked to indicate on a 5-point scale to what extent the statement can be applied to them (1 = "does not describe me very well"; 5 = "describes me very well"). A mean score for each subscale was calculated.

*The Relationship Structure Questionnaire*

The Relationship Structure Questionnaire (ECR-RS; Fraley, Heffeman, Vicary, & Brumbaugh, 2011) is a previously validated, modified version of the Experiences in Close Relationships scale, containing nine of the original 36 items (five of which are reverse scored). The same nine items are asked in relation to various important figures in the participant's life: parents, romantic partner and close friend. For the purposes of this study, the nine items were asked in relation to the participant's mother (or mother- like figure) and father (or father-like figure). Items included statements such as "It helps to turn to this person in times of need" and "I don't feel comfortable opening up to this person." Participants were asked to rate to what extent they agree/disagree with each item (1 = "Strongly Disagree"; 7 = "Strongly Agree"). A mean score was computed for each parent.

*The Autism Quotient (short version)*

The Autism Quotient Short version (AQ-S; Kloosterman, Keefer, Kelley, Summerfeldt, & Parker, 2011) is an adapted 28-item version of the original 50-item Autism Quotient

(Baron-Cohen, Wheelwright, Skinner, et al., 2001). Items relate to five subscales: social skills, mind reading, restricted and repetitive behaviour, imagination and attention to detail. Fourteen items are reverse scored. Items include statements such as "I find social situations easy" and "I find it difficult to work out people's intentions." Participants were asked to rate to what extent they agree/disagree with each item (1 = "Definitely Agree"; 4 = "Definitely Disagree"). A mean for each subscale was calculated.

*Procedure*

Prior to the study participants were instructed to abstain from alcohol for 24 hours, and caffeine for an hour prior to testing. Participants were only allowed to drink water during the study and if any food had been consumed before the start of a session, participants were asked to rinse their mouths thoroughly before any saliva samples were taken. All testing was carried out between 09:00 and 12:00 in order to control for circadian rhythms in other hormones that can be affected in both clinical groups.

On arrival participants' height and weight were measured so that their BMI could be calculated. After a brief period (approximately 10 minutes) of acclimatization to the testing facility participants were asked to provide their first saliva sample. They then completed 30 minutes of testing (including all questionnaires) after which participants provided their second saliva sample, followed by a further 30 minutes of testing before being debriefed. The results relating to the experimental tasks included were discussed in Chapters 6 and 8, and will not be discussed further here.

*Oxytocin Sampling and Analysis*

Participants were asked to produce 2ml of passive drool for each saliva sample. Samples were collected in pre-chilled tubes, stored on ice during the study, and frozen in a -80˚C freezer immediately after the second saliva sample was provided: All samples were frozen

within 60 minutes of collection. Samples were then thawed and centrifuged at 4˚C at 1600 x g for 15 min after which 1ml of supernatant was transferred to a new tube before being frozen again at -80˚C. Once all samples had been collected and centrifuged, they were lyophilized and stored in a -20˚C freezer until analysis.

Samples were analyzed using a 96-well OT ELISA kit (Enzo Life Sciences, Exeter, UK). Importantly, this ELISA kit has a cross-reactivity with AVP of < 0.02% ("Product Manual: Oxytocin ELISA kit," 2013), thereby providing confidence that despite the structural similarity of AVP and OT, the results obtained will not be artificially high in the CDI group as a result of their desmopressin medication. Samples were reconstituted in 250µl of assay buffer, thereby achieving a four-fold concentration in order to ensure samples were above the minimum sensitivity of the kit (15pg/ml). All samples were processed in accordance with the ELISA manual's protocol ("Product Manual: Oxytocin ELISA kit," 2013), with an overnight incubation of 19 hours. Samples were read at 405nm and concentrations were calculated from the standard curve. Finally, the international correction for OT concentrations, devised by the National Institute for Biological Standards and Control and the World Health Organisation was applied. For full details of saliva sampling and analysis, see Chapter 2.

The ELISA manual ("Product Manual: Oxytocin ELISA kit," 2013) reports that intra-assay and inter-assay coefficients of variability are 12.6 – 13.3% and 11.9 – 20.9%, respectively. The present study obtained intra-assay and inter-assay coefficients of <4% and 10.8 – 15.2%, respectively. Accepted values for coefficients of variability are <10% for intra-assay and <15% for inter-assay variability ("Inter- and intra-assay coefficients of variability," 2014).

*Data Analysis*

A 3 (Group: CDI vs HP vs HC; between-subjects) x 2 (Samples: 1 vs 2; within- subjects) mixed ANOVA was conducted to assess the effect of group on participants' OT concentrations. This was repeated with several potential covariates, including, age, medication and BMI. Age was included as a covariate to control for any potential differences in OT production across the lifespan. A 3 (Group: CDI vs HP vs HC; between-subjects) x 2 (Gender: male vs female; between-subjects) x 2 (Samples: 1 vs 2; within-subjects) ANOVA was carried out to assess the effect of group and gender on participants' OT concentrations. Finally, exploratory correlations were carried out to investigate any relationships between OT concentrations and personality measures.

## Results

*Descriptives*

Table 4.2 summarizes the medical characteristics of the CDI and HP clinical groups. By design the majority (75%) of CDI patients had co-morbid diagnoses indicative of reduced hormone production in the anterior pituitary gland. All HP patients had anterior hypopituitarism to some degree, but, the majority (80%) were diagnosed with partial anterior hypopituitarism. The range of tumours removed during surgery is broadly reflective of the diverse aetiology of both CDI and hypopituitarism: 45% of CDI patients had surgery for craniopharyngioma (a common cause of CDI), compared to just one HP patient; 25% of CDI patients had tumours producing either growth hormone, prolactin, or both, compared to 40% of HP patients; and 10% of CDI patients had non-functioning pituitary adenomas, compared to 33% of HP patients. This diverse aetiology was also reflected in the range of medications clinical patients were prescribed: 20% of CDI patients had a full complement of hormone replacement therapy, while the remaining 80%

had a combination of some of these medications. Only two HP patients had a full complement of (relevant) hormone replacement therapy, with the remaining 86% of HP patients having a combination of hormone replacement.

Where possible, a note was also made when patients had been prescribed oestrogen or testosterone replacement: Five women were reported to be taking a contraceptive pill; seven men were reported to be taking a testosterone supplement.

The age at which patients underwent surgery did not differ significantly between clinical groups, $t(31) = -1.65$, $p = .109$, and ranged from 2 to 72 years of age; the mean age at which patients underwent surgery was 36 years of age.

Finally, a two-way ANOVA revealed a significant difference in BMI between groups, $F(2, 48) = 3.729$, $p = .031$, $\eta_p^2 = .134$.

*Oxytocin Analysis*

There was a trend towards a significant main effect of group on oxytocin concentrations, $F(2, 52) = 2.567$, $p = .086$, $\eta_p^2 = .090$ (CDI – 86.11 pg/ml; HP – 86.59 pg/ml; HC – 131.47 pg/ml), but no main effect of sample, $F(1, 52) = .118$, $p = .733$, $\eta_p^2 = .002$, and no significant interaction, $F(2, 52) = .595$, $p = .555$, $\eta_p^2 = .022$. The analysis was also repeated with age, BMI, medication, tumour type and age of surgery as covariates, none of which were significant.

A follow-up ANCOVA was carried out in which the CDI and HP groups were combined into one hypopituitarism group. This analysis was deemed appropriate because i) there was a similarity between CDI and HP patients in oxytocin concentrations; ii) there was also a similarity between the CDI and HP patients in empathy performance, as reported below; iii) the original analysis only achieved 49% power.

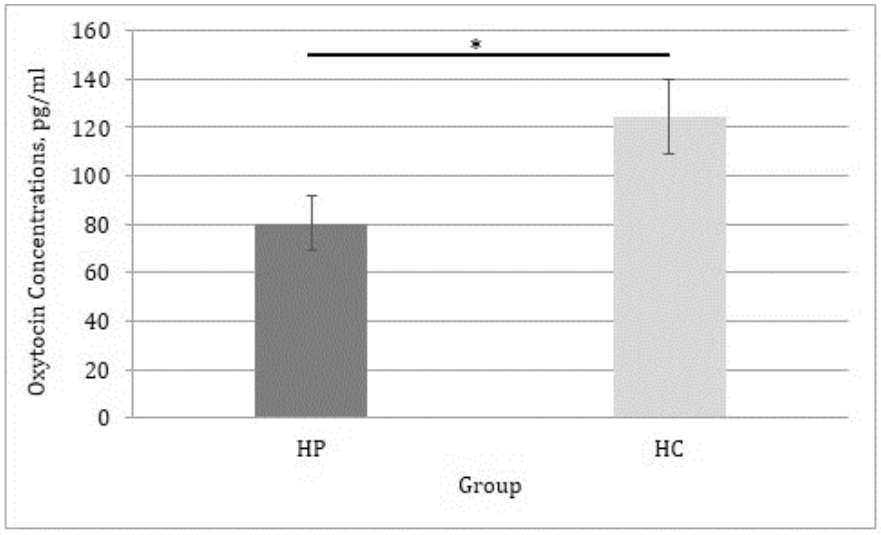
*Table 4.2 – Summary of medical information for CDI and HP patients*

| Medical Information | | CDI Group | HP Group |
|---|---|---|---|
| **Diagnosis** | Panhypopituitarism + CDI | 7 | |
| | Partial hypopituitarism + CDI | 8 | |
| | CDI | 2 | |
| | Partial CDI | 3 | |
| | Panhypopituitarism | | 3 |
| | Partial Panhypopituitarism | | 12 |
| **Tumour** | Non-functioning Pituitary Adenoma | 2 | 5 |
| | Craniopharyngioma | 9 | 1 |
| | Prolactinoma | 2 | 2 |
| | Growth Hormone Adenoma | 2 | 3 |
| | Growth Hormone + Prolactinoma | 1 | 1 |
| | ACTH Adenoma | | 2 |
| | Other | 4 | 1 |
| **Hormone replacement** | Desmopressin, Hydrocortisone, Thyroxine, Growth Hormone | 4 | |
| | Desmopressin, Hydrocortisone, Thyroxine, | 6 | |
| | Desmopressin, Hydrocortisone | 1 | |
| | Desmopressin, Thyroxine | 2 | |
| | ıopressin, Thyroxine, Growth Hormone | 2 | |
| | Desmopressin | 5 | |
| | Hydrocortisone | | 5 |
| | Thyroxine | | 3 |
| | Hydrocortisone, Thyroxine | | 3 |
| | cortisone, Thyroxine, Growth Hormone | | 2 |

Normality analysis revealed an outlier in the hypopituitarism group which was removed, and also that the data did not meet the assumption of normality. A log transformation was carried out, after which the transformed values met the assumption of normality. All statistical analyses reported below were carried out on the transformed data. For ease of interpretation untransformed means and standard errors are reported.

There was a significant main effect of group, $F(1, 46) = 4.922$, $p = .031$, $\eta_p^2 = .097$, hypopituitary patients having significantly lower oxytocin concentrations compared to HC participants (Figure 4.1). Replicating the findings of the previous analysis, there was no main effect of sample, $F(1, 46) = .193$, $p = .662$, $\eta_p^2 = .004$, no interaction, $F(1, 46) = .082$, $p = .776$, $\eta_p^2 = .002$, and age and gender were not significant covariates.

*Figure 4.1 - Average OT concentrations as a function of clinical group (± SE)*




Finally, exploratory correlation analyses did not reveal any consistent correlations between participants' OT concentrations and personality measures.

## Discussion

The present study investigated whether patients with acquired CDI, characterised by a deficiency in AVP, would also demonstrate a deficit in AVP's sister peptide OT. We tentatively conclude that the data presented are consistent with this hypothesis, and that they also indicate that patients with disruptions to anterior pituitary hormone production (as a result of transsphenoidal surgery) also presented with low OT concentrations, compared to an age- and gender-matched HC group.

Despite both clinical groups presenting with lower OT concentrations, compared to HC participants, it is important to note that their concentrations were not abnormally low. Average CDI (86.1 pg/ml) and HP (86.5 pg/ml) concentrations of OT were higher than baseline values reported in Chapters 2 and 3 (approximately 40 pg/ml). Although we had no predictions concerning the extent to which CDI patients would demonstrate an OT deficiency, it is quite surprising that their concentrations were relatively high, given the neuroanatomical similarities between AVP and OT synthesis on which the hypothesis was based. Because all patients included in the study had acquired CDI after surgery, and therefore presumably lost or damaged enough pituitary tissue to cause significant disruption to AVP release, it could reasonably be anticipated that they would exhibit an equivalent and significant decrease in OT release.

However, these initially surprising results may also be explained as a result of the similarity between AVP and OT production and release. Animal model research (Bernal, Mahía, & Puerto, 2016) has found that OT is able to bind to a subset of AVP receptors, demonstrating that OT and AVP systems are able to interact. It may therefore be the case that the desmopressin taken by CDI patients, in order to replace natural AVP production may be interacting with OT production. Indeed one study (Weisman, Schneiderman,

Zagoory-Sharon, & Feldman, 2013a) found that intranasal administration of OT led to a brief increase in salivary AVP, thereby demonstrating that these systems interact albeit demonstrating that OT impacts AVP, as opposed to AVP impacting OT as hypothesised here.

Although the main effect of group was only statistically significant when combining both clinical groups, and we are therefore keen not to overstate the implications of the results, there are several reasons why we believe it is justifiable to regard the results as generalisable. First, the study was intended as a pilot to investigate the validity of the hypothesis presented, and because we were investigating a novel research question our *a priori* power calculations were, by necessity, based on estimates of the anticipated effect size. SPSS power estimates suggest that the original 3 group analysis achieved only 49% power (for the main effect of group); the current study is therefore underpowered for this particular analysis but does suggest that the near-significant trend towards a main effect of group reflects what would be a significant effect in an appropriately powered study (which would be a logical next step to take). Second, the data are consistent with a key finding from earlier chapters of this thesis, namely that there are considerable individual differences in the OT system, and that these are reflected in both baseline concentrations and responses to intranasal OT. These individual differences may, to some extent, mask group differences, by adding to the variance within each group.

The prediction that CDI patients would have significantly lower OT concentrations than HP patients was not supported. Thus even if desmopressin accounts for higher than anticipated OT concentrations observed in CDI patients, it would still not account for the comparable OT concentrations in HP patients, none of whom were taking desmopressin. This result lends itself more readily to the theoretical explanation that pituitary hormones

as a group influence each other. Comparable interactions occur in other hormone systems, e.g., excess testosterone in the body is converted to oestrogen (Naftolin, Ryan, & Petro, 1971). Although it may not be the case that AVP and OT can be converted directly from one to another, evidence (Naftolin et al., 1971) does suggest that some endocrine systems interact. Further research is required to determine the possible influence of anterior pituitary hormones on posterior pituitary function. Finally, it should not be ruled out that the present study observed similar OT concentrations in CDI and HP groups whose conditions had been treated by surgery. It is possible that HP patients, for whom it was the intention to remove anterior pituitary tissue only, may also have had some posterior pituitary tissue removed, and/or tissue that disrupted the neurones relaying AVP and OT from hypothalamic nuclei to the posterior pituitary, thereby explaining similar levels of OT to the CDI patients. This hypothesis is supported by findings from Daubenbüchel et al. (2016) who concluded that hypothalamic lesions after surgery resulted in lower OT concentrations after fasting in paitents with childhood-onset craniopharyngiomas.

Although a precise understanding of all the hormonal consequences of CDI is a key research question with regard to medical treatment, we suggest that arriving at an understanding of which hormones are influenced in specific medical conditions is also essential in order to identify new clinical groups that may be at risk of psychological effects. Recent research has begun to link psychopathologies that are characterised by social and emotional behavioural deficits to altered OT responses (Jobst et al., 2015), OT genetics (LoParo & Waldman, 2015), or low OT concentrations (Demirci et al., 2016). In Chapters 6 and 8 of this thesis we demonstrated that CDI patients (and HP patients) performed significantly worse on empathy-related tasks, compared to HC participants.

Anecdotally, in the course of conducting the present study, behavioural differences between clinical and healthy participants, and also between CDI and HP patients, were

evident. A higher proportion of CDI patients were accompanied to the study by a carer, were off work, and several CDI patients (and their carers) reported that they had noticed a difference in their behaviour pre- and post-surgery. Specifically, patients remarked that they had noticed differences in their emotional behaviour, describing themselves as 'more emotional' and sensitive, with several patients commenting that they felt like 'emotional time bombs'. Such observations are anecdotal reports made by patients in the context of a study designed to investigate emotional behaviour. Whether such reflections would have been made if the study had been investigating different behaviours is unknown. Nonetheless these observations are worthy of careful consideration, especially because they are supported by empirical evidence of differences in empathic ability, which is known to have a significant impact on social relationships (Fischer & Manstead, 2008; Stephan & Finlay, 1999) and mood (Van Kleef, 2009; Van Kleef et al., 2010). Consistent with this reasoning, a previous study (Kao, Stargatt, & Zacharin, 2015) demonstrated that adults who had childhood onset multiple pituitary hormone deficiencies have significantly lower quality of life, compared to their physically healthy peers.

*Conclusions*

In conclusion, the present study found that CDI and HP patients had significantly lower OT concentrations compared to HC participants. An obvious next step would be to conduct a larger replication study to establish whether the difference is also observed in larger groups of hypopituitary patients.

# Chapter 5

## Salivary oxytocin predicts increased prosocial behaviour towards an excluded outgroup member: A Cyberball study


### Abstract

It is known that the neuropeptide oxytocin (OT) can increase an individual's awareness of social cues, and that the social effects of OT depend upon the social context. In a double-blind, randomized, placebo controlled, between-subjects trial, 40 male participants played the virtual ball-tossing game Cyberball. Here they witnessed either an unknown ingroup or outgroup member being excluded by other players (under placebo [PL] or OT conditions). After the game, participants were asked to report how both they and the excluded player felt. We hypothesized that participants in the OT condition would display more prosocial behaviour towards the excluded individual, compared to the PL condition, and that this effect would be moderated by the group membership of the excluded individual. We further predicted that this would be related to OT concentrations. Participants in the OT condition (but not in the PL condition) were more prosocial towards the excluded outgroup member. Interestingly, participants' OT concentrations also positively correlated with their own negative affect when witnessing an ingroup member (but not an outgroup member) being excluded, thereby showing a dissociation between participants' behaviour during the game and their reported affect. These results add to current knowledge by showing that the social effects of OT (a) extend to third-party interactions, (b) affect both behavioural and emotional outcomes, and (c) are moderated by contextual factors.

## Introduction

It is well established that experiencing ostracism elicits strong negative feelings and changes in behaviour (Hartgerink, van Beest, Wicherts, & Williams, 2015; Williams, 2007). It has also been found that the neuropeptide OT plays a significant role in various aspects of social and emotional behaviour (Bartz, Zaki, Bolger, et al., 2010; Domes, Heinrichs, Michel, et al., 2007; Ferguson, Young, & Insel, 2002; Insel & Young, 2001). The broad range of OT effects on social behaviour has led to the hypothesis that OT increases awareness of social cues (Bartz et al., 2011), which in turn suggests that the effects of OT are dependent upon both individual differences and contextual factors. Several studies have investigated how OT affects individuals' responses to directly experiencing ostracism. The present seeks to add to our knowledge by focusing on the effect of OT on emotional and behavioural responses to witnessing someone else being excluded.

After directly experiencing ostracism, individuals report strong negative feelings and violations of basic psychological needs (Eisenberger, Lieberman, & Williams, 2003; Gonsalkorale & Williams, 2007; Zadro, Williams, & Richardson, 2004). Alvares, Hickie, and Guastella (2010) investigated whether OT buffers these negative consequences of exclusion, using the simulated online ball-throwing game, Cyberball. Contrary to their predictions, OT did not reduce the immediate negative affect associated with ostracism. Participants who were ostracized still reported significantly higher levels of negative affect, compared to those who were included in the game. However, OT did increase motivation in participants who were socially included: OT increased their desire to play, which is consistent with the hypothesis that OT facilitates social approach (Domes, Heinrichs, Gläscher, et al., 2007; Guastella, Mitchell, & Dadds, 2008).

Given the influence of OT on social cognition, there has been increasing interest in the therapeutic potential of OT in Autism Spectrum Disorder (ASD), a neurodevelopmental disorder. Individuals with high functioning ASD typically display impairments in social processing and interaction (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). One study investigated whether OT would increase social interaction in 13 high-functioning ASD patients during Cyberball (Andari et al., 2010). Patients and healthy control participants played a 4-player version of Cyberball in which the probability of confederates throwing the ball to participants was manipulated to represent three distinct player profiles. The 'good player' threw the ball 70% of the time to the participant; the 'neutral player' did this 30% of the time; and the 'bad player' did this 10% of the time.

In PL conditions, ASD participants' behaviour showed that they did not react to the different player profiles, in that there was no difference in the number of times ASD participants threw the ball to each player. However, under OT conditions, ASD participants did discriminate between the player profiles, just as control participants did under PL conditions. In the OT condition, both control and ASD participants threw the ball significantly more to the good player, compared to the neutral or bad players. These results suggest that OT increased ASD participants' awareness of the social cues present during the game, consistent with the theory proposed by Bartz et al. (2011), resulting in increased prosocial behaviour towards a friendly player.

To investigate whether OT influenced prosocial behaviour when witnessing others being ostracized, Riem et al. (2013) asked female participants to play Cyberball with two unknown confederates and one known player (a confederate with whom participants had previously interacted). Participants played three rounds of Cyberball. In the first round all three confederates were instructed to behave fairly towards all players; in the second and

third rounds, the two unknown confederates were instructed to exclude the known confederate. In addition, the researchers were interested in whether maternal parenting style would affect social behaviour, and whether such differences would moderate the potential effects of OT.

OT did give rise to more prosocial behaviour; participants compensated for the exclusion by increasing the ratio of throws to the excluded individual, compared to the fair round. This effect was moderated by maternal parenting style. Only participants who reported low levels of 'maternal love withdrawal' demonstrated more prosociality in the OT condition than in the PL condition. This is consistent with research demonstrating that the social effects of OT are often moderated by other factors.

De Dreu et al. (2010) also argue that the effects of OT on social behaviour are moderated. Specifically they predict that these effects are moderated by whether the behaviour in question is directed at ingroup or outgroup members. They propose that OT increases 'ingroup love,' but does not increase 'outgroup hate;' this has been termed the "tend-and-defend" hypothesis, and relates closely to the concept of parochial altruism (the idea that individuals only exhibit altruistic acts towards members of their ingroup). Support for this hypothesis was found in two studies (De Dreu, Greer, Van Kleef, et al., 2011) in which participants were presented with moral dilemmas, in which there was an opportunity to save a group of individuals by sacrificing a lone individual. The lone individual was either an ingroup (Dutch) or outgroup member. In one study the outgroup member was an Arab, in the other he was a German. In both studies, participants in the OT condition were less likely to sacrifice the ingroup member, compared to those in the PL condition. Thus in these studies OT increased parochially prosocial behaviour, and thereby tended to defend their ingroup.

*The Present Study*

In the present study we aimed to investigate the effect of OT on both social and emotional behaviours when witnessing an unknown individual being ostracised during Cyberball. We predicted that OT would increase the ratio of throws to the excluded player, thereby indicating increased prosocial behaviour. We also predicted that this effect would be moderated by whether the excluded individual was an ingroup or outgroup member, in keeping with the tend-and-defend hypothesis. We further predicted that participants' OT concentrations, following IN-OT administration, would predict their prosocial behaviour during the game. Moreover, we were interested to see whether the anticipated prosocial effect of OT would be related to measures of empathy, as reflected in ratings of Player 4's negative affect following exclusion. Finally, we investigated whether participants' emotional responses were moderated by group membership. In this way the study was able to assess the effect of OT on indirect social behaviour, the influence of social context, and individual differences.

## Method

*Participants and Ethics*

Forty male students ($M_{age}$ = 20.98; *SD* = 4.55) at Cardiff University took part in the double-blind, PL controlled, randomised, between-subjects study. Due to a technical issue (in which a software malfunction within Cyberball meant the program could not be launched), some participants did not complete one or other of the Cyberball conditions (inclusion vs exclusion) during one of the two drug conditions (OT vs PL). Participants with incomplete data sets were dropped from the appropriate analyses: there were 30 and 32 participants in the PL and OT game ANOVAs; a minimum of 31 participants included

in the multiple regressions; 37 and 36 participants in the PL and OT affect ANOVAs, respectively; and a minimum of 15 participants in the correlational analyses (see D*ata Analysis* for further details).

The majority of participants were Psychology students; other participants included students of Chemistry, Engineering and Journalism. Psychology students were awarded course credits; non-Psychology students received financial compensation. All participants were British, with typically sounding White-British names. One participant had the same name as a confederate, so for this participant the ingroup name was changed to another typical White-British name. Participants were told that the aim of the study was to assess the effect of OT on emotion processing.

The study was approved by the School of Psychology Ethics Committee at Cardiff University, and the Research and Development Office at Cardiff and Vale University Health Board. All participants completed medical pre-screening forms and signed statements of health before leaving both testing sessions, and were cleared to participate in the study by a medical professional. Participants gave written informed consent at both testing sessions, and were fully debriefed after their second session (see Appendix 1 for a flow diagram of the study).

*Materials*

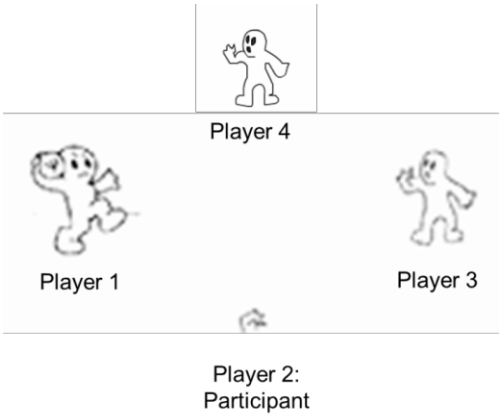
*Cyberball*

Cyberball (Williams, Cheung, & Choi, 2000) is a simulated ball-throwing game, in which players throw a virtual ball to each other. It was designed to research the effects of ostracism, although the flexibility of the software enables researchers to tailor the game to address specific research questions. In the present study, the software was coded for a 4-player version: the participant, and three 'others' who were simulated via the program (see

Figure 5.1 for a schematic of Cyberball).

Each participant was instructed to play two rounds of the game during a test session. Each round consisted of 30 throws. In the first round, the game was programmed such that all players received the ball equally often. Each of the other three players (Players 1, 3 and 4) threw the ball an equal number of times to the other players, while the participant (Player 2) could behave freely. This was the *inclusion* round, and served as a control. In the second round, Players 1 and 3 ostracized Player 4 by not throwing the ball to him; they threw the ball equally often to each other and to the participant. Again, the participant (Player 2) could behave freely, and Player 4 could throw the ball to anyone. This *exclusion* round provides the basis for assessing the effect of witnessing ostracism.

*Figure 5.1 - Schematic representation of the Cyberball paradigm*




To compare the behaviour of the participant towards Player 4 in the inclusion and exclusion rounds, a ratio was calculated that reflected the number of throws from the participant to Player 4 divided by the total number of throws made by the participant. To investigate the effect of whether Player 4 was a fellow ingroup or outgroup member the name of the Player 4 was varied. In the ingroup condition, participants witnessed 'Ben' (a

typical White-British name) being excluded. In the outgroup condition, participants witnessed 'Ahmed' (a typical Middle-Eastern name) being excluded. This group manipulation was chosen i) to replicate the race-based group manipulation used in previous research (De Dreu, Greer, Van Kleef, et al., 2011), and ii) deemed appropriate for replication in Cardiff (as opposed to Amsterdam where the original research was conducted) because of the ethnic diversity of both the city and university populations. This group membership manipulation was a within-subjects factor, and was counterbalanced for order. Because the order in which participants were exposed to this variation in group membership of the excluded player was confounded with the order of drug administration, for certain analyses this resulted in smaller group sizes. However, these are comparable to those in the study reported by Andari et al. (2010).

*Post-Cyberball questionnaires*

After the Cyberball game, participants completed two questionnaires (see Appendix 3). The first of these recorded self-reported affect and contained two sections. Participants first rated the extent to which they thought Player 4 felt several emotions; they then rated how they themselves felt. In each case participants were asked to rate 10 emotions on a 5-point scale (1 = not at all; 5 = very much). The emotions included seven negative (*anger, sad, pain, upset, fearful, scared,* and *hurt*), two positive (*happy, cheerful*), and one neutral (*surprised*). Reliability analysis demonstrated good internal consistency for each subscale (Self-Negative, $\alpha = .85$; Self-Positive, $\alpha = .91$; Player 4- Negative, $\alpha = .79$; Player 4-Positive, $\alpha = .84$).

The second questionnaire was intended to check whether participants had noticed that Player 4 had been ostracized. Participants were asked to circle one of three response options to describe how each player behaved during the ostracism round. The three

response options were: 'involved everyone equally,' 'excluded certain players,' and 'was excluded by others.' Data revealed that 51% of participants reported that they noticed Player 4 being excluded in both rounds (ingroup and outgroup), and a further 34% of participants reported that they noticed Player 4 being excluded in at least one of the two rounds.

*Saliva Samples*

Participants produced six saliva samples during each session: at baseline, and 30, 60, 90, 105, 108 minutes after OT/PL administration. Samples were collected in pre-chilled tubes, stored on ice throughout the study and frozen at -80°C as soon as possible. Samples were subsequently centrifuged, lyophilized, and analysed using the ELISA method, providing a measure of salivary OT at each time point. Full details of sampling and analysis procedures are described in Chapter 2. Participants completed Cyberball immediately after the 90 minute sample. An 'Area Under the Curve' corrected for baseline value up to 90 minutes was calculated for each participant (AUCi90). The formula used [formula 6 from Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003)] controls for individual differences in baseline concentrations. Therefore each participant had one AUCi90 value indicating their OT concentrations over the testing session.

*Procedure*

Participants completed two testing sessions, each lasting approximately 3 hours. The sessions were scheduled to be 2 weeks apart (seven participants had to be tested at later dates; the longest interval was 35 days). Participants completed a questionnaire booklet (including the Interpersonal Reactivity Index (Davis, 1983), Parental Bonding Inventory (Parker, Tupling, & Brown, 1979), State-Trait Anxiety Inventory (Speilberger, 1983), and

the Youth Psychopathic Trait Inventory (Andershed, Ker, Stattin, & Levander, 2002)) before producing the baseline saliva sample. Participants then self-administered a PL (which was matched for all compounds were identical, except for OT) or 24 IU (three puffs per nostril) of synthetic OT both of which were manufactured by St Mary's Pharmaceutical Unit, Cardiff (http://www.wales.nhs.uk/sites3/home.cfm?orgid=828). A doctor was present during administration, and for the next 15 minutes. After a 30-minute wait period to allow the drug to take effect, participants produced a second saliva sample and completed 60 minutes of tasks that are reported elsewhere (Hubble, 2015; Hubble et al., 2016), these included watching short video clips to assess empathic responses and completing the Facial Expression Recognition task (this task is described in Chapter 8) to assess emotion recognition skills. Ninety minutes after drug administration participants produced another saliva sample, and then completed two rounds of the Cyberball game (one inclusion round and one exclusion round). In order to provide the illusion that participants were playing against real people, a cover story stated that three undergraduate research associates had been recruited to help with the study and that they were logged on remotely. The game was described as a mental visualisation task, and participants were instructed to think about their fellow players during the game.

After Cyberball participants were asked to complete the two previously described questionnaires; at the end of the second testing session, participants also completed a further question asking whether or not they believed they had been playing against real people on a 5-point scale (1 = not at all, 5 = very). Sixty percent of participants (with scores ranging from 3 to 5) reported that they believed they were playing against real people. Because there was no effect of 'believability' on participants' behaviour it will not be discussed further.

*Data Analysis*

Two mixed ANOVAs (one for each drug condition) were carried out to assess the effect of Player 4's group membership and round type on participants' behaviour during Cyberball. Session was also included in the analysis to investigate whether participants having the PL spray in the first or second testing session had a significant effect on behaviour. Multiple regression analyses were then carried out to assess whether participants' OT concentrations predicted their prosocial behaviour during Cyberball. Two ANOVAs were carried out (one for each drug condition) to explore whether ratings of Player 4's negative affect depended on the group membership of Player 4. These exploratory analyses were followed up by correlations in order to investigate any further relationships between game behaviour, drug condition and empathy.

## Results

*Game Behaviour - Placebo Condition*

A 2 (Group membership: ingroup vs outgroup; between-subjects) x 2 (Round: inclusion vs exclusion; within-subjects) x 2 (Session: first vs second; between-subjects) ANOVA revealed a significant main effect of round on participants' behaviour, $F(1, 26) = 8.815$, $p = .006$, $\eta^2_p = .25$. Participants threw the ball more to Player 4 when he was excluded ($M = .42$, $SE = .03$), than when he was included ($M = .34$, $SE = .02$). There was no main effect of group membership, $F(1, 26) = 2.178$, $p = .152$, $\eta^2_p = .08$.

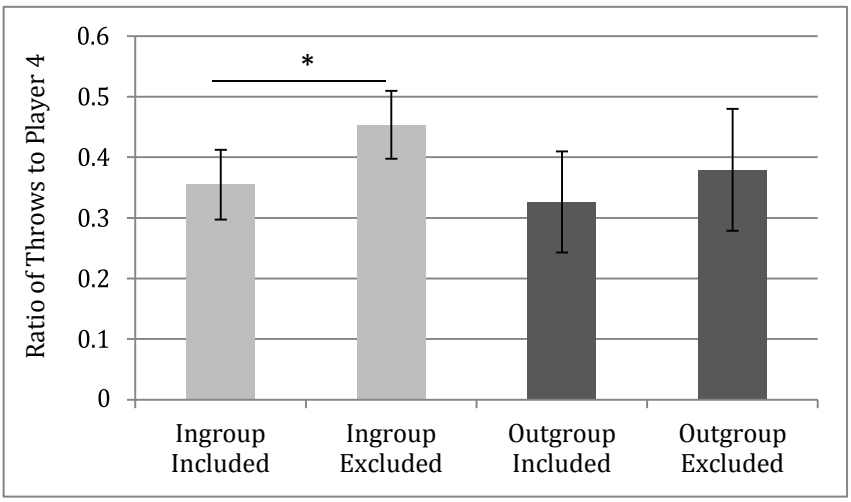
Although the interaction between round type and group membership was not significant ($F(1, 26) = .548$, $p = .466$, $\eta^2_p = .02$), planned *t*-tests revealed that participants were more prosocial towards an ingroup Player 4 when he was excluded ($M = .46$, $SE = .04$), than when he was included ($M = .36$, $SE = .03$), $t(16) = -2.90$, $p = .01$, 95% CI [-.16, -

.04]. When Player 4 was an outgroup member, there was no significant difference in participants' behaviour between inclusion and exclusion rounds, $t(12) = -1.78$, $p = .101$, 95% CI [-.11, .00] (see Figure 5.2).

*Game Behaviour - Oxytocin Condition*

A similar 2 x 2 x 2 ANOVA also revealed a significant effect of round on participants' behaviour, $F(1, 28) = 4.641$, $p = .040$, $\eta^2_p = .15$. Participants threw the ball more to Player 4 when he was excluded ($M = .44$, $SE = .03$), than when he was included ($M = .37$, $SE = .02$). There was no main effect of group membership, $F(1, 28) = .760$, $p = .391$, $\eta^2_p = .03$, and no interaction, $F(1, 28) = 1.886$, $p = .181$, $\eta^2_p = .06$.

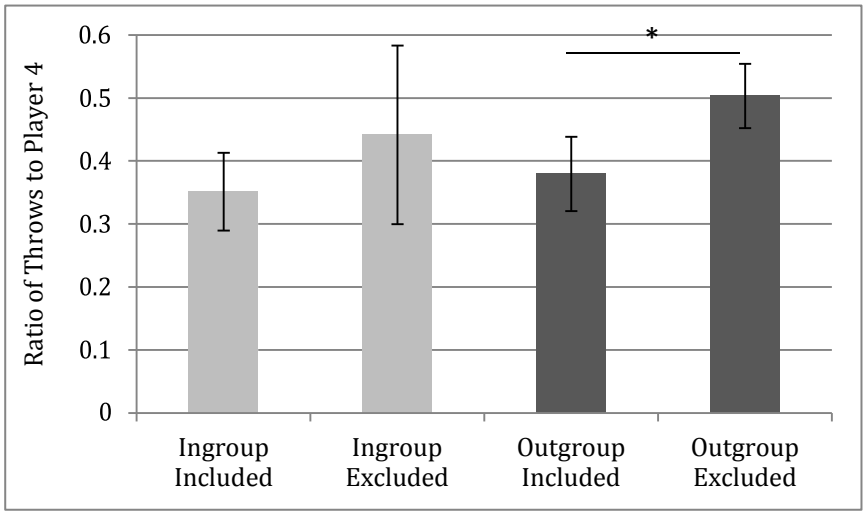
*Figure 5.2 - Ratio of throws to Player 4 as a function of group membership and round (PL condition; 95% CI)*




Planned *t*-tests revealed that participants were more prosocial towards an outgroup Player 4 when he was excluded ($M = .48$, $SE = .04$), than when he was included ($M = .36$, $SE = .03$), $t(15) = -3.95$, $p = .001$, 95% CI [-.19, -.07]. When Player 4 was an ingroup member, there was no significant difference in participants' behaviour between inclusion

and exclusion rounds, $t(15) = -.51$, $p = .616$, 95% CI [-.14, .10] (see Figure 5.3).

*Figure 5.3 - Ratio of throws to Player 4 as a function of group membership and round (OT condition; 95% CI)*




*Oxytocin Concentrations and Prosocial Behaviour*

We analysed the relation between OT concentrations[2] and prosocial behaviour by regressing the ratio of throws made to Player 4 on salivary OT concentrations as measured by AUCi90.[3] The results of these analyses, which are summarized in Table 5 .1, showed that in the OT condition salivary OT was a significant predictor of the ratio of throws made to Player 4 only when Player 4 was an excluded outgroup member. The higher a participant's salivary OT was, the more prosocial he was towards the excluded outgroup member. Salivary OT concentrations did not predict participants' behaviour during the game in the placebo condition.

[2] There was a significant effect of intranasal OT administration on salivary OT concentrations (see Daughters et al. [2015] for details)

[3] Participants' OT concentrations at 90 minutes, as opposed to total OT concentrations up to 90 minutes, was also entered as a predictor which produced an identical pattern of results (see Supplementary Information 2, p. 88).

*Table 5.1 - Summary of regression analyses in which proportion of throws to Player 4 was regressed onto OT concentrations in the OT and PL condition when Player 4 was an ingroup or outgroup member, included or excluded during the game*

| Group | Game (Model) | $R^2$ | *F* | df | *p* | Drug Condition | β | *p* |
|---|---|---|---|---|---|---|---|---|
| Ingroup | Inclusion (1) | .02 | .36 | (2,32) | .699 | PL | .14 | .433 |
| | | | | | | OT | .04 | .831 |
| | Exclusion (2) | .01 | .14 | (2,31) | .868 | PL | -.02 | .936 |
| | | | | | | OT | .10 | .597 |
| Outgroup | Inclusion (3) | .07 | .95 | (2,28) | .401 | PL | -.26 | .184 |
| | | | | | | OT | .05 | .780 |
| | Exclusion (4) | .17 | 2.87 | (2,31) | .073 | PL | -.14 | .419 |
| | | | | | | OT | .40 | .026 |

*Affect*

Two 2 (Scale: positive vs negative; within-subjects) x 2 (Group membership: ingroup vs outgroup; between-subjects) mixed ANOVAs, one for each drug condition, were carried out on ratings of Player 4's perceived affect during the exclusion round.

In the PL condition, there was a main effect of scale type, $F(1, 35) = 20.281$, $p < .001$, $\eta^2_p = .367$, such that ratings of Player 4's affect were higher on the positive scale ($M = 2.75$, $SE = .182$) than on the negative scale ($M = 1.55$, $SE = .119$). There was no main effect of group membership, $F(1, 35) = 1.212$, $p = .278$, $\eta^2_p = .033$, and no significant interaction, $F(1, 35) = 1.081$, $p > .306$, $\eta^2_p = .030$. In the OT condition, there was a main effect of scale type, $F(1, 34) = 14.089$, $p < .001$, $\eta^2_p = .293$, showing the same pattern found in the PL condition: ratings were higher on the positive scale ($M = 2.46$, $SE = .158$)

than on the negative scale ($M$ = 1.63, $SE$ = .105). There was also a near-significant effect of group membership, $F(1, 34) = 3.781$, $p = .060$, $\eta^2_p = .100$, such that participants tended to rate the outgroup Player 4 as feeling more emotion ($M$ = 2.196, $SE$ = .110), compared to the ingroup Player 4 ($M$ = .104, $SE$ = .104). However, there was no significant interaction with scale type, $F(1, 34) = 2.753$, $p = .106$, $\eta^2_p = .075$.

*Game Behaviour and Affect*

Correlation analyses revealed that participants who were more prosocial in their behaviour towards Player 4 during the exclusion round under PL conditions tended to have higher self-reported positive affect. This suggests that the more participants compensated the excluded player (by throwing the ball to them more often), the more likely they were to report greater positive affect. However, this relationship was only significant when Player 4 was an outgroup member (Ingroup: $r(17) = -.04$, $p = .879$; Outgroup: $r(17) = .64$, $p = .005$). There were no significant correlations between prosocial behaviour and self-reported negative affect, or between prosocial behaviour and Player 4's perceived affect.

In the OT condition, correlation analyses revealed that participants who behaved more prosocially towards Player 4 during the exclusion round tended to report that Player 4 felt less negative, but this relationship was only significant when Player 4 was an ingroup member (Ingroup: $r(16) = -.52$, $p = .041$; Outgroup: $r(16) = .34$, $p = .193$). In addition, there was a significant positive relationship between participants' OT concentrations (as measured by AUCi90) and their self-reported negative affect. This relationship suggests that the higher a participant's OT concentrations were, the more negative he felt when witnessing Player 4 being excluded, thereby suggesting a positive relationship between OT concentrations and empathy. However, this relationship was only significant when participants witnessed an ingroup member being excluded (Ingroup:

$r(18) = .49$, $p = .04$; Outgroup: $r(17) = .03$, $p = .921$). There were no significant relationships between prosocial behaviour and ratings of Player 4's positive affect; nor were there any relationships between self-reported emotion and prosocial behaviour.

## Discussion

We investigated the effects of IN-OT administration on behavioural and emotional responses to the Cyberball social exclusion paradigm. This study is the first to investigate the relationship between OT concentrations and Cyberball behaviour when witnessing ostracism. Although there is one other study in which OT concentrations were measured while using Cyberball (Jobst et al., 2015), in that study OT was assessed in participants who directly experienced social exclusion (in a 3-player design); as a result, the researchers were unable to assess the relation between endogenous OT and behaviour *during* Cyberball (because the participants were excluded, they played a purely passive role during the game); instead, they investigated the relationship between individual difference measures and OT concentrations. Furthermore, Jobst et al. did not administer IN-OT, but rather measured 'natural' endogenous OT concentrations. Thus the principal novel finding of the current study is that higher levels of salivary OT resulting from IN-OT administration significantly predicted increased prosocial behaviour towards an unknown excluded outgroup member. The results indicate that the higher participants' OT concentrations were, the more prosociality they demonstrated towards the excluded outgroup member. These results were also reflected in other analyses, where it was found that participants were more prosocial towards an excluded ingroup member under PL conditions, but were more prosocial to an excluded outgroup member under OT conditions.

Although previous research (Riem et al., 2013) demonstrated that OT increases females' prosocial behaviour towards an excluded individual who is known to the participant, our study extends this finding by demonstrating that under OT conditions male participants compensated for the social exclusion of an unknown outgroup member by throwing the ball to him more often. Because endogenous OT has been shown to affect social behaviour in both males and females (Neumann, 2008), a study directly comparing males and females is still needed (see Chapter 6).

Our finding that OT increased prosocial behaviour, but only towards certain individuals, is consistent with those of previous studies (De Dreu, Greer, Van Kleef, et al., 2011) in showing that the effects of OT on social behaviour are moderated by contextual factors: OT does not enhance prosocial behaviour towards all individuals in all contexts.

Taken at face value, however, these findings are not consistent with the tend-and-defend hypothesis (De Dreu et al., 2010), in that OT did not selectively increase prosocial behaviour towards ingroup members. However, De Dreu (2012b) notes that several factors are required for OT to instigate the tend-and-defend response; one of these is the perception that the outgroup poses a threat to the ingroup (De Dreu, Greer, Handgraaf, et al., 2011; De Dreu et al., 2012; Ten Velden, Baas, Shalvi, Kret, & De Dreu, 2014). Because the manipulation of Player 4's group membership in the current research was implicit (it was not directly referenced in any way), and because the game was not presented as a competitive one, it seems unlikely that participants would have perceived the outgroup member as a threat. Indeed given the potential '3-against-1' nature of the exclusion round (three persons with White British names excluding one person with a Middle-Eastern name), it is possible that the outgroup member was seen as being in a vulnerable position, rather than a threat.

Thus it seems that in the absence of a competitive/threatening relationship with the

outgroup, OT does not enhance the tendency to engage in parochial norms. Although this is the first study to demonstrate that OT can lead to an increase in prosocial behaviour towards the outgroup, and therefore would need to be replicated in future before any strong theoretical conclusions can be drawn, the results suggest that the original tend-and-defend hypothesis could be refined: OT increases prosocial behaviour towards the ingroup in competitive or threatening contexts, but in the absence of this, or indeed the opposite, when the outgroup is placed in a vulnerable context, OT may increase prosocial behaviour towards the outgroup. This amended theory also supports Bartz et al.'s (2011) hypothesis that OT increases our awareness of social cues: in addition to the intergroup context becoming more salient under OT conditions, the competitive/vulnerable context between groups is also more salient and thus may also moderate OT related behaviours.

A second noteworthy finding is that prosocial behaviour during Cyberball and rated affect in self and other tended to be inversely related. In the OT condition, despite the fact that participants acted more prosocially towards the excluded outgroup member during Cyberball, they showed less empathy with him in their affective ratings. Correlation analyses revealed a significant negative relationship between prosocial behaviour towards the excluded ingroup member and ratings of his affect in the OT condition: The more participants threw the ball to the excluded ingroup player, the more likely they were to report that he felt less negative (this relationship was not significant in the PL condition). This pattern is compatible with recent findings (Nozaki, 2015) that participants with high "emotional competence" were more prosocial towards an excluded individual, and that this was associated with the participant's motivation to relieve the (excluded) individual's sadness; supporting emotion theories stating that empathy leads to an increase in prosocial behaviour (Eisenberg & Miller, 1987; Hoffman, 2008).

A further novel finding is that participants' OT concentrations were positively

correlated with ratings of their own negative affect when they witnessed an ingroup member being excluded. In line with our hypotheses, this suggests that higher OT levels in the OT condition were associated with greater affective empathy. However, this relationship was only observed when participants witnessed an ingroup (rather than an outgroup) member being excluded (this pattern was not evident in the PL condition), thereby providing further evidence that the effects of OT are dependent on contextual factors.

The apparent dissociation between prosocial behaviour and empathy might reflect differences in participants' awareness of their affect ratings and their Cyberball behaviour. Cyberball behaviour is free-flowing and spontaneous, whereas ratings of an excluded player's affective state were made in response to an explicit request. Our results lend themselves to the theoretical explanation proposed by Nozaki (2015) based on emotion theories (Engen & Singer, 2013; Singer & Lamm, 2009; Van Kleef, De Dreu, & Manstead, 2010) which state that when sensory information is limited (e.g., a participant is not provided with the facial expressions of their fellow players) individuals incorporate contextual information to inform a inferential route of cognitive empathy (i.e., even though a participant cannot see a negative facial expression, by factoring in their exclusion and group membership they may more accurately infer a player's emotional state). This theoretical explanation represents a top-down process which may explain why higher OT concentrations (after IN-OT) significantly predicted an increase in spontaneous prosocial behaviour towards the excluded outgroup member, but greater empathy for the excluded ingroup member as reflected in more explicit measures of cognitive empathy.

There are some limitations to the present research that should be acknowledged. Although every participant took part in all conditions, participants only received each drug once, and were exposed to each group membership manipulation once. Because of

this confound it was not possible to assess the effect of drug condition in the same statistical analysis; our conclusions (drawn from the ANOVAs) are therefore limited to the comparison that the significant increase in participants' prosocial behaviour under PL conditions occurred in the ingroup condition, but under OT conditions occurred in the outgroup condition. We note, however, that this interpretation of the data is supported in the multiple regression analysis: salivary OT concentrations, under OT conditions, predicted increased prosocial behaviour towards an excluded outgroup member. In addition, the present study also reported follow-up *t*-tests of a non-significant interaction; these analyses were deemed appropriate because we had specific *a priori* hypotheses about the moderating nature of group membership on participants' behaviour. However, we acknowledge that as a result of these analytical approaches the risk of type one error has increased. To avoid this in future, and specifically to incorporate both drug conditions in one analysis, a future study would use a fully within-subjects design, with participants witnessing both an ingroup and an outgroup member being ostracized in both drug conditions. This would enable OT and PL conditions to be compared directly, and provide greater statistical power for detecting differences in behaviour and affect ratings, an issue that has been raised recently in the OT literature (Walum, Waldman, & Young, 2015). In a related issue, certain analyses in the present study had a relatively small sample size (albeit comparable to that in previous studies [e.g., Andari et al. (2010)]). Thus future research should seek to incorporate a larger sample size.

Future research could also investigate the consequences of making the group membership manipulation more explicit and more competitive. Although the race-based group manipulation was successful in terms of producing parochial behaviour, and deemed contextually appropriate for the sample recruited, changing the group

manipulation as suggested would provide a direct test of the notion that the reason we did not observe a relationship between OT concentrations and increased prosocial behaviour towards the excluded ingroup member (in the OT condition) was the absence of any significant threat posed by the outgroup. We anticipate that participants' behaviour would be more in line with the tend-and-defend hypothesis in the context of a more competitive intergroup relationship. A final point is that the present study used an all-male sample; future research could use a mixed gender sample to assess whether there are gender differences in prosocial behaviour towards an excluded outgroup member under OT conditions.

*Conclusions*

In conclusion, this is the first study to demonstrate that salivary OT concentrations following IN-OT administration significantly predicts prosocial behaviour during Cyberball. Higher OT concentrations were associated with more prosocial behaviour towards an unknown excluded outgroup player, but not towards an unknown excluded ingroup player. We conclude that in the absence of any threat posed by the outgroup, OT selectively enhances prosocial behaviour towards an excluded outgroup person. By contrast, participants in the OT condition displayed greater empathy for the excluded ingroup member, in line with the tend-and-defend hypothesis. These findings provide support for the influence of contextual factors and individual differences on the social effects of OT, and that these effects extend to third-party behaviour.

## Supplementary Information 2

*SI 1 - Regression analyses in which proportion of throws to Player 4 was regressed onto oxytocin concentrations at 90 minutes in the oxytocin (OT) and placebo (PL) condition when Player 4 was an ingroup or outgroup member, and included or excluded.*

| Group | Game (Model) | $R^2$ | $F$ | df | $p$ | Drug | β | $p$ |
|---|---|---|---|---|---|---|---|---|
| Ingroup | Inclusion (1) | .07 | 1.16 | (2,33) | .328 | PL | .002 | .991 |
| | | | | | | OT | .26 | .197 |
| | Exclusion (2) | .04 | .64 | (2,32) | .537 | PL | .15 | .471 |
| | | | | | | OT | -.23 | .278 |
| Outgroup | Inclusion (3) | .02 | .26 | (2,29) | .772 | PL | -.15 | .478 |
| | | | | | | OT | .07 | .735 |
| | Exclusion (4) | .14 | 2.38 | (2,32) | .109 | PL | -.19 | .329 |
| | | | | | | OT | .42 | .037 |

# Chapter 6

## Cranial Diabetes Insipidus patients respond comparably to clinical and healthy controls when witnessing social exclusion


### Abstract

The social effects of the neuropeptide oxytocin (OT) have been shown to be moderated by contextual and individual difference factors. To date this body of research has focused on the influence of contextual factors after administering intranasal-oxytocin (IN-OT) to healthy volunteers. The present study recruited a novel patient group with an anticipated OT deficit to investigate whether their social and emotional responses under natural conditions (i.e., without IN-OT administration) would demonstrate the same sensitivity to moderating factors. Fifty-five participants ($M_{age}$ = 46.54; *SD* = 16.30; 62% female) played the virtual ball-throwing game Cyberball. Here they witnessed both an unknown ingroup and outgroup member being excluded by two other players. After the game participants were asked to report how they and the excluded player felt. We hypothesised that the social and emotional responses of patients with Cranial Diabetes Insipidus (CDI) would not be moderated by the group identity of the excluded individual. We also aimed to replicate previous findings that participants' OT concentrations would predict their prosocial behaviour towards the excluded individual. CDI patients did not behave significantly differently during Cyberball from a clinical control (patients with anterior hypopituitarism) group and a healthy control group. Results demonstrate that CDI patients are sensitive to contextual factors, but future studies should replicate this finding in a larger sample size before drawing strong conclusions.

## Introduction

The ability to express emotion is functional because it enables individuals to communicate important social information to others; less intuitively obvious, perhaps, is the functionality of being able to regulate one's own emotions. Research has shown that emotion regulation is important in order to avoid emotional 'burn-out' (Grandey, 2000; Grandey, Foo, Groth, & Goodwin, 2012), and dysfunctional emotion regulation is central to several psychological disorders (Aldao, Nolen-Hoeksema, & Schweizer, 2010; Phillips, Drevets, Rauch, & Lane, 2003). Integral to the concept of emotion regulation is the ability to generate appropriate or adaptive appraisals of emotional and social stimuli; maladaptive appraisals or hypersensitivity to social and emotional cues are often evident in those with psychological disorders (Beck, 1979; Watts, 1992). The neuropeptide OT plays an important role in interpreting social and emotional cues, but its influence on such interpretations is likely to be moderated by contextual factors within the social environment and by individual difference factors. The present study investigated the extent to which patients with an anticipated OT deficit respond comparably to a clinical control group and a healthy control group when witnessing an unknown individual being socially excluded, and whether their response to the exclusion is moderated by the group identity of the excluded individual.

Previous research has demonstrated that the social effects of OT extend to third-party behaviour, as well as direct social interactions (Hu et al., 2016). In Chapter 5, we demonstrated that participants with higher OT concentrations (after IN-OT administration) acted more prosocially towards an excluded individual during the computer-based ball throwing game Cyberball. Importantly, this effect was only found when the excluded individual was an outgroup member, providing support for the growing body of literature (for a recent review see Shamay-Tsoory and Abu-Akel (2016)) demonstrating that the

social effects of OT are moderated by contextual factors.

In addition, research has shown that responses to exclusion are moderated by individual differences. Pfundmair et al. (2014) found that participants' responses to being excluded, after receiving IN-OT, were moderated by their social values. Participants who had a horizontal collectivistic orientation (cooperation among equals) and were given OT reported reduced negative consequences of experiencing ostracism, compared to those with an individualistic orientation. Thus the need for social togetherness reduced the negative consequences of exclusion, but only among participants who received IN-OT. The present study seeks to extend this research by investigating whether individual differences (in empathy, autistic-like traits, and OT concentrations) moderate responses to witnessing exclusion.

As stated previously, a common characteristic of several psychopathologies is dysfunctional emotion regulation (Phillips et al., 2003), and several of these disorders have now been associated with low or altered OT (Husarova et al., 2016; Jobst, Dehning, et al., 2014; Jobst et al., 2015). To the best of our knowledge, however, only a handful of studies have investigated the behavioural consequences of low OT concentrations in patients with these disorders. Andari et al. (2010) found that Asperger's Syndrome (AS) and high-functioning autism (HFA) patients were unable to interpret social cues presented during a 4-player version of Cyberball under placebo (PL) conditions, but that they performed comparably to healthy controls when given IN-OT (for a full description see Chapter 5). Jobst and colleagues (Jobst, Albert, et al., 2014; Jobst et al., 2015) found that patients with Borderline Personality Disorder (BPD) and Chronic Depression (CD) had a significant reduction in OT concentrations immediately after experiencing exclusion during Cyberball. Moreover, CD patients reported greater negative affect as a result of the exclusion, and subsequently revealed greater sensitivity to ambiguous social threat

scenarios, demonstrating that patients with dysfunctional emotion regulation experienced adverse emotional outcomes and a significant drop in OT concentrations during Cyberball that had a sustained effect on their ability to interpret social cues. Thus research has demonstrated that patients with several psychological disorders associated with low OT concentrations displayed impaired interpretation of social cues (either during or after Cyberball) that had effects on their subsequent OT and/or emotional responses.

*The Present Study*

The present study investigated whether CDI patients, who were expected to be OT deficient, would behave differently compared to both a clinical control (HP) group and healthy control (HC) group. CDI patients are diagnosed with a deficiency in Arginine Vasopressin (AVP) production; because AVP and OT are synthesised and released into the blood stream in the same way (Swaab et al., 1975), we hypothesised that patients with CDI would also present with an OT deficiency. Evidence supporting this hypothesis was reported in Chapter 4. We hypothesised that CDI patients would display significantly less prosocial behaviour and less empathy towards the excluded player (compared to HP and HC groups); and that CDI patients' behaviour during Cyberball and their emotional responses afterwards would not be moderated by the group identity of the player (but that this factor would moderate HP and HC participants' behaviour and empathy, thereby replicating previous findings in healthy volunteers). We also aimed to replicate the finding that salivary OT concentrations significantly predict prosocial behaviour during Cyberball in healthy volunteers, and to examine whether this is was also true for clinical patients. Again, we hypothesised that this effect would be moderated by the group identity of the unknown excluded player. Finally, given the use of a within-subjects design, we also expected that participants would display more prosocial behaviour towards the excluded

player during the second exclusion round, such that there would be significant order effects. In this way the design of the study enabled us to assess whether contextual factors had the same effect on social and emotional behaviour in three different groups.

## Method

*Participants and Ethics*

Fifty-five white British adults ($M_{age}$ = 46.54; *SD* = 16.30) took part in this clinical study. Participants were recruited to one of three groups: the CDI group, the HP group, and the HC group. Inclusion criteria for the CDI group were that patients had acquired CDI after transsphenoidal surgery. All CDI patients were currently taking desmopressin to compensate for their arginine vasopressin (AVP) deficiency. Ideally, CDI patients were also diagnosed with anterior hypopituitarism; however, due to logistical constraints on recruitment, some patients (n = 5) only had a diagnosis of CDI. CDI patients were matched by age and gender to HP patients, who could have a diagnosis of either full or partial anterior hypopituitarism, as long as they had complementary hormone replacement therapy for the necessary hormones. Finally, HC participants were also recruited (via word of mouth) and matched on age and gender to the CDI group. The target was to recruit 20 participants to each group, although it proved to be impossible to recruit all 20 HP patients within a reasonable timeframe while continuing to accurately match groups; thus only 15 HP patients were tested. The final numbers of males and females recruited for each group were as follows, CDI male – 8, female –12; HP male – 6, female – 9; HC male – 7, female – 13. A one-way ANOVA revealed that there was no significant difference in age between the three groups, $F(2, 52) = .983$, $p = .382$, $\eta^2_p = .039$.

The study was approved by the Research and Development Office at the Cardiff

and Vale University Health Board and the Cambridge Central Research Ethics Committee. All participants read a detailed information sheet and gave written informed consent at the start of the experiment, and were fully debriefed at the end. Participants were financially compensated £20 for their participation (see Appendix 2 for a flow diagram of the study).

*Materials*

*Cyberball*

Participants completed a modified, within-subjects version of the Cyberball task (Williams et al., 2000), as described in detail in Chapter 5. In the present study, participants took part in a 4-player version of the game, consisting of the participant and three 'others.' The others were simulated via the program to throw the virtual ball to other players equally during inclusion rounds, but two of these others (Players 1 and 3) excluded the third other (Player 4) during exclusion rounds. Participants (who were designated as Player 2) were allowed to behave as they wished throughout the game. The number of throws made by the participant to Player 4, divided by their total number of throws in a round, served as an indication of the participant's behaviour towards Player 4. By comparing scores from the inclusion and exclusion rounds, one can assess participants' responses to witnessing social exclusion. Moreover, by manipulating the group identity of Player 4, one can assess whether group identity moderated these responses. In order to make group identity a within-subjects factor participants completed four rounds in total, witnessing an inclusion and exclusion round for both an ingroup and outgroup Player 4. The order in which they witnessed either an ingroup or outgroup Player 4 was counterbalanced within participant groups.

Results from a pilot study (see Supplementary Information 3, p. 108, for full details of the pilot study methods and results) indicated that female participants were more

prosocial during the exclusion round when playing in an all-male group compared to an all-female group. However, this was only true when Player 4 was an ingroup member; all other rounds showed no significant difference in behaviour between all- male and all-female groups. This provided a basis for the decision to conduct an exact replication of the Cyberball game as used in Chapter 5 (despite now using a mixed gender sample). Thus all participants saw Tom (Player 1) and Chris (Player 3) interacting with Ben (ingroup Player 4) or Ahmed (outgroup Player 4).

Participants also completed the post-Cyberball questionnaires (see Chapter 5 for full details). The first questionnaire recorded self-reported affect. Here participants reported the extent to which they thought Player 4 felt each of 10 emotions during the exclusion round, and also how they themselves felt while watching the exclusion. These emotions were categorised into positive and negative affect subscales. All subscales demonstrated satisfactory internal consistency (see Table 6.1). The second questionnaire recorded whether participants noticed the exclusion, by asking them to circle one of three options in response to questions about the other players: 'involved everyone equally,' 'excluded certain players,' and 'was excluded by others.' The data revealed that 54% of participants reported that they noticed that Player 4 was excluded, and 53% of participants reported that they noticed Players 1 and 3 had excluded certain individuals.

*Table 6.1 - Cronbach's alpha values for the post-Cyberball questionnaire*

| Scale | Subscale | Alpha |
|---|---|---|
| **Ingroup** | Other Negative | .906 |
| | Other Positive | .966 |
| | Self Negative | .922 |
| | Self Positive | .969 |
| **Outgroup** | Other Negative | .927 |
| | Other Positive | .914 |
| | Self Negative | .889 |
| | Self Positive | .943 |

*Saliva Samples*

Participants produced two saliva samples during the study, with an average inter- sample interval of 33 minutes. Samples were collected in pre-chilled tubes, stored on ice throughout the study and frozen at -80°C as soon as possible. Samples were subsequently centrifuged, lyophilized, and analysed using the ELISA method. Full details of sampling and analysis can be found in Chapters 2 and 4, respectively. Participants played the Cyberball game immediately after providing their second saliva sample. An average of the two saliva samples was calculated, thereby creating a baseline OT concentration for each participant.

*Procedure*

Participants were asked to abstain from alcohol for 24 hours and from caffeine for an hour prior to testing. They were only allowed to drink water during the study and if they had consumed any food before the start of a session they were asked to rinse their mouths thoroughly before any saliva samples were taken. All testing was carried out between 09:00 and 12:00 to control for circadian rhythms in other hormones that might be affected in the two clinical groups.

On arrival participants' height and weight were measured so that their BMI could be calculated. After a brief period (approximately 10 minutes) of acclimatization to the testing facility, participants were asked to provide the first saliva sample before completing 30 minutes of testing (the tasks completed during this time, and after Cyberball, are not relevant to the current study, but are reported in Chapter 8) before providing the second saliva sample. Immediately thereafter participants completed two rounds of the Cyberball game (one inclusion and one exclusion round) and the first post-Cyberball questionnaire. Participants then played another two rounds of the game and completed both the first and second post-Cyberball questionnaires. The second post-Cyberball questionnaire was administered only after the fourth round in order to avoid revealing the purpose of the task. After the second post-Cyberball questionnaire, participants completed a final task before being debriefed.

To provide the illusion that participants were playing against real people, the cover story stated that three research associates had been recruited to help with the study and that they were logged on remotely. The game was described as a mental visualisation task, and participants were instructed to think about their fellow players during the game. Forty-two percent of participants (with scores ranging from 3 to 5) reported that they believed they were playing against real people. Because there was no effect of 'believability' on participants' behaviour it will not be discussed further.

*Data Analysis*

A mixed ANOVA was conducted to assess the effect of group, Player 4's group identity and round type on participants' behaviour during Cyberball. The order in which participants saw an ingroup member being excluded was also included in the analysis to investigate whether there were significant order effects. Finally, the analysis was repeated,

this time controlling for age and gender[4]. A similar mixed ANOVA was carried out to investigate the effect of group and Player 4's identity on participants' empathic responses to Cyberball.

Multiple regression analyses were carried out to assess whether participants' OT concentrations predicted their prosocial behaviour during Cyberball. Finally, correlation analyses were performed to investigate any further relationships between group, game behaviour, affect and OT response.

## Results

*Game Behaviour*

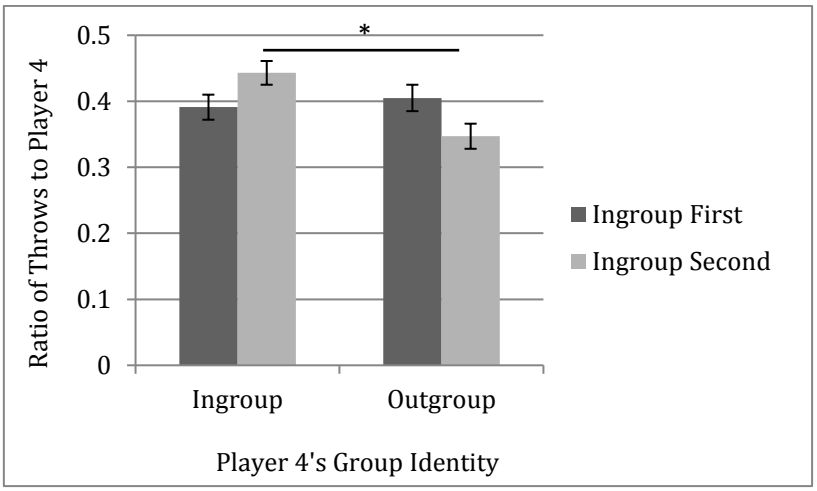
A 3 (Group: CDI vs HP vs HC; between-subjects) x 2 (Identity: ingroup vs outgroup; within-subjects) x 2 (Round: included vs excluded; within-subjects) x 2 (Order: ingroup first vs outgroup first; between-subjects) mixed ANOVA revealed a significant main effect of identity, $F(1, 44) = 6.249$, $p = .016$, $\eta^2_p = .124$, reflecting the fact that participants threw the ball more to Player 4 when they were ingroup ($M = .417$, $SE = .013$) compared to outgroup ($M = .376$, $SE = .014$); and a significant main effect of round, $F(1, 44) = 30.935$, $p < .001$, $\eta^2_p = .413$, reflecting the fact that participants threw the ball more to Player 4 when he was excluded ($M = .450$, $SE = .016$) compared to when he was included ($M = .343$, $SE = .013$). There was no main effect of group, $F(2, 44) = .329$, $p = .722$, $\eta^2_p = .015$, and no main effect of order, $F(1, 44) = .027$, $p = .871$, $\eta^2_p = .001$. The interaction between group and order was not significant, but there was a significant interaction between identity and order, $F(1, 44) = 11.225$, $p = .002$, $\eta^2_p = .203$. This reflected the fact that participants

[4] Age was added as a covariate to control for any differences in response to the game due to the development of the social brain (Mills, Lalonde, Clasen, Giedd, & Blakemore, 2014).

were significantly more prosocial towards an ingroup member when they saw him being excluded in the second game, compared to an outgroup member, $F(1, 44) = 18.054$, $p < .001$, $\eta^2_p = .291$ (see Figure 6.1). All remaining interactions were not significant.

A 3 x 2 x 2 mixed ANCOVA controlling for age and gender was carried out. There was no effect age, $F(1, 42) = 1.152$, $p = .289$, $\eta^2_p = .027$, or gender, $F(1, 42) = .167$, $p = .685$, $\eta^2_p = .004$; all main effects found in the first analysis remained significant.

*Figure 6.1 - Ratio of throws to Player 4 as a function of group identity and order (± SE)*




*Oxytocin Concentrations and Prosocial Behaviour*

We analysed the relation between OT concentrations and prosocial behaviour by regressing the ratio of throws made to Player 4 on salivary OT concentrations alone (Step 1) and then adding group to the equation (Step 2). The results of these hierarchical regressions are presented in Table 6.2. Participants' OT concentrations alone did not predict prosocial behaviour. When adding participants' group (CDI, HP, or HC) as well as their OT concentrations, participants' OT concentrations were a near significant predictor

of prosocial behaviour towards an excluded ingroup member, *ß* = .285, *p* = .061.

*Table 6.2 - Summary of hierarchical regression analyses in which the proportion of throws to Player 4 was regressed on OT concentrations and group*

| DV | Model | $R^2$ | *F* | df | *p* | Predictors | β | *p* |
|---|---|---|---|---|---|---|---|---|
| Ingroup included | 1 | .038 | 1.900 | (1, 49) | .174 | OT | .195 | .174 |
| | 2 | .050 | 1.237 | (2, 49) | .300 | OT | .157 | .303 |
| | | | | | | Group | .116 | .446 |
| Ingroup excluded | 1 | .053 | 2.667 | (1, 49) | .109 | OT | .229 | .109 |
| | 2 | .078 | 1.984 | (2, 49) | .149 | OT | .285 | .061 |
| | | | | | | Group | -.168 | .262 |
| Outgroup included | 1 | .001 | .004 | (1, 50) | .951 | OT | -.009 | .951 |
| | 2 | .004 | .095 | (2, 50) | .910 | OT | -.030 | .844 |
| | | | | | | Group | .066 | .668 |
| Outgroup excluded | 1 | .001 | .029 | (1, 50) | .029 | OT | .024 | .865 |
| | 2 | .036 | .883 | (2, 50) | .883 | OT | -.040 | .762 |
| | | | | | | Group | .198 | .194 |

*Affect*

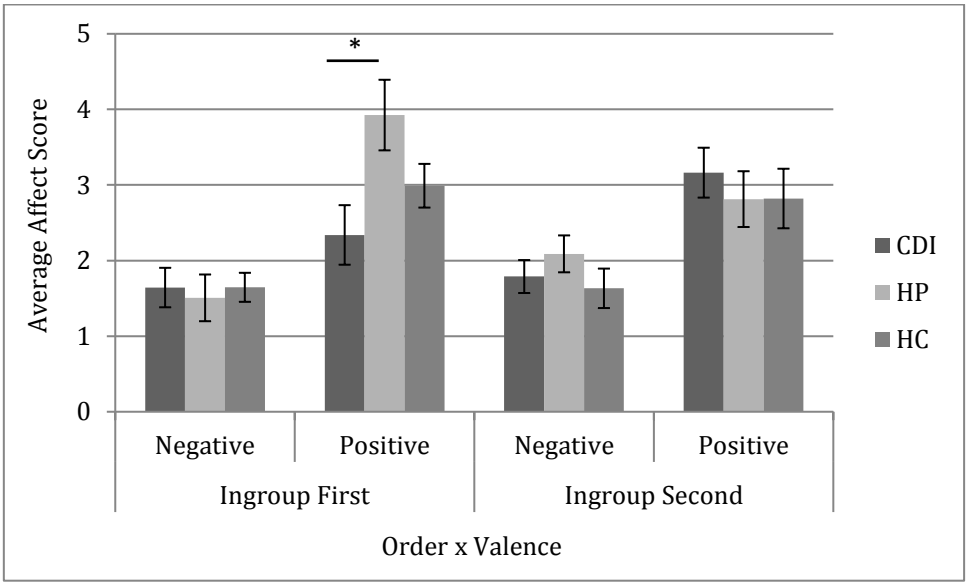
A 3 (Group: CDI vs HP vs HC; between-subjects) x 2 (Identity: ingroup vs outgroup; within-subjects) x 2 (Person: Player 4 vs self; within-subjects) x 2 (Scale: negative vs positive; within-subjects) x 2 (Order: ingroup first vs outgroup first; between-subjects) mixed ANOVA revealed a significant main effect of identity, $F(1, 44) = 4.667$, $p = .036$, $\eta^2_p = .096$, showing that participants rated the excluded outgroup member as feeling more emotion ($M = 2.417$, $SE = .094$) than the excluded ingroup member ($M = 2.309$, $SE = .107$); and a

significant main effect of valence, $F(1, 44) = 54.806$, $p < .001$, $\eta^2_p = .555$, such that participants reported more positive affect ($M = 3.009$, $SE = .154$) than negative affect ($M = 1.718$, $SE = .102$). The main effects of group, $F(2, 44) = 3.998$, $p = .327$, $= .050$, and order, $F(1, 44) = .048$, $p = .827$, $\eta^2_p = .001$, were not significant, and there was no significant interaction, $F(2, 44) = 1.345$, $p = .271$, $\eta^2_p = .058$. There was a significant interaction between identity and order, $F(1, 44) = 5.583$, $p = .023$, $\eta^2_p = .113$. Simple effects analysis revealed that participants who saw an outgroup member being excluded during the first game reported that this outgroup member felt more emotion ($M = 2.497$, $SE = .129$) compared to a similarly excluded ingroup member ($M = 2.272$, $SE = .146$), $F(1, 44) = 10.951$, $p = .020$, $\eta^2_p = .199$. There was no effect of identity for participants who saw an ingroup member being excluded during the first game, $F(1, 44) = .019$, $p = .890$, $\eta^2_p = .001$. Finally, there was a significant interaction between valence, order and group, $F(2, 44) = 3.597$, $p = .036$, $\eta^2_p = .141$. Simple effects analysis revealed that CDI patients who saw an ingroup member being excluded during the first game reported significantly less positive affect compared to HP patients (see Figure 3.2), although neither patient group differed significantly from HC participants, $F(2, 44) = 3.369$, $p = .044$, $\eta^2_p = .133$. All other interactions were not significant.

A 3 x 2 x 2 x 2 mixed ANCOVA was conducted controlling for age and gender. There was no main effect of gender, $F(1, 42) = .121$, $p = .729$, $\eta^2_p = .003$, but there was a near significant effect of age, $F(1, 42) = 13.699$, $p = .054$, $\eta^2_p = .085$. In total there were four significant interactions with age and gender that incorporated identity, person and scale. Correlations were carried out to investigate the effect of age and gender. Here it was found that older female participants tended to report that the excluded ingroup member felt less positive, $r(30) = -.521$, $p = .003$), and older men tended to report that they

personally felt less positive affect when an ingroup, $r(17) = -.649$, $p = .005$, and outgroup, $r(17) = -.493$, $p = .044$, member was excluded.

*Figure 6.2 - Average affect score as a function of order, valence and group (± SE)*




*Group, Game Behaviour, Affect and Oxytocin Response*

Separate correlation matrices were calculated for each group, to identify whether relations between these variables differed as a function of group. Because no consistent pattern of results was found within or between groups, these analyses will not be discussed further. All significant (and trending towards significant) correlations are presented in Supplementary Information 3, p. 112.

## Discussion

In this study we investigated whether the social and emotional behaviour of a clinical group that we expected to have an OT deficit would demonstrate the same sensitivity to

contextual factors and individual difference factors, relative to a clinical and healthy control group. There was no difference in the behaviour of participants in the CDI, HP or HC groups; CDI patients' behaviour was moderated by group identity to the same extent as that of HP patients and participants in the HC group. CDI patients therefore demonstrated the same sensitivity to contextual information as HP patients and HC participants. CDI patients also reported emotional reactions to witnessing social exclusion that were not statistically different from those reported by participants in the other two groups, and these reactions were also sensitive to the group identity of the excluded player. Thus the results are not consistent with those from previous studies in which it was found that patient groups with low OT or altered OT responses, for example ASD patients, were less able to detect the social cues presented during a Cyberball game (Andari et al., 2010; Jobst et al., 2015). It should be noted, however, that the present study assumes that CDI patients had low OT concentrations or altered OT response patterns compared to healthy controls. If CDI patients retain a functional OT system, and are therefore able to interpret social cues just as effectively as HC participants, this would explain why no significant group effects were observed (this question was addressed in Chapter 4).

However, the present results do replicate previous findings in that participants threw the ball more to an ingroup Player 4, compared to an outgroup Player 4, and were more prosocial towards Player 4 when he was excluded compared to when he was included. Although the hypothesised main effect of order was not present, participants did throw the ball more to an ingroup member (across both rounds) after first witnessing an outgroup member being included and excluded. Participants did not throw the ball more to the outgroup member (across both rounds) after first seeing an ingroup member being included and excluded, suggesting that the salience of exclusion enhanced the moderating effect of group identity: Participants were more prosocial towards an ingroup member

when the salience of exclusion was high. This finding fits the concept of parochial altruism, which states that prosocial acts should be limited to ingroup members, and is also consistent with the tend-and-defend hypothesis (De Dreu, 2012a) and the theoretical explanation explored in Chapter 5 highlighting the importance of 'salience of threat' in activating the tend-and-defend response. Previous research has demonstrated that social exclusion threatens basic psychological needs (Eisenberger et al., 2003; Gonsalkorale & Williams, 2007). It follows that witnessing someone else being socially excluded should increase the salience of this potential threat, triggering tend-and-defend related behaviours as an ingroup protection mechanism (see Chapter 7 for further discussion).

The present results also extend the findings reported in Chapter 5 by demonstrating that participants' behavioural responses generalize across age and gender. Although Chapter 5 demonstrated the effect of group identity on social behaviour in Cyberball, that study was carried out using an all-male, undergraduate sample. The present study replicated the finding observed there using a mixed gender sample consisting of participants ranging from 22 to 74 years of age. Moreover, the effect sizes were medium to large, suggesting that the effect of group identity on responses to witnessing social exclusion in the Cyberball paradigm is a robust finding.

Consistent with our hypothesis, and further replicating the findings from Chapter 5, participants' OT concentrations significantly ($p$ = .061) predicted their prosocial behaviour. In the present study, however, participants' OT concentrations (tended to) predict their prosocial behaviour but only towards an excluded ingroup member, in line with the original hypothesis made in Chapter 5 (whereas the Chapter 5 results demonstrated that OT concentrations only predicted participants' prosocial behaviour towards an excluded outgroup member). In the present study we did not administer IN-OT, so the present study is best seen as a replication of the placebo condition of the study

reported in Chapter 5, where it was found that participants were significantly more prosocial towards the excluded ingroup member. The results presented here extend this finding by demonstrating that this biased behaviour in favour of the ingroup was associated with endogenous OT concentrations (the corresponding association was not significant in Chapter 5). We note, however, that this effect was only marginally significant, and then only when group was included in the regression model.

As noted previously, there was no group difference in participants' emotional responses to witnessing social exclusion. Participants did report, however, that outgroup members felt more affect compared to ingroup members, replicating a trend demonstrated in the study reported in Chapter 5. This perceived affect was sensitive to order: participants reported that an excluded outgroup member felt more affect when they witnessed him first and an ingroup member second; participants did not report that an excluded ingroup member felt more affect when they saw him first and an outgroup member second. In contrast to participants' behavioural responses, there was a trend towards an effect of age on participants' emotional responses and several interactions with age and gender, reflecting the fact that older women were more likely to report that the excluded individual felt less positive, and older men were more likely to report that they themselves felt less positive. Because there was no significant main effect of gender on either behavioural or emotional responses, and given that the literature on the reporting of emotions (as opposed to the experience of emotion) is gender-biased (Eisenberg & Lennon, 1983; Manstead, 1992), these correlational analyses may not be representative of a genuine gender difference, a conclusion consistent with the results of a recent meta-analysis of Cyberball studies, which found no effect of gender or age (Hartgerink et al., 2015). We therefore conclude that both behavioural and emotional responses are generalizable across age and gender.

Despite the strengths of the present study (for example, its use of a within-subjects, mixed gender design), some limitations need to be acknowledged. The most obvious limitation concerns the sample size. Although we were able to recruit 20 participants for the CDI and HC groups, it was only possible to recruit 15 HP patients (due to time and logistical constraints). Conclusions, especially in relation to HP patients, should therefore be treated with caution. Ideally a future study would replicate the current design using a larger sample, in order to ensure that the results reported here are reliable. However, it needs to be recognised that recruiting NHS patients who have undergone pituitary surgery and who are matched on both age and gender with another NHS patient group who have undergone pituitary surgery that resulted in an additional condition is a challenging task, even before taking account of geographical and time constraints. We therefore believe that the data reported here make a worthwhile initial contribution to the literature. Finally, and in contrast to Chapter 5, because we did not administer IN-OT in the present study, results regarding the effect of OT on social and emotional behaviour may also be true in the reverse: We are unable to identify cause and affect relationships. Furthermore, there is are justifications for more research to investigate the association between endogenous concentrations of OT and social behaviour, (a) to provide evidence for use in future therapeutic practices, (b) to avoid a recent critique that the conclusions drawn from the current OT literature focus on the effects of supraphysiological levels of OT (Leng & Ludwig, 2015), and thus are of limited use.

*Conclusions*

In this study we found that CDI patients' behavioural and emotional responses to witnessing social exclusion during Cyberball were similar to those of HP patients and HC participants. We also found that participants' OT concentrations trended towards a

significant positive predictor of prosocial behaviour towards an excluded ingroup member, in line with the tend-and-defend hypothesis. Replicating previous findings, participants also reported moderated emotional responses to witnessing exclusion. In conclusion, CDI patients demonstrated comparable sensitivity to contextual cues presented during a social exclusion paradigm to both HP and HC participants, suggesting that this group may not present with the same social and emotional difficulties as those demonstrated in other psychological disorders.

## Supplementary Information 3

## Cyberball Pilot Study

## Method

*Participants and ethics*

Sixty-one first and second year psychology undergraduates from Cardiff University took part in the pilot study. Of these, only 5 were male; a meaningful analysis including gender was therefore not possible and their data were removed from the dataset. Three further participants were removed from the dataset due to potentially conflicting ingroup/outgroup identity.

The study was approved by the School of Psychology Ethics Committee at Cardiff University. Participants received course credits for their participation in the study. All participants provided written informed consent and were fully debriefed at the end of the study.

*Procedure and Materials*

Participants completed a modified, within-subjects version of the Cyberball task described in Chapter 5. Participants completed four rounds in total, witnessing an inclusion and exclusion round for both an ingroup and outgroup Player 4. The order in which they witnessed either an ingroup or outgroup Player 4 was counterbalanced. In order to assess whether female participants would respond differently to an all-male group (i.e., a precise replication of the Chapter 5 Cyberball task) compared to an all- female group (i.e., a conceptual replication of the Chapter 5 Cyberball task) female participants were randomly assigned to one of these two conditions. Participants in the all-male group played with Tom, Chris and Ben/Ahmed (as before), whereas participants in the all-female group

played with Jo, Claire and Emma/Afia.

Participants also completed the Post-Cyberball questionnaires described in Chapter 5; however, participants only completed the first of these questionnaires after the first set of inclusion exclusion rounds, and completed both questionnaires after the second set. Affect data could then be collected for both groups. It was deemed appropriate to only include the manipulation check questionnaire after the second round, so as not to reveal the purpose of the task.

*Data Analysis*

A mixed ANOVA with within-subjects factors of round (included vs excluded) and group (ingroup vs outgroup) and between-subjects factors of condition (all-male group vs all-female group) and order (ingroup/outgroup vs outgroup/ingroup) was carried out to assess the effect of these variables on participants' play behaviour. As previously stated, because there were only 5 males, analyses were only carried out on female participant data. A second mixed ANOVA with within-subjects factors of affect (positive vs negative), person (self vs Player 4), and group (ingroup vs outgroup), and the same between-subjects factors as previously mentioned was carried out in order to assess the effect of the same variables on participants' affect. Scale analysis revealed that all subscales demonstrated satisfactory internal consistency (Self-Negative, $\alpha = .94$; Self-Positive, $\alpha = .91$; Player 4-Negative, $\alpha = .91$; Player 4-Positive, $\alpha = .87$); however all negative subscales were found to violate the assumption of normality. A log transformation was therefore conducted to reduce the skew. Log transformed values were used in the analysis.

Manipulation checks revealed that only 43% of participants believed they were playing against real people (scores ranging from 3-5 on a 5-point scale); that 55% of

participants reported that Player 4 “was excluded by others”; and that on average 60% of participants reported that Player 1 and Player 3 “excluded certain players”.

## Pilot Study Results

*Game*

There was a significant main effect of round, $F(1, 47) = 4.198$, $p = .046$, $= .082$, showing that participants threw the ball to Player 4 more in the inclusion round ($M = .412$, $SE = .013$) compared to the exclusion round ($M = .382$, $SE = .009$); and a significant main effect of group, $F(1, 47) = 51.200$, $p < .001$, $= .521$, showing that participants threw the ball to Player 4 more when they were an outgroup member ($M = .453$, $SE = .011$) compared to when they were an ingroup member ($M = .341$, $SE = .012$). There was no main effect of condition, $F(1, 47) = 1.467$, $p = .232$, $= .030$, or order, $F(1, 47) = 1.866$, $p = .178$, $= .038$. The only significant interaction was between condition and order, $F(1, 47) = 4.267$, $p = .044$, $= .083$. A follow-up ANOVA revealed that this was driven by a significant difference in prosocial behaviour between participants who played in an all-male group, and those in an all-female group, $F(1, 47 = 5.835$, $p = .020$, $= .110$. Female participants who played in an all-male group threw the ball significantly more to the ingroup person when he was excluded ($M = .50$, $SE = .020$) compared to female participants playing in an all-female group ($M = .43$, $SE = 0.19$). All other rounds showed statistically similar behaviour.

*Affect*

There was a significant main effect of affect, $F(1, 47) = 585.403$, $p < .001$, $= .926$, confirming findings from Chapter 5 that participants reported greater positive affect ($M =$

3.012, *SE* = .111) compared to negative affect (*M* = .145, *SE* = .016). All other main effects were non-significant. There was a significant interaction between group and affect, $F(1, 47) = 7.413$, $p = .009$, $= .136$, showing that participants reported less positive affect and more negative affect for games with an ingroup Player 4 ($M_{positive}$ = 2.907, *SE* = .125; $M_{negative}$ = .200, *SE* = .020) compared to an outgroup Player 4 ($M_{positive}$ = 3.117, *SE* = .125; $M_{negative}$ = .091, *SE* = .016). There was a significant interaction between person and group, $F(1, 47) = 5.401$, $p = .024$, $= .103$, which was further qualified by a three-way interaction between person, group and affect, $F(1, 47) = 8.151$, $p = .006$, $= .148$, replicating the previous pattern that female participants reported that they felt less positive and more negative when witnessing an ingroup Player 4 being excluded, compared to an outgroup Player 4 being excluded. Finally there was a marginally significant four-way interaction between person, group, order and condition, $F(1, 47) = 3.777$, $p = .058$, $= .074$. Female participants who played in an all-male group tended to report greater affect in similar patterns to those previously described, compared to female participants who played in an all-female group.

## Cyberball Results

*Group, game behaviour, affect and OT response*

*SI 1 – Significant correlations for CDI patients*

| Category | Variables | *r* | n | *p* |
|---|---|---|---|---|
| Game behaviour | Outgroup excluded – Self-reported positive affect | .471 | 17 | .056 |

*SI 2 – Significant correlations for HP patients*

| Category | Variables | *r* | n | *p* |
|---|---|---|---|---|
| Game behaviour | Ingroup included - Self-reported negative affect | -.661 | 13 | .014 |
| | Ingroup excluded – Other-reported positive affect | -.657 | 13 | .015 |
| | Ingroup excluded – Self-reported positive affect | -.689 | 13 | .009 |
| OT | OT – Ingroup excluded | .819 | 13 | .001 |
| | OT – Ingroup other-reported positive affect | -.634 | 13 | .020 |
| | OT – Ingroup self-reported positive affect | -.689 | 13 | .009 |
| | OT – Outgroup self-reported positive affect | -.576 | 13 | .039 |

SI 3 - Significant correlations for HC patients

| Category | Variables | *r* | n | *p* |
|---|---|---|---|---|
| Game behaviour | Outgroup included – Outgroup other-reported positive affect | .429 | 20 | .059 |
| | Outgroup included – Outgroup self-reported positive affect | .568 | 20 | .009 |
| OT | OT – Ingroup excluded | .434 | 19 | .063 |

# Chapter 7

## Oxytocin modulates the sanctioning of selfish and generous behaviour within and between groups

Adapted from:

Daughters, K., Manstead, A. S. R., Ten Velden, F. S., & De Dreu, C. K. W. Oxytocin Modulates the Sanctioning of Selfish and Generous Behaviour Within and Between Groups. *Psychoneuroendocrinology*.

### Abstract

Human groups function by virtue of their members' cooperative contributions to group-living, including their willingness to reward others' cooperation and punish their non-cooperation. It is possible that such third-party sanctioning of others' non-cooperation is modulated by the same neurobiological mechanisms that support group rather than self-serving behaviour. Here we examined this possibility by testing whether the neuropeptide oxytocin (OT) motivates costly sanctioning of social exchanges that benefit or hurt ingroup members. Healthy males and females ($N$ = 100) self-administered a placebo (PL) or 24 IU of OT in a randomized, double- blind, between-subjects design: Participants witnessed social exchanges between ingroup (outgroup) members investing generously or fairly in ingroup (outgroup) trustees, who reciprocated generously, fairly or selfishly. For each exchange participants could, at a personal cost, sanction the investor and/or the trustee. Punishment (reward) was more likely for selfish (generous) behaviour when (a) investors were ingroup rather than outgroup, and (b) trustees were ingroup rather than outgroup, especially when (c) participants received OT rather than PL. Thus by motivating the punishment (rewarding) of ingroup harming (benefitting) behaviour, OT shapes parochial norms that enforce specific behaviours in response to ingroup or outgroup members. These results demonstrate that the social effects of OT are moderated by contextual factors and extend to third-party behaviour.

## Introduction

Humans are social animals and much of their evolutionary success has been attributed to their capacity to cooperate with others in social groups (Axelrod & Hamilton, 1981). Relative to other species, humans are more likely to cooperate with unfamiliar and genetically unrelated others who go on to form cohesive groups (Bowles & Gintis, 2003; Hill et al., 2011) with distinct, group-serving norms, traditions, and cultural practices (Fehr & Fischbacher, 2004a; Mesoudi, 2016; Seyfarth & Cheney, 2014). Indeed no matter how distinct group norms and traditions may be, one common function underlying many of these aspects is to steer group members away from self- interests and towards group-serving, cooperative behaviour (Jetten et al., 1996, 1997; Tyler & Fagan, 2008). Accordingly, norm abiding and ingroup benefitting behaviour is commonly appreciated and sometimes rewarded, whereas norm violations and selfishness are typically frowned upon and often punished (Balliet & Van Lange, 2013).

Group-living provides fitness functionality to its individual members, and it stands to reason that over evolutionary time humans have become biologically prepared for group-serving behaviour, such as costly cooperation and norm compliance (Axelrod & Hamilton, 1981; Burnham & Johnson, 2005; Fehr & Fischbacher, 2004a). Resonating with this theory is research linking group-serving behaviour to OT, an evolutionarily ancient neuropeptide that plays an important role in social bond formation and maintenance (Carter, 2014; Donaldson & Young, 2008; Meyer- Lindenberg, Domes, Kirsch, & Heinrichs, 2011). In group-living species such as prairie voles, meerkats, and primates (including humans), elevated OT concentrations are associated with an increased ability to discriminate between familiar and unfamiliar others (De Dreu, 2012b; Ferguson et al., 2002; Macbeth, Lee, Edds, & Young, 2009; Rimmele, Hediger, Heinrichs, & Klaver, 2009), prosocial approach especially towards those seen as familiar and ingroup (as

opposed to unfamiliar or outgroup) (De Dreu, Greer, Van Kleef, et al., 2011; De Dreu & Kret, 2016; Declerck, Boone, & Kiyonari, 2010), and with enhanced willingness to protect and defend one's group and territory (Bosch, 2013; De Dreu et al., 2012; Goodson, Schrock, & Kingsbury, 2015).

Whereas evidence suggests that OT shifts an individuals' focus from their self-interests towards those of their group (Fehr & Fischbacher, 2004a), it is unknown whether (and indeed how) OT also modulates the willingness to police and enforce such group-serving behaviours in others, and in particular in one's ingroup. In general, such norm-enforcing tendencies are well-documented and functional for group-living, especially within-group cooperation. By policing behaviours that are disadvantageous to, or defy the social norms of, the group, group members are kept from straying into selfish or exploitive behaviour that endangers the functionality of the group and reduces group efficiency (Gintis, 2000; Gintis, Bowles, Boyd, & Fehr, 2003).

Experimental work on third-party punishment supports the possibility that humans are willing to engage in such policing and norm-enforcing behaviour (Fehr & Fischbacher, 2004b; Nikiforakis & Mitchell, 2014). In these experiments, participants typically witness an exchange between two other individuals, one of whom is exploiting (or benefitting) the other. Participants are given an endowment that is valuable to them, and allowed to use parts or all of this endowment to punish the perpetrator (and sometimes to reward the victim). The participant is not personally involved, and there are no consequences of the observed social exchange, except that using their endowment to punishment (or reward) is personally costly. In purely economic terms then it is not in the individual's immediate self-interest to punish others for selfishness, or to reward others for their generosity. Nevertheless there is converging evidence from different lines of research that participants do punish, at a personal cost, selfishness in others, and to a lesser extent, reward

cooperation and generosity in others (Almenberg, Dreber, Apicella, & Rand, 2011; Fehr & Fischbacher, 2004b; Fehr & Gächter, 2002; Hu et al., 2016; Nikiforakis & Mitchell, 2014). This third-party punishment can increase within-group levels of cooperation and reduce group members' tendencies to defect (Dreber, Rand, Fudenberg, & Nowak, 2008; Egas & Riedl, 2008; Fehr & Gächter, 2002; Yamagishi, 1986). Furthermore, such third-party punishment may be ingroup biased: Costly punishments are given more readily when the 'victim' of the selfish behaviour is an ingroup rather than outgroup member (Baumgartner et al., 2013; McAuliffe & Dunham, 2016; Mifune, Hashimoto, & Yamagishi, 2010; Shinada, Yamagishi, & Ohmura, 2004).

### *The Present Study*

The present study aimed to replicate this general effect, and to explore whether similar patterns emerge when individuals acting as third-parties can punish selfishness and reward generosity. Second, and of greater interest, we examined whether the sanctioning of others' selfish and generous behaviour within and between groups is modulated by OT. We predicted that the extent to which individuals given OT (versus PL) engage in costly punishment (and reward) would be moderated by the target's group membership, in that they would be more motivated to sanction ingroup targets than outgroup targets.

## Methods

### *Participants and Ethics*

One hundred participants took part in the study carried out at the University of Amsterdam; one participant was dropped from the analysis due to missing data, leaving 49 participants in the OT condition, and 50 participants in the PL condition, with a mean

age of 21.83 years (*SD* = 3.12). Age did not differ between conditions, $t(97) = .863$, $p > .250$. To estimate the required sample size for this study, we relied on effect sizes reported in earlier studies on OT and ingroup bounded cooperation (De Dreu et al., 2010, Experiment 1 and 2, [partial] eta-squared = 0.154 and 0.122, respectively; Ten Velden et al., 2016: [partial] $\eta^2 = 0.048$). Using these observed eta-squared as inputs in G-Power 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007), with $\alpha = 0.05$ and $\beta = 0.80$, yielded a required sample for between-within interactions in ANOVA of 62, 88, and 108, respectively. Since the last power estimate was based on a study involving male and female participants, and the current study also targeted a mixed gender sample, we aimed to recruit at least 100 participants. This fits the power estimate and sample size of another recent study in which both male and female participants carried out evaluations/assessments rather than a decision-making task (De Dreu, Kret, & Sauter, 2016) following intranasal administration of OT or a PL.

We used stratified sampling in the present study to achieve an almost equal ratio of females to males across conditions (OT = 32:17 vs. PL = 32:18). Female participants' menstrual cycle and oral contraceptive use as self-reported during medical screening (Follicular phase: n = 24; Luteal phase: n = 35; female participants on oral contraceptives: n = 37) did not influence results or conclusions, and will not be discussed further.

Participants were recruited via an online system, which described the study as investigating the effects of medication on decision making. Participants were offered a monetary reward of €10 for their time, in addition to any earnings accrued during the study. Participants' earnings were determined by decisions made by fellow participants, a fact that was made salient as a result of group testing; however participants were made aware in the instructions that although their financial pay-off from the game was dependent on real decisions, the partner with whom their answers would be matched

might not be present in the lab at the same time, and thus participants were paid at a later date. Exclusion criteria were having a significant physical or psychiatric illness, assessed by medical screening prior to participation (see Appendix 4 for a flow diagram of the study set up).

The study was approved by the University of Amsterdam Ethics Committee (file 2015-WOP-4100), and adhered to the Declaration of Helsinki. Participants gave written informed consent prior to the study, and received full debriefing upon completion of the experiment. The study did not involve deception and was fully incentivized.

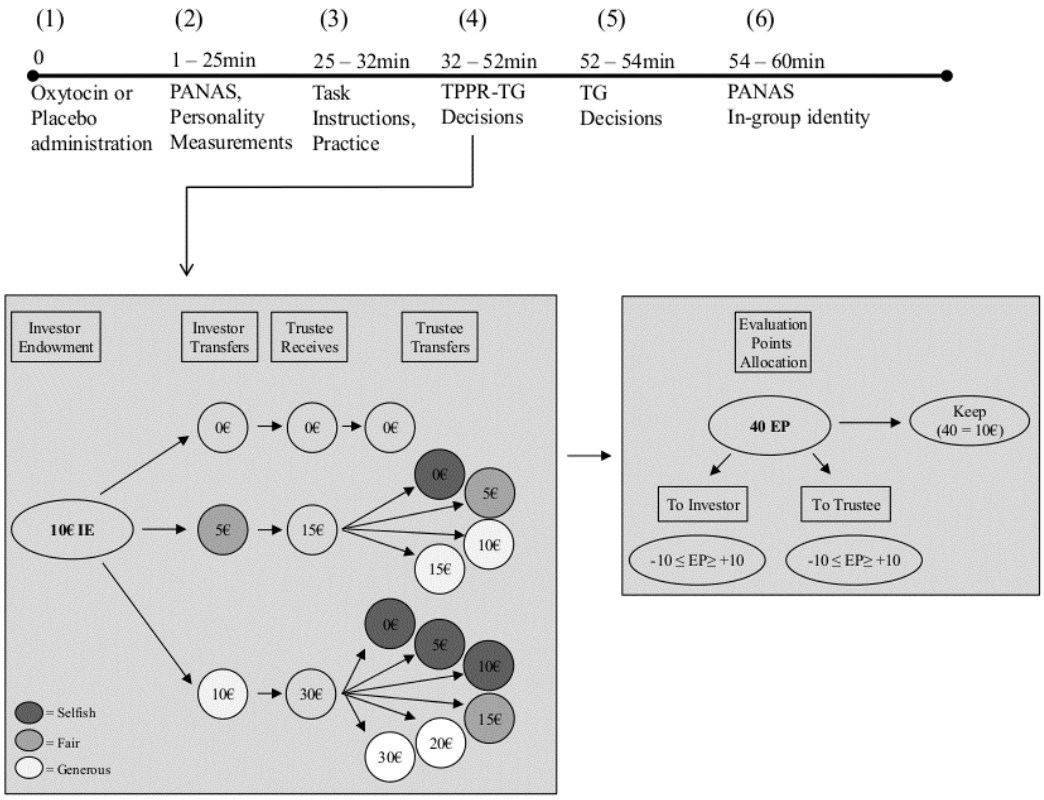
*Materials*

*Third-Party Sanctioning Task*

The computerized instructions began by randomly assigning participants to one of two groups, "Team A" or "Team B". Next, the Third-Party Punishment and Reward Trust Game (TPPR-TG) was explained (see Figure 7.1), and participants completed three practice trials to ensure they understood the decision-making task. A decision trial consisted of a participant being shown an exchange between an investor (i.e., the transfer) and the trustee (i.e., the back-transfer). Participants were then given the opportunity to assign plus (reward) or minus (punishment) points, first to the investor and then to the trustee. For each decision, the participant received a 20-point endowment (from -10 to +10), with each point being worth €0.25. Assigning points was therefore costly: if participants assigned 0 points their pay-off from the trial would be €5, but if they assigned ±10 points their pay-off would be only €2.50. Points assigned were tripled by the experimenter, converted into their financial value, and then added to (or subtracted from) the target's account. The target account was calculated and paid after the study. Therefore, assigning punishing or rewarding points had financial implications for both the participant

and the target.

*Figure 7.1 - A graphical representation of the study procedure and the Third-Party Punishment and Reward Trust Game (TPPR-TG)*




Each decision trial involved an ingroup (or outgroup) investor and an ingroup (or outgroup) trustee, yielding four possible pairings. In addition, investors' transfers and trustees' back-transfers could be generous, fair or selfish. Because there were four dyads, two additional members of the group (i.e., A1 and A2), and 11 possible exchange types (see below), for which the participant had to make two sanctioning decisions (one for the investor and one for the trustee), participants completed 132 sanctioning decisions. For

intragroup dyads participants completed 1 trial (and therefore 2 decisions) per exchange type. For intergroup dyads participants completed 2 trials (and therefore 4 decisions) per exchange type and we computed the average across these exchange types.

The 11 exchange types were as follows: when the investor transferred 0 and the trustee back-transferred 0; when the investor transferred 5 and the trustee back-transferred 0, 5, 10 or 15; and when the investor transferred 10 and the trustee back-transferred 0, 5, 10, 15, 20, or 30; see Figure 7.1.

To incentivize the task and avoid deception, participants completed the experimental task by making six decisions in a direct Trust Game – two as the investor (once playing with an ingroup trustee, once playing with an outgroup trustee), and four as the trustee (with a generous [or fair] ingroup [or outgroup] investor). As described in the participant's instructions, behaviour in the Trust Game would be coupled to a randomly chosen third-party decision trial that matched the exchange in question, so each participant's pay-off was dependent on decisions made by other participants.

*Personality Measures*

Participants completed a series of questionnaires including the Interpersonal Reactivity Index (IRI; Davis, 1983; Empathic Concern $\alpha = 0.61$; Fantasy $\alpha = 0.75$; Perspective Taking $\alpha = 0.76$; Personal Distress $\alpha = 0.76$), the State-Trait Anxiety Inventory (STAI; Spielberger, 1983; State STAI $\alpha = .89$; Trait STAI $\alpha = 0.93$), the Positive and Negative Affect Schedule (PANAS; Watson et al, 1988; PA $\alpha = 0.74$; NA $\alpha = 0.85$), and the Ingroup Identity questionnaire (Leach et al., 2008; Cronbach's alpha = .95) . There were no effects of condition on any personality measure (see Supplementary Information 4, p. 136, for details).

*Motivations*

Based on previous literature (Baumgartner et al., 2013), participants were asked about the

motivation behind their sanctioning behaviour. Participants were asked to rate on a 7-point scale (1 = not motivated by this at all, 7 = very motivated by this) to what extent their sanctioning was motivated by the following reasons: 'to improve future behaviour'; 'in retaliation'; 'out of sympathy'; or 'to achieve fairness'.

Given that participants completed 132 trials (and in line with Baumgartner et al.), participants were only asked to rate their motivations after two specific exchanges: after a fair transfer with a generous back-transfer; and after a generous transfer with a selfish back-transfer. Participants were asked to report their motivations for these exchanges for all four group dyads, resulting in a total of 48 trials.

*Procedure*

Participants were asked to refrain from consuming drugs or alcohol the night before the study, and from smoking or drinking caffeine in the 2 hours prior to the study. Using a double-blind procedure, participants were assigned to either the OT or the PL condition. They self-administered a PL or 24 IU (3 puffs of 4 IU per nostril) of Syntocinon (synthetic OT spray, Novartis). The PL spray matched the OT spray with respect to all ingredients apart from the synthetic OT (De Dreu et al., 2010).

On arrival, participants were seated in individual cubicles so that they could not see or speak to one another. After providing informed consent, they self-administered the nasal spray under the supervision of the experimenter, who then unlocked the computer; the remainder of the experiment was self-guided. In the first 25 minutes, participants completed a series of questionnaires, including the IRI, STAI and the first measure of PANAS. This 'wait period' is the typical length used in OT administration studies (Kret & De Dreu, 2013; Shalvi & De Dreu, 2014; Ten Velden et al., 2014), and research has demonstrated physiological effects of intranasal-oxytocin (IN-OT) after this load time

(Daughters et al., 2015; Weisman, Zagoory-Sharon, & Feldman, 2012). The computer automatically began the instructions for the task after this wait period. After completing the task participants completed the second PANAS measure and the Ingroup Identity questionnaire.

*Data Analysis*

Two mixed ANOVAs, one for sanctioning behaviour towards the investor and one for sanctioning behaviour towards the trustee, were carried out to assess the effect of condition, group membership and exchange type on participants sanctioning behaviour. A mixed ANOVA was carried out to assess the effect of condition, group membership and exchange type on participants' motivations for their sanctioning behaviour. Finally, independent t-tests were carried out to assess the effect of condition on participants transfers and back-transfers as an investor and trustee in the Trust Game, respectively.

## Results

Exploratory analyses including participants' gender were conducted. Although gender influenced third-party decision-making, gender did not interact with condition. Accordingly, findings for condition are similar for male and female participants and the models including main and interaction effects for gender are described in Supplementary Information 4, p. 139. Analyses in which we controlled for personality measures did not change the results or conclusions, and are not considered further.

*Third-Party Sanctioning of Investors*

A 2 (Condition: oxytocin vs placebo) x 2 (Investor group: ingroup vs outgroup) x 2

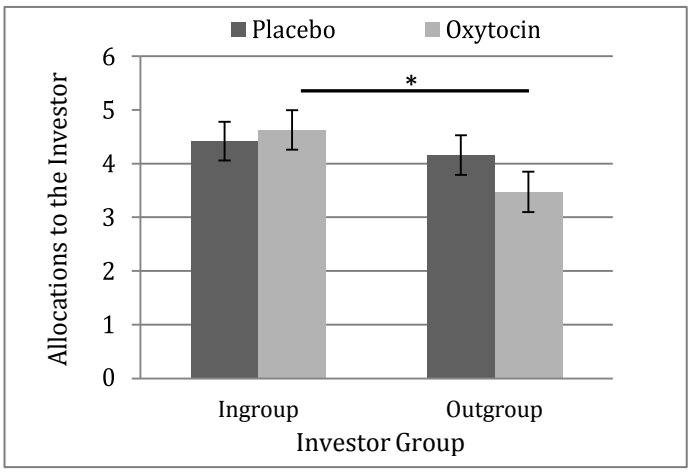
(Trustee group: ingroup vs outgroup) x 2 (Investor's transfer: generous vs fair) x 3 (Trustee's back-transfer: generous vs fair vs selfish) mixed ANOVA, with the first two factors being between-subjects, and the remaining factors being within-subjects was carried out on participants sanctioning decisions for investors. Interaction effects were decomposed using simple effects analysis that preserve the overall error term and degrees of freedom (Winer, Brown, & Michels, 1971). Accordingly, *p*-values do not have to be corrected for multiple testing as we fitted the data only once, and the most robust estimate of specific contrasts is obtained (Rosenthal & Rosnow, 1985; Tatsuoka & Lohnes, 1988; Winer et al., 1971). Because in several interactions involving within- subjects factors the Mauchly's Test of Sphericity was significant (*p*s < 0.0001) the hypothesis that within-factor error terms are correlated could not be rejected. We thus relied on the more robust yet also more conservative multivariate rather than mixed- model *F*-tests (Tatsuoka & Lohnes, 1988).

There was a main effect of transfer, $F(1, 97) = 100.399$, $p < .001$, $\eta^2_p = .514$, generous transfers were rewarded more ($M = 5.587$, $SE = .317$) than fair transfers ($M = 2.752$, $SE = .251$); and a main effect of back-transfer, $F(1.47, 139.42) = 31.724$, $p < .001$, $\eta^2_p = .250$, investors received higher rewards when their trustee's back-transfers were selfish ($M = 4.827$, $SE = .240$) rather than fair ($M = 4.131$, $SE = .266$) or generous ($M = 3.550$, $SE = .287$); and a main effect of ingroup group membership, $F(1,97) = 15.58$, $p < .001$, $\eta^2 = .138$, ingroup investors were rewarded more ($M = 4.523$, $SE = 0.256$) than outgroup investors ($M = 3.816$, $SE = 0.264$).

The main effect for investor group membership was qualified by an interaction with condition, $F(1, 95) = 6.101$, $p = .015$, $\eta^2 = .059$ (see Figure 7.2). Simple effects analysis revealed that whereas participants in the PL condition did not discriminate between ingroup and outgroup investors, $F(1, 95) = 1.10$, $p = .297$, those who received

OT rewarded ingroup investors more than outgroup investors, $F(1, 95) = 20.385$, $p < .001$. The condition x investor's group membership interaction was further qualified by an interaction between condition, investor's group membership, trustee's group membership, and trustee's back-transfer, $F(2, 94) = 4.593$, $p = .012$, $\eta^2 = .087$. In keeping with the nature of the condition x investor's group membership effect, we decomposed this complex effect using simple effects analysis for the (interactions among) condition, investor's group membership, and trustee's group membership within each level of the trustee's back-transfer. Because effects were tested three times (for each level of back-transfer), we corrected for multiple comparisons by setting $\alpha = 0.05/3 = 0.015$ as the critical p-value.

*Figure 7.2 - Mean allocations to investors as a function of investor group and condition (± SE)*




When the trustee's back-transfers were selfish, ingroup investors received more when their selfish trustee was outgroup rather than ingroup (investor's x trustee's group membership, $F[1, 95] = 6.73$, $p = .001$). Furthermore, ingroup investors were rewarded more than outgroup investors, $F(1, 95) = 14.71$, $p = .001$, $\eta^2 = .226$, only when

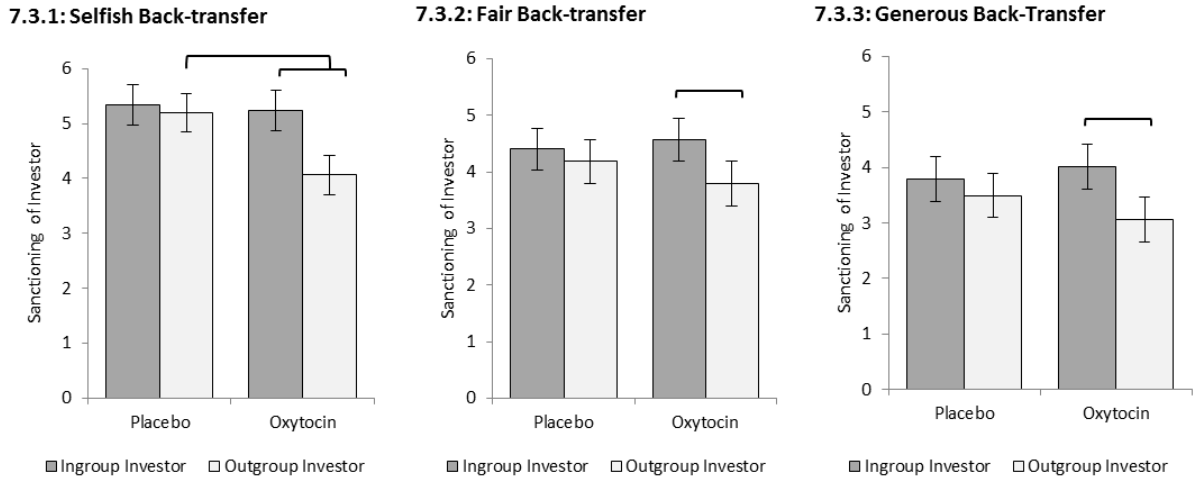
participants received OT, $F(1, 95) = 15.85$, $p = 0.001$, and not when they received PL, $F(1, 95) = 0.24$, $p = .625$ (Figure 7.3.1; overall investor's group membership x condition, $F[1, 95] = 8.18$, $p = .005$).

When the trustee's back-transfers were fair, the interaction among investor's and trustee's group membership was not significant, $F < 1$. However, as with selfish back-transfers, ingroup investors were rewarded more than outgroup investors, $F(1, 95) = 11.78$, $p = .001$, again only when participants received OT, $F(1, 95) = 9.32$, $p = .003$, and not when they received PL, $F(1, 95) = 0.79$, $p = .376$ (Figure 7.3.2; overall investor's group membership x condition, $F[1, 95] = 3.84$, $p = .053$; marginal).

When the trustee's back-transfers were generous, the interaction among investor's and trustee's group membership was again not significant, $F < 1$. Yet here too, ingroup investors were rewarded more than outgroup investors, $F(1, 95) = 17.05$, $p = .001$, when participants received OT, $F(1, 95) = 18.18$, $p = .001$, and not when they received PL, $F(1, 95) = 1.84$, $p = .178$ (Figure 7.3.3; overall investor's group membership x condition, $F[1, 95] = 5.40$, $p = .022$).

Taken together, participants who received OT rather than PL rewarded ingroup investors more than outgroup investors. This condition x investor's group membership interaction was nominally significant at all three levels of the trustee's back-transfer, yet strongest (and surpassed the Bonferonni-corrected threshold) when back-transfers were selfish. Thus, when trustee's back-transfers were selfish, participants given OT compensated their investors but less so when investors were from the outgroup.

*Figure 7.3 - Sanctioning of investors as a function of investor group membership and condition for each level of trustee back-transfer (± SE)*




*Third-Party Sanctioning of Trustees*

The same 2 x 2 x 2 x 2 x 2 x 3 mixed ANOVA was carried out on participants' sanctioning decisions for trustees. There was a main effect of trustee group members, $F(1, 95) = 11.51$, $p = .001$, $\eta^2 = .108$, such that outgroup trustees were rewarded less ($M = 0.989$, $SE = 0.207$) than ingroup trustees ($M = 1.623$, $SE = 0.215$); a main effect of transfer, $F(1, 95) = 124.90$, $p < .001$, $\eta^2 = .568$, generous transfers were rewarded more ($M = 2.161$, $SE = 0.213$) than fair transfers ($M = 0.452$, $SE = 0.195$); and a main effect of back-transfer, $F(2, 94) = 199.687$, $p < .001$, $\eta^2 = .753$, such that generous back- transfers were rewarded more ($M = 5.914$, $SE = 0.304$) than fair back-transfers ($M = 2.219$, $SE = 0.298$), which were rewarded more than selfish back-transfers ($M = -4.213$, $SE = 3.29$).

These main effects were qualified in two two-way interactions among investor's group membership and trustee's back-transfer, $F(2, 94) = 8.397$, $p = .001$, $\eta^2 = .113$, and trustee's group membership and trustee's back-transfer, $F(2, 94) = 4.34$, $p = .016$, $\eta^2 = .062$. These were further qualified in two three-way interactions among investor's group

membership, trustee's back-transfer, and condition, $F(2, 94) = 3.074$, $p = .051$, $\eta^2 = .049$, and trustee's group membership, trustee's back-transfer, and condition, $F(2, 94) = 2.586$, $p = .081$, $\eta^2 = .036$ (marginal) and finally in a four-way interaction among investor's group membership, trustee's group membership, trustee's back-transfer, and condition, $F(2, 94) = 3.850$, $p = .025$, $\eta^2 = .062$. As with results for sanctioning of investors, we probed the nature of these effects with simple effects for (interactions among) investor's group membership, trustee's group membership, and condition within each level of the trustee's back-transfer. Because simple main and interaction effects were estimated three times (for each level of back-transfer), we corrected for multiple comparisons by setting $\alpha = 0.05/3 = 0.015$ as the critical p-value.

When back-transfers were selfish, selfish trustees were punished more when they were from the outgroup rather than from the ingroup, $F(1, 95) = 13.10$, $p = .001$, $\eta^2 = .115$. Furthermore, trustees were punished more when their investor was from the ingroup, rather than the outgroup, $F(1, 95) = 8.63$, $p = .004$, $\eta^2 = .084$. Although the condition x investor's group membership was not significant, $F(1, 95) = 1.58$, $p = .212$, it can be seen in Figure 7.4.1 that effects of investor's group membership were strong and significant when participants received OT, $F(1, 95) = 9.28$, $p = .003$, rather than PL, $F(1, 95) = 1.35$, $p = .247$.

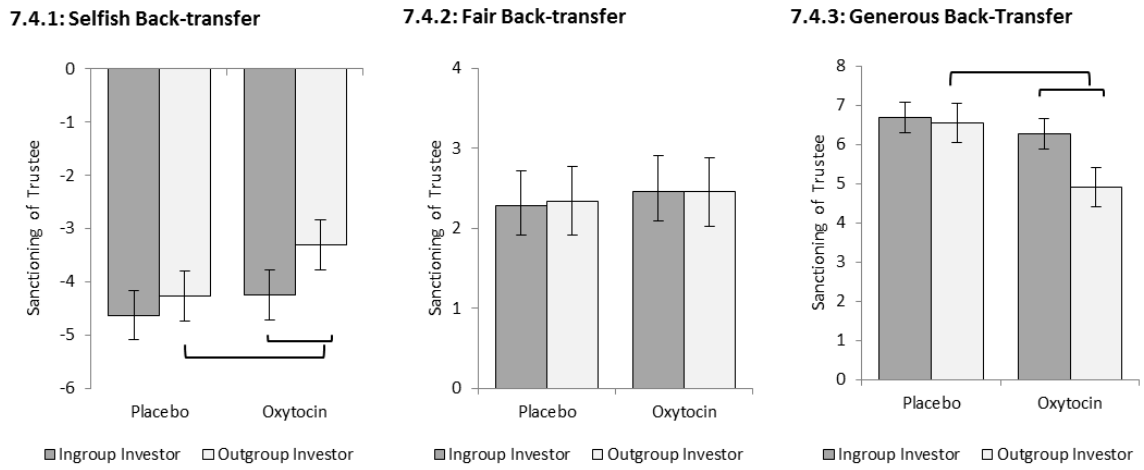
When back-transfers were fair, ingroup trustees were rewarded more than outgroup trustees, $F(1, 95) = 10.47$, $p = .002$, $\eta^2 = .086$; there was some evidence that this effect was particularly strong when investors were ingroup and participants received PL, and when investors were outgroup and participants received OT, $F(1, 95) = 4.43$, $p = .038$, $\eta^2 = .027$ (Figure 7.4.2). However, the effect falls above the Bonferroni-corrected threshold and none of the underlying contrasts were (Bonferroni- corrected) significant. We refrain from further interpreting this finding.

When back-transfers were generous, trustees were rewarded more when they faced

ingroup rather than outgroup investors, F(1,95) = 12.22, $p$ = .001, $\eta^2$ = .098, but only when participants received OT, F(1,95) = 17.24, $p$ = .001, and not PL, F(1,95) = 0.17, $p$ = .680 (Figure 7.4.3; overall investor group membership x condition, F[1,95] = 6.86, $p$ = .010, $\eta^2$ = .068).

Taken together, trustees were sanctioned less when they were from the ingroup, and when interacting with an ingroup rather than an outgroup investor. OT modulated this when back-transfers were generous and, to a lesser extent, when they were selfish. When trustee's back-transfers were generous, participants given OT rewarded these trustees but less so when the generously treated investors were from the outgroup.

*Figure 7.4 - Sanctioning of trustees as a function of investor group membership and condition for each level of trustee back-transfer (± SE)*




*Motivations for Third-Party Sanctioning*

A 4 (Motivation: improve future behaviour vs retaliate vs fairness vs sympathy) x 2 (Investor group: ingroup vs outgroup) x 2 (Trustee group: ingroup vs outgroup) x 2 (Exchange type: fair vs unfair) x 2 (Condition: oxytocin vs placebo) mixed ANOVA was carried out, with all factors apart from condition being within-subjects factors.

There was a main effect of motivation, $F(3, 97) = 14.384$, $p < .001$, $\eta^2_p = .129$, participants were significantly more motivated to achieve fairness ($M = 5.491$, $SE = .107$) compared to improving future behaviour ($M = 4.986$, $SE = .150$), retaliation ($M = 4.566$, $SE = .137$) or sympathy ($M = 4.770$, $SE = .135$); a main effect of exchange type, $F(1, 97) = 60.300$, $p < .001$, $\eta^2_p = .383$, participants were more motivated for unfair ($M = 5.234$, $SE = .109$) compared to fair ($M = 4.672$, $SE = .099$) exchanges; and a significant interaction between the two, $F(3, 291) = 57.223$, $p < .001$, $\eta^2_p = .371$, participants were significantly more motivated to improve future behaviour, achieve fairness and in retaliation for unfair exchanges, but there was no difference in sympathy. There was no main effect of investor group ($F(1, 97) = 2.605$, $p = .110$, $\eta^2_p = .026$), trustee group ($F(1, 97) = 1.162$, $p = .284$, $\eta^2_p = .012$) or condition ($F(1, 97) = .683$, $p = .411$, $\eta^2_p = .007$). There was a significant interaction between trustee group and exchange type, $F(1, 97) = 4.982$, $p = .028$, $\eta^2_p = .049$; and a trend towards a significant interaction between investor group and condition, $F(1, 97) = 3.784$, $p = .055$, $\eta^2_p = .038$.

These lower-order interactions were qualified by a number of higher-order interactions, which ultimately ended in a significant 4-way interaction between motivation, exchange type, investor group and condition, $F(3, 291) = 6.762$, $p < .001$, $\eta^2_p = .065$. In keeping with the same analytic approach for sanctioning behaviour and the (trend towards a significant) interaction between investor group and condition, we decomposed this 4-way interaction using simple effects for the interactions among condition, investor group and exchange type for each type of motivation. Because there were four different motivations we corrected for multiple comparisons by setting $\alpha = 0.05/4 = 0.020$.

Participants who were given OT were less motivated by sympathy, $F(1, 97) =$

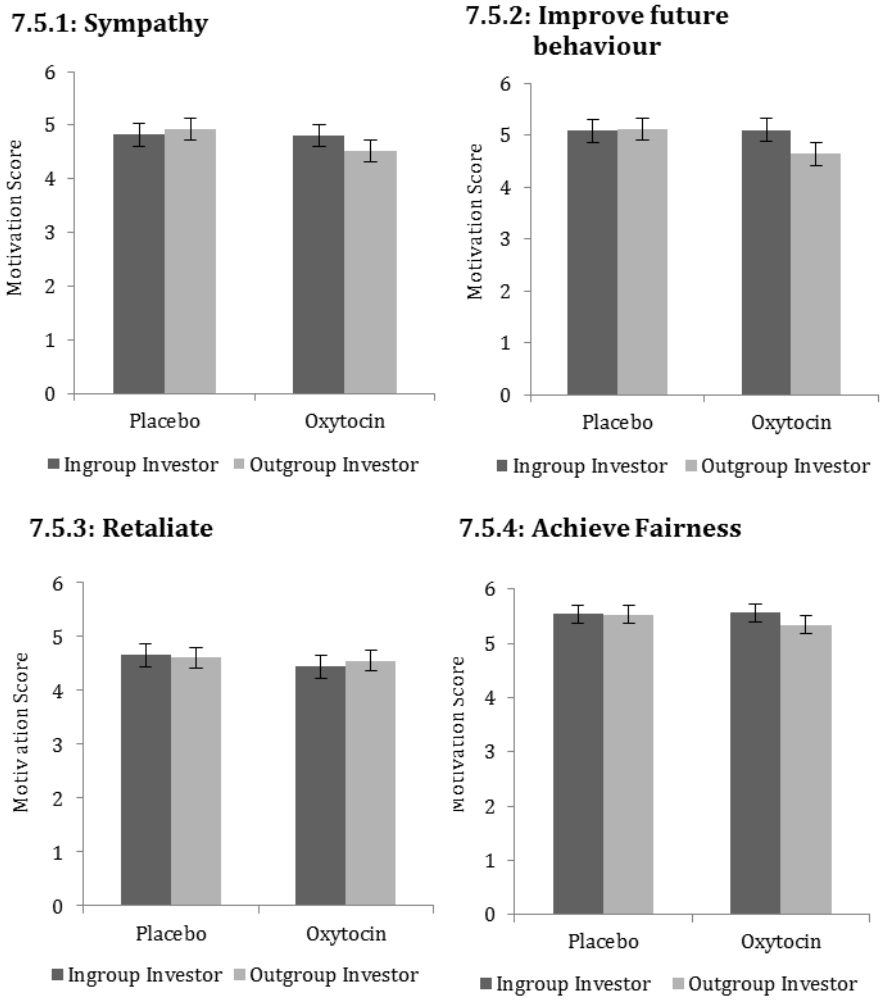
4.273, $p = .041$ (Figure 7.5.1), however this was not significant after correction, but were significantly less motivated to improve the future behaviour, $F(1, 97) = 14.953$, $p < .001$ (Figure 7.5.2), of an outgroup investor compared to an ingroup investor. Participants given OT were less motivated to achieve fairness when the investor was an outgroup members compared to an ingroup member, however this also did not reach statistical significance, $F(1, 97) = 3.375$, $p = .069$ (Figure 7 .5.4). Finally, participants given OT did not differ in their motivation to retaliate, $F(1, 97) = 0.733$, $p = .394$ (Figure 7.5.3). Participants who were given PL were equally motivated across all motivations for ingroup and outgroup investors (sympathy: $F(1, 97) = 0.544$, $p = .463$; improve future behaviour: $F(1, 97) = 0.102$, $p = .750$; fairness: $F(1, 97) = 0.015$, $p = .903$; retaliate: $F(1, 97) = 0.112$, $p = .738$).

Taken together, OT had an effect on the motivations behind participants sanctioning behaviour. Participants who received OT were less motivated for exchanges with outgroup investors compared to ingroup investors, except for retaliation when participants given OT reported greater motivation for exchanges with outgroup compared to ingroup investors.

*Investment Behaviour in the Trust Game*

There was no effect of condition on participants average transfer made as an investor, during the Trust Game, $t(97) = -.977$, $p > .250$ (OT M = 2.35, SD = .50; PL M = 2.45, SD = .55). Replicating the same descriptive analysis reported in the original Kosfeld et al. (2005) paper there were also a similar number of participants giving the highest investment (in this study the maximum invest made was 3 out of 10) in each condition: 33% in the OT condition and 38% in the PL condition (see Figure 7.6); compared to 45% in the OT condition and 21% in the PL condition reported in Kosfeld et al. (2005).

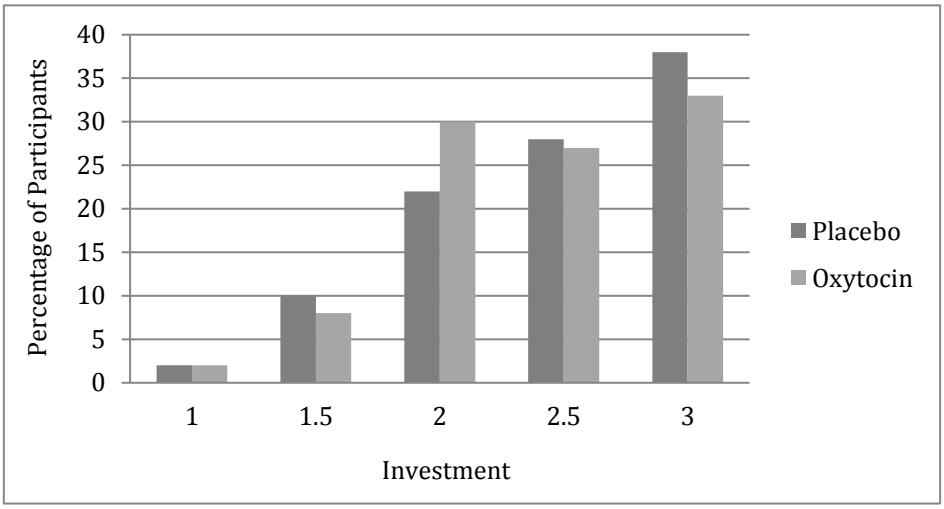
*Figure 7.5 - Motivations behind sanctioning as a function of investor group membership and condition for each motivation (± SE)*




There was also no effect of condition on participants average back-transfer as a trustee, $t(97) = 1.00$, $p > .250$ (OT M = 3.14, SD = .64; PL M = 3.01, SD = .68). The maximum return made was 4.5, however, most participants returned less than this. Therefore participants were grouped into those that returned a low amount, and those that returned a high amount (median split). The frequency of participants offering a high offer was almost identical for participants in the PL condition, 74%, and participants in the OT condition, 73.5%. Kosfeld et al. (2005) did not report trustee behaviour thus no

comparable values can be reported.

*Figure 7.6 –Frequency of investors transfers as a function of condition*




## Discussion

We investigated whether OT moderated participants' sanctioning of the generous and selfish behaviour of others, and how this was affected by group membership. In line with our predictions, OT (rather than PL) increased ingroup bounded sanctioning, such that participants who were given OT punished selfish behaviour and rewarded generous behaviour that harmed or benefitted (respectively) the ingroup to a greater extent than participants who were given a PL. This effect is driven by a relative indifference to exchanges involving two outgroup members compared to exchanges between ingroup and outgroup members. We therefore conclude that OT increases ingroup bounded sanctioning, and thereby promotes group-serving as opposed to self-serving behaviours.

Findings provide further evidence that OT does not make people ubiquitously more prosocial. This would have manifested in overall reduced punishment and increased reward under OT, which we did not observe. Neither did we observe increase investment

or return under OT during the original Trust Game. In addition, current findings clarify that OT does not induce unconditional prosocial treatment of ingroup members. This would have manifested in reduced punishment and increased rewarding of ingroup members only, something we did not observe. Instead, findings support the idea that the function of third-party sanctioning is to regulate people's adherence to group norms (Chavez & Bicchieri, 2013; Fehr & Fischbacher, 2004b), that OT enhances intergroup discrimination (Ten Velden et al., 2014), and that OT shifts the individual's focus towards the ingroup (De Dreu, 2012a; De Dreu & Kret, 2016; Stallen, De Dreu, Shalvi, Smidts, & Sanfey, 2012; Ten Velden, Daughters, & De Dreu, 2016). Findings also demonstrate that this 'OT shift' is activated in a minimal group paradigm, providing support for the evolutionary function of OT in group behaviour via a biological mechanism. It follows that the same pattern of results should also be observed in a real groups paradigm, a potential study for future research.

In addition results present an interesting theoretical overlap with previous findings regarding ingroup bounded sanctioning. Baumgartner et al. (2013) found that such effects were diminished by disruption to the right Temporo-Parietal Junction (rTPJ), whereas in the present research these effects were enhanced by administering IN-OT. This suggests that there may be a functional overlap between the neuropeptide and the rTPJ, a possibility that recent research has found supporting evidence for (Hu et al., 2016), but one that could also be investigated in future research. Importantly, the present study extends the work of Baumgartner and Hu by incorporating IN-OT into a third-party paradigm that included an ingroup/outgroup factor, thereby enabling us to investigate the effects of OT on the behaviour of a third-party in an intergroup context.

We find that OT has a similar effect on motivations for sanctioning (in line with our predictions). OT decreased motivation to improve players' future behaviour if the

exchange involved an outgroup member. Similarly, OT decreased participants' sympathy as a motive for their sanctioning when the exchange involved an outgroup investor, however this did not survive correction. To sum, OT also increased ingroup bounded motivation.

Despite the strengths of our study, it also has some limitations. The study included a direct manipulation of OT, via nasal spray, but did not include a measure of endogenous OT. Although this method permits conclusions about cause and effect relationships, which would not be possible if one were correlating endogenous OT with sanctioning behaviour. The disadvantage, however, is that the neurophysiological pathways through which IN-OT affects brain activity and behavioural responses are not fully understood. Although there is good evidence that IN-OT increases the concentration of endogenous OT found in blood plasma and saliva (Daughters et al., 2015; Gossen et al., 2012; Weisman et al., 2012), the evidence that IN-OT crosses the blood-brain barrier is limited (Neumann et al., 2013; Paloyelis et al., 2014; Striepens et al., 2013). In addition to a direct effect on the brain, IN-OT may also affect brain and behavioural responses through its peripheral effects on the body (e.g., by affecting heart rate, or cortisol responses). Detailing these pathways is an important question for future research. Such new research may also consider the notion that individual differences exist in the peripheral responses to intranasal administration of OT (Daughters et al., 2015). For example, one could investigate whether such individual differences, in turn explain individual differences in the extent to which people pursue ingroup bounded cooperation and uphold and enforce ingroup serving norms. Future research could also consider adding a greater number of trials for each exchange type and dyad to further improve the reliability of the findings. Finally, participants in the current study were undergraduates. Because recent neuroimaging studies suggest that the regions of the brain involved in social behaviour

(including the TPJ) are still developing into early adulthood (Mills, Lalonde, Clasen, Giedd, & Blakemore, 2014), our results may not be generalisable to younger or older populations. Investigating whether the effects of OT in intergroup contexts are independent of the development of the 'social brain' is another important avenue for future research.

*Conclusions*

In conclusion, our results provide important evidence that OT increases the propensity to uphold parochial norms. These results support the tend-and-defend hypothesis (De Dreu, 2012b) and more generally the theory regarding the role of third-party behaviour in enforcing social norms (Fehr & Fischbacher, 2004b). Participants who were given OT demonstrated ingroup bounded sanctioning and motivations for their sanctioning. Our results fit the theory that humans are biologically prepared for group-living, and that OT is co-opted to promote parochial norms and thereby increasing the effectiveness of groups.

## Supplementary Information 4

*Effects of mood and personality measures*

A MANOVA was conducted on both subscales of the STAI and PANAS and revealed no multivariate effects for the gender by treatment interaction, $F(4, 90) = 1,94$, $p = 0.11$, or the gender and treatment main effects, $F(4, 90) = 1.45$, $p = 0.223$ and $F(4, 90) = 1.21$, $p = 0.331$. It follows that treatment alone or in interaction with gender did not produce pre-experimental task differences in mood states.

A MANOVA was also conducted on all four IRI subscales and showed no multivariate treatment x gender effect, $F(4, 90) = 1.20$, $p = 0.315$. Although the multivariate treatment effect was not significant, $F(4, 90) = 2.033$, $p = 0.096$, participants given oxytocin scored higher on empathic concern ($M = 5.18$ vs. $M = 4.83$; $F[1, 95] = 4.803$, $p = 0.031$), and perspective taking ($M = 5.00$ versus $M = 4.76$, $F[1, 95] = 5.42$, $p = 0.22$). MANOVA revealed a strong effect for gender, $F(4, 90) = 4.828$, $p = 0.001$: Females scored higher on empathic concern ($M = 4.86$ versus $M = 5.09$, $F[1, 95] = 16.26$, $p= 0.001$) and personal distress ($M = 4.14$ versus M = 4.60, $F[1, 95] = 7.68$, $p = 0.007$). To see whether treatment effects on empathy and perspective taking relate to sanctioning tendencies in the experimental task, we correlated within each treatment condition empathy and perspective taking with sanctioning of in-group and out-group investors when back-transfers were selfish, fair, and generous . Table S1 shows no significant correlations emerged whatsoever. Table S2 reports the same analyses for sanctioning of trustees and with two exceptions, shows no significant relations either.

Finally, there were no effects on in-group identification, all $F(1, 95) < 1$, $p$s $< 0.50$ (range 1 – 5; overall $M = 3.14$, $SD = 1.18$). The mean level on this scale suggests that, overall, participants had moderate levels of identification with their in-group.

*Table S1*. Correlations between pre-task empathy and perspective taking scores and sanctioning of in-group and out-group investors when back-transfers were selfish, fair, or generous.

| | Back Transfer | | | | | |
|---|---|---|---|---|---|---|
| | Selfish | | Fair | | Generous | |
| Investor | In-Gr | Out-Gr | In-Gr | Out-Gr | In-Gr | Out-Gr |
| Oxytocin | | | | | | |
| IRI-Empathy | 0.179 | 0.050 | 0.175 | 0.201 | 0.186 | 0.202 |
| IRI-Perspective | -0.160 | -0.007 | -0.178 | 0.029 | -0.156 | -0.057 |
| Placebo | | | | | | |
| IRI-Empathy | -0.004 | 0.143 | -0.060 | 0.094 | -0.156 | -0.008 |
| IRI-Perspective | 0.038 | 0.089 | 0.066 | 0.069 | -0.050 | -0.060 |

*Table S2*. Correlations between pre-task empathy and perspective taking scores and sanctioning of in-group and out-group trustees when back-transfers were selfish, fair, or generous.

| | Back Transfer | | | | | |
|---|---|---|---|---|---|---|
| | Selfish | | Fair | | Generous | |
| Investor | In-Gr | Out-Gr | In-Gr | Out-Gr | In-Gr | Out-Gr |
| Oxytocin | | | | | | |
| IRI-Empathy | 0.074 | 0.085 | 0.175 | 0.227 | 0.177 | 0.150 |
| IRI-Perspective | -0.044 | -0.017 | -0.025 | -0.073 | -0.014 | -0.052 |
| Placebo | | | | | | |
| IRI-Empathy | -0.366* | -0.24 | -0.062 | 0.053 | 0.243 | -0.049 |
| IRI-Perspective | -0.134 | 0.165 | 0.076 | 0.216 | 0.044 | -0.156 |

*Note* * $p < 0.015$ (uncorrected)

*Gender interactions with third-party sanctioning of investors*

As noted in the main text, treatment and gender never interacted. However, gender did influence sanctioning behaviour of investors, as shown in the significant interaction between gender and transfer, $F(1, 95) = 4.729$, $p = 0.032$, $\eta_p^2 = 0.047$. Male participants ($M = 2.125$, SE = .403) rewarded fair investors less compared to female participants ($M = 3.380$, SE = .298); there was no gender difference for generous investors. This effect was qualified by a significant four-way interaction between gender, transfer, treatment, and investor group, $F(1, 95) = 5.117$, $p = 0.026$, $\eta_p^2 = 0.051$. The relevant means and standard errors are presented in Table S3. As can be seen, especially males given oxytocin discriminate between in-group and out-group investors who are generous. Finally, there was a complex and difficult to interpret four-way interaction between gender, back-transfer, investor group and trustee group, $F(1, 95) = 3.453$, $p = 0.035$, $\eta_p^2 = 0.035$ (see also Table S4).

*Gender interactions with third-party sanctioning of trustees*

There were no significant two-way or three-way interactions, but there were several four-way interactions with gender. There was a significant interaction between gender, investor group, trustee group and transfer, $F(1, 95) = 4.865$, $p = 0.030$, $\eta_p^2 = 0.049$ and gender, investor group, trustee group and back-transfer, $F(1, 95) = 4.009$, $p = 0.020$, $\eta_p^2 = 0.040$. There was also a four-way interaction between gender, transfer, back-transfer and treatment, $F(1, 95) = 3.281$, $p = 0.042$, $\eta_p^2 = 0.033$. These effects were neither predicted not very robust, and we refrain from interpreting them. For the reader's convenience we provide descriptive statistics in Table S5 and S6.

*Table S3.* Means and standard errors relating to the four-way interaction between gender, transfer, treatment, and investor group.

| Treatment | Transfer | Investor Group | Male | Female |
|---|---|---|---|---|
| **Placebo** | Fair | In-group | 1.903 (.607) | 3.686 (.455) |
| | | Outgroup | 1.380 (.618) | 3.622 (.463) |
| | Generous | In-group | 6.257 (.711) | 5.829 (.533) |
| | | Outgroup | 6.016 (.761) | 5.625 (.571) |
| **Oxytocin** | Fair | In-group | 3.137 (.625) | 3.469 (.455) |
| | | Outgroup | 2.078 (.636) | 2.742 (.463) |
| | Generous | In-group | 6.245 (.731) | 5.658 (.533 |
| | | Outgroup | 3.780 (.784) | 5.285 (.571) |

*Table S4.* Means and standard errors relating to the four-way interaction between gender, back-transfer, investor group and trustee group.

| Back-Transfer | Investor Group | Trustee Group | Male | Female |
|---|---|---|---|---|
| **Selfish** | In-group | In-group | 4.624 (.463) | 5.352 (.342) |
| | | Outgroup | 5.426 (.413) | 5.529 (.305) |
| | Outgroup | In-group | 4.281 (.429) | 5.066 (.317) |
| | | Outgroup | 3.138 (.551) | 5.203 (.407) |
| **Fair** | In-group | In-group | 4.311 (.476) | 4.492 (.352) |
| | | Outgroup | 4.285 (.451) | 4.695 (.334) |
| | Outgroup | In-group | 3.495 (.454) | 4.191 (.336) |
| | | Outgroup | 3.002 (.563) | 4.578 (.416) |
| **Generous** | In-group | In-group | 3.736 (.512) | 3.809 (.379) |
| | | Outgroup | 3.931 (.484) | 4.088 (.357) |
| | Outgroup | In-group | 3.366 (.474) | 3.334 (.350) |
| | | Outgroup | 2.601 (.556) | 3.539 (.411) |

*Table S5.* Means and standard errors relating to the four-way interaction between gender, investor group, trustee group and transfer.

| Transfer | Investor Group | Trustee Group | Male | Female |
|---|---|---|---|---|
| **Fair** | In-group | In-group | .825 (.468) | 1.099 (.346) |
| | | Outgroup | -.858 (.361) | .586 (.267) |
| | Outgroup | In-group | .421 (.381) | 1.363 (.282) |
| | | Outgroup | -.712 (.430) | .893 (.318) |
| **Generous** | In-group | In-group | 1.901 (.418) | 2.998 (.309) |
| | | Outgroup | 1.699 (.403) | 2.502 (.298) |
| | Outgroup | In-group | 1.806 (.403) | 2.575 (.298) |
| | | Outgroup | 1.086 (.443) | 2.720 (.327) |

*Table S6.* Means and standard errors relating to the four-way interaction between gender, investor group, trustee group and back-transfer.

| Back-Transfer | Investor Group | Trustee Group | Male | Female |
|---|---|---|---|---|
| **Selfish** | In-group | In-group | -4.110 (.714) | -3.823 (.528) |
| | | Outgroup | -5.744 (.513) | -4.525 (.379) |
| | Outgroup | In-group | -3.667 (.650) | -3.079 (.481) |
| | | Outgroup | -4.660 (.612) | -4.099 (.453) |
| **Fair** | In-group | In-group | 2.199 (.539) | 3.188 (.438) |
| | | Outgroup | 1.366 (.551) | 2.223 (.408) |
| | Outgroup | In-group | 2.069 (.535) | 2.957 (.396) |
| | | Outgroup | .872 (.582) | 2.875 (.431) |
| **Generous** | In-group | In-group | 5.999 (.508) | 6.781 (.375) |
| | | Outgroup | 5.639 (.491) | 6.934 (.363) |
| | Outgroup | In-group | 4.937 (.670) | 6.029 (.495) |
| | | Outgroup | 4.348 (.611) | 6.645 (.452) |

# Chapter 8

## The relationship between oxytocin and empathy: Consequences for patients with hypopituitarism


### Abstract

The neuropeptide oxytocin (OT) has been found to influence cognitive empathy. Consequently there is interest in utilising OT as a therapeutic tool in psychopathologies that are characterised by social and emotional difficulties. Taking this line of investigation further, the present study investigated whether a clinical group (CDI) with an anticipated OT deficiency would perform significantly worse on two cognitive empathy tasks, the Reading the Mind in the Eyes Task (RMET) and Facial Expression Recognition (FER), compared to a clinical control (HP) group and a healthy control (HC) group. CDI patients performed significantly worse overall and on easy RMET items compared to HC participants, a finding which was supported by regression analyses showing that CDI patients' OT response during testing predicted their accuracy at identifying easy items. Interestingly, HP patients also displayed cognitive empathy deficits, in particular during the FER. Both clinical groups demonstrated biases towards over-reporting fearful and angry expressions in the FER (regardless of the expression presented). The results add to the discussion about the influence of OT on cognitive empathy and identify two novel clinical groups. In light of these and recent findings, it is important that future research identifies new groups that may be at risk of low or altered OT concentrations and investigate the impact of this on their emotional behaviour.

## Introduction

The ability to empathise is an important skill in order to engage in successful social interactions. Empathy can be broken down into two key components: cognitive empathy and affective empathy (Smith, 2010). Cognitive empathy, sometimes referred to as mentalising (Domes, Heinrichs, Michel, et al., 2007) and a subcomponent of theory of mind (Premack & Woodruff, 1978), in its' most basic form is the ability to correctly identify the emotional states of others, and its' more advanced form the ability to understand why others may be feeling this emotion; affective empathy refers to the embodied experience of the same emotion as that experienced by others. The hormone OT has been found to play a role in empathy (Shahrestani, Kemp, & Guastella, 2013); however, there is conflicting research evidence concerning which component of empathy OT affects (Bartz, Zaki, Bolger, et al., 2010; Hurlemann et al., 2010; Wu, Li, & Su, 2012). The current study aimed to investigate the influence of OT on cognitive empathy (focusing on the more basic emotion recognition form), using two previously validated measures, in a clinical population we anticipated would present with an OT deficiency.

The Reading the Mind in the Eyes Task (RMET) (Baron-Cohen, Wheelwright, Hill, et al., 2001) is a well-established psychological task measuring an individual's cognitive empathy by asking them to identify the mental state of an actor using only the eye region of the face. Several studies have now shown that OT increases participants' accuracy during the RMET. Domes, Heinrichs, Michel, et al. (2007) first established this in a within-subjects design study using 30 healthy males, finding that intranasal OT (IN-OT) improved their overall RMET score, but that when this was broken down by item difficulty (easy vs difficult expressions), OT was only found to have a beneficial effect for difficult faces.

This finding was replicated in a larger ($n$ = 71 males) between-subjects study (Feeser et al., 2015), which investigated the relationship between an individual's trait empathy and their RMET score. There was no effect of IN-OT on RMET scores for participants who were high in trait empathy (as measured by the Empathy Quotient (Baron-Cohen & Wheelwright, 2004)), compared to participants in the placebo (PL) condition. However, there was an effect of OT for participants who were low in trait empathy, such that OT enhanced their cognitive empathy, compared to those in the PL condition. These results are consistent with other findings showing that the social effects of OT are moderated by individual difference factors (Bartz et al., 2011; Shamay-Tsoory & Abu-Akel, 2016).

Although the RMET is a widely used task, it does have some limitations, the most obvious one (although it is arguably also a strength, depending on the research question at hand) being that because participants are only presented with the eye region of the face, this is not be the most ecologically valid way of measuring cognitive empathy. The Facial Emotion Recognition (FER) task (Bowen, Morgan, Moore, & van Goozen, 2014) presents participants with a series of faces displaying a range of basic emotional facial expressions. The advantage of this measure is that participants can use cues from the whole face to identify the emotion, making it more ecologically valid, but in addition the paradigm only uses basic emotions. The RMET uses a wide range of words that are not necessarily 'emotions' (see Chapter 1 of Niedenthal, Krauth-Gruber, and Ric (2006) for a discussion). Thus by using emotions that are typically thought to be 'basic emotions' (Ekman, 1992; Niedenthal et al., 2006), the FER also has greater construct validity.

Hubble et al. (2016) investigated the effect of IN-OT on FER performance in healthy male undergraduates. Participants were faster at identifying the correct emotion when they were given OT compared to when they were given a PL. A similar study also

found that OT lowered the intensity at which participants could accurately identify facial expressions of emotions (Lischke et al., 2012), thereby supporting conclusions drawn in a recent meta-analysis which found that IN-OT administration significantly improved recognition of facial expressions of emotions (Shahrestani et al., 2013).

Deficits in cognitive empathy can be a defining characteristic in several clinical populations, including autism spectrum disorder (ASD), mood disorders, and those with antisocial behaviour; there has been increasing interest in the therapeutic potential of OT in these clinical groups, specifically an interest in using OT to combat cognitive empathy deficits. Both the FER and RMET have been used in populations who present with a deficit in cognitive empathy. Baron-Cohen, Wheelwright, Hill, et al. (2001) found that participants with Asperger's Syndrome (AS) and high-functioning ASD (HFA) had significantly lower scores on the RMET, compared to healthy participants and IQ-matched healthy participants, consistent with previous findings reported by Davies, Bishop, Manstead, and Tantam (1994). In addition, they also found an inverse correlation between AS and HFA participants' scores on the Autism Quotient (AQ; Baron-Cohen, Wheelwright, Skinner, Martin, and Clubley (2001)) and their RMET score: the more autistic-like traits a participant self-reported (as indicated by a higher AQ score) the lower their RMET score. Similar results were also found in a later replication study (Guastella et al., 2010). Finally, Bowen et al. (2014) found that young offenders displayed deficits in recognising high intensity fear expressions and low intensity anger expression during the FER, but similar levels of accuracy for happy expressions compared to a matched participant group. Thus prior research has shown that specific clinical populations show a deficit in cognitive empathy, and that cognitive empathy can be moderated by OT.

*The Present Study*

The present study investigated whether lower salivary OT concentrations, anticipated in a clinical group, would have an effect on cognitive empathy. We hypothesised that patients with Cranial Diabetes Insipidus (CDI) would have significantly lower scores on the RMET and FER tasks, compared to both an age- and gender-matched clinical control (HP) group and a healthy control (HC) group. Given the similarity in tasks, we also anticipated that there would be a learning effect, such that all participants would perform better on the second task, regardless of the order of tasks.

We further hypothesised that both contextual and individual difference factors would affect performance in each task. We hypothesised that CDI patients would report more incorrect responses on difficult items on the RMET, compared to clinical and healthy participants; and that CDI patients would also report more incorrect responses in the FER task for lower intensity (25% and 50%) items, compared to clinical and healthy participants. Finally we explored the relationship between trait empathy (as measured by the Interpersonal Reactivity Index) and autistic-like traits (as measured by the Autism Quotient – Short version) and participants' performance on both tasks. Thus the present study was able to investigate whether OT affects cognitive empathy, and if so whether this effect is moderated by individual differences.

## Method

*Participants and Ethics*

Fifty-five adults ($M_{age}$ = 46.54; *SD* = 16.30) took part in the clinical study conducted at the University Hospital Wales. Participants were recruited to one of three groups: the CDI group, the HP group, and the HC group. Twenty participants were recruited to each group;

however, it was only possible to recruit 15 patients to the HP group. For further details, see Chapter 4.

The study protocol was approved by the Research and Development Office at the Cardiff and Vale University Health Board and by the Cambridge Central Research Ethics Committee (see Appendix 2 for a flow diagram of the study's progress). All participants read a detailed information sheet and gave written informed consent at the start of the experiment, and were fully debriefed at the end. Participants were financially compensated £20 for their participation.

*Materials*

*Reading the Mind in the Eyes (RMET) Task*

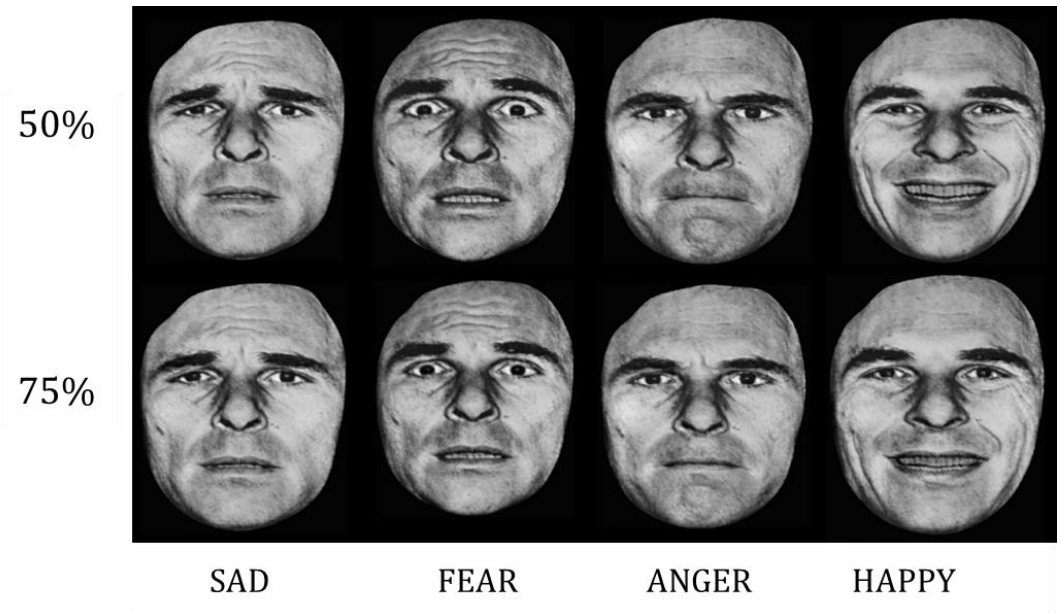
The RMET is a pre-existing validated measure of cognitive empathy created by Baron-Cohen, Wheelwright, Hill, et al. (2001) in which participants are presented with 36 faces displaying a range of facial expressions. Participants are only presented with the eye region of the face, and are therefore only able to use the eyes to infer the mental state of the actor (see Figure 8.1). The faces included male and female actors. For each face there are four response options and participants are asked to select the word that they feel best describes the face. As the response options are diverse (e.g., despondent, aghast etc.), participants are provided with a list of definitions to ensure that the meaning is clear. Participants are instructed to work through the task at their own pace, and to refer to the definitions at any time. A percentage of each participant's total number of correct responses was calculated, in addition to two subscale scores of the number of correct responses for easy and difficult items (Domes, Heinrichs, Gläscher, et al., 2007).

*Figure 8.1 - Example stimulus from the Reading the Mind in the Eyes Test from Baron-Cohen et al. (2001)*


*Facial Emotion Recognition (FER) Task*

A modified version of the FER task (Bowen et al., 2014) was used to assess participants' accuracy at identifying facial expressions of emotion. Participants saw an equal mix of male and female faces from the Ekman and Friesen (1975) series, representing four so-called 'basic' emotions (happiness, anger, fear and sadness) and also neutral faces. Participants saw each expression six times at four different intensities (25%, 50%, 75%, and 100%; differing intensities were created by morphing each expression with the actors' neutral face), seeing a total of 96 faces. The hair and background were masked so that only the facial features remained (see Figure 8.2). For each face participants were asked "What emotion is this person showing?" and asked to select the number corresponding to that emotion (1 = happy, 2 = anger, 3 = fear, 4 = sad, 5 = neutral). Participants were instructed to work through the task at their own pace. A percentage of each participant's correct responses was calculated. An error bias was also calculated for each expression by summing the number of times participants incorrectly identified a face as expressing a particular emotion (e.g., all the times a participant reported fear, when the expression was another emotion).

*Figure 8.2* - Example stimuli of the FER from Bowen et al. (2014)




*Interpersonal Reactivity Index*

The Interpersonal Reactivity Index (IRI; Davis, 1983) is a well established questionnaire, containing four subscales: empathic concern, fantasy, personal distress and perspective taking. There are 28 items in total, seven for each subscale; nine items are reverse scored. For each item, participants are asked to indicate on a 5-point scale the extent to which the statement could be applied to them (1 = "does not describe me very well; 5 = "describes me very well"). A mean for each subscale is calculated. All subscales obtained satisfactory internal consistency (empathic concern: $\alpha = .682$; fantasy: $\alpha = .722$; personal distress: $\alpha = .767$; perspective taking: $\alpha = .814$).

*The Relationship Structure Questionnaire*

The Relationship Structure Questionnaire (ECR-RS; Fraley, Heffeman, Vicary, & Brumbaugh, 2011) is a previously validated, modified version of the Experiences in Close Relationship scale, containing just nine of the original 36 items (five of which are

reverse scored). The same nine items are asked in relation to various important figures in the participant's life: parents, romantic partner and close friend. For the purposes of this study, the nine items were asked in relation to the participant's mother (or mother- like figure) and father (or father-like figure). Items included statements such as "It helps to turn to this person in times of need" and "I don't feel comfortable opening up to this person". Participants are asked to rate to what extent they agree/disagree with each item (1 = "Strongly Disagree"; 7 = "Strongly Agree"). A mean score is created for each parent. Both scales obtained satisfactory internal consistency (mother: $\alpha = .876$; father: $\alpha = .890$).

*The Autism Quotient (short version)*

The Autism Quotient short version (AQ-S; Kloosterman, Keefer, Kelley, Summerfeldt, & Parker, 2011) is an adapted 28-item version of the original 50-item Autism Quotient (Baron-Cohen, Wheelwright, Skinner, et al., 2001). Items relate to five subscales: social skills, mind reading, restricted and repetitive behaviour, imagination and attention to detail. Fourteen items are reverse scored. Items included statements such as "I find social situations easy" and "I find it difficult to work out people's intentions." Participants are asked to rate to what extent they agree/disagree with each item (1 = "Definitely Agree"; 4 = "Definitely Disagree"). A mean for each subscale is created.

All subscales apart from the restricted and repetitive behaviour subscale obtained satisfactory internal consistency (social skills: $\alpha = .752$; mind reading: $\alpha = .702$; restricted and repetitive behaviour: $\alpha = .429$; imagination: $\alpha = .629$; attention to detail: $\alpha = .706$).

*Saliva Samples*

Participants produced two saliva samples during the study, with an average interval of 33 minutes. Samples were collected in pre-chilled tubes, stored on ice throughout the study, and frozen at -80°C as soon as possible. Samples were subsequently centrifuged,

lyophilized, and analysed using the ELISA method. Full details of sampling and analysis can be found in Chapters 2 and 4, respectively. Participants' first saliva sample was subtracted from their second to create a measure of participants' OT response during the session.

*Procedure*

Participants were instructed to abstain from alcohol for 24 hours and caffeine for an hour prior to testing. Participants were only allowed to drink water during the study and if any food had been consumed before the start of a session, participants were asked to rinse their mouths thoroughly before any saliva samples were taken. All testing was carried out between 09:00 and 12:00 in order to control for circadian rhythms in other hormones that could be affected in both clinical groups.

On arrival participants' height and weight were measured in order that their BMI could be calculated. After a brief period (approximately 10 mins) of acclimatization to the testing facility participants were asked to provide their first saliva sample before completing either the FER or RMET task. Given the similarity of these tasks, the order in which they were presented was counterbalanced to control for any learning affects that might arise from the first task. The second task was completed at the end of the testing session, approximately 35 minutes later, after which participants were fully debriefed before leaving.

*Data Analysis*

An ANCOVA was carried out to investigate any differences in total RMET scores between groups, while controlling for age and gender. In line with previous research, a second ANOVA was carried out to investigate any differences between groups in

interpreting easy versus difficult items, while controlling for age and gender.[5] The internal consistency of the difficulty subscales achieved acceptable, albeit not high, internal consistency scores (easy subscale: $\alpha = .605$; difficult subscale: $\alpha = .627$); reliability was improved by dropping one item from each subscale (new easy subscale: $\alpha = .622$; new difficult subscale: $\alpha = .666$), thus items 4, 'insisting', and 23, 'defiant', were dropped from the easy and difficult subscales, respectively. Analyses reported below were conducted on the revised subscales. These analyses were then repeated while controlling for (a) the order in which participants completed the empathy tasks, (b) trait empathy, (c) attachment style and (d) autistic-like trait scores.

To investigate the relationship between RMET performance and OT concentrations, a hierarchical regression was performed (step 1: OT; step 2: group). To investigate whether (any) relationships were dependent on item difficulty, participants' easy and difficult subscale scores were regressed on OT concentrations, which were split by group in order to tease out any group differences.

A mixed ANOVA was carried out to assess the influence of different facial expressions, expression intensity, and group on participants' accuracy during the FER. This analysis was repeated using participants' OT concentrations as a covariate to identify whether OT concentrations were responsible for any observed group effects. These analyses were repeated with gender, task order, trait empathy, attachment style and autistic-like traits as covariates. Finally, a mixed ANOVA was carried out to assess whether there was an effect of any propensity of participants in a given group to over-report emotions during the FER, subsequently termed 'error biases'.

[5] Valence subscales were also calculated but did not achieve acceptable reliability (positive subscale: $\alpha = .494$; negative subscale: $\alpha = .491$; neutral subscale: $\alpha = .664$). They were not analysed further

# Results

*Personality Measures*

A 4 (IRI subscale: Empathic Concern vs Fantasy vs Perspective Taking vs Personal Distress; within-subjects) x 3 (Group: CDI vs HP vs HC; between-subjects) mixed ANOVA was carried out. There was a significant main effect of IRI subscale, $F(3, 150) = 33.485$, $p < .001$, $\eta^2_p = .401$, such that participants scored more highly on the Empathic Concern subscale ($M = 3.056$, $SE = .082$) compared to Fantasy ($M = 2.070$, $SE = .106$), Perspective Taking ($M = 2.389$, $SE = .103$) and Personal Distress ($M = 1.756$, $SE = .116$); there was no difference between Fantasy and Perspective Taking scores; and Fantasy scores were higher than Personal Distress scores. There was also a main effect of group, $F(2, 50) = 3.639$, $p = .033$, $\eta^2_p = .127$, such that HP patients ($M = 2.195$, $SE = .111$) and CDI patients ($M = 2.220$, $SE = .102$) had lower scores compared to HC participants ($M = 2.538$, $SE = .096$). Finally there was a significant interaction between group and subscale, $F(4.867, 121.677) = 3.460$, $p = .006$, $\eta^2_p = .122$. Simple effects analysis revealed that there was no difference between groups on the Empathic Concern ($F(2, 50) = 1.069$, $p = .351$) or Personal Distress ($F(2, 50) = .554$, $p = .578$) subscales, however CDI patients scored significantly lower on the both Fantasy ($F(2, 50) = 7.368$, $p = .002$) and Perspective Taking ($F(2, 50) = 4.812$, $p = .012$) subscales compared to HC participants, while HP patients only scored significantly lower compared to HC participants on the Fantasy subscale (see Table 8.1).

*Table 8.1 - Means and standard errors relating to the group by IRI subscale interaction*

| Group | IRI Subscale | Mean | SE |
|---|---|---|---|
| **CDI** | Empathic Concern | 3.111 | .139 |
| | Fantasy | 1.837 | .180 |
| | Perspective Taking | 1.976 | .175 |
| | Personal Distress | 1.921 | .198 |
| **HP** | Empathic Concern | 2.886 | .153 |
| | Fantasy | 1.714 | .197 |
| | Perspective Taking | 2.476 | .192 |
| | Personal Distress | 1.705 | .217 |
| **HC** | Empathic Concern | 3.171 | .132 |
| | Fantasy | 2.621 | .171 |
| | Perspective Taking | 2.714 | .166 |
| | Personal Distress | 1.643 | .188 |

A 2 (ECR-RS Subscale: Mother vs Father; within-subjects) x 3 (Group: CDI vs HP vs HC; between-subjects) mixed ANOVA was carried out. There was no main effect of subscale, $F(1, 50) = 2.210$, $p = .143$, $\eta^2_p = .042$, or group, $F(2, 50) = .553$, $p = .579$, $\eta^2_p = .022$, and no significant interaction, $F(2, 50) = 2.358$, $p = .105$, $\eta^2_p = .086$.

Finally a 4 (AQ-S Subscale: Social Skills vs Mind Reading vs Imagination vs Attention to Detail; within-subjects) x 3 (Group: CDI vs HP vs HC; between-subjects) mixed ANOVA was carried out. There was a main effect of subscale, $F(3, 153) = 19.676$, $p < .001$, $\eta^2_p = .278$, such that participants scored more highly on the Imagination subscale ($M = 3.118$, $SE = .085$) compared to Social Skills ($M = 2.758$, $SE = .086$, Mind Reading ($M = 2.769$, $SE = .083$), and Attention to Detail ($M = 2.241$, $SE = .097$); there was no difference between Social Skills and the Mind Reading scores, but both scores were greater than the Attention to Detail subscale. There was a significant main effect of group,

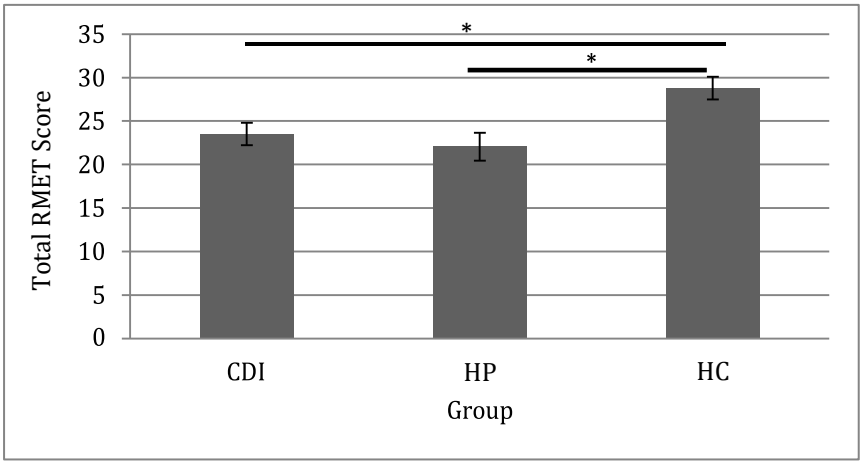
$F(2, 51) = 6.269$, $p = .004$, $\eta^2_p = .197$, such that HP patients ($M = 2.502$, $SE = .095$) scored significantly lower compared to HC participants ($M = 2.952$, $SE = .085$), but there was no difference between CDI patients ($M = 2.710$, $SE = .090$) and HP patients or HC participants. There no significant interaction, $F(5.033, 128.350) = 1.398$, $p = .229$, $\eta^2_p = .052$, however simple effects analysis did reveal a significant difference between groups on the Attention to Detail subscale, $F(2, 51) = 7.083$, $p = .002$, such that HP patients ($M = 1.750$, $SE = .177$) scored significantly lower, and CDI patients ($M = 2.333$, $SE = .167$) trended towards lower scores, compared to HC participants ($M = 2.640$, $SE = .159$).

*RMET – Group Effects*

A one-way ANCOVA was carried out to investigate the effect of group (CDI vs HP vs HC) on total RMET scores, while controlling for any effects of gender and age. There was a significant main effect of group, $F(2, 42) = 5.557$, $p = .007$, $\eta^2_p = .209$; further analysis showed that both CDI and HP patients had significantly lower RMET scores compared to HC participants (see Figure 8.3), but there was no difference between CDI and HP scores. Age ($F(1, 42) = .004$, $p = .950$, $\eta^2_p = .001$) and gender ($F(1, 42) = .118$, $p = .733$, $\eta^2_p = .003$) were not significant covariates.

A 2 (Scale: Easy vs Difficult; within-subjects) x 3 (Group: CDI vs HP vs HC; between-subjects) mixed ANCOVA with age and gender as covariates also revealed a significant main effect of group, $F(2, 43) = 6.294$, $p = .004$, $\eta^2_p = .226$. Bonferroni corrected pairwise comparisons revealed that CDI patients ($M = 11.017$, $SE = .547$) had significantly lower scores than HC participants ($M = 13.657$, $SE = .561$), while there was also a trend for HP patients ($M = 11.396$, $SE = .726$) to have significantly lower scores compared to HC participants. There was also a main effect of scale, $F(1, 43) = 18.731$, $p < .001$, $\eta^2_p = .303$, such that (as expected) participants performed better on easy items ($M = 12.874$, $SE = .362$) compared to difficult items ($M = 11.172$, $SE = .444$).

*Figure 8.3 - RMET score as a function of clinical group (± SE)*

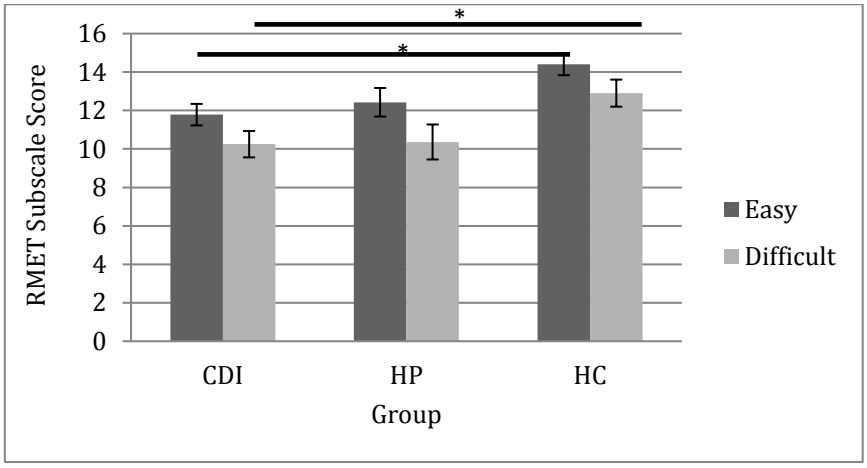



Although the interaction was not significant, $F(2, 43) = .174$, $p = .841$, $\eta^2_p = .008$, hypothesis-driven simple effects analyses revealed a significant effect of group on both easy items, $F(2, 43) = 5.675$, $p = .006$, $\eta^2_p = .209$, and difficult items, $F(2, 43) = 4.304$, $p = .020$, $\eta^2_p = .167$; in both cases CDI patients scored significantly lower than HC participants, and there was no difference between HP and CDI patients or between HP patients and HC participants. The relevant means are displayed in Figure 8.4. Again age ($F(1, 43) = .148$, $p = .703$, $\eta^2_p = .003$) and gender ($F(1, 43) = .075$, $p = .786$, $\eta^2_p = .002$) were not significant covariates.

*RMET – Individual Difference Effects*

The above analyses were repeated to assess whether the main effect of group was robust when controlling for the order of empathy tasks, trait empathy, attachment style and autistic-like traits. None of these factors were significant covariates and the main effect of group remained significant in all analyses.

*Figure 8.4 - RMET score as a function of difficulty and clinical group (± SE)*




*RMET – Oxytocin Effects*

A regression analysis revealed that even when controlling for group participants' OT response during testing was a significant predictor (β = .034, $p$ = .025) of their performance in the RMET, $R^2$ = .096, $F(1, 50) = 5.314$, $p$ = .025. Further regression models conducted within each participant group revealed that OT response did not predict participants' performance on difficult items; rather, it predicted CDI participants' performance on easy items (see Table 8.2).

*Table 8.2 - OT response specifically predicts CDI patients' performance for easy items, not difficult items, of the RMET*

| Scale | Group | $R^2$ | F | df | *p* | β |
|---|---|---|---|---|---|---|
| **Easy** | CDI | .277 | 6.513 | (1,17) | .021 | .526 |
| | HP | .214 | 2.730 | (1,10) | .129 | .463 |
| | HC | .022 | .396 | (1,18) | .537 | .147 |
| **Difficult** | CDI | .062 | 1.128 | (1,17) | .303 | .249 |
| | HP | .093 | 1.025 | (1,10) | .335 | .305 |
| | HC | .080 | 1.563 | (1,18) | .227 | .283 |

*FER – Group Effects*

A mixed 4 (Emotion: happy vs sad vs fear vs anger; within-subjects) x 4 (Intensity: 25 vs 50 vs 75 vs 100; within-subjects) x 3 (Group: CDI vs HP vs HC; between-subjects) ANOVA was carried out. There was a significant main effect of emotion, $F(3, 153) = 27.797$, $p < .001$, $\eta^2_p = .353$, reflecting the fact that more happy ($M = 80.744$, $SE = 1.501$) facial expressions were correctly identified, compared to sad ($M = 61.779$, $SE = 2.497$), fearful ($M = 61.290$, $SE = 1.586$) and angry ($M = 62.585$, $SE = 1.800$) expressions.

There was also a main effect of intensity, $F(3, 153) = 655.542$, $p < .001$, $\eta^2_p = .928$, reflecting the fact that higher intensity expressions were more often identified correctly (100%: $M = 89.181$, $SE = 0.929$; 75%: $M = 84.390$, $SE = 1.398$; 50%: $M = 68.019$, $SE = 1.767$; 25%: $M = 24.809$, $SE = 1.613$). There was also a significant interaction between emotion and intensity, $F(9, 459) = 5.779$, $p < .001$, $\eta^2_p = .102$. Bonferroni corrected pairwise comparisons revealed that more happy, fearful, and angry facial expressions were identified correctly at 50% intensity, compared to 25%, and at 75% compared to 50%; however, the difference in scores between 75% and 100% was not significant, although this apparent ceiling effect was not present for sad facial expressions (see Table 8.3).

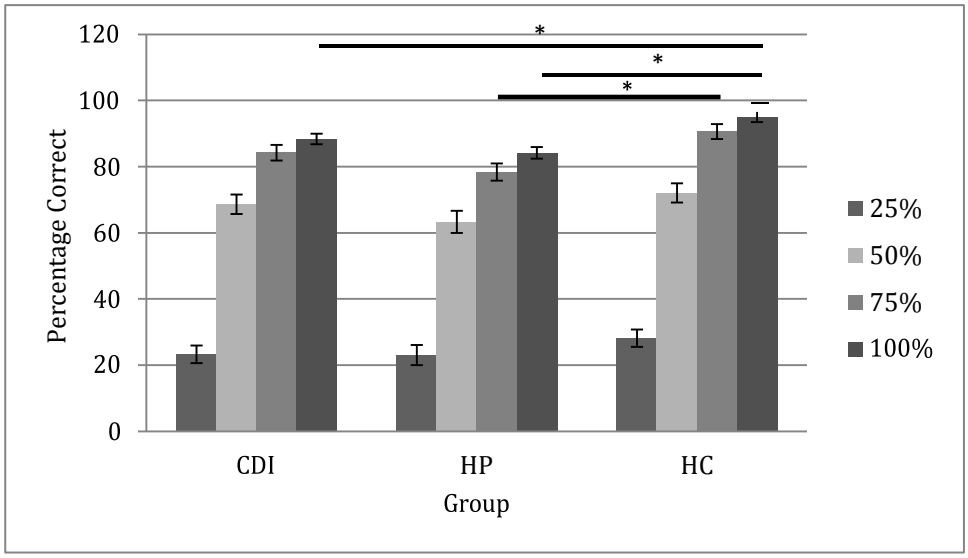
*Table 8.3 - Means and standard errors relating to the emotion by intensity interaction*

| Emotion | Intensity | Mean | SE |
|---|---|---|---|
| **Happy** | 25% | 45.370 | 3.975 |
| | 50% | 87.037 | 2.275 |
| | 75% | 95.062 | 1.502 |
| | 100% | 96.914 | 1.172 |
| **Sad** | 25% | 24.691 | 3.260 |
| | 50% | 60.802 | 3.820 |
| | 75% | 77.469 | 3.120 |
| | 100% | 86.420 | 2.206 |
| **Fear** | 25% | 13.272 | 1.940 |
| | 50% | 64.498 | 2.969 |
| | 75% | 84.877 | 2.761 |
| | 100% | 84.877 | 2.170 |
| **Anger** | 25% | 16.667 | 2.203 |
| | 50% | 61.728 | 3.286 |
| | 75% | 82.407 | 2.622 |
| | 100% | 90.432 | 2.356 |

There was a significant main effect of group, $F(2, 51) = 6.157$, $p = .004$, $\eta^2_p = .194$, such that HP patients ($M = 62.222$, $SE = 2.021$) had significantly lower scores than HC participants ($M = 71.458$, $SE = 1.750$), but there was no difference between CDI patients ($M = 66.118$, $SE = 1.796$) and HP or HC participants. Simple effects analyses were carried out to assess the effect of group on recognition of expressions varying in emotion and intensity. Group had no effect on recognition of happy, sad, or angry expressions, but HP patients ($M = 55.833$, $SE = 2.986$) correctly identified significantly fewer fearful expressions compared to HC participants ($M = 67.292$, $SE = 2.586$), $F(2, 51) = 4.331$, $p =$

.018, $\eta^2_p$ = .145; there was no difference between CDI and HP patients. Unexpectedly, group also had a significant effect on high intensity facial expressions: 100%, $F(2, 51)$ = 11.491, $p < .001$, = .311; 75%, $F(2, 51) = 6.308$, $p = .004$, $\eta^2_p$ = .198. At 100% intensity both HP and CDI patients identified fewer expression correctly compared to HC participants (there was no difference between CDI and HP patients). At 75% intensity HP patients correctly identified fewer expressions compared to HC participants. There was no effect of group on low intensity expressions. The relevant means and standard errors are presented in Figure 8.5.

*Figure 8.5 - FER performance as a function of intensity and clinical group (± SE)*




A 4 x 4 x 3 x 2 mixed ANOVA with age as a covariate and gender as a between-subjects factor revealed no effect of gender, $F(1, 44) = 1.753$, $p = .192$, $\eta^2_p$ = .038, or age, $F(1, 44) = .595$, $p = .445$, $\eta^2_p$ = .013, there were no significant interactions, and all significant main effects and interactions previously reported remained significant.

Finally a 4 (Emotion: happy vs sad vs fear vs anger; within-subjects) x 3 (Group: CDI vs HP vs HC; between-subjects) mixed ANOVA revealed a significant main effect of emotion on error bias, $F(3, 147) = 12.025$, $p < .001$, $\eta^2_p = .197$; participants had a significantly lower bias towards happy ($M = .819$, $SE = .192$) compared to sadness ($M = 4.315$, $SE = .747$), fear ($M = 3.950$, $SE = .354$) and anger ($M = 3.837$, $SE = .391$). There was also a significant main effect of group, $F(2, 49) = 3.962$, $p = .025$, $\eta^2_p = .139$, reflecting the fact that CDI ($M = 3.369$, $SE = .392$) and HP ($M = 3.714$, $SE = .445$) patients had larger error biases than HC participants did ($M = 2.338$, $SE = .372$). Although there was no significant interaction, $F(3.743, 91.699) = 1.524$, $p = .205$, $\eta^2_p = .059$, simple effects analysis revealed a significant difference between the groups for fear bias, $F(2, 49) = 4.002$, $p = .025$, $\eta^2_p = .140$, and anger bias, $F(2, 49) = 5.559$, $p = .007$, $\eta^2_p = .185$: HP patients had a greater fear bias ($M = 5.429$, $SE = .745$) compared to HC participants ($M = 2.750$, $SE = .624$), and both CDI ($M = 4.500$, $SE = .596$) and HP ($M = 5.000$, SE = .676) patients had a greater anger bias compared to HC participants ($M = 2.350$, $SE = .565$). All other pairwise comparisons were non-significant, and there was no effect of group on happy bias, $F(2, 49) = .671$, $p = .516$, $\eta^2_p = .027$, or sad bias, $F(2,49) = .945$, $p = .396$, $\eta^2_p = .037$.

*FER – Oxytocin Effects*

The original 4 x 4 x 3 mixed ANOVA was repeated, this time including participants' OT response during testing as a covariate, to assess whether group differences were related to differences in OT concentrations. The covariate was not significant, $F(1, 50) = .059$, $p = .809$, $\eta^2_p = .001$, and all original main effects and interactions remained significant. Participants' OT response was also added as a covariate to the 4 x 3 mixed ANOVA investigating error biases, but again there was no effect of the covariate, $F(1, 48) = .933$, $p$

= .339, $\eta^2_p$ = .019, and all original main effects and interactions also remained significant.

*FER – Individual Difference Effects*

Three separate mixed ANCOVAs were carried out to investigate whether individual differences in personality measures (trait empathy, attachment style, and autistic-like traits) would act as significant covariates. None of the covariates were significant and they will therefore not be discussed further.

## Discussion

The present study investigated whether patients with CDI, who we anticipated would present with an OT deficiency, would also demonstrate a cognitive empathy deficit relative to HP patients and HC participants. In line with our hypothesis, CDI patients scored significantly lower on the RMET compared to HC participants, and participants' OT responses over the testing session significantly predicted their RMET performance: the greater the OT response, the higher their score. The RMET results only partly supported our secondary hypothesis: CDI patients scored significantly lower on both easy and difficult items, compared to HC participants, although the effect was stronger for easy items. Interestingly, HP patients also tended to have lower overall scores compared to HC participants, but this effect was lost when taking account of item difficulty. These results were supported by regression analyses demonstrating that participants' OT response during testing only predicted CDI patients' performance on easy items, but did not predict the performance of either HP patients or HC participants.

On the FER task, HP patients had significantly lower overall scores than HC participants, and this was driven in particular by a lower ability to identify fearful facial

expressions. The scores of CDI patients tended to fall between the HP and HC scores. Interestingly, and in contrast to hypotheses, CDI patients performed significantly less well than HC participants in identifying 100% intensity expressions, while HP patients also had significantly lower scores than HC participants at both 75% and 100% intensities. CDI and HP patients also demonstrated a greater error bias, compared to HC participants; HP patients demonstrated a fear bias, in that HP patients tended to over report the emotion fear when presented with other emotions, and both HP and CDI patients had an anger bias. In contrast to what was observed on the RMET, participants' OT response during testing did not affect FER performance.

Finally there were no group differences in attachment style (at least when measured using the ECR-RS), however, there were significant group differences in trait empathy and autistic-like traits, such that HP patients scored significantly lower in both personality measures compared to HC participants, and CDI patients scored significantly lower on trait empathy. Interaction effects indicated that hypopituitary (CDI and HP) patients reported a poorer ability to put themselves in the same emotional/mental state as both fictional characters and real people; and that HP patients differed significantly to CDI and HC participants on Attention to Detail scores. However, these personality measures had no significant effect when added as covariates to participants' performance on both the RMET and FER. Similarly, age and gender also had no effect of participants' cognitive empathy performance.

The results are consistent with previous studies finding (a) that a positive association between OT and RMET performance (Domes, Heinrichs, Michel, et al., 2007; Feeser et al., 2015), and (b) that this association is moderated by item difficulty (Domes, Heinrichs, Michel, et al., 2007). However, in contrast to previous findings (Feeser et al., 2015), RMET performance was not moderated by trait empathy , or autistic-like traits

(Baron-Cohen, Wheelwright, Hill, et al., 2001; Guastella et al., 2010). The current study also generated some important new findings: There was evidence of a cognitive empathy deficit in patients with CDI and HP; also, to our knowledge, this is the first study to demonstrate that endogenous OT concentrations (as opposed to IN-OT) predicts RMET performance, and is also the first to do so in a mixed-gender sample.[6]

In contrast to previous literature the present study did not administer IN-OT, and thus a limitation of the study is that one cannot make inferences about causality. While OT concentrations were entered as a predictor into the model based on theoretically grounded hypotheses, one could also say that RMET significantly predicted participants OT concentrations. Indeed research has demonstrated that the association between OT and empathy-related behaviours is bidirectional (Smearman et al., 2016). Furthermore this bidirectional relationship may be advantageous; if psychologists are able to isolate behavioural paradigms that reliably trigger an OT response naturally and thus improve characteristic deficits in social behaviour associated with many psychopathologies, it would reduce the need to administer IN-OT as a treatment. As the longevity of IN-OT effects is between two to seven hours (Van IJzendoorn, Bhandari, Van der Veen, Grewen, & Bakermans-Kranenburg, 2012; Weisman et al., 2012) and there is currently no study investigating the effect of long- term OT administration, a natural paradigm may provide the most ethical and efficient treatment plan.

The results are also consistent with previous research in which it was found that clinical groups are significantly less accurate at detecting fearful facial expressions compared to matched healthy controls (Bowen et al., 2014), and extends previous work (Bowen et al., 2014; Hubble, 2015) by investigating the effect of endogenous OT

[6] One other study has used a mixed-gender sample in an OT and RMET study (Rodrigues et al., 2009), however, that study investigated the effect of OT receptor polymorphisms and empathy, so the present study is the first to investigate the relation between endogenous OT concentrations and RMET in a mixed-gender sample.

concentrations in healthy and clinical groups in a mixed-gender sample. Although Hubble (2016) found that IN-OT increased the speed with which participants' identified the correct response, the present study found no effect of participants' OT concentrations on FER accuracy. A further novel finding relates to HP patients' performance; while HP patients were significantly worse at recognising fearful facial expressions, they also had a tendency to report that other facial expressions depicted fear. This is a particularly interesting and relevant finding because clinical groups who over-report negative (in particular fearful) expressions may be at greater risk of developing mood disorders (Beck, 1979, 2008). Thus an important avenue for future research is to investigate whether patients with hypopituitarism are also at to an increased risk of co-morbid diagnoses of mood disorders.

There are some (further) limitations to the present study. The sample size target for the study was to recruit 20 participants for each group; however, given time restrictions and logistical constraints of recruiting matched NHS patients, it was only possible to recruit 15 HP patients. Consequently, the results pertaining to HP patients need to be treated with caution. Although we managed to recruit 20 participants for the other two groups, which was sufficient (based on a priori power calculations) to test the hypotheses, a future study should aim to use a larger sample size to confirm the generalizability of the present results. A future study may also wish to match participants on intellectual ability to confirm this is not a confounding variable. More patients compared to HC participants were accompanied to the study by a carer or currently off work, while this observation may be related to the patients' medical health, it cannot be ruled out that this may (also) be related to their intellectual ability.

In order to rule this out, therefore, a future study may wish to control for IQ or a history of learning difficulties. Importantly, a strength of the study is the age range of

participants (22–74). This, in combination with the fact that age was not a significant covariate, suggests that age does not moderate any effects of group or OT on cognitive empathy. Another limitation of the current study is the relatively poor internal consistency achieved on the personality measures. Thus while we find no evidence that individual differences moderated the effects of group in the present study, this may nevertheless prove to be an interesting avenue for future research to investigate, provided more reliable measures of the relevant constructs are used.

*Conclusions*

The current study is the first to demonstrate that patients with CDI exhibit a significant cognitive empathy deficit compared to HC participants. In particular, these patients' OT concentrations over the testing session significantly predicted their RMET performance (although it may also be the case that RMET performance predicts participants' OT concentrations). However, HP patients also demonstrated a significant cognitive empathy deficit, and both clinical groups demonstrated biases in over reporting certain negative emotions (fear and anger) regardless of the expression presented. The findings demonstrate the need to identify clinical groups who may be at risk of an OT deficiency and to investigate how this may impact their social and emotional behaviour (see Chapter 4). More generally, the findings add to the current debate concerning the influence of OT on empathy-related behaviour.

# Chapter 9

# Discussion

*Summary of the Thesis*

The aim of the current thesis was to investigate the role of oxytocin (OT) in social and emotional behaviours, specifically examining whether these effects are moderated by contextual factors and individual differences. In Chapters 2, 3, and 4, however, I began by addressing some methodological considerations in OT research. In Chapters 2 and 3 I replicated and extended previous research by conducting the largest studies (at the time of each study) to date to assess the effect of intranasal OT (IN-OT) on salivary OT concentrations. I found a reliable increase in salivary OT after IN-OT administration, and the presence of large individual differences in response to the same intranasal dosage. Finally in Chapter 4 I found an OT deficiency in two novel clinical groups, relative to a matched healthy control group.

In Chapters 6, 7, and 8, I examined the moderating effect of ingroup/outgroup contexts on the social and emotional effects of IN-OT, and investigated whether these effects extended to third-party behaviour. In Chapters 5 and 6, I found that both healthy male undergraduates and mixed gender clinical groups were significantly more prosocial towards an excluded individual, but (and in line with predictions) that this was moderated by the group identity of the excluded player. In Chapter 7 I found that IN-OT increased ingroup bounded sanctioning, and motivations underlying sanctioning, of players during a novel economic game. In Chapter 8 I examined the relationship between OT and cognitive empathy in a novel clinical group, finding that both the clinical group and a clinical control group demonstrated an empathy deficit, and that for the clinical group this was

related to their OT concentrations. These findings will be discussed below, in addition to considering some limitations of the thesis, how the findings might be used to inform future research, and discussing the implications of the results reported in the thesis.

*Methodological Considerations in Oxytocin Research*

While I was conducting the research reported in this thesis a number of critiques of IN-OT research were published. Leng and Ludwig (2015), among others (Churchland & Winkielman, 2012; Leng & Sabatier, 2016; McCullough et al., 2013; Walum et al., 2015), highlight the gaps in our current understanding of the biological mechanisms mediating the effects of IN-OT on behaviour. The issues identified include whether IN-OT crosses the blood-brain barrier (BBB); the efficacy of this administration technique; effects of IN-OT on the peripheral system; the usefulness of (only) investigating the behavioural effects of supraphysiological concentrations of OT; and the presence of a publication bias for positive results. I agree that these are all important issues to address, and will consider each issue below.

Perhaps the most frequently repeated criticism of IN-OT research is one that refers to the 'black box' in our understanding of how IN-OT reaches the brain to exert its effects on social behaviour. There are three reasons why this critique may not be of real concern. First, there are a number of proposed mechanisms for how IN-OT may reach the brain. These are not mutually exclusive, and have been proposed several times in recent years (Evans et al., 2014; Grinevich et al., 2016; Quintana et al., 2015; Veening & Olivier, 2013). Specifically, there are three proposed pathways for IN-OT: the olfactory pathway, the trigeminal pathway, and the peripheral pathway. The olfactory pathway suggests that OT reaches the brain via the olfactory sensory neurones in the nasal mucosa, which connect to the olfactory nerve, leading to the brain. The trigeminal pathway suggests that

OT reaches the brain via trigeminal ganglion cells in the nasal mucosa, leading to the trigeminal nerve and to the brain. Finally the peripheral pathway suggests that OT diffuses into the bloodstream via nasal capillaries, binding with OT receptors (OTRs), triggering a signal to OTRs in the brain, which triggers release of OT from vesicles in the brain, and thereby causing a rise in central. After an extensive summary of the literature Veening and Olivier (2013) concluded "…that OT delivered intranasally gains access to… brain areas via 'direct pathways' form the nasal epithelium…", and "that peripheral OT-levels remain elevated for more than an hour after intranasal administration strongly suggests the 'secondary' involvement of the magnocellular OT system in addition to the central effects of IN-OT." Thus is seems that IN-OT exerts effects on the brain through both direct nose-to-brain pathways and through positive feedback from the periphery.

The second reason why the 'black box' critique may not be of real concern, which is related to the first, is that IN-OT indirectly leads to an increase in central concentrations of OT by diffusing into the peripheral circulation. This mechanism not only deals with the critique that IN-OT cannot cross the BBB (because this mechanism completely avoids this issue) but also explains how a peptide with a relatively short half-life can have a sustained effect on peripheral concentrations of OT. Moreover, Valstad et al. (2017) conducted a recent meta-analysis to test the association between central and peripheral concentrations of OT and found a significant correlation after IN-OT administration. Providing important empirical evidence that peripheral concentrations of OT can be used as a proxy measure to reflect central concentrations of OT. Finally, to quote Leng and Ludwig (2015), significant increases in peripheral OT concentrations following IN-OT administration should not be "assumed to have no behavioural consequences" (Leng & Ludwig, 2015, p. 4). In other words, while the field may not currently agree on how IN-OT reaches the brain, the evidence strongly supports the conclusion that IN-OT, directly or indirectly,

does influence social and emotional behaviour.

A related critique, already referred to above, is that IN-OT cannot cross the BBB. Although several studies have reported significant increases in cerebrospinal fluid (CSF) OT after IN-OT (Dal Monte et al., 2014; Modi et al., 2014), which would suggest that OT can cross the BBB, Leng and Ludwig (2015) recently argued that CSF OT may not be the most valid measure. Discussing the evidence, they note that CSF OT may not be a reliable indicator of OT concentrations in the brain, because OT is degraded more rapidly in the brain compared to the peripheral system. These authors propose instead that neurophysin (a unit of the OT precursor) may be a more reliable measure. With this in mind, conclusions about whether IN-OT can reach the brain may be based on research that underestimates the effect.

This addresses another critique, namely that only 0.002% of IN-OT reaches the brain. Even if one were to assume that this critique is based on a valid measure, the fact that such a small amount results in significant behavioural changes should not be dismissed. Moreover, this critique seems at odds with another common critique regarding the validity of examining the effects of supraphysiological concentrations of OT on behaviour. If <1% of IN-OT reaches the brain, yet this results in supraphysiological levels, any increase in the percentage of IN-OT reaching the brain would do little to overcome this concern. Leng and Ludwig (2015) argue that although intranasal administration studies have been a useful method for investigating the existence and extent of OT's effects on social behaviour, the results are limited by a lack of ecological validity. The current thesis has attempted to address this critique by measuring endogenous concentrations of OT in a novel clinical group, and investigating the relationship between OT concentrations and patients' behaviour.

Future studies may wish to explore how OT fluctuates naturally during various

social interactions, enabling researchers to create naturalistic psychosocial interventions that could prime endogenous OT concentrations. Given the interest in OT's therapeutic potential, devising robust behavioural interventions that have a proven influence on endogenous OT concentrations would be extremely useful. In Chapter 3 I found an increase in OT concentrations in the PL condition between 30 and 60 minutes. During this time participants were engaging in a group-based decision-making task, providing evidence that echoes some of the earliest and simplest hypotheses in OT research: OT is involved in, and may be stimulated by, social interaction. Indeed developmental research has already shown that social interactions between parents and their infants results in OT synchrony (Feldman, Gordon, & Zagoory-Sharon, 2011). This is a potential avenue for future research.

Interestingly, the most valid concern regarding OT research is also the least frequently mentioned and arguably the most poorly understood. This concern regards the validity of measuring OT in different mediums and the validity of different laboratory analyses. Leng and Sabatier (2016) recently published a paper addressing this issue. In the present thesis I attempted to address this issue by reporting three studies in which salivary OT concentrations were measured. The first two chapters report the largest studies to date in which the impact of IN-OT was assessed. Here I found a reliable increase in salivary OT after IN-OT. In Chapter 4, I confirmed the validity of measuring natural concentrations of OT in saliva. Given the difficulties of measuring OT in plasma (Leng & Sabatier, 2016), saliva may be the most valid measure of peripheral OT. However, it should be acknowledged that the current thesis did not examine the difference in validity between ELISA and RIA techniques; nor did I use a chemical extraction process during sample preparation. However, all samples were lypholized prior to analysis, a process that has been found to have a comparable effect to using a chemical extraction process (Carter et

al., 2007; White-Traut et al., 2009). Further research is therefore required to assess the validity of each technique (ELISA versus RIA) in saliva, and whether a chemical extraction process generates the same results as lypholization.

The final critique of OT research refers to the possibility of a publication bias for positive results (Lane, Luminet, Nave, & Mikolajczak, 2016). While this is a current concern for all psychological literature, as far as I am aware there is no evidence that this issue is particularly prevalent in OT research, relative to other fields.

In the current thesis I attempted to address recent concerns wherever possible (e.g., by replicating the impact of IN-OT on salivary OT concentrations), and where this was not possible (e.g., using RIA versus ELISA techniques), I attempted to provide an explanation for why these issues may or may not be of real concern. For the remaining critiques, I have pointed to ways in which future research could address these issues. Resolving these concerns would help to shift the focus back to the reliable and significant effects of OT on social and emotional behaviour, and, in particular, how this effect could be used to improve the well-being of those with psychopathologies.

*The Influence of Contextual Factors on the Social Effects of Oxytocin*

It is well known that OT influences social behaviour. Consistent findings from the same research group demonstrate that these effects are moderated by contextual factors (De Dreu, Greer, Handgraaf, et al., 2011; De Dreu et al., 2010; De Dreu & Kret, 2016). Because much of the research published in this area has come from one research group, one aim of the present thesis was to replicate these findings in a new setting. Furthermore, this research has tended to focus on the effects of OT on social behaviour, and thus a more novel aspect of the research presented in the current thesis is the inclusion of emotional

behaviours, investigating whether OT's effects on participants' emotional responses are also moderated by contextual factors. Finally, in this thesis I sought to extend previous research by investigating whether the well-known effects of OT on social behaviour also extends to third-party behaviour.

In Chapter 5 I investigated whether IN-OT would increase prosocial behaviour towards an excluded individual in the online ball throwing game Cyberball. Here I manipulated whether participants witnessed either an ingroup or outgroup member being excluded while they were in the placebo (PL) or OT condition, to examine whether context would moderate participants' behaviour. After Cyberball participants were asked to report their own emotional response and the perceived emotional response of the excluded individual, to examine whether context moderated participants' emotional responses. Participants who were given OT were more prosocial towards the excluded individual. However, and inconsistent with the prediction derived from the tend-and-defend hypothesis (De Dreu, 2012a), OT increased prosocial behaviour towards an excluded outgroup player, rather than an excluded ingroup player. Furthermore, these results are the first to demonstrate that participants' OT concentrations after IN-OT significantly predicted increased prosocial behaviour towards the excluded outgroup member.

While initially surprising, these results serve to highlight the importance of the role played by 'perception of threat' in the tend-and-defend hypothesis. Because Cyberball is not a competitive game, and given the 3-against-1 nature of the excluded outgroup round, it seems highly likely that participants did not perceive the outgroup member as posing a threat to the ingroup. This appears to be a plausible reason for the fact that the OT condition did not evoke defence-related behaviours. Support for this theoretical argument was subsequently found in the studies reported in Chapters 6 and 7.

I also found that group identity modulated participants' emotional responses to witnessing the exclusion, this time in line with predictions formed by the tend-and- defend hypothesis: Participants who were given OT reported greater empathy for the excluded ingroup member. However, this study used an all-male undergraduate sample that limited the generalizability of the findings, and as a result of logistical issues condition and group membership factors were confounded. Thus a future study was suggested in which a mixed gender sample would participate in a within-subjects version of the Cyberball paradigm. Such a study was reported in Chapter 6.

The study reported in Chapter 6 is a replication and extension of the Cyberball paradigm using a novel clinical group (in addition to a matched clinical control group [HP] and healthy control group [HC]). Cranial Diabetes Insipidus (CDI) patients present with a deficit in arginine vasopressin (AVP) which is the sister peptide of OT (Brownstein, 1983; Swaab et al., 1975). Because AVP and OT are synthesised and released into the bloodstream in the same way, it was hypothesised that CDI patients would also present with an OT deficit. Assuming this was the case (and this assumption was empirically tested in Chapter 4), I investigated whether CDI patients would be responsive to the social cues presented during the game, demonstrating an increase in prosocial behaviour towards an excluded individual, and whether this would be moderated by group identity. This hypothesis was informed by previous research in which it was found that patients with psychopathologies that have been linked with low or altered OT did not detect cues presented during Cyberball under natural conditions, but did do so after IN-OT (Andari et al., 2010).

The results of this study revealed that both the behavioural and emotional responses during and after Cyberball were moderated by contextual factors. This was true for all groups, suggesting that CDI and HP patients were sensitive to the contextual cues

presented during the game. In addition, I replicated the novel finding from Chapter 5 that participants' OT concentrations (this time under natural conditions, rather than IN-OT) also predicted participants' prosocial behaviour, although this finding did not quite reach significance ($p$ = .061). However, in contrast to the findings reported in Chapter 5, the results were in line with the tend-and-defend hypothesis: Participants with greater OT concentrations were more prosocial towards an excluded ingroup member. Finally, the results also supported the theoretical argument discussed in Chapter 5 regarding the importance of the perception of threat in triggering tend-and- defend related behaviours. Participants who were first exposed to an outgroup member, were more prosocial towards the ingroup member (although the reverse did not apply). This suggests that participants were more prosocial towards an ingroup member when the salience of exclusion was high. Because previous research has found that social exclusion threatens psychological needs (Eisenberger et al., 2003), it follows that the increased salience of exclusion may have also increased the salience of the threat of potential exclusion, and thus triggered an ingroup bounded increase in prosocial behaviour, in line with the tend-and-defend hypothesis.

However, because I did not administer IN-OT in the study reported in Chapter 6 (and also Chapter 8) I am unable to infer causality, which is a limitation of the research. Nevertheless, these findings replicate those from an IN-OT study (Chapter 5), thereby providing support for the interpretation that OT concentrations predicted social behaviour, as opposed to social behaviour predicting OT concentrations. Given the current 'controversy' around IN-OT research (as previously discussed in *Methodological Considerations of Oxytocin Research*), it may be appropriate to continue examining the effects of natural concentrations of OT on social and emotional behaviours. This line of research avoids many of the recent criticisms, including studying the effects of

supraphysiological levels of OT; the 'black box' mechanism of IN-OT effects; the efficacy of IN-OT; and (indirectly) sample size (because IN-OT is expensive, there are often financial restrictions on recruitment). More importantly, this line of research may be used to inform interventions for clinical populations. Because the effects of IN-OT are relatively short, its use as a therapeutic tool may be limited. Understanding how to evoke natural increases in OT or adaptive OT responses to social stimuli may be a more sustainable approach to OT therapy. Thus if social behaviour during Cyberball did predict participants' OT response, this would still be a relevant and important finding.

In the study reported in Chapter 7, I created a novel economic game to assess the effect of OT on participants' behaviour as they witnessed, and subsequently sanctioned, economic exchanges between either inter- or intragroup dyads. This paradigm was also used to increase the salience of threat posed by the outgroup by creating competition for limited resources. Again, participants' behavioural and motivational responses were moderated by the group identity of players presented during the game; in line with the tend-and-defend hypothesis participants given IN-OT invested less, both in terms of financial investment and motivational investment, in outgroup members than in ingroup members. In addition, the results also support the theory presented in Chapter 1 of this thesis regarding the role of OT in a biological mechanism that may have formed over evolutionary time to promote group-serving as opposed to self-serving behaviour, in order to increase the functionality of one's group and therefore indirectly one's own fitness.

There are also limitations of this study; although the study employed a double-blind, between-subjects design and a large, mixed gender sample, and allowed me to infer cause and effect relationships, the study did not include a measure of endogenous OT concentrations. Consequently I was unable to investigate whether, as seen in Chapters 5 and 6, participants with higher OT concentrations would also demonstrate greater ingroup

bounded sanctioning. In addition, this study used an undergraduate sample, thereby limiting the generalizability of the findings.

Chapters 5 – 7 therefore all found moderating effects of group context on OT related behaviours in line with hypotheses formed on the body of literature by De Dreu and colleagues centred on the tend-and-defend hypothesis. Although the findings from each chapter can be used to support the tend-and-defend hypothesis, taking the findings together the results support the need to update the original hypothesis. The tend-and-defend hypothesis proposes three ways in which OT modulates cooperation: i) OT enables categorization of ingroup and outgroup members; ii) OT dampens fear responses to promote trust and cooperation, iii) OT motives non-cooperation towards potentially threatening outgroups. Results from the present thesis challenge the final prediction of the tend-and-defend hypothesis.

In Chapter 5 I found the opposite effect of IN-OT that would be expected based on the third prediction: IN-OT increased prosocial behaviour towards an outgroup member. As previously stated, this finding serves to highlight the importance of 'threat' in evoking behavioural outcomes that are compatible with the tend-and-defend hypothesis. Therefore a more nuanced hypothesis might state 'OT motivates non-cooperation towards potentially threatening outgroups, but cooperation towards vulnerable outgroups'. However, future research is needed to replicate these findings.

In Chapter 7 I created a competitive intergroup context (by introducing competition for limited financial resources) and found that IN-OT increased ingroup bounded sanctioning. Although the results arguably support the final prediction of the tend-and-defend hypothesis, it could also be argued that the results more accurately reflect 'an indifference' towards outgroup behaviour, as opposed to deliberate non-cooperation. Indeed recent research by Ten Velden, Daughters, and De Dreu (2016) found evidence

suggesting that the ingroup favouritism driven by IN-OT is intuitive rather than deliberated; suggesting that non-cooperation with the outgroup is a consequence of increased cooperation with the ingroup, and is therefore not a deliberate anti-social behaviour. These findings are therefore at odds with conclusion drawn from the original hypothesis that OT can, under certain circumstances, evoke premeditative aggressive behaviour towards threatening outgroups (i.e., De Dreu, 2016).

Taken together I propose that the third prediction of the tend-and-defend hypothesis is not supported in its current form. Recent research supports a more nuanced, revised prediction: 'OT motivates intuitive ingroup bounded behaviour when the outgroup is deemed to pose a threat'. This revision therefore implicitly implies that i) OT does not increase ingroup bounded behaviour in the absence of a threatening outgroup, and more importantly that ii) OT does not deliberately increase defensive behaviour towards outgroups, but rather an indifference towards the potential outcomes for the outgroup. In conclusions, and in light of recent research, a revised tend-and-defend hypothesis may more accurately be termed the 'tend-and-indifference' hypothesis.

*The Influence of Individual Differences on the Social Effects of Oxytocin*

The social effects of OT have been found to be moderated by individual differences in psychological variables. Understanding which variables moderate these effects and whether these changes are positive or negative is essential in order to confirm for whom and under what circumstances OT may provide beneficial effects. This question was addressed in several chapters, but was particularly targeted in the research reported in Chapter 8. In this final behavioural study I investigated the role of individual differences, and the effect of OT on emotion processing, studying whether an anticipated OT deficit in CDI patients would affect their cognitive empathy ability relative to HP patients and HC

participants. The results demonstrated that both CDI and HP patients displayed cognitive empathy deficits. When taking into account the results from Chapter 4 demonstrating that both CDI and HP patients had lower OT concentrations compared to HC participants, these findings support previous literature in which a positive relationship between OT and cognitive empathy (Shahrestani et al., 2013). Indeed CDI patients' OT concentrations predicted their performance during the Reading the Mind in the Eyes Task (RMET), although the reverse may also be true.

Surprisingly, however, individual differences in psychological variables were not found to moderate any social or emotional outcomes investigated in this thesis. In Chapter 8 individual differences in trait empathy, attachment style and autistic-like traits were not significant covariates of participants' performance in two cognitive empathy tasks. In Chapter 7 individual differences in trait empathy, anxiety, ingroup identity, and mood also failed to moderate participants' sanctioning and motivations during an economic game. These results are at odds with a body of research demonstrating that attachment style (Bartz, Zaki, Bolger, et al., 2010; Fang et al., 2014), parenting style (Riem et al., 2013; Van Ijzendoorn et al., 2011), and trait empathy (Feeser et al., 2015; Kret & De Dreu, 2013) moderate the behavioural effects of OT.

There are two possible reasons why the present research failed to replicate these moderating effects. First, the levels of internal consistency of the individual difference measures achieved in both studies were satisfactory but not good. It may therefore be the case that these constructs were not adequately captured by the measures I used. As such, the non-significant findings presented here may not reflect a genuine absence of the moderating effect of these variables. Second, the questionnaires used in the present research to measure the same constructs as those studied in previous research were not always the same as those originally used. This was done deliberately to test the

generalizability of the previous findings. For example, to test the generalizability of Feeser et al.'s (2015) finding that trait empathy moderates participants' performance during the RMET, rather than using participants' scores on the Empathy Quotient (Baron-Cohen & Wheelwright, 2004), participants in Chapter 8 completed the Interpersonal Reactivity Index (Davis, 1983) an alternative measure of trait empathy. Thus the absence of moderating effects may be due to using different measures of individual difference variables that may tap into slightly different constructs than those captured by the original measures. Although using different measures provides an insight into the generalizability of previous findings, it may be more useful at this stage of research to demonstrate replicability of these earlier findings using the same measures in order to ensure that these effects are robust.

Even though individual differences in psychological variables did not moderate the social effects of OT there were nonetheless interesting novel individual differences in salivary OT concentrations. Chapter 2 first demonstrated individual differences in response to the same dosage of IN-OT in 40 male undergraduate students, and this finding was replicated in Chapter 3. In Chapter 3 I investigated whether individual differences in response to IN-OT could be explained by various biological factors commonly referred to in the IN-OT research, including gender, menstrual cycle phase, digit ratio and time of day. Results demonstrated that individual differences in response to IN-OT were found in both male and female students and although there were significant differences in salivary OT concentrations between males and females at baseline, after the study began males and females had statistically similar OT concentrations, in both the PL and OT conditions. There was no difference in OT concentrations between menstrual cycle phases; between high and low digit ratio; or between morning and afternoon testing. These findings suggest that individual differences in OT are not accounted for by biological factors. Thus

opening up the question of whether individual differences in psychological factors might explain this variation. It is important to note that these biological factors may still influence behavioural effects of OT, but the findings do suggest that this influence is not driven by their moderating influence on peripheral responses to IN-OT.

In Chapter 4 I examined whether patients with CDI present with an OT deficiency. In contrast to the hypothesis, both CDI and HP patients had lower, albeit not significantly lower, OT concentrations, compared to age- and gender-matched HC participants. Neither patient groups presented with abnormally low OT concentrations; although I did not make any predictions regarding the extent of the OT deficit, it would not have been surprising if CDI patients had presented with very low OT concentrations, given the cause of their CDI diagnosis and the similarity between OT and AVP production and release. There are several potential explanations for this unexpected finding: (1) although HP patients should only have had anterior pituitary tissue removed during surgery, it is possible that surgery also caused sufficient disruption to the posterior pituitary to result in lower OT production; (2) OT and AVP systems may interact with each other, which could explain why CDI patients taking the synthetic AVP analogue, desmopressin, had higher than anticipated OT concentrations. However, the latter explanation would not account for the comparable OT concentrations observed in HP patients who were not taking desmopressin.

Finally, I examined whether individual differences in psychological variables could account for the individual differences in salivary OT concentrations. Again, no relationships were found. Continuing to investigate the causes of individual differences in salivary OT concentrations is an important avenue for future research.

*Limitations*

Although the research presented in the current thesis avoids many of the limitations in OT

research by using a within-subjects design when possible (to control for individual differences in OT concentrations), double-blind administration (in order to avoid experimenter bias), mixed gender samples (to improve generalizability and test assumptions of gender differences), and empirically confirming the influence of IN-OT on salivary concentrations of OT, there are some more general limitations that should be noted (chapter-specific limitations have been discussed elsewhere).

When considering in isolation the effects of a particular hormone on social behaviour there is the risk of missing similar or interacting effects of other hormones on the same behavioural outcome. Biological systems, especially endocrine systems, are highly interdependent and it may not be ecologically valid to tease out the effects of one hormone when under natural conditions this hormone acts in concert with other endocrine mechanisms. Against this critique, however, it could be argued that at this stage of research, it is prudent to identify the effects of each hormone in isolation before conducting multi-hormone studies.

In order to accurately determine the influence of each hormone, studies should be adequately powered. Although the current thesis tried to address this issue where possible, employing some of the largest sample sizes used in OT research to date, it was not possible to recruit the target sample size for the clinical study of CDI patients. As a result, some analyses were underpowered, as suggested by the power estimate of 49% of the original 3 group analysis reported in Chapter 4. Given the relatively rare incidence of CDI and HP, it was not possible to recruit a larger sample size within the geographical area where the research was conducted; it should nevertheless be acknowledged that the small sample size obtained limits the generalizability of the findings.

Furthermore, a measure of IQ or socio-economic status (SES) was not taken during the clinical study and it is therefore not possible to rule out the influence of these

variables on the observed results. Although IQ and SES are unlikely to affect OT concentrations or behaviour during and after Cyberball, it is possible that these may have had an effect on participants' performance on the cognitive empathy tasks and questionnaires presented in Chapter 8. However, given that several other, more relevant, variables such as trait empathy and attachment style did not influence participants' performance, it seems unlikely that IQ and SES would have an influence over and above these more likely candidates.

*Clinical Implications and Future Research*

In the course of this thesis I studied two novel clinical groups in which to investigate the effects of OT on social and emotional behaviours. Patients with CDI and HP were found to present with lower OT concentrations compared to age- and gender-matched HC participants, although their levels were not significantly lower and were not abnormally low in absolute terms. Importantly, both CDI and HP patients presented with a cognitive empathy deficit relative to their matched HC participants, and for CDI patients this was associated with their OT concentrations. Both CDI and HP patients also tended to over-report fearful and angry facial expressions when presented with other emotional expressions, and HP patients were significantly worse at correctly identifying fearful expressions when they were presented. This is particularly relevant because research suggests that individuals who over-report negative, especially fearful, expressions are at a greater risk of developing mood disorders (Beck, 1979, 2008). Future research should seek to replicate this study with a larger sample size in order to achieve suitable power to determine whether the finding that CDI and HP patients have lower OT concentrations is robust. Given the incidence of CDI, achieving this sort of sample size the study would probably require multi-centre collaboration over a longer period of time. Although this

would be a challenging study to conduct, I believe that this would be an interesting and clinically relevant follow-up study.

The presence of individual differences in response to IN-OT also has clinical implications. Given the continuing therapeutic interest in OT, and IN-OT specifically, it is important to identify the factors that predict whether an individual is going to be a high responder or a low responder and what impact this is likely to have on their social and emotional behaviour. For example, a recent meta-analysis (Mah, 2016) found that of the four studies that investigated the association between OT and post-natal depression, two found that lower endogenous concentrations of OT were associated with greater symptomology, while the two studies that had administered OT found negative effects. In this instance, and for many other psychopathologies, it may be more beneficial to devise a psychosocial intervention that reliably increases endogenous concentrations of OT, thereby avoiding these potential negative effects of IN-OT, or split effects (for example, when participants with high or low attachment avoidance behave in opposing ways after IN-OT administration). A further benefit of using psychosocial interventions (with or without IN-OT) is that the effects are also likely to last longer than those achieved by IN-OT administration alone.

A further implication of the research is that some of the logistical constraints applied to IN-OT studies can be relaxed or even abandoned, given my findings that gender, menstrual cycle phase, digit ratio and time of day do not influence peripheral responses to IN-OT. Relaxing these constraints would not only ease the planning and running of IN-OT studies, but also increase the sample size by not restricting research to unisex samples. Although these factors have been found to influence behavioural outcomes (Kret & De Dreu, 2013) the present findings show that they do not do so via peripheral responses to IN-OT.

Finally, there are a number of future studies that could be carried out with the results of the thesis in mind. In addition to a multi-centre investigation of CDI and HP patients, future research could seek to replicate the Cyberball paradigms reported in Chapters 5 and 6 with a more explicitly threatening outgroup member, in order to investigate whether this would evoke tend-and-defend behaviours. Future research could also replicate the novel third-party punishment and reward paradigm used in the study reported in Chapter 7 with a different (non-student) sample. It would be particularly interesting to use this paradigm, or an adapted version of it, with adolescents or children in order to test the validity of the theory that OT plays an important role in a biological mechanism promoting group-serving behaviour. Such a theory suggests that younger participants should behave in the same way as their older counterparts. If they do not, such a study could establish the age at which this mechanism is 'switched on'.

*Conclusions*

My aims in the current thesis were to investigate the role of OT in social and emotional behaviours and whether these effects are moderated by contextual factors and individual differences; and to address some of the methodological issues that arise in IN-OT research. Whereas previous research has focused on the effect of OT on direct social interactions, the present thesis extends this line of research by investigating the effect of OT on third-party behaviour. In addition, the thesis also reports a study of a clinical group that is novel in the context of OT research. The findings indicate that the social effects of OT do extend to third-party behaviour, and that these effects are moderated by contextual factors, although in contrast to previous research there was no evidence that individual difference factors moderate the effect of OT participants' social or emotional behaviour. This absence of a moderating effect may not reflect a genuine lack of influence, given that

internal consistency values for these individual difference measures were not high, and the measures themselves were not direct replications of those used in previous research. Thus this finding may reflect the fact that different measures capture subtle differences in the underlying constructs. The moderating effect of ingroup/outgroup membership was reliable and lends itself to the theoretical argument that OT plays a role in a biological mechanism, developed over evolutionary time, to promote group-serving as opposed to self-serving behaviour in order to preserve group functioning and therefore provide indirect fitness benefits to the individual. Future research could investigate this theory by using the novel paradigm presented in Chapter 4 in adolescents to investigate the age at which such a mechanism is 'switched on'.

Findings reported in the second half of this thesis provide evidence of a reliable effect of IN-OT on salivary OT concentrations, and the presence of sizable individual differences in response to IN-OT. While the thesis provides evidence that these individual differences in peripheral concentrations of OT in response to IN-OT are not accounted for by various biological factors (such as gender) that often act as logistical constraints in OT research, there was also no evidence that psychological factors could explain these differences. Thus future research should seek to identify which factors determine whether an individual is likely to be a high or a low responder to IN-OT. This is a crucial question to address if we are to establish for whom and under what circumstances IN-OT could be used for patients with psychopathologies.

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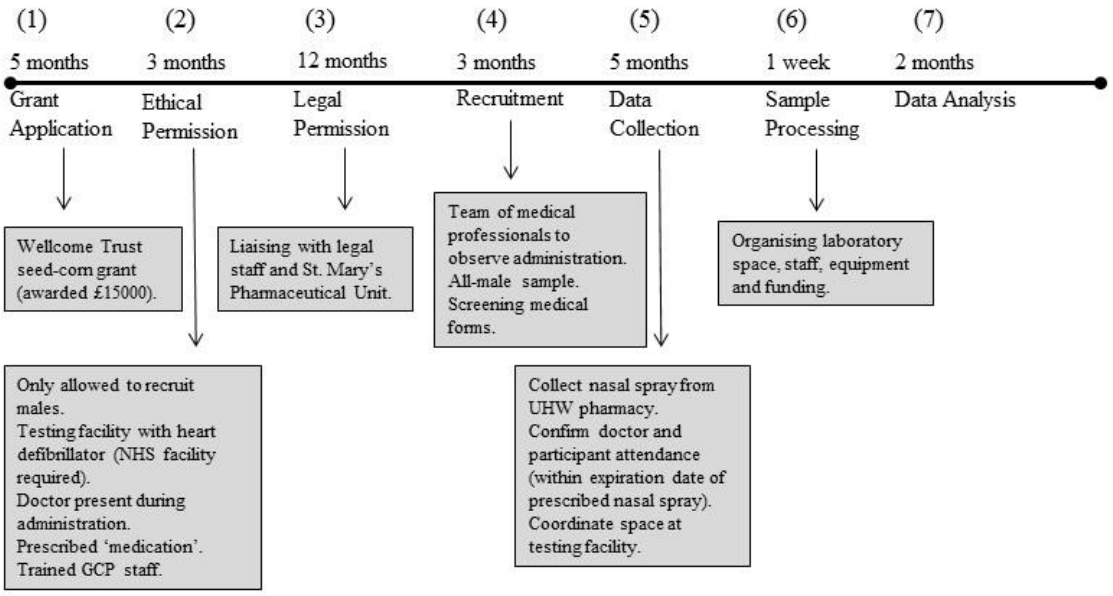
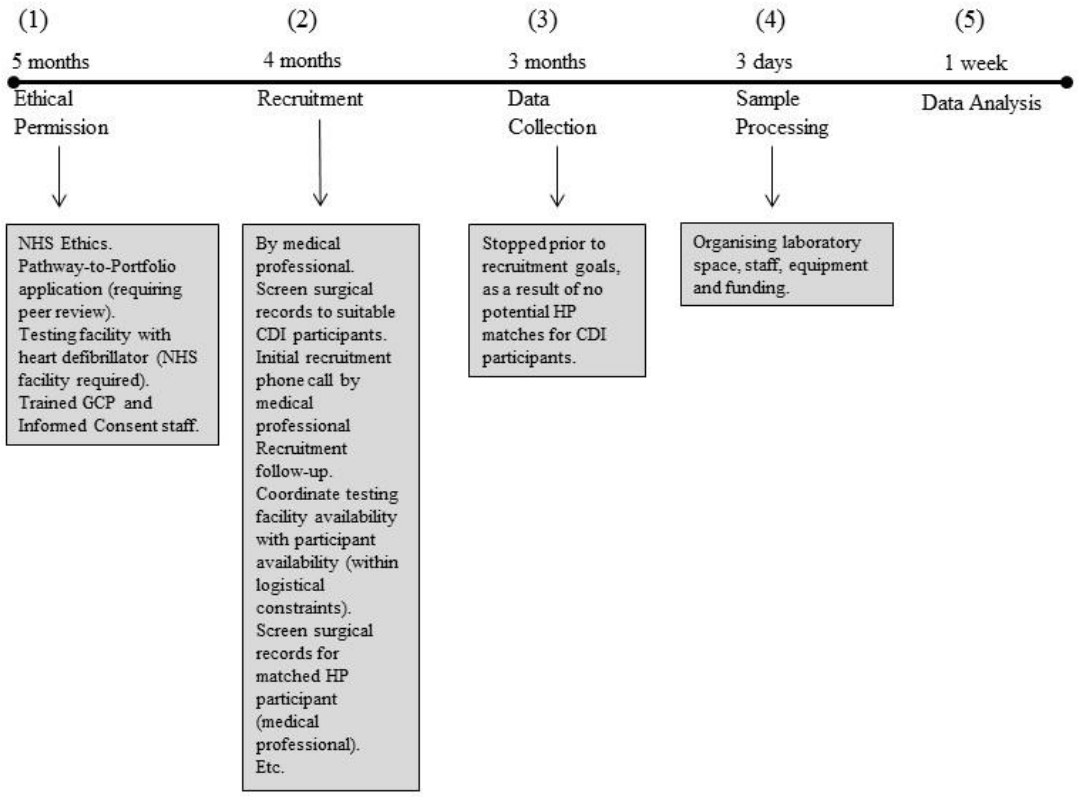
# Appendix 1

*Figure 1 – A schematic representation (with examples) of the OT trial conducted at Cardiff University (Chapters 2 and 6)*



# Appendix 2

*Figure 1 – A schematic representation (with examples) of the clinical trial conducted at Cardiff University (Chapters 3, 5 and 8)*

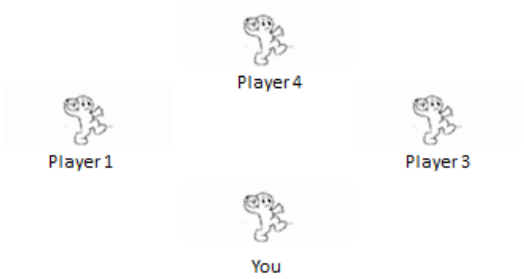


# Appendix 3

**Post Cyberball Questionnaire 1**

Please indicate, by circling the corresponding number, the extent to which **Player 4** feels each of the following emotions:

Schematic of Cyberball:




**Anger**

Not at all 1 2 3 4 5 Very Much

**Sad**

Not at all 1 2 3 4 5 Very Much

**Pain**

Not at all 1 2 3 4 5 Very Much

**Upset**

Not at all 1 2 3 4 5 Very Much

**Fearful**

Not at all 1 2 3 4 5 Very Much

**Happy**

Not at all 1 2 3 4 5 Very Much

**Scared**

Not at all 1 2 3 4 5 Very Much

**Cheerful**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Surprised**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Hurt**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

Please indicate, by circling the corresponding number, the extent to which **you** feel each of the following emotions:

**Anger**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Sad**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Pain**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Upset**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Fearful**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Happy**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Scared**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Cheerful**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Surprised**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

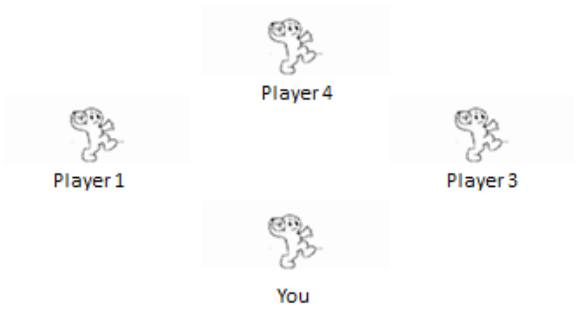
**Hurt**

| | | | | | | |
|---|---|---|---|---|---|---|
| Not at all | 1 | 2 | 3 | 4 | 5 | Very Much |

**Post Cyberball Questionnaire 2**

For each of the other players please underline the option that best describes their behaviour during the second round of the game.

Schematic of Cyberball:




**Player 1**
Involved everyone equally Excluded certain players Was excluded by others

**Player 3**
Involved everyone equally Excluded certain players Was excluded by others

**Player 4**
Involved everyone equally Excluded certain players Was excluded by others

How easy was it for you to believe you were playing against real people?

| Not at all easy | 1 | 2 | 3 | 4 | 5 | Very Easy |
|---|---|---|---|---|---|---|

# Appendix 4

*Figure 1 – A schematic representation (with examples) of the OT trial conducted at the University of Amsterdam (Chapter 7)*